The Molecular Basis of Transcriptional Activation by the CDP/Cux Transcription Factor

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Abstract

The CDP/Cux transcription factor is expressed as a 200 kDa protein that interacts rapidly and transiently with DNA. Proteolytic processing generates a shorter isoform, p110 CDP/Cux, that binds stably to DNA. Processing occurs at the G1/S transition of the cell cycle in normal cells, and constitutively in transformed cells. p110 CDP/Cux stimulates cell proliferation by accelerating entry into S phase. Transgenic mice expressing p110 CDP/Cux are more susceptible to different cancers.

CDP/Cux was originally described as a repressor of transcription. My goal was to verify whether CDP/Cux might also participate in transcriptional activation and characterize the molecular basis for transcriptional activation by CDP/Cux. Using the DNA polymerase α gene promoter as a model system, I showed that stimulation of a DNA pol α reporter correlated with DNA binding. Importantly, p110 CDP/Cux stimulated expression from the endogenous DNA pol α promoter. Linker-scanning analysis of the DNA pol α promoter identified a cis-element that was required for p110-mediated activation, yet was not bound by it. I determined that E2F1 and E2F2 cooperated with p110 in activating the DNA pol α promoter, and did so via this cis-element. Furthermore, CDP/Cux recruited these E2Fs to the promoter in chromatin immunoprecipitation experiments. Location array analysis revealed many targets common to p110 and E2F1. DNA metabolism and cell cycle targets were overrepresented, and further studies showed that p110 and E2F cooperated to activate many cell cycle genes.

I also described a second proteolytic event, which generated an isoform lacking two active repression domains in the C-terminus. Processing was observed in S phase, but not in early G1, suggesting that processing occurs in proliferating cells. I determined that caspases were responsible for this processing, and that this occurs in non-apoptotic conditions. A C-terminally-truncated CDP/Cux protein was a more potent activator of cell cycle-regulated promoters, and accelerated entry of Kit225 T cells into S phase, while uncleavable p110 CDP/Cux proteins were inactive in both assays. These results identified p110 CDP/Cux as a substrate of caspases in proliferating cells, and suggested a mechanism by which caspases may accelerate cell cycle progression.

Résumé

CDP/Cux est un facteur de transcription de 200 kDa interagissant de façon rapide et transitoire avec l'ADN. Son clivage protéolytique génère une isoforme plus courte, appelée CDP/Cux p110, se liant de façon stable à l'ADN. Ce clivage a lieu lors de la transition G1/S du cycle cellulaire dans les cellules normales, et est constitutif dans les cellules transformées. p110 stimule la prolifération cellulaire en accélérant l'entrée en phase S du cycle cellulaire. Ainsi des souris transgéniques exprimant p110 sont plus susceptibles de développer différents cancers.

CDP/Cux a originellement été décrit comme un répresseur transcriptionnel. Le but de mon travail a été de vérifier s'il pouvait également participer à l'activation transciptionnelle ainsi que de caractériser les bases moléculaires de cette activation transcriptionnelle. En utilisant le promoteur du gène de l'ADN polymérase αcomme modèle d'étude, j'ai pu montrer que la stimulation d'un gène rapporteur sous le contrôle du promoteur de l'ADN polymérase α était corrélée à la liaison de CDP/Cux à l'ADN. De plus, p110 stimule l'expression de l'ADN polymérase α endogène. L'analyse séquentielle du promoteur de l'ADN polymérase α a permis l'identification d'un élément cis requis pour l'activation par p110, mais sur lequel p110 ne se lie pas. J'ai déterminé qu'E2F1 et E2F2 coopèrent avec p110 pour activer le promoteur de l'ADN polymérase α, et ceci via cet élément cis. De plus, des expériences d'immunoprécipitation de chromatine ont révélé que CDP/Cux recrute les facteurs E2Fs sur le promoteur. Par des analyses de ChIP-chip, j'ai mis en évidence de nombreux gènes cibles communs à p110 et E2F1. Parmi eux, les gènes impliqués dans le métabolisme de l'ADN et dans la progression du cycle cellulaire sont surreprésentés. Des études complémentaires ont montré que p110 et E2F coopèrent pour activer la plupart des gènes impliqués dans la progression du cycle cellulaire.

J'ai par ailleurs décrit un second événement protéolytique, permettant de générer une isoforme ne possédant pas deux des domaines répresseurs actifs présents dans la région carboxy-terminale de la protéine. Ce clivage est seulement observé au cours de la phase S, et non lors de la phase G1 précoce, suggérant qu'il a lieu dans les cellules en prolifération. J'ai déterminé que les caspases étaient responsables de ce clivage, et ceci de façon indépendante de l'apoptose. Une forme de CDP/Cux tronquée de sa région

carboxy-terminale représente un meilleur activateur des cibles impliquées dans le cycle cellulaire et accélère l'entrée des cellules Kit225 T en phase S, alors qu'une forme non-clivable de p110 CDP/Cux est inactive dans ces deux essais. Ces derniers résultats ont identifié p110 CDP/Cux comme un substrat des caspases dans les cellules en prolifération, et suggèrent un mécanisme par lequel les caspases pourraient accélérer la progression du cycle cellulaire.

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Preface

The guidelines concerning thesis preparation issued by the Faculty of Graduate and Postdoctoral Studies at McGill University reads as follows:

1. Candidates have the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted, for publication, or the clearly-duplicated text (not the reprints) of one or more published papers. These texts must conform to the "Guidelines for Thesis Preparation" with respect to font size, line spacing and margin sizes and must be bound together as an integral part of the thesis. (Reprints of published papers can be included in the appendices at the end of the thesis.)

2. The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next. In order to ensure that the thesis has continuity, connecting texts that provide logical bridges preceeding and following each manuscript are mandatory.

3. The thesis must conform to all other requirements of the "Guidelines for Thesis Preparation" in addition to the manuscripts.

The thesis must include the following:

1. a table of contents;
2. a brief abstract in both English and French;
3. an introduction which clearly states the rational and objectives of the research;
4. a comprehensive review of the literature (in addition to that covered in the introduction to each paper);
5. a final conclusion and summary;
6. a thorough bibliography;
7. Appendix containing an ethics certificate in the case of research involving human or animal subjects, microorganisms, living cells, other biohazards and/or radioactive material.

4. As manuscripts for publication are frequently very concise documents, where appropriate, additional material must be provided (e.g., in appendices) in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.

5. In general, when co-authored papers are included in a thesis the candidate must have made a substantial contribution to all papers included in the thesis. In addition, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. This statement should appear in a single section entitled "Contributions of Authors" as a preface to the thesis. The supervisor must attest to the

accuracy of this statement at the doctoral oral defence. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to clearly specify the responsibilities of all the authors of the co-authored papers.

I have chosen to write my thesis according to these guidelines, with one published manuscript and two manuscripts to be submitted. The thesis is organized into six chapters: (I) a general introduction and literature review, (II-IV) manuscripts, each with their own abstract, introduction, materials and methods, results, discussion and references, (V) a general discussion of all results with references, and (VI) claims to original research.

Publications arising from work of the thesis

First-author publications

Truscott, M., Raynal, L., Premdas, P., Goulet, B., Leduy, L., Bérubé, G., Nepveu, A. CDP/Cux Stimulates Transcription from the DNA Polymerase α Gene Promoter. (2003) *Molecular and Cellular Biology* **23** (8): 3013-3028

Truscott M, Raynal L, Wang Y, Berube G, Leduy L, Nepveu A. The N-terminal region of the CCAAT displacement protein (CDP)/Cux transcription factor functions as an autoinhibitory domain that modulates DNA binding. (2004) J Biol Chem 279: 49787-94

Truscott M., Harada R., Vadnais C., Robert F., Nepveu, A. Co-operation of CDP/Cux With E2F in the Transcriptional Activation of Cell Cycle-Regulated Gene Promoters. (2006). Manuscript submitted.

Truscott M., Denault J.-B., Leduy L., Salvesen G., Nepveu, A. C-terminal Proteolytic Processing of the CDP/Cux Transcription Factor by a Caspase Enables Transcriptional Activation in Proliferating Cells. (2006). Manuscript in preparation.

Other publications

Goulet, B., **Truscott, M.**, Nepveu, A. A Novel Proteolytically Processed CDP/Cux Isoform of 90 kDa Is Generated by Cathepsin L. (2006) Biol Chem in press

Truscott, M. and A. Nepveu. (2005) Homeodomain Transcription Factors. Encyclopedic Refererence of Genomics and Proteomics in Molecular Medicine. Springer-Verlag. http://encref.springer.de/genomics/

Moon, N. S., Premdas, P., **Truscott, M.**, Leduy, L., Bérubé, G., Nepveu, A. S Phase-Specific Proteolytic Cleavage Is Required to Activate Stable DNA Binding by the CDP/Cut Homeodomain Protein. (2001) *Molecular and Cellular Biology* 21: 6332-6345

Santaguida, M., Ding, Q., Bérubé, G., **Truscott, M.**, Whyte, P., and Nepveu, A. Phosphorylation of the CDP/Cux transcription factor by cyclin A/Cdk1 modulates its DNA binding activity in G2. (2001) *Journal of Biological Chemistry* 276 (49): 45780-45790

Contribution of Authors

Raynal L: In manuscript 1, she performed the chromatin immunoprecipitation (ChIP) in Figure 7A and 7B

Premdas P: In manuscript 1, he helped set up the conditions for Figure 2

Goulet B: In manuscript 1, she performed Western blot analysis and EMSA of various short CDP/Cux isoforms in Figure 2C and 2D. In manuscript 2, she constructed one of the mutant CDP/Cux plasmids and carried out the Western blot and EMSA in Figure 5A and 5B.

Leduy L: In manuscript 1, he isolated the DNA pol alpha gene promoter from a library and subcloned it into a reporter plasmid. Lam also performed the luciferase assay for figure 1a. He did the ChIP presented in figure 8 and the infection and RT-PCR in Figure 9A. In manuscript 3, he generated the cell lines and performed the experiment in Figure 6.

Bérubé G: In manuscript 1, she constructed the CDP/Cux expression plasmids. In chapter 3, she generated all, but one CDP/Cux mutant construct.

Harada R: In manuscript 2, she set up the ChIP-chip microarray technique and generated identified the CDP/Cux promoter targets. She also prepared three E2F1 ChIP samples that were used in ChIP-chip.

Robert F.: In manuscript 2, the promoter microarray was designed and constructed by the group of Dr. Francois Robert at the Institut de Recherches Cliniques de Montreal

Denault J.B.: In manuscript 3, he produced the titrated, purified recombinant caspases used in Figure 4.

In Goulet et al., (2006), I performed the luciferase assay and Western blot in Figure 4B.

In Truscott and Nepveu (2005), I assisted in the research and writing of the text.

In Truscott et al. (2004), I purified the recombinant proteins, and performed the EMSA and Western blot in Figure 5. I also performed the reporter assay and Western blot in Figure 6. I assisted in the writing and submitting of the manuscript.

In Moon et al. (2001), I set up the reporter assay conditions used in Figure 8A.

In Santaguida et al. (2001), I generated the plasmid expressing CDP/Cux 1029-1505$^{\Delta Cy}$

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Chapter 1 Introduction and General Literature Review

This literature review will first focus on the regulation of gene expression, particularly at the level of transcriptional regulation. The E2F family of transcription factors will be described. Limited proteolysis and the caspase family of cysteine proteases will then be discussed. Finally, the features and roles of CDP/Cux, a transcription factor involved in cell cycle progression in mammalian cells, will be summarized.

1. Regulation of gene expression

The human genome is thought to contain 20,000-35,000 protein-coding genes and several thousand RNA genes. Genes account for only 2% of the genome, with the remainder regulating the proper spatial and temporal expression of these genes, as well as providing structural integrity to the chromosomes (130). Every cell in an organism contains the same genetic information, yet the same gene can be regulated differently in different cell types and under different circumstances (62).

In eukaryotes, chromosomal DNA is packaged into chromatin, which renders it inaccessible by transcription factors and the RNA polymerase (RNAP) machinery. There are three RNA polymerases: RNAP I, RNAP II, and RNAP III, that transcribe rRNA genes, protein-coding genes, and tRNA, 5sRNA, and small RNA genes, respectively. Each RNAP holoenzyme is made up of different, yet homologous subunits, and is subject to similar regulation. As my studies have involved the regulation of expression of protein-coding genes, I will describe what is known about RNAP II-regulated transcription.

If a gene is within packaged chromatin, it is inaccessible to transcription factors, and will not be expressed. Activation of its expression requires chromatin remodeling of the gene, and the sequences regulating its expression. Upon decondensation of higher-order chromatin, nucleosomes become available for remodeling. Nucleosomes consist of approximately 146 bp DNA wrapped around a core histone octamer, which contains two molecules of each of histone H2A, H2B, H3, and H4. Chromatin structure at all levels is dictated by post-translational modifications to histone tails, according to a "histone code",

as well as by the incorporation of histone variants, which marks chromosomal domains, and this governs gene expression patterns. To facilitate binding of transcription factors, and transcription, nucleosomes are remodeled by chromatin-modeling factors, and enzymatic modification of histones and other proteins. Examples of covalent modification include acetylation, phosphorylation, methylation, ubiquitylation, and ADP-ribosylation. (14, 95, 134, 198).

Gene expression is regulated by the precise arrangement of cis-regulatory regions, which are made up of clusters of several short recognition sites. These regulatory modules, or enhancers, interact with the sequences around the transcription start site, known as the proximal promoter.

Individual sequence-specific factors bind to DNA recognition sites with relatively low affinity. The strength and specificity in the regulation of expression of a particular gene comes from the arrangement of multiple factor recognition sites within the cis-regulatory region. The cooperative recruitment of multiple sequence-specific binding proteins, or transcription factors, results in synergistic activation of transcription. This nucleoprotein complex is referred to as the enhanceosome (reviewed in (31, 150)).

Gene expression is regulated by repression, de-repression and activation. Mechanisms of each are discussed in the following sections.

1.1 Mechanisms of Transcriptional Repression

Transcriptional repression can arise by competition for binding site occupancy. In this situation, a repressor would prevent binding by a transcriptional activator, or general transcription factors (GTFs), thereby preventing the formation of the pre-initiation complex at the transcription start site. Alternatively, an activator and a repressor can bind to adjacent sequences, and, via protein-protein interactions, the repressor can prevent the activator from interacting with the general transcription complex. This mechanism is known as quenching. Direct repression involves DNA-binding by a transcriptional repressor, and interferes with the assembly, or activity of the basal transcription complex, by means of a repression domain. Finally, squelching can occur when a repressor can sequester an activator such that it cannot bind DNA and activate transcription.

Derepression occurs as a result of removal of repressors and corepressors from promoters, as in the case of pocket-protein phosphorylation (see section 2.4.2)

1.2 Mechanisms of Transcriptional Activation

Transcriptional activators can recruit the basal transcription machinery either directly, or through a coactivator intermediate, which aids in the recruitment of general transcription factors to, and stabilization of, the pre-initiation complex on the gene promoter. Alternatively, protein-protein interactions between transcriptional activators and chromatin-modifying enzymes, results in the modification and remodeling of chromatin, such that it is accessible to the basal transcription machinery. These two mechanisms positively regulate the initiation of transcription. Elongation by RNAP II can also be regulated by transcription factors that interact directly with the RNAP II elongation complex (54, 179, 184, 258).

The above mechanisms apply to transcription factors that have both DNA binding domains, and transcriptional activation domains. However, the formation of nucleoprotein complexes involved in activation, or enhanceosomes (see section 1.1), also depends on DNA binding factors that do not have activation domains. These architectural transcription factors facilitate interactions between non-adjacent DNA-bound proteins, by distorting the conformation of the DNA.

1.3 Dual function transcription factors

It was initially believed that transcription factors were either repressors or activators. It is now clear that many transcription factors can both activate and repress transcription. The YY-1 transcription factor, named yin yang 1 for its dual function, has both activation and repression domains. YY1 can repress transcription by competition with activators, by interaction with co-repressors, or by changing the conformation of DNA. This latter mechanism has also been associated with transcriptional activation (reviewed in (203)). In the presence of E1A, YY1 can switch from being a repressor to being an activator. In fact, interaction with a number of other proteins also has this effect. YY1 can also function as transcription initiation factor, by binding near the transcription start site (201, 210).

Alternatively, within a transcription factor family, members with the same DNA binding specificity can function as activators or repressors. This occurs within the E2F family of transcription factors, which is discussed in detail in section 2.

In all the above examples, the promoter context influences the transcriptional outcome. Binding to a specific cis-element is influenced by neighbouring cis-elements and the trans-acting factors that bind them. In addition, the sequence of DNA surrounding the binding site can have an effect on the conformation of a transcription factor upon binding. In this sense, the DNA binding site would be seen to allosterically modulate the protein that binds it (128).

Nuclear receptors activate or repress transcription in a ligand-dependent manner. Upon ligand-binding, corepressors are exchanged for coactivators. The promoter context and tissue type can influence which coregulator is recruited to the same nuclear receptor. Nuclear receptors can also repress transcription by inhibiting other sequence-specific transcription factors (reviewed in (188)). Similarly, upon phosphorylation by PKA, the Hox-Pbx complex switches from being a transcriptional repressor to being an activator, via the exchange of histone deacetylase activity for histone acetylase activity (189).

2. The E2F Family of Transcription Factors

2.1 Discovery of E2F

In 1986, E2F was initially described as a cellular activity that bound and activated the adenovirus E2 promoter (123). E2F is now a collective term used to describe at least nine different E2F transcription factors: E2F1, E2F2, E2F3a, E2F3b, E2F4, E2F5, E2F6, E2F7 and E2F8. DP1 was also cloned by virtue of its sequence-specific binding to the same site on the E2 promoter, and was found to heterodimerize with E2F. A DP1 gene homolog, named DP2, was later cloned. A diagram of the E2F family members is presented in Figure 1.

2.2 E2F, Rb and the cell cycle

Early experiments showed that E2F associated with the retinoblastoma tumour suppressor, pRb. The association of pRB with E2F was disrupted by viral oncoproteins, such as adenoviral E1A, which resulted in abnormal cellular proliferation. These observations suggested that E2F could also regulate cellular genes whose products play roles in regulating proliferative signals and the control of DNA replication. Indeed, E2F sites were found in many gene promoters whose products are required for the regulation of the cell cycle and DNA replication, such as MYC (220) and DHFR (15). E2F activation of these promoters was observed, and was dependent on intact E2F binding sites.

It was soon determined that different E2Fs could be characterized as activators or as repressors. The expression of E2F1-E2F3a, the activator E2Fs, increases at the G1/S transition, and they bind and activate the expression of their targets preferentially in S phase. E2F3b, E2F4, E2F5, E2F6, E2F7, and E2F8 are repressor E2Fs. E2F4 and E2F5 were cloned by virtue of their interaction with p107 and p130 pocket proteins, which function as repressors (see 2.4.2 and 2.5.2). E2F6, E2F7, and E2F8 do not contain transactivation domains and repress E2F responsive genes in overexpression studies.

Interestingly, overexpression of E2F1, and possibly E2F2 and E2F3, can also induce apoptosis ((126), and ref therein: (182), (202), (98), (176), (177), (178), (226), (46), (88). However, the mechanism by which this occurs has not been fully elucidated. A number of E2F targets play roles in apoptosis, including ASK-1 (117), Chk2 (187), APAF-1 (69), p73 (107), p14ARF (12) etc. E2F-induced apoptosis likely involves interaction with pRB via a distinct interaction domain in Rb (48). The E2F1 marked box domain is important for its apoptotic function (88). It can involve the p53 pathway, but it can also occur via p53-independent mechanisms (2), (106).

2.3 DNA binding by E2F

On their own, E2F family members with only one DNA binding domain have weak DNA binding affinity (E2F1-E2F6). However, upon interaction with DP proteins, they form stable heterodimers that bind strongly to DNA (93). E2F7 and E2F8 each

contain two DNA binding domains, which are both used to bind the E2F consensus site: TTT(C/G)(C/G)CGC.

2.4 Regulation of E2F activity

2.4.1 Protein expression

The expression of E2F1, E2F2, E2F3a, with increased expression occurring in late G1 phase. E2F3b, E2F4 and E2F5 proteins are detected throughout the cell cycle (194). While E2F6 is expressed throughout the cell cycle, the highest expression is observed at the G1/S transition (43). E2F7 and E2F8 are constitutively expressed (122).

2.4.2 Interaction with pocket proteins

The pocket protein-binding domain in E2F overlaps with the transactivation domain. pRb, p107, and p130 interact with E2Fs, shielding the E2F transactivation domain, and recruiting repressive chromatin remodeling factors, such as histone deacetylases, methyltransferases, and chromatin remodeling factors, thereby actively repressing transcription. E2F1, E2F2, E2F3a and E2F3b specifically associate with pRb, while E2F4 can interact with all three pocket proteins, and E2F5 preferentially binds p130 (155), (92). In G0, the predominant complexes are p130-E2F, while p107-E2F complexes are observed in S phase, and pRB-E2F in G1 and S, although the latter is observed to a lesser extent (38). Pocket proteins are released from E2F upon phosphorylation of multiple serine and threonine residues by cyclin-dependent kinase complexes, or by competition with viral oncoproteins (34), (119). Upon pocket protein release, E2F target genes are derepressed and/or activated. E2F6, E2F7, and E2F8 lack pocket protein-binding domains and therefore are not subject to this type of regulation.

2.4.3 Post-translational modification

2.4.3.1 Phosphorylation

E2F1, E2F2, and E2F3a contain an N-terminal domain that can bind cyclin A. Upon interaction, cyclin A/CDK2 can phosphorylate E2F and DP1, thus downregulating activator E2F DNA-binding activity, and releasing E2F from DP1 (52), (86). This effect is not observed with E2F4 and E2F5, which do not contain cyclin A-binding domains (53).

Phosphorylation of E2F1 by ATM kinase or ATR kinase interferes with ubiquitination (see 6.4.4), thereby protecting E2F from degradation. This phosphorylation has been proposed to occur as part of the cellular response to DNA damage (136).

2.4.3.2 Acetylation

The CBP/p/CAF acetyltransferase complex can acetylate lysine residues slightly N-terminal to the DNA binding domain. This occurs on E2F that is not complexed with pocket proteins, and results in increased protein stability, as well as DNA binding activity (71).

2.4.4 Proteasome-mediated degradation

While the half-lives of free E2F1 and E2F4 are approximately 2-3 hours, binding of a pocket protein can extend the half-lives of these proteins to 10-12 hours (91), (97), (28). Further studies demonstrated that their degradation was mediated by the ubiquitin-proteasome pathway. $p14^{ARF}$ (the human homologue of mouse $p19^{ARF}$) can bind the E2F1 carboxyl-terminus and flag it for SCF^{Skp2}ubiquitin ligase-mediated polyubiquitination and subsequent proteasome-mediated degradation.

2.4.5 Subcellular localization

While E2F1, E2F2, and E2F3a are localized in the nucleus when overexpressed, E2F4 and E2F5 are found mainly in the cytoplasm. A nuclear localization signal (NLS) was found in the amino-termini of E2F1, E2F2, and E2F3a. It has been suggested that nuclear localization of E2F4 and E2F5, which do not have an NLS, is dependent on their association with pocket proteins. In early phases of the cell cycle, E2F4 and E2F5 are found in the nucleus complexed with pocket proteins and associated with promoters of genes that are repressed until G1 and S phases are reached. At this time, the pocket proteins are phosphorylated and dissociate from E2Fs, thereby allowing activation of transcription (4). Two nuclear export signals (NES) were identified in E2F4 and are important for CRM1-dependent nuclear export. It is thought that, for this reason, E2F4 and E2F5 cannot activate E2F responsive genes (73).

2.5 Transcriptional regulation by E2F

E2Fs interact with specific promoter elements in a manner that is dependent on surrounding sequences and partner proteins.

2.5.1 E2F as a transcriptional activator

E2F1, E2F2, and E2F3a are known as activator E2Fs. They activate a number of cell cycle regulators and genes with roles in DNA replication, and their overexpression can induce S phase entry.

Overexpression of E2F1, E2F2, or E2F3 induces quiescent cells to enter S phase (112). The combined loss of E2F1-3 in mouse embryonic fibroblasts abolishes their ability to enter S phase, undergo mitosis, and to proliferate, providing direct evidence for the essential role of activator E2Fs in proliferation, cell cycle progression, and development (244), (46).

Mutation of E2F sites in a number of cloned promoter fragments has been shown to diminish promoter activity. Examples of such promoters include E2F-1, E2F-2, cyclin A, cyclin E, and DHFR. Downregulation of E2F DNA binding activity by cyclin A-CDK2-mediated DP phosphorylation, as well as degradation of activator E2Fs in S phase, correlates with decreased transcriptional activity of a number of E2F target genes.

A number of coactivators have been shown to interact with E2F1: ACTR (141), ASC-2 (121), PARP-1 (207), (206), p300/CBP (204); (227) and Tip60 (218). These coactivators carry histone acetyltransferase activity and enhance transcriptional activation via their interaction with the activation domain of E2F. These protein-protein interactions are coincident with activation of E2F target genes.

2.5.2 E2F as a transcriptional repressor

E2F3b, E2F4, E2F5, E2F6, E2F7 and E2F8 are known as repressor E2Fs. E2F3b, E2F4 and E2F5 are constitutively expressed, and are localized in the nucleus in G0. E2F3b specifically interacts with Rb in quiescent cells.

As pocket proteins bind E2Fs, consequently masking their transactivation domains, E2Fs have been associated with the repression of transcription. Furthermore, pocket proteins can interact with histone deacetylase activity and histone methylase activity, thereby remodeling chromatin to a repressive state. Mutation of E2F binding

sites in a number of genes normally expressed in S phase results in their aberrant expression in G0/G1. E2F4 and E2F5, the repressor E2Fs, are believed to regulate cell cycle exit and differentiation. Upon release of repressive pocket proteins, the free repressor E2F is thought to be transcriptionally inert.

E2F6 lacks pocket protein-binding and transcriptional activation domains. It mediates repression via recruitment of Polycomb proteins and histone methyltransferases (173); (225) rather than pocket proteins. From chromatin immunoprecipitation studies and comparison of gene expression in wild-type and E2F6-/- mouse embryonic fibroblasts (MEFs), a model was proposed whereby E2F6 down-regulates expression of G1/S-activated E2F target genes specifically in S phase (77). Results also suggest a role for E2F6 in development and differentiation. Gene ablation in mice resulted in a mild homeotic phenotype (215). MEFs isolated from these mice displayed no defect in cell proliferation assays or in the ability to undergo quiescence.

E2F7 and E2F8 each have two distinct DNA binding domains, but no DP-dimerization, pocket protein-binding or transcriptional activation domains. Their expression is cell growth-regulated, with a peak in transcription occurring at S phase. Overexpression of either E2F7 or E2F8 in primary mouse embryonic fibroblasts results in decreased proliferation, accumulation of cells in G1, and repression of activator E2F gene targets (47); (147). E2F7 was found associated with promoters during S and G2 phases of the cell cycle, a time when other E2F/pocket protein complexes have not been observed.

2.6 Transcriptional targets of E2F

2.6.1 Identification of transcriptional targets

Transcriptional targets were initially studied using transient reporter assays. Many of these targets were confirmed in overexpression assays by measuring changes in endogenous gene expression. Correlations were made between the mutation of E2F binding sites and altered expression in G0 versus G1/S. These early studies involved a limited number of selected gene promoters.

With the development of large-scale systematic approaches came the ability to identify gene targets in an unbiased manner. Using E2F-overexpressing cells and DNA oligonucleotide microarrays, hundreds of new targets were identified, and previously

identified targets were confirmed. Chromatin immunoprecipitation (ChIP), using E2F-specific antibodies, enabled researchers to study the binding of specific E2Fs to endogenous gene promoters at different stages in the cell cycle. Similary, location analysis using ChIP-promoter microarray (also referred to as ChIP-chip) is being used for the genome-wide identification of targets.

Gene targets with roles in proliferation, cell cycle, DNA replication, DNA repair, apoptosis, differentiation, development and other physiological processes were identified. Correlations were made between the new gene targets and phenotypes observed in knockout and transgenic mouse models (see 2.7.3).

2.6.2 E2Fs target unique and overlapping gene promoters

Results from activator E2F mouse models (see below) suggest that these E2Fs regulate some common target genes, explaining their partially redundant roles, as well as some unique subsets of genes, which contributes to their unique functions.

2.6.3 Cooperation with other transcription factors

As E2Fs all bind to the same sequence, other factors are necessary to recruit specific E2Fs to their target promoters via cooperative interactions. A number of transcription factors, including NF-Y, Sp1, TFE3, YY1, and B-Myb, have been shown to cooperate with E2Fs in transcriptional regulation (261), (196), (75), (76), (233). By yeast two-hybrid screen, RYBP (Ring1- and YY1-binding protein) was identified as a protein that interacts with E2F2 and E2F3 specifically. The specificity of the interaction was mapped to the E2F marked box domain, a region originally shown to be necessary for E2F to interact with adenoviral E4 orf6/7 gene product. This interaction was necessary for the stable binding of E2F to the adenovirus E2 promoter (113); (171). RYBP cooperated with E2F2 and E2F3, but not E2F1, in the activation of the Cdc6 promoter. These factors bound the endogenous Cdc6 promoter at the G1/S transition, when the gene is expressed (196). Similarly, the E-box binding factor TFE3 interacted specifically with E2F3 and was dependent on the marked box domain. TFE3 and E2F3 cooperated in the synergistic activation of the p68 subunit gene of DNA polymerase alpha. Using ChIP assays with TFE3 or E2F3 knockout MEFs, it was shown that binding of each factor was dependent on the other (75).

Similarly, adjacent transcription factor binding sites in a gene promoter can determine whether an activator or a repressor E2F will bind a given E2F site in a gene promoter. The CCAAT site in the cdc2 promoter is necessary for binding by an activator E2F, while binding of a repressor E2F to a different E2F site is dependent on a (cell cycle homology region) CHR element (261).

2.7 E2F in other organisms

E2Fs are evolutionarily conserved among plants and animals, however there are no E2F homologs in yeast. In general, the more complex the organism, the more E2F factors there are and the more complex their roles.

2.7.1 E2F in *Caenorhabditis elegans*

Mutational analysis in C. elegans suggested that E2F (efl-1), DP (dpl-1) and Rb (lin-35) function as a corepressor complex, and individual mutants each give rise to a synthetic multivulval phenotype, synMUV B (142). lin-35 Rb functions in the negative regulation of G1 progression. efl-1 negatively regulates cell cycle entry, and dpl-1 was shown to function both in positive and negative regulation (22). lin-35/Rb and efl-1/E2F also negatively regulate a component of the anaphase-promoting complex that promotes the progression from metaphase to anaphase (72).

2.7.2 E2F in *Drosophila Melanogaster*

Drosophila possess two E2F genes (dE2F1 and dE2F2), one DP (dDP) and two pocket protein homologs (RBF1 and RBF2). dE2F1 mimics activator E2Fs, while dE2F2 is the repressor E2F. Reduced expression of a number of E2F target genes was observed in dE2F1 mutant embryos, while loss of dE2F2 resulted in increased expression of a number of targets. Interestingly, dDP mutant embryos, which would be predicted to have no E2F DNA binding activity, survive until the pupae stage. Like dDP mutants, dE2F1/dE2F2 double mutants display unregulated expression of E2F target genes. These results suggest that while E2F may not be required for cell cycle progression in Drosophila, it regulates the proper control of proliferation (reviewed in (45).

2.7.3 E2F in *Mus Musculus*

E2Fs 1 through 5 have been knocked out individually and in various combinations. Interpretation of the roles of individual E2Fs is complicated since redundant roles have been proposed for the activator E2Fs and repressor E2Fs. The genetic ablation of DP1 resulted in embryonic lethality prior to E12.5 (120), suggesting that the E2F family is dispensable for early embryonic development.

Disruption of E2F1 resulted in a range of tumours in older mice, and decreased T cell apoptosis (246), (66). E2F2 -/- mice also displayed increased tumourigenesis, and increased proliferation of hematopoietic cells (162). Interestingly, E2F3 gene ablation resulted in partial embryonic lethality, and MEFs displayed reduced cell cycle entry (105). While E2F1-/-E2F2-/- mice were viable (260), E2F1-/-E2F3-/-, and E2F2-/-E2F3-/- mice had more severe phenotypes (37). The different phenotypes highlight specific roles for different E2Fs, such as a pro-apoptotic role for E2F1 and a role for E2F3 in proliferation. MEFs with all three activator E2Fs ablated did not proliferate and were unable to reenter the cell cycle (244). These findings also suggest that redundant roles exist and are necessary for development.

E2F4-/- mice are runt and display hematopoietic, craniofacial, and intestinal defects (105), (185). E2F5-/- mice die shortly after birth due to hydrocephalus (137). Combined gene ablation of E2F4 and E2F5 is embryonic lethal, suggesting partial redundancy during development. However, E2F4-/-E2F5-/- MEFs exhibit normal serum starvation-induced growth arrest, and upon reintroduction of serum, normal kinetics of proliferation are observed. No major defects in E2F target gene regulation were observed (74). E2F6 gene ablation suggested that it functions in long-term somatic silencing of a number of male-germ-cell-specific genes, however, no cell cycle regulation defects were observed (180).

2.8 E2F and cancer

2.8.1 E2F as an oncogene

While E2F is a downstream mediator of the cyclin D/Rb pathway, whose deregulation is implicated in nearly all human cancers, E2F itself is not often mutated in cancer. Mutations in the Rb pathway are more frequently in the upstream regulators,

$p16^{INK4A}$, and cyclin D1. Viral oncoproteins such as adenovirus E1A or the human papilloma virus E7 bind and inactivate pocket proteins, thereby deregulating E2F activity, and allowing transactivation of E2F target genes in the absence of growth factor stimulation (reviewed in (23))

E2F-1 overexpression and gene amplification has been described in a number of erythroleukemia cell lines, however no amplification was observed in primary human acute lymphoid or myeloid leukemias. Amplification of E2F1 was also observed in 4% (1/23) of gastric and 25% (3/12) of colorectal carcinoma samples, while its general overexpression was also frequently observed (217). E2F1 was overexpressed in, and associated with, poor prognosis in non-small cell lung carcinomas (80) and esophageal squamous cell carcinomas (55). E2F3 was amplified and overexpressed in human bladder cancer (63), and was overexpressed in prostate cancer (67).

In vitro, E2F-1 cooperates with activated Ras in soft agar transformation assays. The transformed cells produce tumours in nude mice (111). Overexpression of E2F-1, E2F-2 and E2F-3 was also noted in transformed NIH 3T3 cells (245).

2.8.2 E2F as a tumour suppressor

As mentioned above, E2F1 overexpression induced apoptosis, and gene ablation resulted in increased tumourigenesis in adult mice. E2F3 mutation was shown to suppress pituitary tumour formation in tumour-prone Rb+/- mice (262). Therefore, E2Fs can also function in tumour suppression.

3. Proteolysis

Proteases can terminally degrade their substrates. They can also post-translationally modify their substrates by limited proteolysis. However, contrary to other post-translational modifications, such as phosphorylation and acetylation, proteolysis is irreversible.

3.1 Limited proteolysis

The functional consequences of limited proteolysis, affect the activity, localization half-life and binding interactions (to DNA or other partners) of the target protein. These include the removal of an inhibitory domain, thereby activating the substrate.

Inactivation can also result, often yielding a dominant negative. Alternatively, limited proteolysis can redirect a protein to a different subcellular localization, or change its biochemical activity.

The blood coagulation pathway is a complex cascade of sequential limited proteolytic reactions, ending with cleavage of fibrinogen, by the thrombin protease, yielding insoluble fibrin, which polymerizes and forms a clot (reviewed in (166)).
Another well-characterized cascade of limited proteolysis is the complement cascade, which is triggered by the binding of antibodies to a pathogen. Inactive precursors are cleaved into a large active fragment, which binds to the pathogen and triggers the next cleavage event, and a small peptide fragment that mediates an inflammatory response (224).

3.2 Activation of protease zymogens

Proteases, themselves, are regulated by limited proteolysis. Expression of a zymogen results in an inactive protease precursor that can be activated either by auto-proteolysis, or by an upstream protease, and elicit a quick response.

3.3 Regulation of subcellular localization by proteolysis

Secretory proteins are usually synthesized with an amino-terminal extension called the signal peptide, which is co-translationally cleaved by signal peptidase during translocation across the membrane (240).

Intramembrane proteolysis of membrane receptors can initiate intracellular signaling in response to an extracellular cue. Upon ligand binding, the Notch receptor is cleaved by γ-secretase complex proteases, and the Notch intracellular domain translocates to the nucleus, where it interacts with the CSL (CBF/RBPkJ, Suppressor of Hairless, LAG-1) transcription factor, displacing a co-repressor, thereby converting CSL to a transcriptional activator (7, 110). Sterile Regulatory Element Binding Protein (SREBP), upon proteolysis, translocates to the nucleus, and regulates the expression of genes involved in cholesterol and fatty acid biosynthesis (25). Similarly, the amyloid precursor protein (APP), and the ErbB4 receptor tyrosine kinase, are subject to intramembrane proteolysis, and signaling to the nucleus (29, 169).

3.4 Change of biochemical activity

Protease activated receptors (PARs) are another class of transmembrane receptors that are activated by proteolytic cleavage. Extracellular cleavage of PARs reveals an amino-terminal sequence that initiates transmembrane signaling by interacting with a nearby extracellular loop in the receptor (70).

Prohormones are synthesized as inactive precursors. Upon limited proteolysis, catalyzed often by trypsin-like enzymes, the active hormone is generated (reviewed in (166)).

The p50 subunit of NF-kappaB is generated from a p105 precursor. p105 is proteolytically processed by the proteasome, cotranslationally. Upon endoproteolysis, the C-terminus is selectively degraded by the proteasome in a ubiquitin-dependent manner. A glycine-rich region in p105 prevents complete degradation, thereby allowing for the generation of p50 (160).

3.4.1 Regulation of transcription factor activity

HCF-1 is a transcriptional coactivator that is autocatalytically processed in the nucleus. The proteolytic products associate together (242, 243). This proteolysis regulates its interaction with protein partners, and consequently its function as a transcriptional coactivator. The FHL2 coactivator associates with specifically with uncleaved HCF-1, via its proteolytic processing domain, and coactivates the HCF-1-activated HSV immediate early promoter. Upon proteolysis, the interaction is lost, as is the ability of FHL2 to coactivate HCF-1-regulated gene expression (236). HCF-1 is essential for different stages of the cell cycle (81, 114). Interestingly, the amino-terminal cleavage products promote cell cycle entry from quiescence, while the carboxy-terminal products support cytokinesis (114).

Signal transducer of activated transcription (STAT) transcription factors are activated downstream of cytokines, and regulate cell proliferation, differentiation, and survival. Limited proteolysis of STAT transcription factors by serine and cysteine proteases removes the activation domain, creating a functional dominant negative. Short isoforms are detected in myeloid progenitor cell lineages, but not in mature myeloid cells.

Proteolysis correlates with the loss of expression of STAT-regulated genes in mature myeloid cells (94).

The IRF-1 transcription factor binds and activates the IFN-α gene promoter. Another IRF family member, IRF-2, was shown to displace IRF-1, and repress transcription (89). Proteolytic processing of the IRF-2 increases its DNA binding affinity, and, consequently, is a more potent transcriptional repressor (239)

4. Cysteine Proteases

Depending on which amino acid is acting as the nucleophile in the catalytic triad of the active site, a protease is classified as a cysteine protease, a serine protease, an aspartic acid protease, or a metalloprotease, whose activity depends on a zinc ion.

The cysteine protease 'family' members share the same catalytic cysteine residue, however, their evolutionary origins are very different. Cysteine proteases have been divided into clans. Within a clan, proteases often have low amino acid sequence homology, however their folding patterns are conserved (11).

4.1 Caspases

The caspase family of cysteinyl-aspartate-specific proteases belongs to Clan CD. There are 14 known caspases, which can be divided into three groups, depending on trends in substrate specificity, the length of their prodomain, and, to a certain extent, their function. Their targets always have an aspartate residue in the P1 position, meaning that cleavage follows the aspartate residue. Amino acids in positions P2, P3, and P4 play a role in determining the specificity of recognition by individual caspases (1, 20, 124). Figure 2 shows the different groups and their substrate specificity.

Deregulation of caspases can lead to autoimmunity and immunodeficiency, cancer, neurodegenerative disorders, inflammation, sepsis, and reperfusion injury after ischemic episodes (199, 214, 222, 254).

4.2. Structure of caspases

Caspases are synthesized as zymogens, that have a prodomain, which varies in length, followed by a large subunit, and a small subunit (see Figure 2). Upon activation,

the zymogen is cleaved at specific arginine residues located within the prodomain, and the large and small subunits. The large and small subunits from two caspase molecules form heterotetramers.

Caspase prodomains contain conserved structural motifs that belong to the death domain superfamily, which consists of the death domain (DD), the death effector domain (DED), and the caspase recruitment domain (CARD). These motifs interact with other proteins with the same motif, via homotypic interaction. DEDs and CARDs are found in caspases that function as initiators of apoptotic or inflammatory signals (see Figure 2) (223).

4.3 Regulation of Caspase Activity

Caspases function in apoptosis, inflammation, development, and in proliferation. As most of the studies with caspases are associated with apoptosis, the caspases will be introduced in this context. Non-apoptotic functions will be discussed thereafter.

4.3.1 Activation of caspases in apoptosis

Apoptosis is a form of programmed cell death in which a cell is dismantled in a regulated fashion, without disrupting the extracellular environment. Characteristics of apoptosis include destruction of the cellular architecture, membrane blebbing, chromatin condensation and disassembly of the nuclear envelope, detachment of the cell from the embedding tissue, and clearance reviewed in (20, 222, 223).

The general model follows that initiator caspase zymogens are recruited into protein complexes via homotypic interactions with upstream signaling molecules. Two very well characterized pathways are activated in response to external apoptotic stimuli, or internal apoptotic stimuli.

4.3.1.1 Extrinsic apoptotic pathway

Briefly, following triggering by ligands, members of the tumour necrosis factor (TNF) family (TNFR1, CD95, TRAIL, etc.) that contain DDs, form a death-inducing signaling complex (DISC) containing cytoplasmic adaptor molecules, such as Fas-associated death domain (FADD/MORT1), which recruits procaspase-8 or procaspase-10. According to the "induced proximity" model, high local concentrations of appropriately

oriented procaspases undergo autoproteolytic activation. However, it is the dimerization of the procaspases that imparts activity (19).

4.3.1.2 Intrinsic apoptotic pathway

Similarly to the extrinsic pathway, dimers of the initiator caspase zymogen, procaspase-9, are induced via the formation of the apoptosome complex. This complex forms in response to a number of apoptotic stimuli, such as heat shock, oxidative stress, cytotoxic stress, and DNA damage. These insults induce the release of cytochrome c from the mitochondria, which, in the presence of dATP, catalyzes the oligomerization of Apaf-1 (apoptotic protease-activating factor-1). This results in the recruitment of procaspase-9, facilitating dimerization and activation (20, 223).

4.3.1.3 Initiation of the inflammatory response

Likewise, the inflammasome complex serves as the assembly site for caspase-1 and caspase-5 dimerization and activation. Recruitment of the caspases occurs via CARD domain homotypic interactions with NALP-1 (149).

4.3.1.4 Effector caspases

Effector caspases, such as caspase-3 and caspase-7, are activated downstream of initiator caspases. They are present, usually within the cytosol as inactive dimers, and are activated by limited proteolysis, which allows the activation loop to translocate and form the active site (19). Substrates for these caspases are involved in scaffolding of the cytoplasm, and nucleus, proteins involved in signaling, cell cycle, and DNA repair, and transcription regulatory proteins (see 4.6 for examples). These caspases are often referred to as executioner caspases, and this is thought of as the step after which the cell is committed to die.

4.3.2 Negative regulation of caspase activity

Both precursor and active caspases can be restricted via direct contact by viral and cellular gene products, as well as artificial caspase inhibitors.

4.3.2.1 Natural caspase inhibitors

Under conditions of overexpression, c-FLIP (Flice-like inhibitory protein, where Flice is Fas-associated death domain interleukin 1β-converting enzyme) proteins can inhibit the activation of procaspase 8, by blocking its recruitment to the DISC. However,

at low concentrations, cFLIPL can heterodimerize with procaspase 8, facilitating its activation (129).

Inhibitor of Apoptosis (IAP) proteins were first discovered in baculoviruses, based on their ability to prevent host cell death upon viral infection. Homologs in yeasts, *C. elegans*, *Drosophila melanogaster* and vertebrates have since been described (reviewed in (230)). IAPs contain a minimum of one baculoviral IAP repeat (BIR), and can prevent apoptosis upon overexpression. Whether each IAP can function as a caspase inhibitor in physiological conditions has been a subject of debate. It is well accepted that XIAP binds and inhibits the catalytic activity of caspase 3, caspase 7, and caspase 9 (32, 102, 186, 213). However, while cIAP1 and cIAP do provide protection against apoptosis, they do so by a mechanism other than inhibition of caspase activity (56). Evidence suggests that survivin, another IAP family member, may play a role in cytokinesis, rather than in the regulation of apoptosis (131).

p35 is another baculoviral protein that can inhibit caspases in vivo. It can inhibit caspases 1, 3, 6, 7, 8, and 10 with high efficiency. While some caspase inhibitors inhibit non-caspase proteases, p35 could not (26, 259). After cleavage by a caspase at Asp87, the cleaved subunits form an inhibitory complex with the caspase, which can be dissociated by SDS (259).

The Cowpox virus product Cytokine Response Modifier A (CrmA), was first described with regards to its ability to inhibit caspase 1 to prevent cleavage of interleukin-1β, thereby preventing an inflammatory response. CrmA can also bind caspase-8 with high affinity, and could inhibit these two caspases *in vivo*. Inhibition is brought about as CrmA functions as a pseudosubstrate that binds the active proteins, thereby inactivating them. CrmA variants have been made by replacing its tetrapeptide pseudosubstrate region, LVAD, with tetrapeptides that are good substrates for different caspases, including DQMD from the p35 protein. These variants target different caspases (59).

Bcl-2 family members have been suggested to act both upstream, and downstream of caspase activation. The best-known model suggests that antiapoptotic Bcl-2 family members preserve mitochondrial integrity, thereby preventing the release of cytochrome c from the mitochondria, the assembly of the apoptosome, and caspase 9 activation, followed by caspase 3 activation (247). Bcl-2, and its antiapoptotic family member Bcl-

XL, have each been detected in a complex with the endoplasmic reticulum-localized protein, p28Bap31, and procaspase-8 (168). In addition, Bcl-2 interacted with activated caspase-3, thereby preventing caspase-3 activity, via the K7 Kaposi's sarcoma-associated herpes virus protein (237). Interestingly, Bcl-2 also delays cell cycle re-entry of quiescent cells, although it does not influence cycling cells (172). Overexpression of Bcl-2 in colon carcinoma cells led to senescence (42). This function is genetically separable from its antiapoptotic role, since mutation of Tyr28 abolished the cell cycle constraint, but not apoptosis prevention (101).

Alternatively-spliced, catalytically inert mRNAs for caspase 2, caspase 6, caspase 8, and caspase 9 have been detected, and were suggested to prevent activation of procaspases (5, 50, 64, 100, 200, 212). Phosphorylation of procaspase-9, or the large subunit, by the serine/threonine kinase, Akt, inhibited its proteolytic activity (30).

Cleavage of procaspase-7, procaspase-8, or procaspase-9 by calpains, in a manner different from one that activates them, results in their inactivation inactivates (35).

4.3.2.2 Synthetic caspase inhibitors

Caspase inhibitors are based upon substrate cleavage sites, and act as pseudosubstrates for active caspases. The peptides are linked to a chemical group, such as halo-methyl ketones, that influence permeability and irreversibility (reviewed in (58)). However, while they can be highly reactive, these inhibitors can inhibit other proteases (197). As such, conclusions as to the identity of a proteolytic activity cannot be made based on observations made using these inhibitors.

However, new inhibitors are continually being developed. Activity-based probes for caspases-3, -7, -8, and –9 were recently described, and allowed for the identification of a novel, partially cleaved, caspase-7 (13).

4.4 Subcellular localization

The identification of the subcellular localization of caspases is complicated by the nature of their regulation. Caspases are first expressed as zymogens. Upon activation, or in response to different cues or interacting partners, they could relocate to a different compartment. The induction of apoptosis leads to disruption of the nuclear membrane,

complicating the analysis even more. It is not surprising that conflicting results were obtained by different laboratories, as methods of analysis and reagents vary, as do the cell or tissue types analyzed, and the apoptotic stimuli.

In one study, wild-type and catalytically inactive amino-terminally-GFP-tagged caspases were transiently expressed in cells. The subcellular localization of the caspases was observed in living cells. Caspase-1, -3, -6, -7, and -9 were mainly cytoplasmic, with some degree of nuclear localization. Caspase-2 was primarily nuclear, and caspase-8 and caspase-10 were cytoplasmic (205).

Another study assessed subcellular localization by cellular fractionation and Western blot analysis of endogneous caspases. This method distinguished between proforms, and cleaved forms of the caspases. In untreated Jurkat T lymphocytes, procaspases-2, -3, and -9 were localized in the cytosol and mitochondria. Procaspase-2 was also found in the nucleus, while procaspases-7 and -8 were cytosolic. In apoptotic cells, caspase-3 was found in the cytosol, mitochondria, and nucleus (257).

4.5 Caspase Activity in Non-Apoptotic Conditions

Non-lethal roles for caspases in immune functions have been known for a long time. The first caspase to be described, caspase 1, was originally named ICE, or interleukin 1β-converting enzyme, for its role in processing interleukin 1β in inflammation (221). See below, as well as Figure 2 for non-apoptotic roles of caspases, that were predicted from mouse knockout phenotypes.

4.5.1 Differentiation

Caspase activity has been detected during sperm formation in *Drosophila melanogaster*. Sperm individualization involves the encapsulation of individual sperm by an independent plasma membrane, and the elimination of cytoplasm. This process requires the activities of DRONC, an apical caspase, and DRICE, an effector caspase (104).

Upon induction of osteoblastic differentiation, active caspase fragments are detected by Western blot, and inhibition of caspase activity with inhibitors blocks exit from the cell cycle (156).

Caspase activation also occurs in the differentiation of red blood cells and lens fiber cells. This is accompanied by some of the morphological changes associated with apoptosis, such as chromatin condensation, and nuclear destruction (108, 161, 253). Similarly, skeletal muscle differentiation is dependent on caspase activity, and leads to actin fiber reorganization, and myosin light chain kinase, which plays a role in membrane blebbing (65). Therefore, apoptosis and induction of differentiation of some tissues may use common cellular mechanisms. However, it is not known how caspase activity is controlled, such that apoptosis does not occur.

The differentiation of monocytes, specifically into macrophages, and not into dendritic cells, is inhibited by treatment with caspase inhibitors, suggesting a requirement for caspases(211). In addition, the terminal differentiation of keratinocytes is also correlated with the activation of caspases, as evidenced by antibodies specific for activated caspases, and increased expression levels of caspase-14 (57, 138).

4.5.2 T cell and B cell activation

An inherited mutation in humans, causing defects in the activation of T, B, and NK (natural killer) cells, was mapped to caspase-8 (36). While targeted disruption of caspase 8 in mice is embryonic lethal, insight into its function in T cells was gained by targeted deletion of caspase 8 in the T cell lineage. This resulted in fewer than normal peripheral T cells, and an inability to mount an immune response following infection with choriomeningitis virus. In addition, ex vivo, the ability of T cells to respond to activation stimuli was impaired. Results from this study revealed that caspase 8 plays an essential role in T cell homeostasis and T cell-mediated immunity (190).

Results from a number of studies suggested that caspases were activated following T cell stimulation with PHA (154), or with IL-2 and mitogens (241), where no evidence of cell death was detected. Levels of cleavage of procaspase 3 into its active form were higher than what is normally observed upon induction of apoptosis (154). This suggested that caspase activity could be regulated following activation. It was later shown that granzyme B, or another aspartate-specific protease, was activated post-lysis, and was responsible for the cleavage of procaspase 3(250). However, following this study, another group showed that caspase 3 was activated following PHA-stimulation of T cells.

Furthermore, IL-2 release from activated T cells was blocked in the presence of peptide-based caspase inhibitors (181).

Fas-associated death domain protein (FADD) was originally described as being involved in the recruitment of caspase-8 to the DISC following stimulation of the extrinsic apoptotic pathway. However, similarly to caspase-8 knockout mice, ablation of FADD led to hematopoietic precursor cells with impaired colony-forming ability, and fewer T cell progenitors, as well as impaired heart muscle development (248). In mice that lack FADD function, either due to gene ablation, or expression of a dominant negative, T cells are defective in activation-induced proliferation (167, 255). Furthermore, T cells expressing dominant negative FADD arrest at G0/G1 of the cell cycle (167). Mice expressing a FADD mutant, in which serine 191 was mutated to aspartate, were smaller, anemic and presented splenomegaly. No apoptotic defects were found, however, their T cells were defective in cell cycle progression. Interestingly, FADD is differentially phosphorylated throughout the cell cycle, at serine 194, which is equivalent to serine 191 in mice, further suggesting that post-translational modification could modulate its function (195).

4.5.3 Non-autonomous, apoptosis-independent function

In the normal, developing *Drosophila melanogaster* wing disc, a large number of cells are lost (40-60%). However, this death induces compensatory proliferation, and may be mediated by the stimulation of the mitogen wingless. Activation of the DRONC caspase has been shown to be necessary and sufficient for compensatory proliferation, which suggested that caspases can function, nonautonomously, in the induction of proliferation (103).

5. The CDP/Cux Transcription Factor

The CDP/Cux/Cut family of proteins is conserved through evolution. cDNAs for homologs of the original member, *Drosophila melanogaster* Cut, have been isolated from many species including human (165), dog (6), mouse (231), rat (249), and C. elegans (NCBI accession number U28993). There is no known yeast homolog. The mammalian

homologs will be referred to, in general, as CDP/Cux. The CDP/Cux/Cut proteins contain two or more DNA binding domains: one, two, or three Cut repeats, and Cut homeodomain.

The CCAAT Displacement Protein was first described in the sea urchin, *Psammechinus miliarus*, as a factor that prevented the binding of a CCAAT-binding factor to the sperm-specific histone H2b-1 gene in tissues where the gene is not expressed (10). The gene coding for this activity was cloned and found to resemble the *Drosophila* cut gene.

5.1 *Drosophila melanogaster* Cut

The name 'Cut' comes from the cut wing phenotype of a viable mutation at the cut locus in *Drosophila*. Like other homeotic selector genes, *cut* is involved in the cell fate specification in many different tissues. It is expressed in, and is necessary for the normal differentiation and development of tissues in the embryo (16, 17, 21). In the adult, cut expression was the same, but was also expressed in additional tissues, suggesting that it is also required for the maintenance of the tissues in which it is expressed (18).

Interestingly, in follicle cells and at the wing margin, Cut was shown to have non-autonomous effects in cells adjacent to those in which it was expressed. The mechanism likely involves cut-mediated regulation of the expression of molecules involved in intercellular signaling (44, 151). This function could be evolutionarily conserved, as both murine Cux and human CDP homologs complemented some *cut* mutants in *Drosophila* (143).

5.2 Mammalian CDP/Cux

Drosophila Cut and mammalian CDP/Cux share 5 regions of homology: a coiled-coil (CC) domain, three Cut repeats (CR, CR2, and CR3), and a Cut homeodomain (HD). Full-length CDP/Cux is a ubiquitously expressed 200kDa, 1505 amino acid protein, referred to as p200. Mammalian CDP/Cux proteins contain a putative nuclear localization signal (NLS), two active repression domains within the carboxy-terminus, as

well as an inhibitory domain located at the extreme amino-terminus, that inhibits DNA binding by CDP/Cux (reviewed in (164)). These domains are illustrated in Figure 3.

The human CUTL1 (Cut-like 1) gene is located on chromosome 7, band 22. It extends over 340 kb, includes 33 exons and codes for two proteins: CDP-1 and CASP (cut alternatively spliced product) (256), (78). Five different CDP transcripts have been detected (see Figure 4, (256), (85)). CDP transcripts that generate a full-length protein contain one of two alternate first exons, and are all spliced to a common exon 2. These proteins are coded for by exons 2 to 24. In addition, two different splice acceptor sites exist in exon 15, and further splicing has been detected, in which exon 16 was absent. The effect of the latter splicing event is unknown.

The transcript that generates the CASP protein is generated by the splicing of exon 14 to exon 25. It codes for the amino-terminal coiled-coil domain, but none of the DNA binding domains. The CASP protein contains a transmembrane domain and is located in the Golgi apparatus (78).

An additional CDP/Cux transcript, which is initiated within intron 20, is detected in the placenta, thymus, CD4+/CD8+ and CD4+ T cells. While expression in normal human mammary epithelial cells and breast tissues is weak or undetected, many breast tumor cell lines and breast tumor samples show higher expression of this transcript. This intron 20-initiated mRNA generates a shorter CDP/Cux isoform, p75, that contains amino acids 1062-1505, within which is CR3, Cut HD, and the carboxy-terminal domain (85).

A third CDP/Cux isoform, p110 CDP/Cux isoform has been characterized in proliferating cells. It is generated in a cell cycle-dependent manner in normal cells by proteolytic processing by a nuclear Cathepsin L isoform at the G1/S transition. p110 CDP/Cux contains CR2, CR3, the Cut HD, and two carboxy-terminal active repression domains (158).

An additional isoform has been identified in epithelial cells: p90 CDP/Cux. Like p110, it contains CR2, CR3, the Cut HD, and the C-terminal domain, and is the product of proteolytic processing by Cathepsin L (84).

A second human CDP gene, CUTL2, maps to 12q.23.13. Cux2 is expressed, late in fetal development, in neural tissue (183). It also functions in organogenesis during embryonic development (109). Like CUTL1, it expresses a protein with four DNA

binding domains. DNA binding activities were similar to those of the corresponding CDP-1 isoforms. As shorter Cux2 isoforms were not detected, it is thought to function only as a transcriptional repressor (79).

5.3 DNA Binding by CDP/Cux

CDP/Cux contains four DNA binding domains: three Cut Repeats (CR) and a cut homeodomain (HD), that function in pairs (see Figure 3) ((90), (157)).

Evidence from *in vitro* DNA binding studies suggested that, in the context of the full-length p200 CDP/Cux protein, DNA binding is mediated by the N-terminal two DNA binding domains, CR1 and CR2. This DNA binding activity was transient, with rapid on- and off-rates. The preferred DNA binding site was two C(A/G)AT sites, organized as direct of inverted repeats ((90), (157)). CR3 and the HD were shown to function together (90), allowing stable binding to an ATCGAT motif (158).

A number of DNA binding sites were determined by gene promoter regulation studies. CDP/Cux was shown to bind CCAAT, ATCGAT, Sp1 sites, and AT-rich matrix attachment regions ((238), (33), (49), (140)). Altogether, evidence suggests that CDP/Cux can bind a wide range of sequences.

5.4 Modulation of CDP/Cux Activity

5.4.1 Proteolytic Processing

A portion of p200 CDP/Cux is proteolytically processed at the G1/S transition of the cell cycle, generating an amino-terminally truncated isoform, p110 CDP/Cux. This proteolysis is catalyzed by a nuclear isoform of the Cathepsin L cysteine protease that is produced by downstream translation initiation (82). The DNA binding of p110 CDP/Cux is mediated by CR2, CR3, and the HD, is stable, while that of p200 CDP/Cux is rapid and transient ((158), (82)). A second isoform, p90 CDP/Cux is also generated by Cathepsin L and displays the same DNA binding activity. Like p110 CDP/Cux, it is amino-terminally truncated (84).

5.4.2 Inhibitory domain

An inhibitory domain was mapped to the extreme amino-terminus of p200 CDP/Cux and was shown to inhibit CDP/Cux DNA binding activities. This autoinhibitory function is independent of post-translational modification and other factors, as its effect was observed with proteins expressed in and purified from bacteria. However, we predict that this activity could be modulated by a post-translational mechanism in vivo. Amino-terminal proteolytic processing removes the autoinhibitory domain (228).

5.4.3 Phosphorylation

CDP/Cux is a substrate for a number of kinases, as well as for the Cdc25A phosphatase. Effects of phosphorylation include down-modulation, or maintenance of DNA binding, inhibition of proteolytic processing. Conversely, dephosphorylation of the homeodomain by Cdc25A stimulates DNA binding activity in the S phase of the cell cycle (40). In the G1 phase of the cell cycle, the phosphorylation of atypical serine residues by the Cyclin D/cdk 4 kinase delayed the amino-terminal proteolytic processing of CDP/Cux (Santaguida, manuscript in preparation).

The phosphorylation of DNA binding domains inhibits DNA binding ((39), (41), (192)). While cyclin A/cdk1 has this effect, which servs to downregulate CDP/Cux activity in the G2 phase of the cell cycle, cyclin A/cdk2 phosphorylates different sites and does not inhibit DNA binding or transcriptional activities, rather it permits CDP/Cux activity in S phase (193). Protein kinase A (PKA) also phosphorylates the CDP/Cux homeodomain, and as predicted, inhibits DNA binding (152).

5.4.4 Acetylation

The homeodomain of CDP/Cux is acetylated by the p/CAF histone acetyltransferase in vitro and in vivo. However, the consequence of this is debated. One group showed that this inhibited DNA binding and transcriptional repression (132), however this could not be repeated in Dr. Nepveu's laboratory. Instead, p/CAF-mediated acetylation prevented phosphorylation by cyclin A/Cdk1 in vitro, which inhibits CDP/Cux DNA binding ((163), and Muzzin et al, manuscript in preparation). In addition, acetylation enhanced amino-terminal proteolytic processing of CDP/Cux (163).

5.5 Transcriptional Properties of CDP/Cux

5.5.1 Transcriptional Repression

CDP was cloned by virtue of its CCAAT-displacement activity. By binding to a CCAAT site, CDP prevented binding of transcriptional activators, thereby repressing transcription. The CCAAT displacement activity is mediated by the full-length p200 CDP/Cux protein, likely via CR1 and CR2 (157).

A second mechanism of transcriptional repression was identified: active repression. CDP/Gal4 DNA binding domain fusion proteins were tested for transcriptional activity on a Gal4 reporter plasmid. Two regions, both downstream of the HD, repressed transcription (146). Evidence suggested that CDP/Cux could interact with pRb, which has been associated with repression of cell cycle-regulated transcription (87). The carboxy-terminal domain (CTD) was later shown to recruit histone deacetylase activity (133). Furthermore, CDP recruited G9a histone lysine methyltransferase activity to the $p21^{WAF1}$ gene promoter, upon overexpression of both proteins, in ChIP experiments.

CDP/Cux functions as a repressor of developmentally regulated genes, preventing their expression prior to terminal differentiation. Such targets include the myeloid cytochrome heavy chain gene, gp91-phox ((209), (135), (144)), g-globin (148), b-globin (174), b-MHC gene (6), NCAM (231), Lactoferrin (116), Human Papillomavirus Type 6 Long Control Region ((175), (3), (170)), neutrophil collagenase (125), cystic fibrosis transmembrane conductance regulator (133), osteocalcin (234), immunoglobulin heavy chain enhancer (238), and tryptophan hydroxylase (219). Upon terminal differentiation, CDP/Cux DNA binding activity is lost, and expression of those targets is turned on.

p200 CDP/Cux was also proposed to repress the thymidine kinase, c-myc, and c-mos genes in proliferating cells ((51), (118), (96)).

In addition, CDP/Cux can repress transcription by binding to matrix attachment regions (MAR) and remodeling chromatin ((9), (33), (238), (140), (216), (115)). In so doing, on some promoters CDP/Cux can compete with nuclear matrix proteins such as SATB1 (special AT-rich DNA-binding protein 1) ((9), (33)). Interestingly, in so doing, overexpression of CDP/Cux could also stimulate expression from the mouse mammary tumour virus (MMTV) long terminal repeat (139). Alternatively it could repress

transcription by cooperating with other MAR-interacting proteins, such as SMAR1 (scaffold/matrix associated region 1) (115).

5.5.2 Transcriptional Activation

The Gal4 fusion protein method described above can also, in some situations, reveal transactivation domains. No such domain was identified in CDP/Cux (146). However, there was some evidence that CDP/Cux could participate in the activation of transcription. Binding of CDP2, the rat CDP/Cux ortholog, to the rat tyrosine hydroxylase (TH) enhancer enhanced the binding of rITF2 (rat immunoglubulin transcription factor 2). Coexpression of these two proteins in transient reporter assays stimulated expression from TH promoter.

CDP/Cux was shown to bind to positive-acting CCAAT sites in *Xenopus* histone H2A, H2B, H3, and H4 gene promoters (60), as well as sequences in the human histone H1, H3, and H4 gene promoters (232). Later, CDP/Cux was defined as the DNA-binding component of the histone nuclear factor D (HiNF-D) complex that binds to cell cycle-regulated histone gene promoters (235). In normal cells, DNA binding by HiNF-D is regulated in a cell cycle-dependent manner, where it is increased in S phase, while in transformed cells, it is constitutively elevated throughout the cell cycle (99). The other components of the HiNF-D complex are CDC2, cyclin A, and pRB (235). Studies using the G1/S-activated histone H4 gene, FO108, revealed that sequences necessary for binding by CDP/Cux were also necessary for its correct temporal expression (8). However, transient reporter assays using p200 CDP/Cux showed transcriptional repression (235) and recently, that pRb functioned as a co-repressor of p200 CDP/Cux in the repression of the H4 gene (87). From this, it was proposed that, in late S phase, pRb and CDP/Cux, in the context of HiNF-D, repressed histone gene promoters (87)

A recent report suggested that p200 CDP/Cux could also function in transcriptional activation of the gp91-phox gene promoter, by displacing SATB1, which repressed transcription (68).

5.6 CDP/Cux Activity During the Cell Cycle

p200 CDP/Cux is expressed in proliferating cells, and, as mentioned above, its CCAAT displacement activity functions to repress the expression of genes in precursor cells, prior to differentiation, upon which CDP/Cux DNA binding activity is no longer detected, and the target gene is expressed (reviewed in (163), (157)(156)).

p110 CDP/Cux is generated in a cell cycle-dependent manner, as a result of proteolytic processing, by a nuclear isoform of the cathepsin L cysteine protease (83). Upregulation of CDP/Cux DNA binding is observed late in G1 phase, following processing by Cathepsin L, and dephosphorylation by the Cdc25A phosphatase ((40), (158)). Following S phase, p110 CDP/Cux DNA binding activity is downregulated, as a result of phosphorylation by cyclin A/Cdk1 (192).

NIH 3T3 cells stably expressing p110 CDP/Cux display shorter G1 phase and accelerate entry in to S phase following a number of cell cycle synchronization methods. Furthermore, p110-expressing wild-type MEFs proliferate faster, and reach a higher saturation density than their $Cutl1^{z/z}$ (CDP/Cux-null) counterparts (191). However, the expression of p110 CDP/Cux is not sufficient to induce S phase entry of quiescent cells in the absence of growth factors, and it is not essential for cell survival following gene ablation. As no increase in apoptosis was observed following overexpression of p110, it was proposed that CDP/Cux overexpression serves to impart a growth advantage, consistent with its role in cancer ((191); see below).

5.7 Knockouts and Transgenic Mice

5.7.1 Cux gene ablation in mice

Four CDP/Cux knockout mouse models have been described. Deletion of CR1 resulted in homozygous Cux-1ΔCR1 mutant mice with curly vibrissae and wavy hair. Mutant mothers lost 50% of their litter shortly after birth, irrespective of their genotype, due to a lactation defect (229). Cux-1ΔHD and $Cux\text{-}1\Delta C^{-/-}$ mice expressed a Cux protein truncated after CR3, thereby lacking the HD and carboxy-terminus. Homozygous mutant mice displayed partial neonatal lethality ((208), (145)). Surviving mice had a deficit in T cells and B cells, and a surplus of myeloid cells (208), had stunted growth, hair follicle defects and reduced fertility (145). Studies using mutant MEFs, as well as adult tissues

from mutant mice, revealed a loss in HiNF-D DNA binding activity, and reduced expression levels of histone H4.1 and H1 genes (145). $Cutl1^{z/z}$ mice were generated by targeted mutagenesis, in which the exons coding for CR3, and HD were replaced, in frame, with the lacZ gene. The CDP-lacZ fusion protein was detected in the cytoplasm and was transcriptionally inactive. Homozygous mutant mice from an inbred strain died at birth due to respiratory failure caused by retarded differentiation of the lung epithelia. Homozygous mutant mice from an outbred background survived, but displayed retarded growth. Hair follicle development was impaired, and gave rise to sparse, abnormal hair (61).

5.7.2 Transgenic CDP/Cux mice

CDP/Cux transgenic mice displayed contrasting phenotypes. Transgenic mice that constitutively overexpressed Cux-1 presented multi-organ hyperplasia and organomegaly (127). Studies of adult transgenic kidneys revealed an increased number of proliferative cells, which was correlated with the reduced expression of the $p27^{kip1}$ cyclin-dependent kinase inhibitor. Further studies revealed the development of glomerulosclerosis and interstitial fibrosis, as well as increased mesangial proliferation (24).

Overexpression of p75 CDP/Cux under the control of the mouse mammary tumour virus long-terminal repeat (MMTV-LTR) caused a myeloproliferative-disease-like myeloid leukemia with a long latency, an average of 20 months, in approximately 33% of mice. Observations that lead to this conclusion included hepatomegaly, splenomegaly and infiltration of leukocytes into non-hematopoietic organs, such as the lungs and kidneys (27).

5.8 CDP/Cux and Cancer

Earlier studies predicted that CDP/Cux was a tumour suppressor. The CUTL1 gene lies in a chromosomal region, 7q22, which is frequently rearranged, or deleted in cancers ((251), (252)). Polymorphic markers within and adjacent to CUTL1, were present in a frequently deleted region in seven of 50 uterine leiomyomas tested, and CUTL1 mRNA levels were reduced in eight out of 13 tumour samples (251). In addition, at least one of the aforementioned polymorphic markers was deleted in breast cancers and

was associated with increased tumour size. Immunocomplexes of CUTL1 and the Polyomavirus (PyV) large T (LT) antigen were detected in leiomyomas and mammary tumours from PyV LT transgenic mice, which suggested that CUTL1, like pRB and p53 could be a tumour suppressor targeted by the PyV LT oncoprotein (252).

In contrast increased expression of p110 CDP/Cux and p75 CDP/Cux has been associated with tumourigenesis. Western blot analysis of uterine leiomyomas revealed increased expression of shorter CDP/Cux isoforms in 11 of 16 samples, but not in the surrounding, normal, myometrium (159). The increased expression of short isoforms correlated with an increase in DNA binding activity. Furthermore, sequencing of CUTL1 isolated from uterine leiomyomas revealed no mutations, suggesting that CUTL1 was not the tumour suppressor on 7q22 (159). p75 CDP/Cux was expressed weakly, or not at all, in normal breast tissues, but was detected in breast tumour samples and breast tumour cell lines. Expression of p75 was correlated with invasive breast tumours. Upon culturing in collagen, T47D cells stably expressing p75 CDP/Cux, solid, undifferentiated aggregates of cells formed, rather than tubule structures that are normally observed (85).

Therefore, CDP/Cux is likely not a tumour suppressor, and shorter isoforms may function as oncoproteins. To explore this possibility, transgenic mice expressing short CDP/Cux isoforms under the control of the MMTV LTR were generated, which drives expression in mammary epithelial cells, as well as in other cells, including the hematopoietic system (27). As discussed above, these p75 transgenic mice developed a myeloproliferative disease-like myeloid leukemia (see 5.7.2) (27).

Further support for the role of CDP/Cux as an oncoprotein was provided by the finding that CUTL1 expression correlates with poor prognosis in breast cancer. In addition, increased expression of CUTL1 was observed in high-grade breast and pancreatic cancers. Results from RNAi microarray analysis revealed many targets that promote growth, motility, and invasion, which are events necessary for metastasis. CDP/Cux expression enhances migration and invasion in *in vitro* and *in vivo* assays (153). Therefore, in addition to its function in the cell cycle, favouring proliferation, CDP/Cux may be a regulator of cell motility and invasion.

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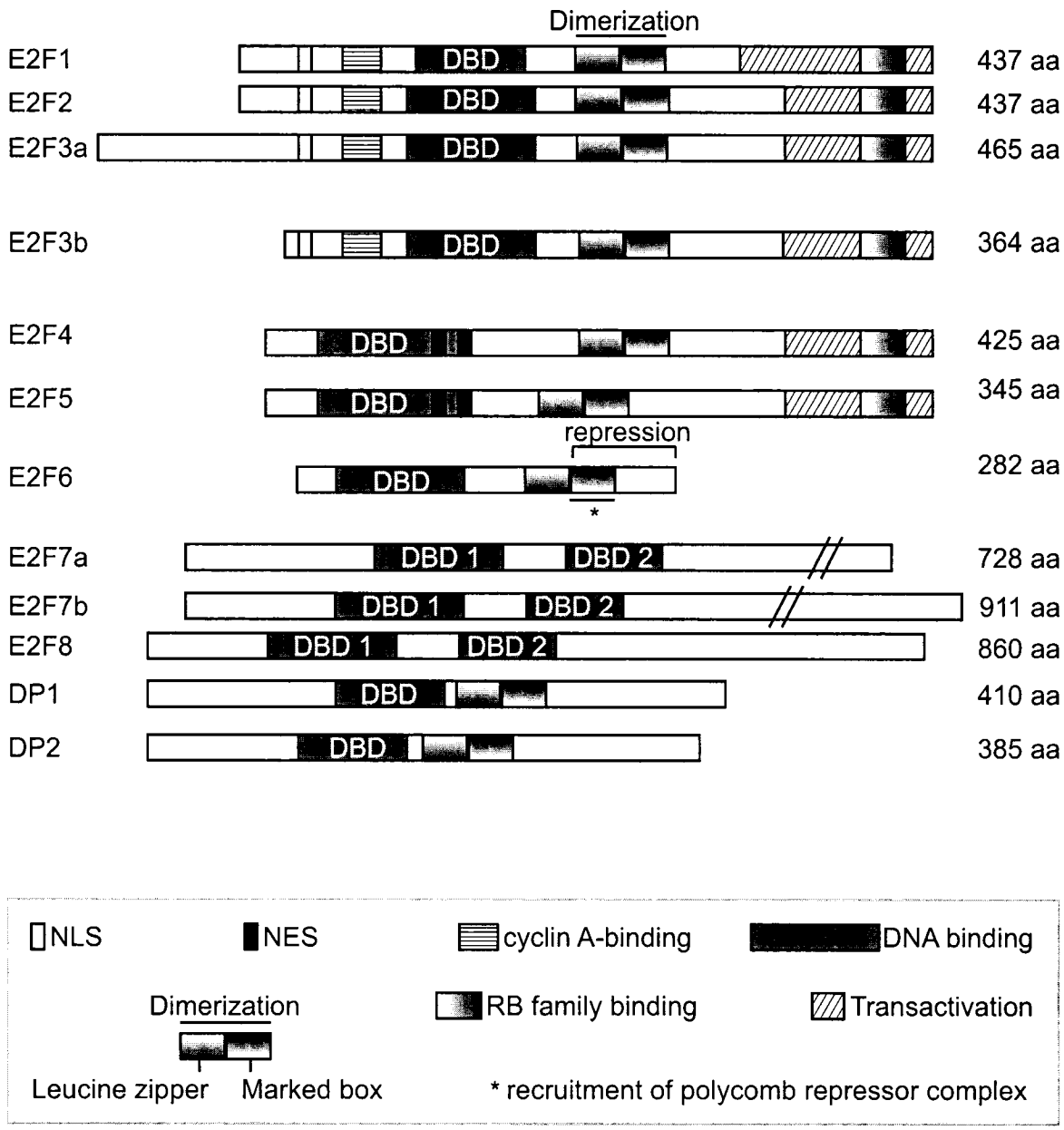
Figure 1: The E2F family of transcription factors

There are 10 members of the E2F family. E2F1-E2F3a function as transcriptional activators of cell cycle regulated genes. E2F3b-E2F8 are transcriptional repressors. DP1 and DP2 are heterodimerization partners of E2F1-E2F6. Pocket proteins regulate E2F1-E2F5 via interactions at the carboxy-terminus, within the transactivation domain.



Modified from Dimova and Dyson (2005) Oncogene 24: 2810 and
Trimarchi and Lees (2002) Nature Reviews in Molecular and Cellular Biology 3: 11

Figure 2: Caspase family members, substrate specificity, and *in vivo* models

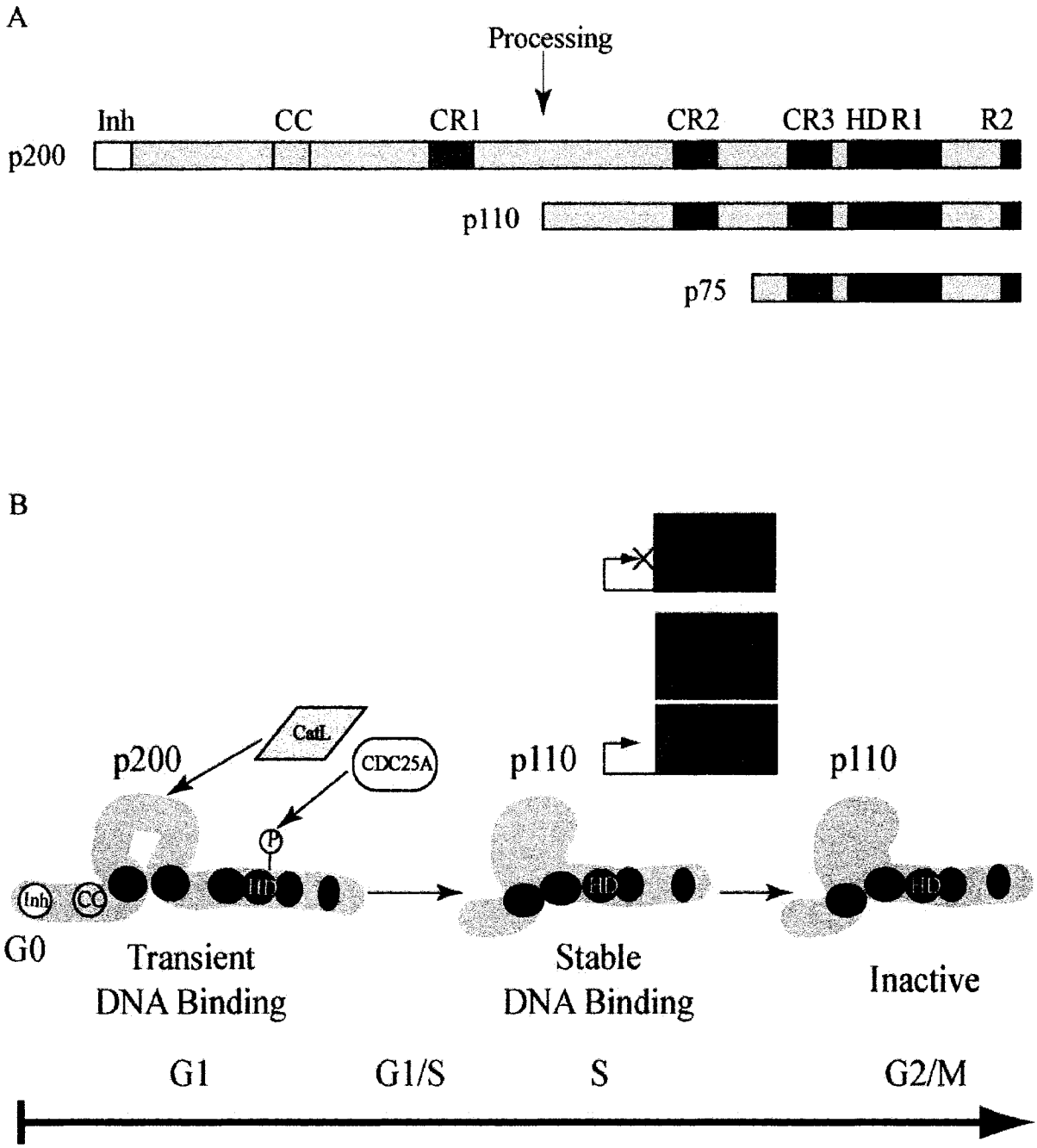
There are 14 known caspases. Group I caspases have similar structure and substrate specificity. These caspases are known for their roles in inflammation. Group II caspases are the apoptotic initiator caspases. Group III are the effector caspases, which are activated by limited proteolysis.

	Substrate specificity	Mutant phenotype	Cell death phenotype	Other roles
Group I				
 Caspase 1	WEHD	Knockout. Normal development	Defects in death receptor-mediated apoptosis	Defects in production of IL-1α and IL-1β
 Caspase 4	(W/L)EHD			
 Caspase 5	(W/L)EHD			
 Caspase 11 (murine)		Defects in oligodendrocyte-mediated cell death		Defects in production of IL-1α and IL-1β
 Caspase 12		Fibroblasts defective response to ER stress stimuli; sepsis		Block inflammatory response by caspase-1(sepsis)
 Caspase 13	WEHD			
 Caspase 14	WEHD	No		Terminal differentiation in keratinocytes
Group II				
 Caspase 2	DEHD	Knockout. Animals have excess oocytes	Oocytes resistant to cell death	None identified
 Caspase 8	LETD	Knockout. Embryonic lethality; impaired heart muscle development; decreased pool of hematopoietic precursors; familial mutation (human)	Defects in Fas and TNF-initiated cell death	T cell function
			Defects in death receptor-mediated apoptosis (human)	Defects in activation of T, B, and NK cells (human)
 Caspase 9	LEHD	Knockout. Perinatal lethality; excess brain tissue	Defects in brain apoptosis, cell death in response to UV or γ-irradiation	None identified
 Caspase 10	LEXD	familial mutation (human); autoimmune lymphoproliferative syndrome type II	Defects in death receptor-mediated apoptosis	None identified
Group III				
 Caspase 3	DEVD	Knockout. Perinatal lethality; excess brain tissue; smaller	Defects in brain apoptosis	Skeletal muscle differentiation
 Caspase 6	VEHD	Knockout. Normal development	Not determined	Not determined
 Caspase 7	DEVD	Knockout. Embryonic lethality	Not determined	Not determined

Modified from Lavrik et. al, 2005 The Journal of Clinical Investigation 115(10): 2665-2672, Figure 1; and Abraham et. al, 2004 Trends in Cell Biology 14(4): 184-193, Table 1

Figure 3: Alternative modes of DNA binding and regulation of CDP/Cux during the cell cycle

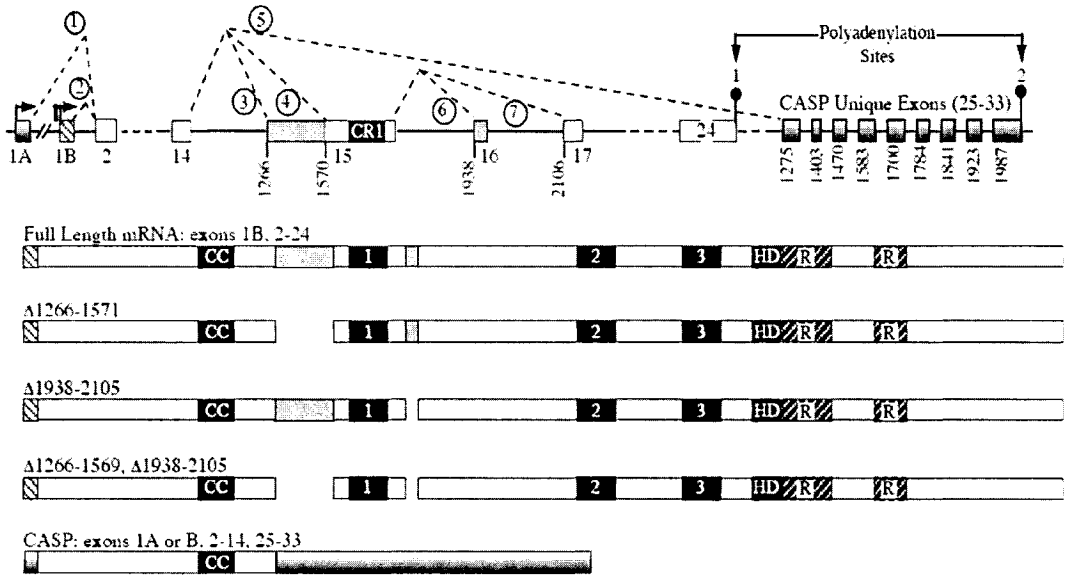
p200 CDP/Cux is expressed throughout the cell cycle. It binds DNA transiently via CR1 and CR2. During the G1/S transition, the homeodomain (HD) is dephosphorylated by the Cdc25A phosphatase. Also, a nuclear isoform of Cathepsin L proteolytically processes p200. This generates p110 CDP/Cux, which is truncated after CR1. p110 CDP/Cux binds stably to DNA. This isoform regulates the expression of cell cycle-regulated genes. At the G2 phase of the cell cycle, DNA binding is downregulated as a result of phosphorylation by cyclin A/cdk1.



from Santaguida, 2005

Figure 4: CUTL1 splice variants

The human CUTL1 gene is located at 7q22. Within this locus are 33 exons. CUTL1 codes for two proteins: CDP-1 and CASP. Different splicing events lead to the potential generation of five different CDP proteins and one CASP protein.



from Zeng et al., 2000 Gene 241: 75-85

Chapter II - CDP/Cux Stimulates Transcription from the DNA Polymerase α Gene Promoter

Preface

At the beginning of this project, there was some evidence suggesting that CDP/Cux, a transcriptional repressor, could also activate transcription. CDP/Cux was identified as the DNA binding subunit of the HiNF-D complex that bound cell cycle regulated histone gene promoters in S phase. However, in transient reporter assays, CDP/Cux repressed transcription of a histone promoter. Dr. Nam Sung Moon had recently identified a new CDP/Cux isoform that was generated by proteolytic processing at the G1/S transition of the cell cycle. This truncated isoform, p110 CDP/Cux bound DNA stably. In addition, Dr. Olivier Coqueret had recently shown that the $p21^{WAF1}$ was a transcriptional target of CDP/Cux. My first objective was to determine if CDP/Cux could stimulate transcription from the DNA polymerase alpha gene promoter. This promoter contains seven CDP/Cux consensus sites, and is regulated in a cell cycle-dependent manner.

The results from this chapter show a correlation between DNA binding by p110 CDP/Cux and transcriptional activation of the DNA polymerase alpha gene promoter. They also show that CDP/Cux can bind to, and stimulate expression of the endogenous DNA polymerase alpha gene.

Abstract

CDP/Cux (CCAAT-displacement protein/cut homeobox) contains four DNA binding domains: 3 Cut repeats (CR1, CR2 and CR3) and a Cut homeodomain (HD). CCAAT-displacement activity involves rapid but transient interaction with DNA. More stable DNA binding activity is up-regulated at the G1/S transition and was previously shown to involve an N-terminally truncated isoform, CDP/Cux p110, that is generated by proteolytic processing. CDP/Cux has been previously characterized as a transcriptional repressor. However, here we showed that expression of reporter plasmids containing promoter sequences from the human DNA polymerase α, CAD and cyclin A genes are stimulated in co-transfections with N-terminally truncated CDP/Cux proteins but not with full length CDP/Cux. Moreover, expression of the endogenous DNA pol α gene was stimulated following the infection of cells with a retrovirus expressing a truncated CDP/Cux protein. Chromatin immunoprecipitation (ChIP) assays revealed that CDP/Cux was associated with the DNA pol α gene promoter specifically in S phase. Using linker scanning analyses, *in vitro* DNA binding and ChIP assays, we established a correlation between binding of CDP/Cux to the DNA pol α promoter and the stimulation of gene expression. Although we cannot exclude that stimulation of gene expression by CDP/Cux involved the repression of a repressor, our data support the notion that CDP/Cux participates in transcriptional activation. Notwithstanding its mechanism of action, these results establish CDP/Cux as an important transcriptional regulator in S phase.

Introduction

CDP/Cux (CCAAT-displacement protein/cut homeobox) belongs to a family of transcription factors present in all metazoans and involved in the control of proliferation and differentiation (reviewed in (49)). In *Drosophila melanogaster*, the gene was named *cut* after the "cut wing" phenotype more than 50 years ago (27). Overall, genetic studies in *Drosophila* suggested that *cut* plays a role, late in development, in determining cell-type specificity in several tissues (8-10, 13, 19, 31, 32, 41, 42, 46). In higher, vertebrates, there are two CDP/Cux genes called CDP-1 and CDP-2 in human, and Cux-1 and Cux-2 in mouse and chicken (50, 57, 66). The cux-1 knockout mice displayed phenotypes in various organs including curly whiskers, growth retardation, delayed differentiation of lung epithelia, altered hair follicle morphogenesis, male infertility, a deficit in T and B cells and a surplus of myeloid cells (21, 44, 59, 65). In contrast to the small size of the cux-1 knock-out mice, transgenic mice expressing Cux-1 under the control of the CMV enhancer/promoter displayed multi-organ hyperplasia and organomegaly (38). Thus, from genetic studies both in Drosophila and the mouse, it is clear that the CDP/Cux/Cut gene plays an important role in the development and homeostasis of several tissues.

At the molecular level, CDP/Cux is a complex protein with four evolutionarily conserved DNA binding domains: three Cut repeats (CR1, CR2 and CR3) and a Cut homeodomain (HD) (1, 2, 26, 50). The full length protein, that we refer to as CDP/Cux p200, was found to be proteolytically processed at the G1/S transition of the cell cycle, thereby generating the CDP/Cux p110 isoform that contains three DNA binding domains, CR2, CR3 and HD (48). In addition, two alternate, tissue-specific mRNA species were found to code for a CDP/Cux p75 isoform that contains only two DNA binding domains: CR3 and HD (23, 73). Despite early claims made by us and others on the basis of results obtained with GST fusion proteins (1, 2, 25, 26), individual Cut repeats cannot bind to DNA on their own but need to cooperate with a second Cut repeat or with the Cut homeodomain (47). CR1CR2 was found to make a rapid but transient interaction with DNA, whereas CR2CR3HD and CR3HD bound more slowly, but stably, to DNA (47). Predictably, CDP/Cux p110 and p75 exhibited DNA binding properties similar to that of CR2CR3HD and CR3HD. However, somewhat surprisingly, CDP/Cux p200 behaved like CR1CR2 and made an unstable interaction with DNA, suggesting that DNA binding

by CR3HD is inhibited in the context of the full-length protein (47, 48). The carboxy-terminal domain (CTD) of the protein was found to contain two active repression domains, and the CTD was reported to recruit the HDAC-1 histone deacetylase (39, 45). The protein was shown to repress transcription by at least two mechanisms: competition for binding site occupancy and active repression (45). CDP/Cux was reported to repress a large number of genes, in particular those genes expressed in precursor cells prior to terminal differentiation (6, 17, 20, 28, 33-35, 37, 39, 43, 51, 53, 55, 60, 63, 64, 68, 78). In addition, the binding of CDP/Cux to a number of matrix attachment regions (MARs) raises the possibility that the protein is involved in higher order chromatin organization or may be able to target certain regulatory loci to specific regions of the nucleus (5, 16, 40, 63, 75).

A number of studies demonstrated that CDP/Cux is regulated in a cell cycle-dependent manner and may have a specific function in S phase. The histone nuclear factor D (HiNF-D), which was later found to include CDP/Cux as its DNA binding partner, was shown to be up-regulated in S phase in normal cells (29, 69, 71, 72, 77). The up-regulation of stable DNA binding at the G1/S transition was shown to involve at least two post-translational modifications: dephosphorylation of the Cut homeodomain by the Cdc25A phosphatase, and proteolytic cleavage of CDP/Cux p200 between CR1 and CR2 to generate CDP/Cux p110 (17, 48). Later in the cell cycle, DNA binding was found to decrease in G2 following phosphorylation by cyclin A/Cdk1 of two serines residues in the region of the Cut homeodomain, S1237 and S1270 (58). The rise and decline of CDP/Cux' stable DNA binding activity at the beginning and at the end of S phase suggests that the CDP/Cux p110 isoform plays a role in S phase. We reported that CDP/Cux was able to repress a reporter plasmid carrying the promoter of the $p21^{WAF1/CIP1}$ gene (17). Moreover, inhibition of CDP/Cux expression in S phase, by way of an antisense vector, restored expression of the $p21^{WAF1/CIP1}$ reporter to the higher-level observed in G1 (17). Interestingly, expression of another cyclin kinase inhibitor, p27, was shown to be down-regulated in the CMV/Cux-1 transgenic mice (38).

The binding of HiNF-D to the promoter of several S phase-specific histone genes at the same time in the cell cycle when these genes are induced is consistent with the notion that HiNF-D functions as a transcriptional activator (3, 4, 36, 71, 72, 77). However,

co-transfection of CDP/Cux with a reporter containing the promoter of the FO108-H4 histone gene did not lead to the activation of this reporter, but rather to its repression (71). In contrast, in another study co-transfection of CDP/Cux with the ITF2 transcription factor led to the activation of a reporter containing the tyrosine hydroxylase gene promoter (79). These results may indicate that CDP/Cux needs to cooperate with other proteins in order to mediate activation. Another difference that might explain the discrepancy between these results was that the latter study utilized an incomplete rat CDP/Cux cDNA clone that expressed an N-terminally truncated CDP/Cux protein equivalent to the p110 processed isoform (79). The latter two possibilities are not mutually exclusive. Indeed, one can envision a complex interdependent mechanism in which transcriptional activation requires both stable DNA binding by CDP/Cux p110 as well as cooperation with other proteins.

In the present study, we show that CDP/Cux p110, but not CDP/Cux p200, was capable of stimulating expression of a reporter containing the promoter of the DNA polymerase α (DNA pol α) gene. Moreover, the introduction of a truncated CDP/Cux protein by retroviral infection led to an increase in DNA pol α mRNA level. Using *in vitro* mutagenesis and DNA binding assays, we were able to establish a correlation between the binding of CDP/Cux to DNA pol α promoter sequences and the stimulation of the DNA pol α reporter plasmid. The potential mechanisms by which CDP/Cux may stimulate expression of the DNA pol α reporter plasmid are discussed.

Materials and Methods

Plasmid construction.

The DNA pol α -1561/+47 reporter plasmid was constructed as previously described (48). 5' deletion constructs were made as follows: -1158/+47 was inserted into pGL3-Basic, both digested with HindIII and NcoI; -402/+47 was constructed via insertion of the BssHII/NcoI promoter fragment into MluI/NcoI of pGL3-Basic; the promoter was digested with SphI, the overhang was removed with T4 DNA polymerase, followed by digestion with NcoI, yielding –248/+47, which was cloned into SmaI/NcoI of pGL3-Basic; -116/+47 was made by digestion of the promoter with SacII/NcoI, and ligated with pGL3-Basic digested with SacI/NcoI; -65/+47 was made by digestion of DNA pol α with EagI/NcoI, and ligation into SmaI/NcoI of pGL3-Basic. Linker scanning mutants were made by PCR using pGL3-pol α (-65/+47) as a template: 5'-most primer: 5'AGGTACGGGAGGTACTTGGAGCGG3'; 3'-most primer: 5'ATGTCGTTCGCGGGCGCAACTGCAACTC3'. The sequences of the inner primers can be made available upon request. Briefly, inner primers used to generate the upstream fragments had the following tail sequence: 5'GACTTGAAGCTTTC. Inner primers for the downstream fragments had the following tail sequence: 5'GACTGAAAGCTTCA. Upstream fragments were digested with NotI/HindIII, downstream fragments with HindIII/BstBI. Upstream and downstream fragments were ligated together with pGL3-Basic digested with NotI/BstBI. Constructs were sequenced to verify the absence of mutations. Sequences and/or maps will be provided upon request for CDP/Cux 831-1505, 831-1505, 659-1192+NLS, CR2CR3HD constructs. All other CDP/Cux constructs have been described in our previous studies (48), (58).

Expression and purification of CDP/Cux fusion proteins. The full-length CDP/Cux protein was expressed in SF9 insect cells using a Baculovirus vector as previously described (47). All truncated CDP/Cux protein were otherwise expressed in bacteria using the pET-15b vector (Novagen). The expression plasmids were introduced into the BL21 (DE3) strain of *E. coli* and induced with 1mM IPTG for 1.5 hours. Proteins were purified on nickel nitrilotriacetic acid-agarose (Qiagen) according to the manufacturer's instructions.

Cell culture, transfection and synchronization. HeLa and C33A are human epithelial cell lines derived from cervical carcinomas (18). HS578T, T47D and MCF-7 are human epithelial cell lines derived from breast carcinomas (11, 24, 76). T98G is a human fibroblastic cell line derived from a gliobastoma multiforme tumor (62). NIH3T3, C33A and HeLa cells were grown in DMEM medium supplemented with 10% fetal bovine serum (FBS). HS578T, T47D and MCF-7 cells were grown in DMEM medium supplemented with 5% FBS. T98G cells were grown in EMEM medium supplemented with 10% FBS. Synchronization in G1/S was performed by two methods. Serum starvation/stimulation: post-transfection, cells were maintained in DMEM plus 0.4% FBS for 36h, followed by 18h in DMEM plus 10% FBS. Thymidine block: Post-transfection, cells were cultured overnight in DMEM plus 10% FBS supplemented with 2 mM thymidine and harvested. Transient transfections were performed with ExGen500 (MBI Fermentas) according to the manufacturer's instructions.

Luciferase assay. Cells were plated in 12-well plates to be approximately 50% confluent on the day of transfection. A total of 750ng DNA (250ng reporter + 500ng effector) was transfected. Cells were either synchronized (see above) or harvested 24-48 hours later. Luciferase assays were performed as described previously (48). Because the internal control plasmid is itself often repressed by CDP/Cux, as a control for transfection efficiency the purified β-galactosidase protein (Sigma) was included in the transfection mix, as previously described (30). The luciferase activity was then normalized based on β-galactosidase activity.

The GST fusion proteins used for the generation of antibodies contained the following regions:

CDP/Cux antibodies and Western blot analysis. Antibodies 861 and 1300 have previously been described (23, 48). To generate polyclonal antibodies against various regions of CDP/Cux (1505 a.a.), rabbits were injected with 500 μg of purified bacterial fusion protein containing various regions of CDP/Cux in Freund's complete adjuvant. The α23 was raised against amino acids (a.a.) 23 to 50; α403, a.a. 403 to 449; α510, a.a. 510 to 541; α861, a.a. 861 to 936; α1300, a.a. 1300 to 1402. The animals were boosted twice with 250 μg of protein, and serum was collected 10 days after the last boost. Polyclonal antibodies were purified by affinity chromatography. The serum was passed through two

GST affinity columns and the flow-through was then applied to a GST-CDP/Cux affinity column to isolate antibodies.

Preparation of nuclear extracts and Western blot analysis. Nuclear extracts were prepared as described previously(48). For Western blot, indicated quantities of nuclear extracts were recovered as described above, and were resuspended in Laemli buffer. Proteins were then boiled for 5 min and loaded on an SDS-polyacrylamide gel. The gels were equilibrated for 10 min in 0.1 M Tris, 0.192 M glycine, 20% (v/v) methanol, and the proteins were electro-transferred to PVDF membranes overnight at 40V at 4°C. The membranes were then washed with TBS (10 mM Tris pH 8, 150 mM NaCl) and blocked in TBS supplemented 5% milk and 2% BSA for 2 hours at room temperature. The indicated antibodies were diluted 1:1000 in TBS supplemented with 0.1% Tween (TBST), and membranes were incubated for 1 hour at room temperature. Following 5 washes with TBST, HRP-conjugated secondary antibodies (Santa Cruz) were diluted 1:4000 in TBST and membranes were incubated 40 minutes at room temperature. Membranes were washed 5 times with TBST followed by 2 washes with TBS. Proteins were detected using the ECL kit from Amersham Pharmacia Biotech.

Electrophoretic Mobility Shift Assay (EMSA). EMSA was performed with the indicated quantity of purified protein. Samples were incubated at room temperature for 20 minutes in a final volume of 30 µl of 25 mM NaCl, 10 mM Tris, pH 7.5, 1 mM MgCl2, 5 mM EDTA, pH 8.0, 5% glycerol, 1 mM DTT, 3 µg BSA with 0.2 pmol of radiolabeled oligonucleotides. Samples were loaded on a 5% polyacrylamide (29:1), 0.5X TBE gel and separated by electrophoresis at 8V/cm in 0.5X TBE. Gels were dried and visualized by autoradiography.

Calculation of the DNA binding affinity. To determine the dissociation constant (K_D), EMSA were performed essentially as described above, but using a fixed amount of DNA ($\leq$10 pM) and a wide range of protein concentrations and with the following modifications: less than 10 pM of DNA was used, and protein and DNA were incubated for 15 min at room temperature. The binding affinity (K_D) was calculated using the method described by Janet Carey (14, 15). The amount of free and bound DNA was quantitated by scanning of the autoradiograms on a Phosphoimager (Amersham Pharmacia Biotech Typhoon 8600) and verified by scintillation counting of the excised bands in an independent

experiment. The data was plotted as the fraction of free DNA vs. log of protein concentration. Since the protein concentrations did not take into account the fraction of inactive proteins, our data are referred to as the apparent dissociation constant (K_D(app.)).

Oligonucleotides. The sequences of oligonucleotides used in this study are are follows: TCGAGACGATATCGATAAGCTTCTTTTC (universal CDP/Cux consensus binding site); TCGAGACGGTATCGATAGCTTCTTTTC (ATCGAT); GGGCCGCTGATTGGCTTTCAGGCTGGCGCCTCGA (DNA pol α –40/-14); GGGCCGCTGAAAGCTTCACAGGCTGGCGCCTCGA (DNA pol α –40/-14 mut –35/-26). Underlined sequences represent mutations introduced in linker/scanning analysis.

DNaseI Footprinting. The DNA pol α fragment –116/+47 was used for this analysis. The plasmid was ^{32}P labeled at the NcoI site with the Klenow fragment of DNA Polymerase I, and cleaved with SacII. After electrophoresis through a 5% polyacrylamide gel, the labeled fragments were purified by passive elution in 10 mM Tris-HCl (pH 7.5), 1 mM EDTA. DNase footprinting analysis was performed as previously described (25). End-labeled DNA (8,000 cpm per tube) was incubated with of purified bacterially expressed fusion proteins for 15 min at room temperature in a final volume of 75 µl in 10 mM Tris (pH 7.5), 25 mM NaCl, 1 mM $MgCl_2$, 1 mM dithiothreitol, 5% glycerol, 4% (wt/vol) polyvinyl alcohol. 225 µl of 10 mM $MgCl_2$, 5 mM $CaCl_2$ was added for 90 s. Various dilutions of DNase I were added and samples were then incubated for 90 s. At that time 270 µl of DNase stop solution (20 mM EDTA, 1% sodium dodecyl sulfate, 0.2 M NaCl) was added and the solution mixed by vortexing. Following phenol-chloroform extraction and ethanol precipitation, samples were electrophoresed through a 6% denaturing polyacrylamide (40:1) gel in 1X Tris borate-EDTA. Gels were dried and visualized by autoradiography.

Chromatin immunoprecipitation. Chromatin was prepared as described (52) with the following exception. Sonication was performed for 5 sec followed by a 2 min incubation on ice and this was repeated a total of 4 to 6 times. A single aliquot was retained for chromatin quality assessment/ chromatin quality control. We estimated that 15-20 X 10^6 cells per aliquot would ensure similar titers of chromatin in all subsequent immunoprecipitations. To ensure quality control between experiments Protein A agarose beads from the ChIP Assay Kit #17-295 (Upstate Biotechnology) were used for the immunoprecipi-

tations. The extract was first incubated with 30 µl of beads for 1 hour. After centrifugation, the supernatant was transferred to a new tube and was incubated overnight at 4°C with 2 µg purified CDP/Cux antibody. The next day, 25 µl of beads were added and incubation was continued for at least 1 hour at 4°C. The supernatant was removed and the beads were washed 2 times with low salt buffer, 2 times with high salt buffer and then once with TE. Elution and DNA purification followed the protocol of Nissen and Yamamoto, 2000, with the addition of an additional phenol-chloroform extraction (52). The presence of binding to 3 different regions of the DNA pol α gene promoter in the immunoprecipitated chromatins was analyzed by PCR with specific sets of oligonucleotides: region 1 (PCR 1) 5'CCCTCAGCTCTAGCTTTTCCCTAAGGGG 3' and 5'CATGGTCCCGAATCTCCCGATTCC 3'; region 2 (PCR 2) 5'GGTTCTCTCCTGGTTGGAAAAGCTTG 3' and 5'TTGCCCACATGCTTATTGATCCCTTC 3'; region 3 (PCR 3) 5'GGTGCCTTATTGCTCTGTTCTCACATGG 3' and 5'CAGCTGATTACTTCCCACATGCCCG 3'. PCR reactions, 50 µl, were done with Taq polymerase (MBI Fermentas) for a total of 37 cycles. The temperature of hybridization corresponded to the value of the Tm of the oligonucleotides for the first 6 cycles. The hybridization temperatures were decreased until 5°C below the Tm for the rest of the PCR.

***In vivo* DNA binding to transfected reporter plasmids.** HS578T cells were transfected with pGL3-Pol α (-65/+47), pGL3-Pol α (-65/+47) mut (-35/-26), and either pXJ42 or pXJ42/CDP/Cux CR2CR3HD. DNA was extracted approximately 24 hours post- transfection. The chromatin immunoprecipitation protocol described above was used except that samples of extracted DNA were not sonicated. Primers used in PCR are as follows: 5' CCGAGCCGCTGATTGGCTTT 3' (WT) or 5' CCGAGCCGCTGAAAGCTTCA 3' (mut –35/-26) was used with 5' AGCGGTTCCATCTTCCAGCGGATAGA 3'

Retroviral infections and RT-PCR. HS578T cells were infected by the addition of virus-containing supernatant from 293VSV producer cells (54). Cells were harvested 48 hours post-infection. To minimize the extent of proteolytic processing of the full-length CDP/Cux protein, cells had been plated so as to reach near-confluence 48 hours post-infection. Real-time RT-PCR was performed with a Light Cycler using the Fast Start

DNA Master SYBR Green I Kit (Roche) and the following primers: For DNA pol α: sense primer: 5'-GCTTCACCGAATCCTTTCTCTGTG-3' (mRNA position 581-604); antisense primer: 5'-TTCCTCATCTGCCCCTTTTACC-3' (1030-1009). DNA pol α RNA was normalized to the amount of GAPDH (see (23) for primer sequence) RNA amplified.

Results

CDP/Cux stimulates the DNA pol α gene promoter during S phase in NIH 3T3 cells. A search of the promoter database using the CDP/Cux consensus binding site revealed that the proximal promoter sequences of the DNA pol α gene contained several putative CDP/Cux binding sites in both *Drosophila melanogaster* and human (Fig. 1). The DNA pol α gene was previously shown to be up-regulated at the transcriptional level in S phase (56). Using reverse-transcriptase polymerase-chain-reaction, we confirmed that DNA pol alpha mRNA expression was up-regulated in S phase following re-entry of NIH3T3 cells into the cell cycle (data not shown). To determine whether CDP/Cux could regulate the human DNA pol α gene promoter, NIH 3T3 cells were co-transfected with a luciferase reporter plasmid containing sequences -1561 to +47 of the human DNA pol α gene, and either an empty vector or a vector expressing CDP/Cux 817-1505 (Fig. 1). This recombinant protein corresponds to the 110 kDa isoform, CDP/Cux p110, that is generated by proteolytic processing in S phase of the cell cycle (48). CDP/Cux p110 had little or no effect on the expression of the DNA pol α reporter when transfected NIH3T3 cells were allowed to grow asynchronously (Fig. 1 A and B). In contrast, expression of the DNA pol α reporter was stimulated in the presence of CDP/Cux p110 when NIH3T3 cells were synchronized in S phase either by thymidine block (Fig. 1A) or by serum starvation/re-stimulation (Fig. 1B). The same assay was repeated using a panel of transformed cell lines that were allowed to grow asynchronously. Significant stimulation of the DNA pol α reporter was observed in HS578T, T47D, and T98G cells, and moderate levels of stimulation were observed in C33A, MCF-7 and HeLa cells (Fig. 1C). The levels of activation may vary due to differences in transfection efficiency or levels of endogenous CDP/Cux, which may affect the response to its overexpression. In summary, CDP/Cux p110 was able to stimulate expression of the DNA pol α reporter in several cell lines, however, in NIH3T3 cells this stimulatory effect was dependent upon the cells being synchronized in S phase.

N-terminal truncation of CDP/Cux is necessary for stimulation of the DNA pol α reporter. To investigate the mechanism by which CDP/Cux was able to stimulate expression of the DNA pol α reporter, the reporter assay was repeated using effector plasmids expressing CDP/Cux recombinant proteins with progressive N-terminal truncations.

HS578T cells were utilized for these assays as our preliminary experiments revealed that this line consistently displayed the highest level of stimulation by CDP/Cux. Full-length CDP/Cux protein was unable to stimulate expression and, in fact, produced what appeared to be weak repression (Fig. 2A). Yet, the protein expressed from this plasmid was functional since it was able to repress a reporter plasmid carrying the promoter of the $p21^{WAF1/CIP1}$ gene, as previously published (Fig. 2B) (17). In contrast to full-length CDP/Cux, all N-terminally truncated CDP/Cux proteins were able to stimulate expression of the DNA pol α reporter (Fig. 2A). This stimulatory effect correlated well with the ability of CDP/Cux proteins to bind to a consensus binding site that is specific for CR3HD or CR2CR3HD (Fig. 2D, lanes 3-6). In contrast, the full length CDP/Cux protein, whose expression was confirmed by Western blot (Fig. 2C, lane 1), interacted only weakly with this sequence (Fig. 2C, lane 2). This data is in agreement with previous studies showing that full length CDP/Cux only binds to DNA transiently and exhibits a preference for sequences containing not just one but two CAAT or CGAT motifs (47, 48). Thus we conclude that p110, but not p200, is able to stimulate expression of the DNA pol α reporter.

The carboxy-terminal domain (CTD) is expendable, but the Cut homeodomain is required, for stimulation. Removal of the carboxy-terminal domain of CDP/Cux had no effect on the reporter assay, whereas removal of the Cut homeodomain prevented stimulation of the DNA pol α reporter (Fig 3A). All CDP/Cux proteins were expressed at high levels as observed by Western blot analysis (Fig. 3B). All recombinant proteins bound to DNA efficiently with the exception of CDP/Cux 659-1192, in which the Cut homeodomain was deleted (Fig. 3C, compare lane 5 with 2-4). We conclude that the Cut homeodomain is required for the stimulation of the DNA pol α reporter. In summary, results from mapping analysis demonstrated that amino acids 1 to 1061 and 1301 to 1505 are dispensable. Moreover, the presence of the N-terminal portion of the protein, from a.a. 1 to 659, and the absence of the Cut homeodomain will prevent the stimulatory function of CDP/Cux. While the mapping data presented were obtained in the HS578T cell line, similar results were obtained in NIH3T3 cells (data not shown). These results are consistent with the notion that CDP/Cux must be able to make a stable interaction with DNA in order to stimulate expression from the DNA pol α reporter.

CDP/Cux can stimulate the expression of reporter plasmids containing the promoter sequences of other S phase-specific genes. To verify whether stimulation of gene expression by CDP/Cux was unique to the DNA pol α promoter, we tested promoter sequences from a number of genes whose expression is up-regulated in S phase. As a control, we also tested a reporter containing the core promoter of the $p21^{WAF1/CIP1}$ gene. This reporter was previously shown to be repressed by CDP/Cux in co-transfection assays (17, 58). Co-transfection with CDP/Cux CR2CR3HD did not affect the expression of the $p21^{WAF1/CIP1}$ reporter. It is likely that repression of the $p21^{WAF1/CIP1}$ promoter by CDP/Cux requires the action of the active repression domains present in the CTD. In contrast, reporter plasmids carrying the promoters from the dihydrofolate reductase (DHFR), carbamoyl-phosphate synthase /aspartate carbamoyltransferase/dihydroorotase (CAD), and cyclin A genes were stimulated by CDP/Cux CR2CR3HD, albeit to a lesser extent than what was observed with the DNA pol α reporter (Fig. 4).

CDP/Cux can stimulate the core promoter of the DNA pol α gene. To identify the DNA pol α promoter sequences that are required for stimulation by CDP/Cux, a series of reporter plasmids with progressive 5' deletions were tested in the co-transfection assays. CDP/Cux stimulated the expression of all reporter plasmids, including one plasmid carrying DNA pol α sequences from -65 to +47 (Fig 5A). We conclude that the core promoter of the DNA pol α gene contains sequences that allow its stimulation in the presence of CDP/Cux.

Linker scanning mutations were introduced into the -65/+47 DNA pol α reporter plasmid. These mutations consisted in the serial replacement of 10 bp sequences with the sequence GAAAGCTTCA. Two replacement mutations, at position -35/-26 and -25/-16, significantly reduced the ability of CDP/Cux CR2CR3HD to stimulate gene expression (Fig. 5B).

CDP/Cux CR2CR3HD can bind to the core DNA pol α gene promoter *in vitro*. DNase footprinting analysis was performed to verify whether CDP/Cux proteins containing the two DNA binding domains CR2, CR3 along with the Cut homeodomain (CR2CR3HD), or just CR3HD, would interact with the core DNA pol α gene promoter. A DNA fragment was end-labeled at position +47, incubated with purified bacterially expressed his-CR2CR3HD or his-CR3HD protein, and treated with DNase 1. A protected

region was observed between nt -14 to -40 of the coding strand (Fig 6A, lanes 2, 6-7). Interestingly, an inverted CCAAT motif resides within this interval, at position -30 to -34. Electrophoretic mobility shift assay (EMSA) using double-stranded oligonucleotides corresponding to nt -40/-14 confirmed that the purified his-CR2CR3HD protein could form a strong retarded complex with this sequence (Fig 6B, lane 2). In contrast, the full-length CDP/Cux protein was unable to make a stable interaction with the DNA pol α sequence (Fig. 6D, lanes 6-9).

A mutation that reduces stimulation of expression *in vivo* also reduces DNA binding *in vitro*. Two assays were performed to verify whether the replacement mutation at position -35/-26 would reduce the affinity of his-CR2CR3HD for this sequence. In the first assay, wild type or mutated -40/-14 oligonucleotides were used as cold competitors in EMSA with the wild type -40/-14 probe. Whereas a 100-fold excess of the wild type oligonucleotides completely eliminated the retarded complex, the oligonucleotides with the -35/-26 replacement mutation did not compete as efficiently (Fig 6B, lanes 3 and 4). In the second assay, oligonucleotides with the -35/-26 replacement mutation were used as an EMSA probe. The his-CR2CR3HD protein was able to form a generate a complex, although the intensity of the complex appeared weaker than that of the wild type sequence (Fig 6B, compare lanes 2 and 6). Moreover, as we had seen previously, the wild type oligonucleotides were more efficient competitors than the mutated ones (Fig. 6B, lanes 7 and 8).

The DNA binding affinity for the wild type and mutated oligonucleotides was assessed by performing EMSA with a fixed amount of DNA (≤10 pM) and a wide range of protein concentrations (Fig. 6C). The apparent dissociation constants (K_D) were measured as described in Materials and Methods. His-CR2CR3HD exhibited apparent dissociation constants (K_D) of 5.8×10^{-8} M and 1.3×10^{-7} M for the wild type and mutated oligonucleotides, respectively (Fig. 6C). Thus, the -35/-26 replacement mutation reduced the affinity of CR2CR3HD for the core DNA pol α promoter by a factor of approximately 2.2-fold. In other experiments, we found that the -25/-16 replacement mutation that also reduced the stimulatory effect of CDP/Cux on the DNA pol α reporter, did not affect the interaction of CDP/Cux with the DNA pol α promoter sequences *in vitro* (data not shown). We postulate that this mutation interferes with the binding of another

protein that participates in the transcriptional activation of the DNA pol α promoter and is required for the stimulatory effect of CDP/Cux.

In summary, a CDP/Cux protein containing CR2CR3HD was able to stimulate expression of a reporter containing sequences -65/+47 from the DNA pol α gene promoter. This stimulatory effect was abolished by the replacement of sequences -35/-26 or -25/-16. *In vitro*, a purified CDP/Cux protein containing CR2CR3HD was able to interact with the core DNA pol α promoter sequences, however the replacement of sequences -35/-26 diminished the affinity of CDP/Cux for the DNA pol α promoter. Thus, a correlation was established between the stimulation of the core DNA pol α gene promoter *in vivo* and the interaction of CDP/Cux with DNA pol α promoter sequences *in vitro*.

CDP/Cux binds the DNA pol α gene promoter *in vivo*, specifically during the S phase of the cell cycle. Chromatin immunoprecipitation (ChIP) assays were performed to investigate whether endogenous CDP/Cux proteins bind to the promoter of the DNA pol α gene *in vivo*. Primers were designed to amplify three different regions of the DNA pol α gene promoter and one region upstream of the glyceraldehyde phospho-dehydrogenase (GAPDH). Using total chromatin as a template, each pair of primers amplified a DNA fragment of the expected molecular weight (Fig. 7A, lane 3). Using chromatin obtained after immunoprecipitation with the anti-CDP/Cux antibody 861, an amplified fragment was observed for the regions -1179/-843 and -173/+47 (Fig. 7A, lane 2, PCR1 and 2). In contrast, no fragment was obtained using primers for the GAPDH gene promoter or the upstream region of the DNA pol α gene promoter between nt -1505 and -1229 (Fig. 7A, lane 2, GAPDH and PCR3). Controls consisted of template chromatin that was obtained following immunoprecipitation with either anti-IgG or anti-HA antibodies. No amplified fragment was observed with any of the primer pairs (Fig. 7A, lanes 4 and 5). We conclude that CDP/Cux can interact with the DNA pol α gene promoter *in vivo*. We note also that a more intense signal was obtained in the region -1179/-843 than in the proximal promoter region (Fig. 7A, lane 2, compare PCR1 and 2). The reason for this is not entirely clear but may involve the fact that this region of the promoter contains a higher concentration of sequence motifs that match the CDP/Cux consensus binding site (see the map in Fig. 1). To verify whether the association of CDP/Cux with the DNA pol α gene promoter is regulated during the cell cycle, we performed ChIP assays on synchronized

HS578T cells. Binding to PCR regions 1 and 2 was detected specifically in S phase (Fig. 7B, lane 2). CDP/Cux did not interact with the DNA pol α gene promoter in G2/M or G1 (Fig 7B, lane 3, 4).

To verify whether the full-length CDP/Cux protein can interact with the DNA pol α gene promoter *in vivo*, ChIP assays were performed using a panel of antibodies that recognize various regions of CDP/Cux (see diagram in Fig. 7C). Three of these antibodies, 23, 403 and 510 recognize only the full-length protein, while antibodies 861 and 1300 can bind to both the full-length and the processed isoform (Fig 7C, rightmost panel). As a control, ChIP were performed with IgG. The signal obtained with this sample was taken as background and attributed a value of 1. Significantly stronger signals were observed with samples obtained with the 861 and 1300 antibodies. In contrast, samples obtained with antibodies that recognize only the full-length protein did not generate a stronger signal than background. We cannot exclude that the epitope recognized by an antibody is masked when the protein is bound to DNA. However, it becomes difficult to evoke this possibility to explain the absence of amplification with three different antibodies. The results rather suggest that the full-length CDP/Cux protein does not make a stable interaction with the DNA pol α gene promoter.

Reduction in stimulation of gene expression correlates with a decrease in DNA binding *in vivo*. Since we observed a correlation between a decrease in DNA binding *in vitro* and a reduction in the stimulation of gene expression in transfection assays with the linker scanning mutant -35/-26, we considered the possibility that the decreased affinity of CDP/Cux for this mutant might preclude the recruitment of CDP/Cux to the reporter plasmid following transfection into cells. To test this hypothesis, we designed oligonucleotide primers that would specifically amplify sequences from either the wild type or the -35/-26 mutant reporter plasmid. In preliminary experiments, the wild type primers were found to efficiently amplify the wild type, but not the mutated sequence, whereas the mutated primers amplified the mutated but not the wild type sequence (Fig. 8A). HS578T cells were co-transfected with both the wild type and the mutated reporter plasmids together with either an empty vector or a vector coding for CDP/Cux CR2CR3HD. Using total chromatin, a fragment of the expected molecular weight was amplified with each pair of primers to verify that the cells had each received the two plasmids as ex-

pected (Fig. 8B, lanes 2-3 and 8-9). Faint amplified fragments were observed when using as a template chromatin obtained by immunoprecipitation with the pre-immune serum or chromatin derived from cells that had received the empty effector plasmid (Fig. 8, lanes 4-7 and 10-11). We think this is due to the fact that a small, background amount of CDP/Cux is immunoprecipitated with beads alone, regardless of the buffer used. However, when the chromatin was first subjected to immunoprecipitation with the anti-CDP/Cux antibody, a fragment of strong intensity was amplified by the wild type primers but not by the mutated primers (Fig. 8, lanes 12 and 13). This result indicates that in cells containing both the wild type and the mutated reporter plasmids, the recombinant CDP/Cux protein was able to interact efficiently with the wild type reporter plasmid but not with the mutated plasmid.

CDP/Cux can regulate the endogenous DNA pol alpha gene. The experiments described above demonstrated that expression of a DNA pol α reporter could be stimulated in the presence of N-terminally truncated CDP/Cux protein. These experiments, however, did not establish whether CDP/Cux could regulate the endogenous DNA pol α gene. To address this question, we infected HS578T cells with high-titer retroviral vectors expressing either the full-length or a truncated CDP/Cux protein. Cells were harvested 48 hours following infection. RNA and proteins were purified from 70% and 30% of the cells, respectively. Expression of the recombinant CDP/Cux proteins was verified by Western blot analysis (Fig. 9B). Reverse-transcriptase polymerase chain reaction (RT-PCR) analysis was used to quantitate DNA pol α mRNA (Fig. 9A). When compared with cells infected with an empty retrovirus, the amount of DNA pol α mRNA was increased 4-fold on average in cells infected with the retrovirus expressing the truncated CDP/Cux protein. However, no increase in DNA pol α expression was observed in cells infected with the full-length CDP/Cux vector. Again, the stimulation in gene expression by the truncated CDP/Cux protein could result from true activation or repression of a repressor. Nonetheless, these results clearly demonstrate the endogenous DNA pol α gene can be regulated in response to CDP/Cux.

Discussion

The CDP/Cux transcription factor was originally characterized as a transcriptional repressor (6, 17, 20, 28, 33-35, 37, 39, 43, 45, 51, 53, 55, 60, 63, 64, 68, 78). Recently, it was shown that an N-terminally truncated isoform, CDP/Cux p110, is generated by proteolytic processing at the G1/S transition of the cell cycle. Results from the present study revealed that CDP/Cux p110 is able to stimulate transcription from a reporter plasmid containing the DNA pol α promoter (Fig 1-5). Moreover, expression of the endogenous DNA pol α gene was stimulated in a population of cells infected with a retrovirus expressing a truncated CDP/Cux protein (Fig 9). These results suggest, yet do not demonstrate, that CDP/Cux p110 can function as a transcriptional activator. Stimulation of transcription, whether of a reporter or an endogenous gene, is consistent with a number of possible mechanisms. CDP/Cux p110 might directly activate transcription. It is unlikely that CDP/Cux p110 functions like a classical transcriptional activator with a DNA binding domain and an activation domain. No region of CDP/Cux was found to function as an activation domain in the Gal4 fusion assay, in which various regions of a protein are fused to the DNA binding domain of the Gal4 transcription factor and are assayed together a Gal4 reporter plasmid (45). Therefore, we envision that CDP/Cux p110 might contribute, perhaps as an architectural factor, to the formation of a larger complex or enhanceosome. This mode of action would be consistent with what we know of the HiNF-D complex, which includes CDP/Cux as well as a number of other proteins, and whose presence on the promoter of histone genes coincides with their induction in S phase (3, 4, 36, 67, 69-72).

In light of the well-characterized role of CDP/Cux as a transcriptional repressor, we cannot exclude the possibility that CDP/Cux p110 represses the expression of another repressor that down-modulates the DNA pol α promoter. Indeed, as cells were harvested 48 hours post-transfection or infection, any regulatory effect might be direct or indirect. However, we did not obtain any evidence in support of a repression mechanism. DNA fragments containing sequences -65/+47 of the DNA pol α promoter were tested in EMSA and DNase footprinting analyses. Using protein extracts from cells overexpressing CDP/Cux, we did not observe a decrease or disappearance of a retarded complex (data not shown). It still remains possible that CDP/Cux p110 interferes with the

binding of a repressor that could not be detected in unfractionated nuclear extracts. In contrast to the lack of evidence in favor of a repression mechanism, we were able to establish a correlation between transcriptional stimulation and the ability of CDP/Cux p110 to bind to the DNA pol α promoter sequences in EMSA and ChIP assays. These results strongly suggest, but yet do not prove, that CDP/Cux p110 functioned as a true activator. More direct evidence that CDP/Cux p110 may function as a transcriptional activator would be provided if the addition of CDP/Cux p110 to an *in vitro* transcription system led to transcriptional activation. Again, however, in a crude *in vitro* system we could not exclude that CDP/Cux p110 stimulates transcription by competing with, and displacing, a repressor. Therefore, we do not think that experimental evidence from a single assay will be sufficient to demonstrate one mechanism of action at the expense of another. Instead, the accumulation of evidence from a panoply of assays and experimental conditions will gradually build a case in favor of one mechanism.

A recombinant CDP/Cux protein corresponding to CDP/Cux p110 was able to stimulate transcription, but the full length CDP/Cux isoform was not (Fig. 2 and 9). These results indicate that stimulation of transcription is a specific property of CDP/Cux p110 that is not shared with CDP/Cux p200. As CDP/Cux p110 is capable of making a stable interaction with DNA, whereas CDP/Cux p200 is not, the need for N-terminal truncation is likely to reflect the requirement for stable DNA binding. This would be compatible with the two mechanisms cited above: direct activation or repression of a repressor. CDP/Cux was previously shown to repress by two mechanisms: active repression and competition for binding site occupancy (45). While competition can be accomplished via transient or stable DNA binding, active repression was shown to involve the recruitment of a histone deacetyltransferase (39). Similarly, transcriptional activation has been associated with the recruitment of HATs, chromatin-remodeling machines, general transcription factors and/or the stabilization of other site-specific transcription factors (7, 12, 61, 74). Although this has not been formally tested, it is generally assumed that a DNA-binding transcription factor must be able to make a stable interaction with DNA in order to participate in transcriptional activation or active repression. In the case of CDP/Cux, it is clear that proteolytic processing modifies its DNA binding properties. It is

possible that another consequence of processing is to change the ability of CDP/Cux to interact with other proteins.

One linker mutation between nt –25 and –16 abolish transcriptional stimulation by CDP/Cux but did not affect its affinity for the core promoter (Fig. 5B and data not shown). It is likely that this mutation interferes with the binding of another protein that participates in the transcriptional activation of the DNA pol α promoter and is required for the stimulatory effect of CDP/Cux. This protein could be another transcription factor or one of the components of the pre-initiation complex.

Results from ChIP assays showed that two regions of the DNA pol α gene promoter could be immunoprecipitated with CDP/Cux, the core promoter and a region approximately 1 Kbp upstream. The latter was immunoprecipitated more efficiently, yet we found that the core promoter of the DNA pol α gene was sufficient to allow its stimulation in reporter assays. This result does not exclude that the upstream sequences may also contribute to the recruitment of CDP/Cux to the DNA pol α gene promoter. We envision that the core promoter, which contains a low affinity binding site for CDP/Cux p110, was able to recruit CDP/Cux p110 when the protein was over-expressed in transfected cells, but it is possible that the upstream sequences play an important role in the recruitment of CDP/Cux p110 when the protein is expressed at physiological levels. In agreement with this notion, we found that a substantial fraction of purified CDP/Cux p110 elutes as a multimeric complex on a size exclusion column (Leduy and Nepveu, unpublished observations). Whether CDP/Cux p110 can multimerize *in vivo* and how this process is regulated should be addressed in future studies.

Earlier studies on the HiNF-D factor pointed to a role of CDP/Cux in the S phase of the cell cycle (29, 69, 71, 72, 77). More recently, various post-translational modifications of CDP/Cux were shown to regulate its DNA binding activity in a cell cycle dependent manner (17, 48, 58). Results presented herein have revealed a novel activity of this transcription factor that pertains to its role in cell cycle progression: CDP/Cux p110 is capable of stimulating, directly or indirectly, the promoters of DNA pol α and other genes that are induced in S phase. Future studies should ascertain the role of CDP/Cux p110 as a transcriptional activator. Another important issue will be to evaluate the role of CDP/Cux in the regulation of the cell cycle in various cell types. Unless the cux-1 and

cux-2 genes were partially redundant, the fact that cux homozygous knock-out mice did not exhibit embryonic lethality indicates that cux-1 is not an essential gene (21, 44, 59). Yet, some phenotypes of the cux-1 homozygous knockout mice, like the smaller size of the mice, their defect in hair growth and their reduced number in B and T cells, are compatible with a role of cux-1 in the proliferation of at least certain cells:. Interestingly, these phenotypes are in striking contrast with the multi-organ hyperplasia and organomegaly displayed by a cux-1 transgenic mouse (38). The identity of the cells in which CDP/Cux contributes to proliferation, and the developmental signals to which CDP/Cux responds should be the subject of intense investigation in the future.

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Fig. 1. CDP/Cux stimulates the DNA pol α gene promoter.

(A) & **(B)** NIH3T3 cells were co-transfected with a DNA pol α reporter construct (pGL3-pol α (-1561/+47)) and either an empty vector or a vector expressing a CDP/Cux protein containing a.a. 817-1505 (pXM/HSCDP-817-1505). Cells were either left unsynchronized or were synchronized in S phase by thymidine block (A) or by serum starvation-re-stimulation (B), as described in Materials and Methods. Cytoplasmic extracts were prepared and processed to measure luciferase activity. Results are expressed as relative light units (RLU) normalized to β-galactosidase activity from an internal control, and are representative of the mean of a minimum of two separate experiments.

(C) Various tumor cell lines were co-transfected as in (A) and were left unsynchronized. Results are expressed as fold activation when CDP/Cux is transfected relative to transfection of empty vector.

(D) HS578T cells were co-transfected with the DNA pol α -1561/+47 reporter construct and either an empty vector or increasing amounts of the pXM/HSCDP-817-1505 vector. Luciferase activity was expressed as relative light units (RLU) normalized to β-galactosidase activity from an internal control, and are representative of the mean of three separate experiments.

A diagrammatic representation of the reporter and effector plasmids is shown at the bottom. The bars within the DNA pol α promoter sequences represent putative CDP/Cux binding sites.

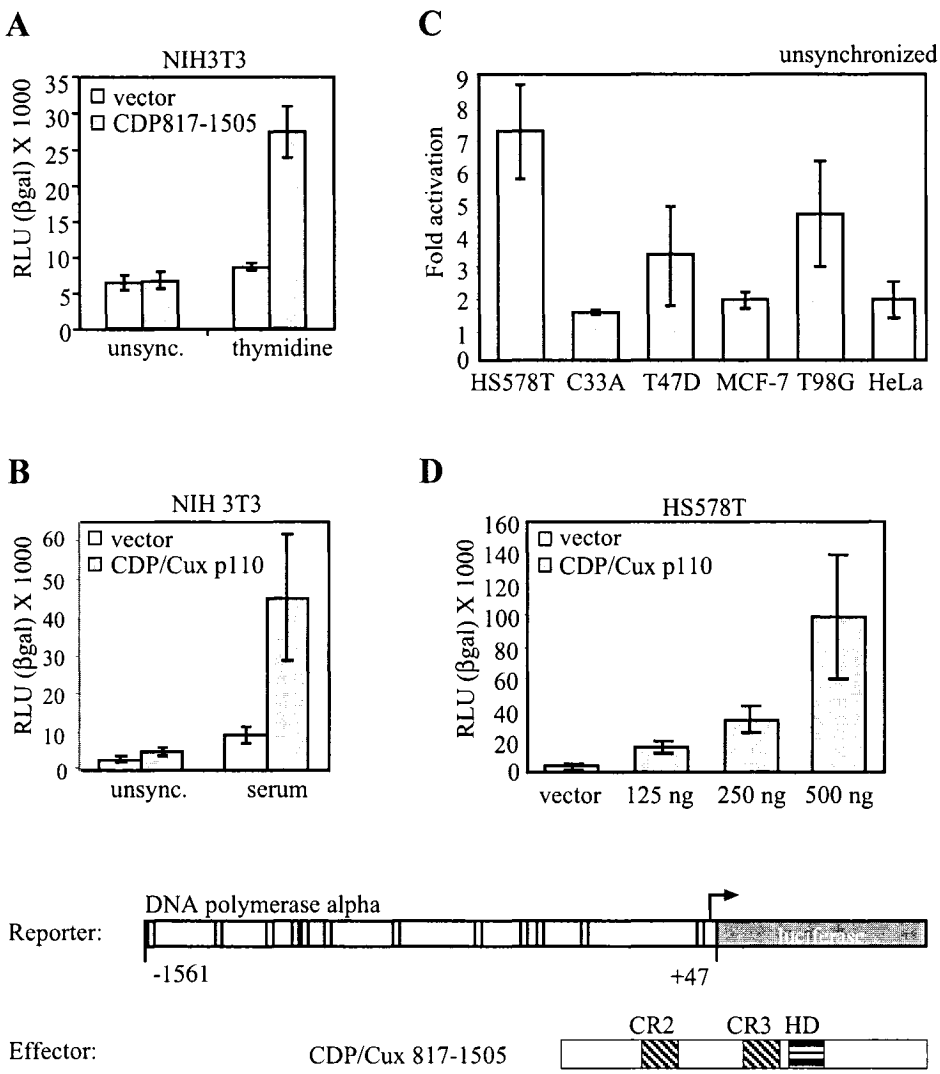

A
NIH3T3
RLU (βgal) X 1000
vector
CDP817-1505
unsync.
thymidine
C
unsynchronized
Fold activation
HS578T C33A T47D MCF-7 T98G HeLa
B
NIH 3T3
RLU (βgal) X 1000
vector
CDP/Cux p110
unsync.
serum
D
HS578T
RLU (βgal) X 1000
vector
CDP/Cux p110
vector 125 ng 250 ng 500 ng
Reporter:
DNA polymerase alpha
-1561
+47
Effector:
CDP/Cux 817-1505
CR2
CR3 HD

Fig. 2. Amino-terminal truncation of CDP/Cux is necessary for stimulation of DNA pol α gene expression.

(A) HS578T cells were co-transfected with the DNA pol α -1561/+47 reporter construct (see Fig. 1), and vectors expressing CDP/Cux proteins with progressive N-terminal truncations, as indicated. 48 hours post-transfection, cells were harvested and cytoplasmic and nuclear extracts were prepared. Cytoplasmic extracts were analyzed for luciferase activity as in Fig. 1A.

(B) HS578T cells were co-transfected with a reporter plasmid carrying the promoter of the $p21^{WAF1/CIP1}$ gene and a vector expressing the full-length CDP/Cux proteins. 48 hours post-transfection, cytoplasmic extracts were prepared analyzed for luciferase activity as in Fig. 1A.

(C) Nuclear extracts were separated on an 8% SDS-polyacrylamide gel. Proteins were transferred to PVDF membrane and analyzed by Western blot using an α HA antibody that recognizes the C-terminus.

(D) Nuclear extracts were analyzed by EMSA with oligonucleotides encoding a CDP/Cux consensus binding site.

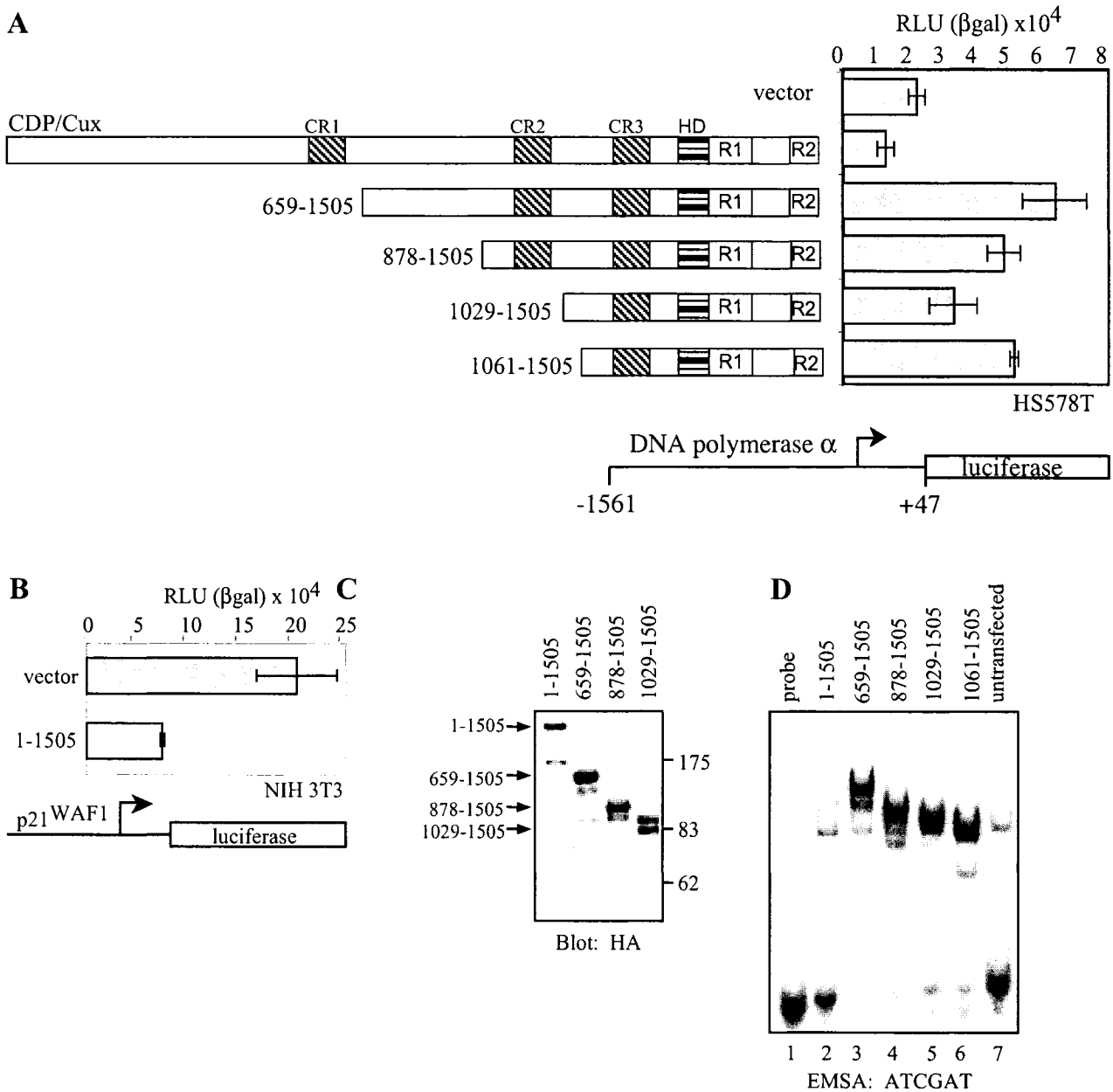

A
RLU (βgal) x10^4
0 1 2 3 4 5 6 7 8
vector
CDP/Cux
CR1
CR2
CR3
HD
R1
R2
659-1505
878-1505
1029-1505
1061-1505
HS578T
DNA polymerase α
luciferase
-1561
+47
B
RLU (βgal) x 10^4
0 5 10 15 20 25
vector
1-1505
NIH 3T3
p21WAF1
luciferase
C
1-1505
659-1505
878-1505
1029-1505
175
83
62
Blot: HA
D
probe
1-1505
659-1505
878-1505
1029-1505
1061-1505
untransfected
1 2 3 4 5 6 7
EMSA: ATCGAT

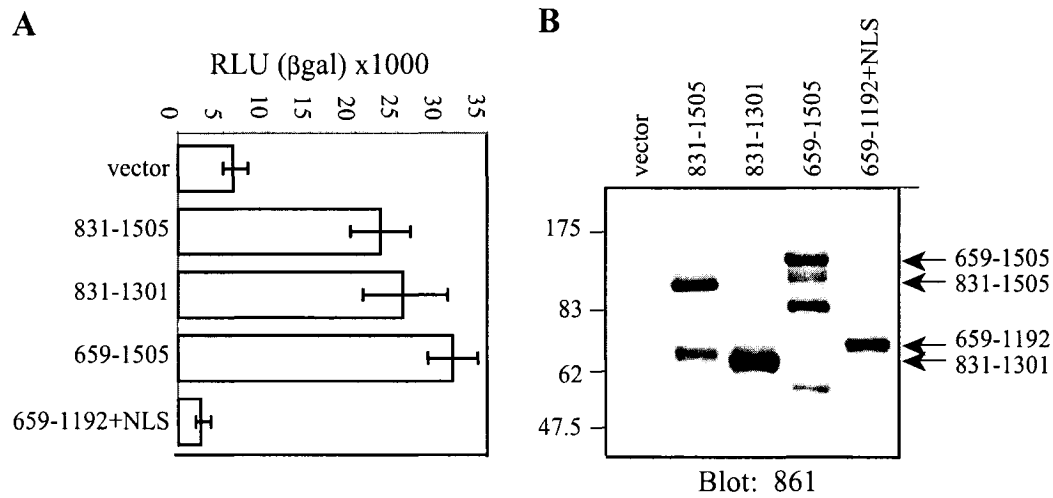
Fig. 3. The Cut homeodomain, but not the carboxy-terminal domain of CDP/Cux is required for stimulation. HS578T cells were co-transfected with the DNA pol α -65/+47 reporter construct (see Fig. 5), and vectors expressing N- and C-terminally truncated CDP/Cux proteins, as indicated. 48 hours post-transfection, cells were harvested and cytoplasmic and nuclear extracts were prepared.

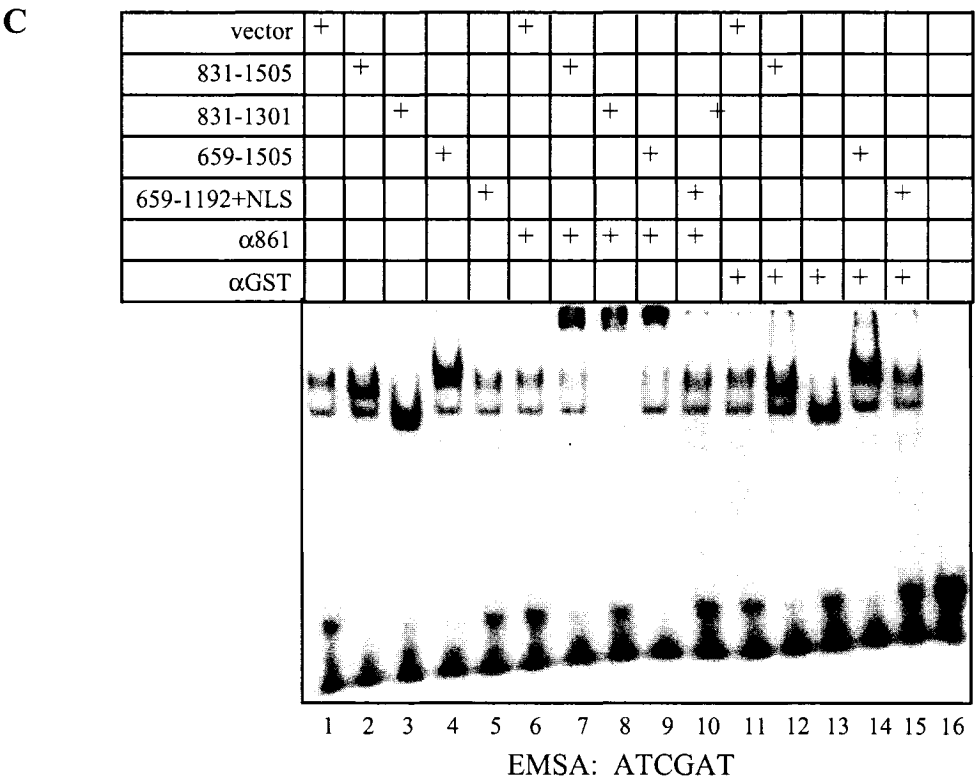
(A) Cytoplasmic extracts were analyzed for luciferase activity as in Fig. 1A. The means of 3 transfections are shown.

(B) Nuclear extracts were submitted to Western blot analysis using the α 861 antibody.

(C) Nuclear extracts were analyzed by EMSA with oligonucleotides encoding a CDP/Cux consensus binding site as in Fig. 2C. Complexes containing CDP/Cux were supershifted with the 861 antibody (lanes 6-10), but not with a non-specific antibody (lanes 11-15).

A diagrammatic representation of the effector plasmids, and the region recognized by the 861 antibody are shown at the bottom.


A
RLU (βgal) x1000
0
5
10
15
20
25
30
35
vector
831-1505
831-1301
659-1505
659-1192+NLS
B
vector
831-1505
831-1301
659-1505
659-1192+NLS
175
83
62
47.5
659-1505
831-1505
659-1192
831-1301
Blot: 861


C
vector
831-1505
831-1301
659-1505
659-1192+NLS
α861
αGST
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
EMSA: ATCGAT

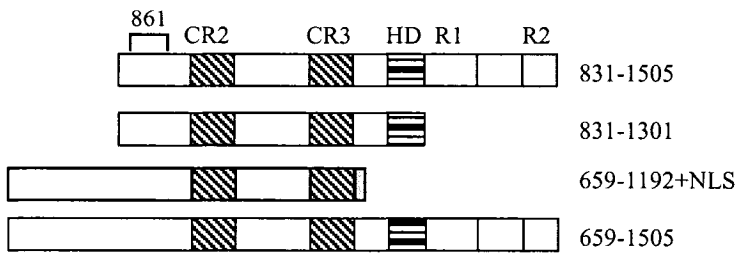

861
CR2
CR3
HD
R1
R2
831-1505
831-1301
659-1192+NLS
659-1505

Fig. 4. CDP/Cux CR2CR3HD stimulates other S phase-specific gene promoters. HS578T cells were co-transfected with the indicated reporter constructs and either empty vector or a vector expressing CDP/Cux CR2CR3HD. Luciferase assays were performed as in Fig 1A. Average fold activation for DNA pol α, p21, DHFR, CAD, and Cyclin A are 12, 1, 12, 8, and 9, respectively.

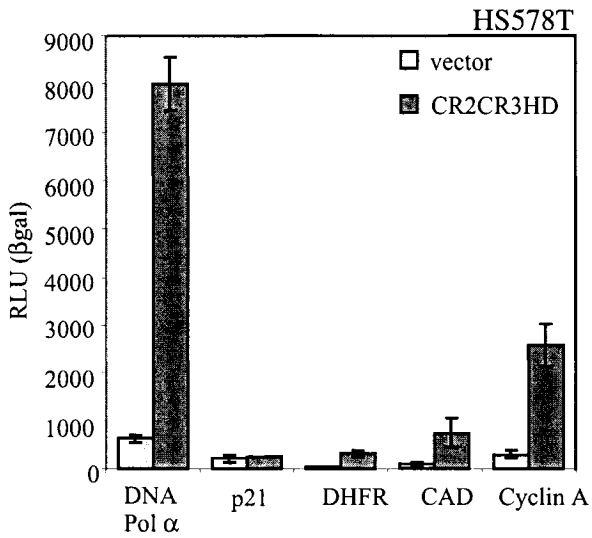
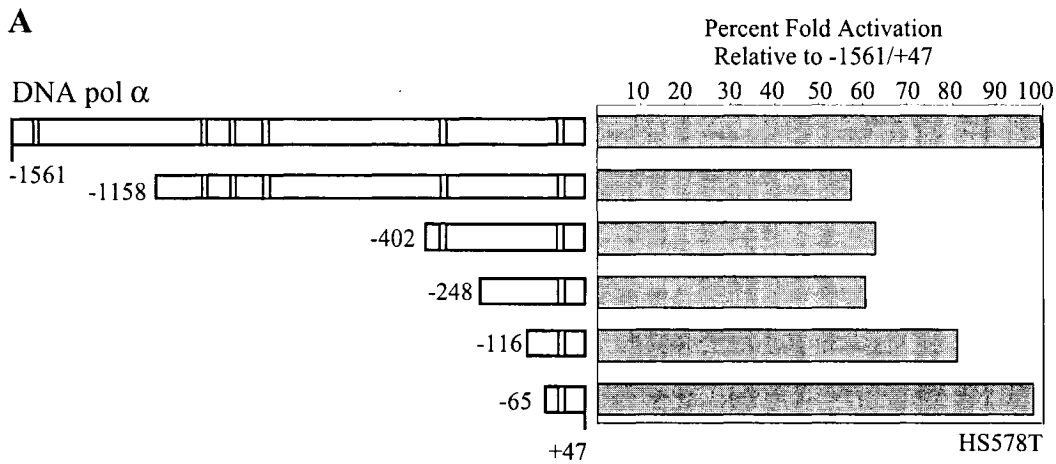

HS578T
vector
CR2CR3HD
RLU (βgal)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
DNA Pol α
p21
DHFR
CAD
Cyclin A

Fig. 5. CDP/Cux can stimulate the core DNA pol α gene promoter.

(A) HS578T cells were co-transfected with DNA pol α reporter constructs with progressive 5' deletions, and either an empty vector or a vector expressing CDP/Cux CR2CR3HD. Luciferase assays were performed as described. Results are expressed as fold activation relative to that of the –1561/+47 reporter, which was assigned a value of 100%.

(B) Linker scanning mutations were introduced at 10 bp intervals within the reporter construct containing the core DNA pol α gene promoter. Mutations consisted in the replacement of the indicated sequence with the sequence GAAAGCTTCA. HS578T cells were co-transfected with the indicated reporter constructs and either an empty vector or a vector expressing CDP/Cux CR2CR3HD. Luciferase assays were performed as described in Fig 1A. Results are expressed as fold activation relative to that of the wild type -65/+47 reporter, which was assigned a value of 100%.


A
Percent Fold Activation
Relative to -1561/+47
DNA pol α
10 20 30 40 50 60 70 80 90 100
-1561
-1158
-402
-248
-116
-65
+47
HS578T

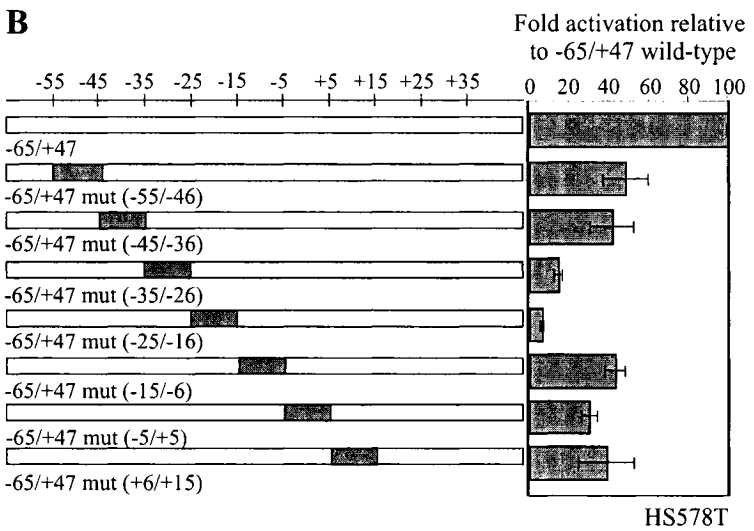

B
Fold activation relative
to -65/+47 wild-type
-55 -45 -35 -25 -15 -5 +5 +15 +25 +35
0 20 40 60 80 100
-65/+47
-65/+47 mut (-55/-46)
-65/+47 mut (-45/-36)
-65/+47 mut (-35/-26)
-65/+47 mut (-25/-16)
-65/+47 mut (-15/-6)
-65/+47 mut (-5/+5)
-65/+47 mut (+6/+15)
HS578T

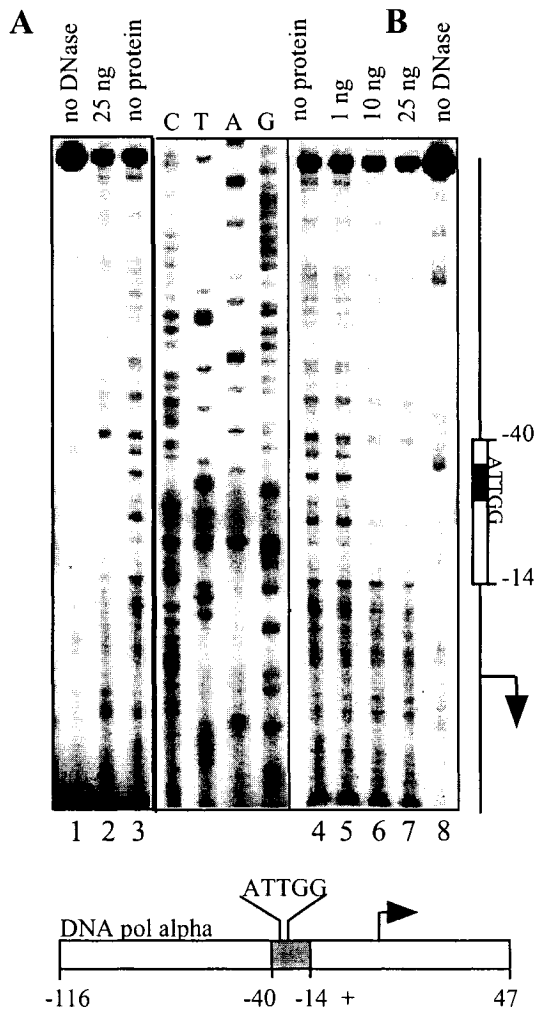
Fig. 6. A truncated CDP/Cux protein can bind to the core DNA pol α gene promoter.

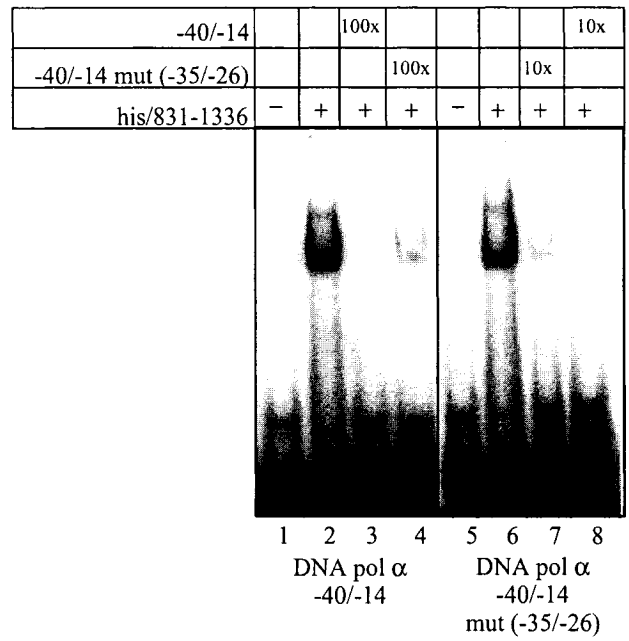
(A) DNase I footprinting analysis of the core DNA pol α promoter. A DNA fragment including sequences -116/+47 of the DNA pol α promoter was end-labeled at position +47 and was incubated with the purified bacterially expressed his-CR2CR3HD (lane 2) or his-CR3HD protein (lanes 5-7). A sequencing reaction was run in parallel.

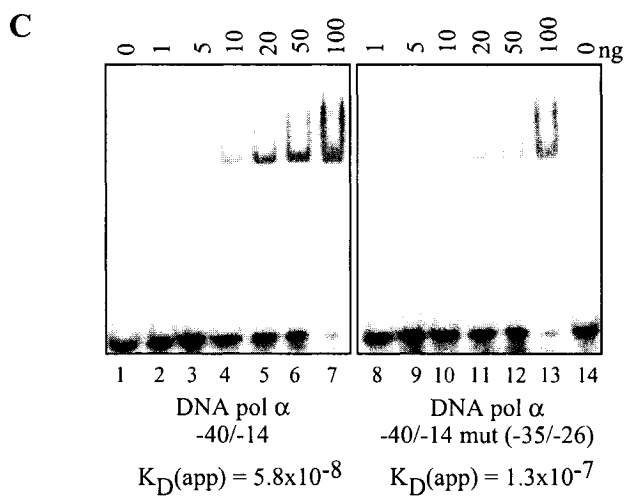
(B) EMSA was performed using radiolabeled oligonucleotides containing nt -40/-14 of the DNA pol α gene promoter, either wild type (left panel) or with the linker replacement mutation at position -35/-26 (right panel), and 20 ng of purified bacterially expressed his-CDP/Cux CR2CR3HD protein. As competitors, the same, cold, oligonucleotides representing the wild type and mutated sequence were added as indicated.

(C) EMSA was performed using the same radiolabeled oligonucleotides as in (B) with increasing amounts of his-CDP/Cux CR2CR3HD and analyzed by EMSA. The apparent K_D was determined as described in Materials and Methods.

(D) EMSA was performed using oligonucleotides containing nt -40/-14 of the DNA pol α gene promoter and varying amounts of the purified histidine-tagged CDP/Cux recombinant proteins, 831-1336 (CR2CR3HD) and 1-1505.


A
no DNase
25 ng
no protein
C T A G
B
no protein
1 ng
10 ng
25 ng
no DNase
-40
ATTGG
-14
1 2 3
4 5 6 7 8
ATTGG
DNA pol alpha
-116
-40
-14
+
47


-40/-14
-40/-14 mut (-35/-26)
his/831-1336
100x
10x
1 2 3 4 5 6 7 8
DNA pol α -40/-14
DNA pol α -40/-14 mut (-35/-26)


C
0 1 5 10 20 50 100
1 5 10 20 50 100 0 ng
1 2 3 4 5 6 7
8 9 10 11 12 13 14
DNA pol α -40/-14
DNA pol α -40/-14 mut (-35/-26)
K_D(app) = 5.8x10^-8
K_D(app) = 1.3x10^-7

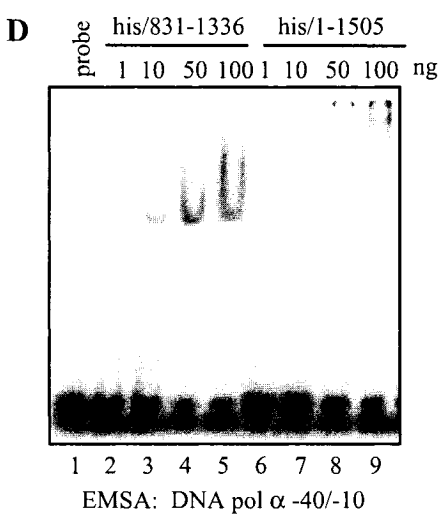

D
probe
his/831-1336
his/1-1505
1 10 50 100 1 10 50 100 ng
1 2 3 4 5 6 7 8 9
EMSA: DNA pol α -40/-10

Fig. 7. CDP/Cux binds the DNA pol α gene promoter *in vivo*, specifically during the S phase of the cell cycle.

(**A**) CDP/Cux binds to the DNA pol α gene promoter *in vivo*. Chromatin immunoprecipitations (ChIP) were performed using HS578T cells and either of the following antibodies: α 861 CDP/Cux antibody (lane2), anti-IgG (secondary) antibody (lane 4), or anti-HA antibody (lane 5). The immunoprecipitated DNA was used as template in polymerase chain reactions (PCR) using the indicated primers from the DNA pol α gene promoter or from the GAPDH promoter (lane 2). As control, the PCR reactions were performed in parallel using total chromatin (lane 3). Shown below is a map of the DNA pol α gene promoter indicating the positions of primers used in PCR reactions 1, 2 and 3 .

(**B**) Binding of CDP/Cux to the DNA pol α gene promoter occurs in S phase only in HS578T cells. HS578T cells were synchronized by double thymidine block as detailed in Materials and Methods. To obtain populations of cells enriched in either S, G2/M or G1 phase, cells were grown for 0, 4, or 10 hours respectively, following the second thymidine block. Cells were harvested and processed for ChIP assay and cell cycle analysis. Cell cycle distribution was monitored by fluorescence-activated cell sorting (FACS) analysis after staining of the DNA with propidium iodide. The FACS profiles are shown at the bottom, together with the calculated proportion of cells in each phase of the cell cycle.

(**C**) Chromatin immunoprecipitations (ChIP) were performed using HS578T cells and the indicated antibodies. The immunoprecipitated DNA was used as template in real-time PCR using either PCR1 or PCR2 primers as indicated. The results are expressed as fold-activation using as a control the αIgG sample which was given a value of 1. In the rightmost panel, nuclear extracts from HS578T cells were submitted to immunoprecipitation with the indicated antibodies, followed by immunoblotting with the 1300 antibody. Below is a schematic representation of CDP/Cux isoforms and the regions recognized by the respective antibodies. The evolutionarily conserved domains are indicated (CC, coiled-coil; CR1, CR2 and CR3: Cut repeats 1, 2 and 3; HD, homeodomain).

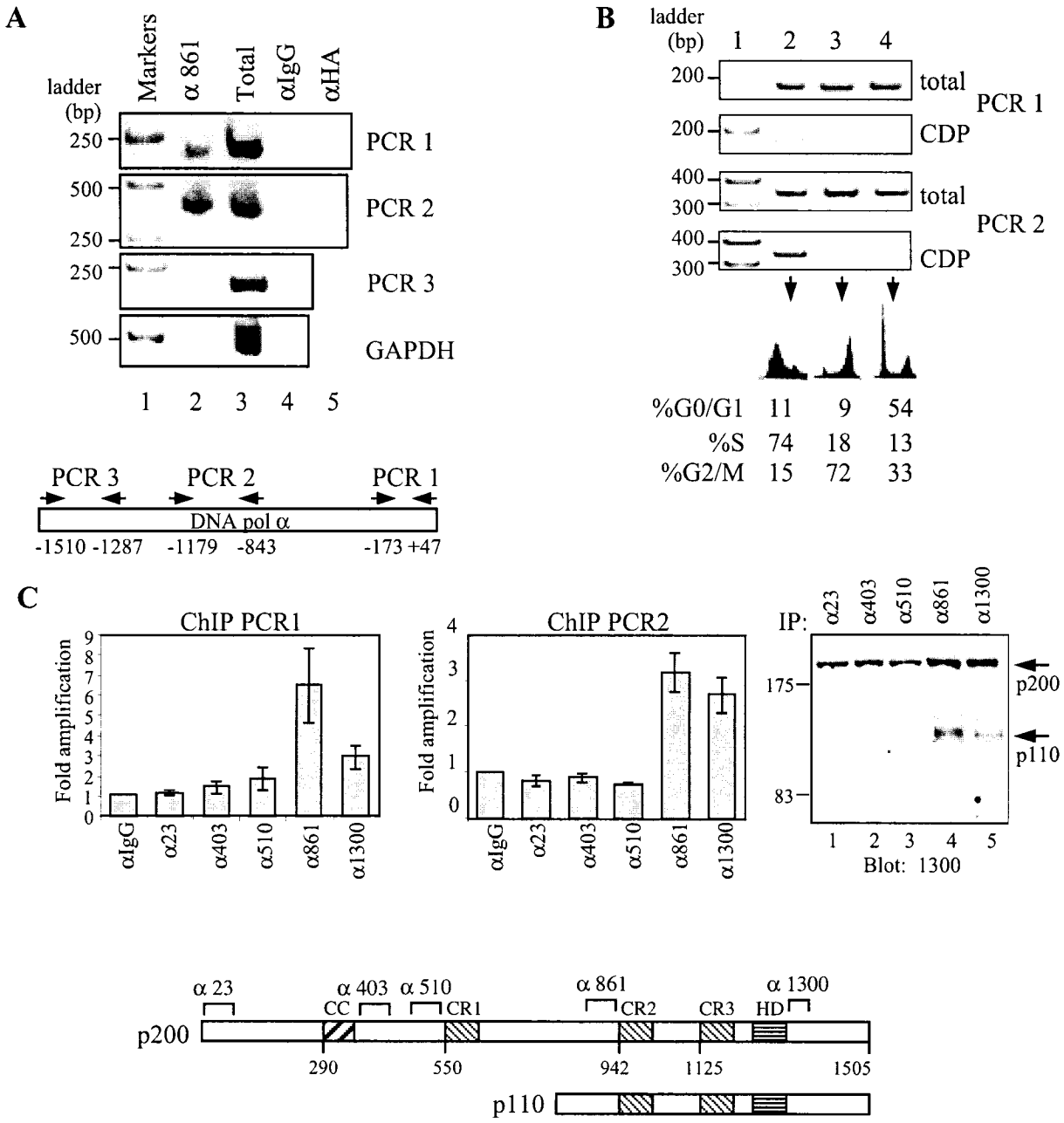
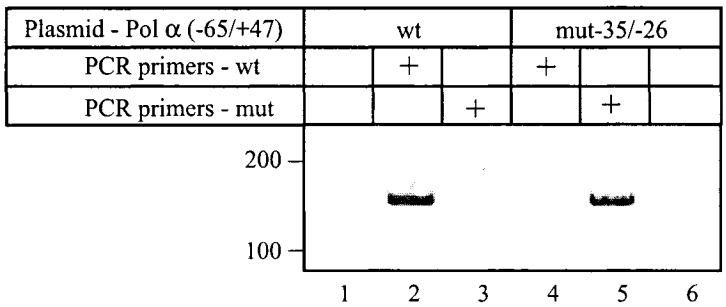

A
Markers
α 861
Total
αIgG
αHA
ladder (bp)
250
500
250
250
500
PCR 1
PCR 2
PCR 3
GAPDH
1 2 3 4 5
PCR 3
PCR 2
PCR 1
DNA pol α
-1510 -1287
-1179
-843
-173 +47
B
ladder (bp)
1 2 3 4
200
200
400
300
400
300
total
CDP
total
CDP
PCR 1
PCR 2
%G0/G1 11 9 54
%S 74 18 13
%G2/M 15 72 33
C
ChIP PCR1
Fold amplification
αIgG
α23
α403
α510
α861
α1300
ChIP PCR2
IP:
175
83
p200
p110
Blot: 1300
α 23
α 403
α 510
α 861
α 1300
CC
CR1
CR2
CR3
HD
p200
290
550
942
1125
1505
p110

Fig. 8. A linker-scanning mutation at positions -35/-26 of the DNA pol α gene promoter prevents the binding of CDP/Cux to the reporter plasmid *in vivo*.

(A) PCR primers (indicated by arrows in the diagram) were designed to specifically amplify either the wild type DNA pol α gene promoter or the mutant promoter containing the linker-scanning mutation at position -35/-26 (see Fig 7). PCR reactions were performed using either wild type or mutated plasmid DNA template and the corresponding primers, as indicated.

(B) CDP/Cux *in vivo* binds to the wild type DNA pol α reporter but not to the -35/-26 mutant reporter. HS578T cells were co-transfected with the wild type and mutant -35/-26 reporter construct and either an empty vector or a vector expressing CDP/Cux CR2CR3HD. After two days, ChIP assays were performed using the indicated primers and antibodies. As control, the PCR reactions were performed in parallel using total chromatin (lane 3). Abbreviations: Pre-I: pre-immune serum; CDP: 1300, a CDP/Cux-specific Ab

A


Plasmid - Pol α (-65/+47)
wt
mut-35/-26
PCR primers - wt
PCR primers - mut
+
200
100
1 2 3 4 5 6

B

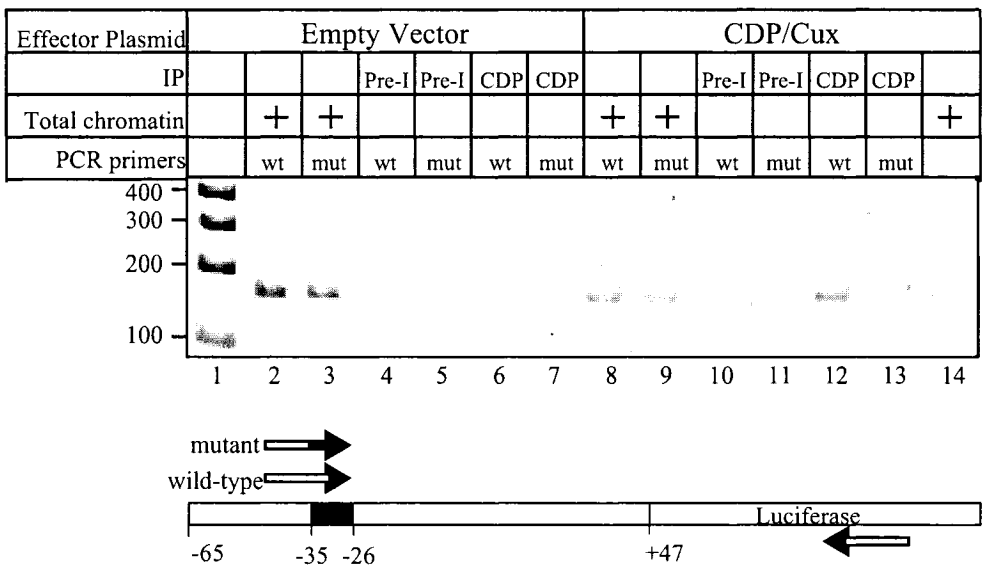
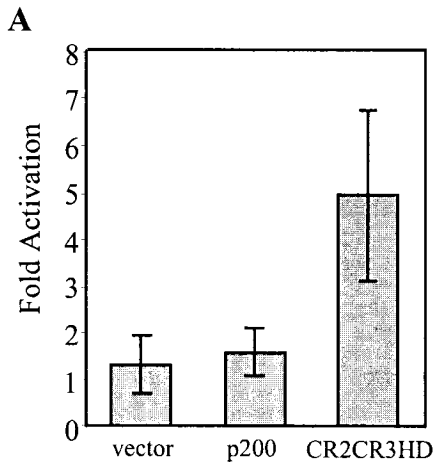
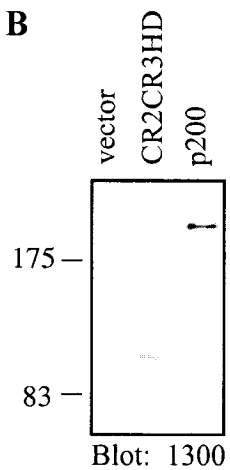

Effector Plasmid
Empty Vector
CDP/Cux
IP
Pre-I
CDP
Total chromatin
PCR primers
wt
mut
400
300
200
100
1 2 3 4 5 6 7 8 9 10 11 12 13 14
mutant
wild-type
Luciferase
-65
-35 -26
+47

Fig. 9. CDP/Cux stimulates the endogenous DNA pol α gene promoter.

(A) HS578T cells were infected with pREV retroviral vectors expressing a full-length or a truncated CDP/Cux protein. To minimize the extent of proteolytic processing of the full-length CDP/Cux protein, cells had been plated so as to reach near-confluence 48 hours post-infection, at which time total RNA and whole cell extracts were prepared. Expression of DNA pol α RNA was determined by Real-Time PCR and was normalized for GAPDH RNA expression. DNA pol α expression in infected cells was compared with that in uninfected cells, and is expressed as fold activation relative to expression in uninfected cells. Values represent the mean of three separate infections.

(B) Nuclear extracts were submitted to Western blot analysis using the α 1300 antibody.


A
Fold Activation
8
7
6
5
4
3
2
1
0
vector
p200
CR2CR3HD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Chapter III - p110 CDP/Cux Cooperates with E2F Transcription Factors in the Transcriptional Activation of Cell Cycle-Regulated Gene Promoters

Preface

I had shown that p110 CDP/Cux could stimulate expression of the DNA polymerase α gene promoter, and that this correlated with DNA binding. Results from my linker-scanning analysis suggested that another transcription factor could cooperate with p110 in the activation of this promoter. One of the mutants, –25/-16, displayed reduced activation by CDP/Cux in reporter assays, yet was not bound by CDP/Cux. I reasoned that another transcription factor would bind there, and would facilitate activation by p110 CDP/Cux

In this chapter, I demonstrated that the –25/-16 mutant prevented binding by E2F transcription factors. E2F1 and E2F2 cooperated with p110 in the activation of cell cycle-regulated gene promoters. Chromatin immunoprecipitation experiments suggested that p110 CDP/Cux could recruit E2Fs to cell cycle-regulated promoters.

ABSTRACT

The processed isoform of the CDP/Cux transcription factor, p110 CDP/Cux, was shown to stimulate cell proliferation by accelerating entry into S phase. Previous studies established that p110 CDP/Cux can function as a transcriptional repressor or activator depending on promoter context. Using the DNA pol α gene promoter as a model system, we investigated the mechanism of transcriptional activation by p110 CDP/Cux. Linker-scanning analysis identified a region that is required for p110-mediated activation but is not bound by it. This region contained a low-affinity E2F binding site, and co-expression with a dominant-negative mutant of DP-1 suggested that endogenous E2F factors, indeed, are needed for p110-mediated activation. Tandem affinity purification, chromatin immunoprecipitation and reporter assays indicated that p110 CDP/Cux can engage in weak protein-protein interactions with E2F1 and E2F2, stimulate their recruitment to the DNA pol α gene promoter and cooperate with these factors in transcriptional activation. Genome-wide location analysis identified 212 targets common to p110 CDP/Cux and E2F1. Validation assays on 16 targets did not uncover any false-positives. Gene ontology analysis revealed a striking overrepresentation of genes involved in DNA metabolism and cell cycle progression. Reporter assays on a subset of genes confirmed that p110 CDP/Cux and E2F1 cooperate in the transcriptional activation of their common targets. Overall, our results show that p110 CDP/Cux and E2F cooperate in the binding to and the regulation of many cell cycle genes.

INTRODUCTION

CDP/Cux (CCAAT-displacement protein/cut homeobox) belongs to a family of transcription factors involved in the control of proliferation and differentiation (reviewed in (44)). The full-length protein, p200 CDP/Cux, interacts transiently with DNA and is expressed throughout the cell cycle. Proteolytic cleavage at the G1/S transition yields an amino-terminally truncated isoform, p110 CDP/Cux, which, following dephosphorylation by Cdc25A, interacts stably with DNA (7, 41). A tissue-specific mRNA is initiated within intron 20 and codes for the p75 CDP/Cux isoform that also binds stably to DNA (48) (21).

Initial studies of mammalian CDP/Cux revealed its role as a transcriptional

repressor that is expressed in differentiating precursor cells, and serves to down-regulate the expression of genes expressed only in terminally differentiated cells (35, 46, 56, 58, 59). CDP/Cux was also reported to regulate the expression cell cycle-regulated genes such as $p21^{WAF1}$ (7), histone H1, H2A, H2B, H3, and H4 (12, 23, 65, 67), and DNA pol α (62). Cux1 gene ablation in mice resulted in high perinatal lethality. Surviving mice exhibited a number of phenotypes, including growth retardation, male infertility, curly whiskers, abnormal hair follicle morphogenesis, male infertility, and a shortage of T and B cells (13, 37, 55, 64). Transgenic mice expressing p200 CDP/Cux exhibited multi-organ hyperplasia and organomegaly (31), whereas those expressing p75 or p110 CDP/Cux displayed enhanced susceptibility to malignancies in various tissues and cell-types ((4), and Cadieux and Nepveu, unpublished data).

The E2F family of transcription factors consists of activator E2Fs (E2F1, E2F2, and E2F3a), repressor E2Fs (E2F3b, E2F4, E2F5, E2F6, E2F7, and E2F8), and DP heterodimerization partners (DP1 and DP2) (reviewed in (36)). Evidence from gene ablation studies has revealed some functional redundancy among E2F family members. While the loss of one E2F can be functionally compensated for by other E2Fs (15, 25, 26, 32), the combined loss of two E2Fs results in a more severe phenotype (6, 33). Loss of E2F1, E2F2, and E2F3 prevents mouse embryonic fibroblasts (MEFs) from re-entering the cell cycle following quiescence (75). Indeed, E2Fs play a critical role in the control of cellular proliferation. In quiescence, pRB pocket protein family members bind E2Fs and repress transcription of target genes (50, 57). Following growth stimulation, cyclin/cyclin-dependent kinase (Cdk) complexes phosphorylate the pocket protein, which then dissociates from the promoter-bound E2F/DP heterodimer. This results in derepression, and allows for the transcriptional activation of numerous genes with roles in DNA replication and cell cycle progression.

Using classical approaches, such as transient reporter assays and overexpression systems, a number of E2F targets were identified, such as DHFR, Cdc6, Orc1L, Cdc25A, B-myb, and cyclin A (reviewed in Bracken 2004). In more recent years, gene expression microarray profiling and chromatin immunoprecipitation-microarray (ChIP-chip) have allowed the unbiased identification of target genes (3, 22, 43, 70, 72). Other studies have explored the promoter occupancy, at different stages of the cell cycle, of different E2F

family members, and pocket proteins (2, 49, 61). The molecular basis for the functional differences among E2F family members has been the subject of many recent studies (17, 18, 22, 54). Whereas no distinction in DNA binding specificity among E2Fs has been determined (76), specific protein-protein interactions were shown to contribute to promoter specificity. For example, cooperativity was documented between E2F3 and TFE3 (17) and both E2F2 and E2F3 and YY1 (54).

We have recently shown that p110 CDP/Cux can stimulate cell proliferation by accelerating entry into S phase (51). Moreover, p110 CDP/Cux was shown to stimulate expression of a DNA pol α gene reporter in transient transfection assays, and to stimulate the expression the endogenous DNA pol α gene following retroviral infection (62). Linker-scanning mutations identified a few regions that are required for CDP/Cux-mediated activation. One region contained an inverted CCAAT sequence that functioned as a low-affinity binding site for CDP/Cux. Using *in vitro* and *in vivo* DNA binding assays, in conjunction with mutated versions of the promoter, a correlation was established between transcriptional stimulation and binding of CDP/Cux to this inverted CCAAT site (62). An adjacent region contained a sequence related to the consensus-binding site for E2F. The homologous region in the mouse DNA pol α gene promoter had been shown to be required for growth-dependent regulation and for E2F1-mediated stimulation (27). That this region was required for transcriptional activation, although CDP/Cux did not bind to it *in vitro*, suggested the possibility that E2F might cooperate with CDP/Cux. In the present study, we investigated this hypothesis. Reporter assays in the presence of a dominant-negative mutant of DP1 suggested that the binding of an E2F factor was necessary for CDP/Cux-mediated activation. Further experiments showed that both E2F1 and E2F2 were able to cooperate with CDP/Cux. In chromatin immunoprecipitation assays, ectopic expression of CDP/Cux enhanced the recruitment of either E2F1 or E2F2 to the DNA pol α promoter. In contrast, increased expression of E2F1 or E2F2 did not improve the binding of CDP/Cux, suggesting that CDP/Cux is the limiting factor in the recruitment of these factors to this promoter. Using ChIP-chip assays, we established a list of cell cycle genes that are co-regulated by CDP/Cux and E2F1. These genes are involved in biochemical activities that take place during S phase,

like DNA replication and DNA repair, but also in functions that are required later during the cell cycle either in G2 or M phases.

MATERIALS AND METHODS

Plasmid construction.

CDP/Cux and luciferase reporter constructs have been described in our previous studies ((52, 62) and (Harada et al., submitted for publication; please, see attached manuscript in appendix)).

Cell culture and transfection and synchronization.

Hs 578T cells were grown in DMEM medium supplemented with 5% fetal bovine serum (FBS)(Gibco). Transient transfections were performed with GeneJuice (Novagen) according to the manufacturer's instructions. For synchronization by thymidine block, Hs 578T cells were cultured overnight in DMEM plus 5% FBS supplemented with 2 mM thymidine and harvested.

Luciferase assay.

Luciferase assays were performed as previously described (41). Because the internal control plasmid is itself often repressed by CDP/Cux, as a control for transfection efficiency the purified β-galactosidase protein (Sigma) was included in the transfection mix, as previously described (24). The luciferase activity was then normalized based on β-galactosidase activity.

Chromatin Immunoprecipitation (ChIP). 2 x10^8 thymidine-blocked Hs 578T cells were used for each ChIP. Immunoprecipitation was performed with affinity-purified CDP/Cux antibodies 861 and 1300 (41), or an E2F1 antibody (#05-379, Upstate). The nuclei were lysed as described in (71), then lysed in RIPA-M buffer (10 mM Tris-HCl pH 8, 1 mM EDTA, 0.5 mM EGTA, 150 mM NaCl, 1% Triton X-100, 0.5% DOC, 0.1% SDS, 1 mM PMSF, protease inhibitors) and sonicated on ice to obtain 250- to 800-bp-long DNA fragments. After preclearing for 1 hour and incubation with antibodies overnight, immunocomplexes were washed 3 times each in wash buffer I (20 mM Tris-HCl pH 8, 2 mM EDTA, 2 mM EGTA, 150 mM NaCl, 1% NP-40, 0.5% DOC, 0.2% SDS), wash buffer II (20 mM Tris-HCl pH 9, 2 mM EDTA, 2 mM EGTA, 500 mM NaCl, 1% NP-40, 0.5% DOC, 0.1% SDS), wash buffer III (50 mM Tris-HCl pH 7.5, 2 mM EDTA, 1 mM EGTA, 0.5M LiCl, 1% NP-40, 0.7% DOC,) then washed once in Tris-EDTA. Crosslinked DNA was eluted with 1% SDS, 10 mM Tris-HCl pH 8, 10 mM EDTA at 65°C for 30 min. After reversal of formaldehyde cross-linking, chipped DNAs

were treated with RNase A and Proteinase K.

Enrichment Calculation. Enrichment levels of genes were determined by real-time PCR using G6PDH as an internal control and chipped DNAs obtained by immunoprecipitation with either CDP/Cux antibodies or no antibody. Specific enrichment of a promoter was calculated as follows: Target (CDP/Cux IP)/Target (no Ab IP)) x ((G6PDH (no Ab IP)/G6PDH (CDP/Cux IP).

Probe generation and microarray hybridization. The generation of labeled DNAs from individual ChAP samples was performed following the protocol of linker-mediated PCR (LM-PCR) as detailed previously (49). Briefly, ChIPped and ChAPped DNAs and input DNA were blunted, ligated to a unidirectional linker and amplified by PCR for 24 cycles to generate a sufficient amount of DNA. ChIP or ChAP, and input DNAs were fluorescently labeled with Cy5 fluorophore and Cy3 fluorophore, respectively, by using BioPrime Array CGH genomic labeling kit following the manufacturer's instructions (Invitrogen). Prior to hybridization, microarray slides were incubated in a blocking solution, 1.6% succinic anhydride in 1-methyl-2-pyrrolidinone, for 20 min at RT. After washing, labeled DNAs were added to the hybridization buffer (25% Formamide, 5x SSC, 0.1% SDS, 0.2% BSA, 0.4ug/ul of human Cot-1 DNA, 0.8ug/ul of yeast tRNA) and hybridized at 55°C for 20 hours. The slides were washed once with 2x SSC, 0.1% SDS for 15 min, twice 2 min with 0.1x SSC, 0.1% SDS, twice 1 min 0.1x SSC and then spun dried. Hybridized slides were scanned with an Axon 4000b scanner and the acquired images were analyzed with the software GenePix Pro, Version 4.1. Each set of hybridizations was performed three times with independent ChIP or ChAP materials.

Microarray design. A microarray containing 19k human promoters was generated as reported (29). In brief, the regions ranging from 800 bp upstream and 200 bp downstream of the transcription start sites from 18,660 human genes were amplified by PCR and QC tested and applied on an Poly-L-lysine glass slides.

Microarray data analysis. The analysis of the ChIP-chip or ChAP-chip results was done as described (45). Promoters were considered 'bound' when the binding P-value in the error model was < 0.005. Functional categories were established using programs from Expression Analysis Systematic Explorer (EASE) at: (http://david.niaid.nih.gov/david/ease.htm). The list of genes from the 19k microarray was

used as the background.

Tandem Affinity Purification and Western blot analysis.

Hs 578T cells stably expressing a recombinant p110-Tag[2] protein, or vector control, were transfected with pCMV/HA-E2F1, pCMV/HA-E2F2, pCMV/HA-E2F3a, or pCMV/HA-E2F5 expression plasmids. 2-4 x 10^8 cells were used for purification by the Taptag purification method (47). Western blots were performed using 861 and 1300 antibodies (data not shown), or an HA-11 antibody (Covance).

***In vivo* DNA binding to transfected reporter plasmids.**

Hs 578T cells were transfected with pGL3-Pol α (-65/+47), or pCADluc, and either pXJ42 or pXJ42/Myc-CDP/Cux 878-1336, and either pcDNA3 or pCMV/E2F1 or pCMV/E2F2. DNA was extracted approximately 24 hours post-transfection, and was processed as described in (62).

RESULTS

The dominant-negative DP1Δ103-126 mutant prevents transcriptional activation by CDP/Cux

Previous experiments using linker-scanning mutations identified distinct regions of the DNA pol α gene promoter that were necessary for the transcriptional activation by p110 CDP/Cux. The -35/-26 and the -25/-16 regions, respectively, contain binding sites for p110 CDP/Cux and E2F (see introduction). The requirement for an E2F binding site raised the possibility that an endogenous E2F factor might participate in the transcriptional activation mediated by p110 CDP/Cux. As a preliminary approach to test this hypothesis, we measured the activity of the DNA pol α gene reporter in the presence of CDP/Cux and a dominant-negative mutant of DP1, DP1Δ103-126. This mutant was previously shown to interact with E2Fs but to be unable to bind DNA, thereby keeping its E2F partners away from DNA (74). Transcriptional activation was reduced from 10-fold down to 2.5-fold in the presence of DP1Δ103-126 (Fig. 1A). These results suggested that functional endogenous E2F factors are necessary for CDP/Cux to transactivate the DNA pol α gene promoter.

CDP/Cux cooperates with E2F1 and E2F2 in the stimulation of the DNA pol α gene promoter.

We then investigated which E2F factors, if any, were able to transactivate the DNA pol α gene promoter. Hs 578T cells were transfected with the reporter construct, and increasing amounts of E2F1-6 expression plasmids. Dose-dependent stimulation was observed for E2F1, E2F2, and E2F4 (Fig. 1B). It should be noted that while E2F4 does have a transactivation domain, it is observed in the nucleus only in G0 and early G1, in a complex with pocket proteins, and is therefore not normally associated with activation of transcription (69).

We next asked if any E2Fs could cooperate with p110 CDP/Cux (Fig. 2C). Hs 578T cells were co-transfected with sub-optimal amounts of various combinations of effector plasmids. With these experimental conditions, on their own p110 CDP/Cux and E2F1 mediated 3.5- and 1.5-fold activation, respectively (Fig. 1C). When the factors were expressed in combination, synergy was observed between CDP/Cux and either E2F1

or E2F2, but not with E2F4 (Fig. 1C). Therefore, directly or indirectly, activator E2Fs, E2F1 and E2F2, cooperate with p110 CDP/Cux in the activation of the DNA pol α gene promoter.

Cooperation between p110 CDP/Cux and E2F1 requires binding sites for both factors.

The above results indicated that some E2F factors were able to potentiate the transcriptional activation mediated by p110 CDP/Cux. The effect of E2F could be indirect or could involve a direct interaction with the DNA pol α promoter. To begin to investigate the mechanism by which E2F and p110 CDP/Cux cooperate, we repeated the reporter assay using linker-scanning mutants in which the CDP/Cux or the E2F binding site, or other sequences, were replaced. Interestingly, when CDP/Cux and E2F1 were co-expressed, transcriptional activation was reduced to 7% and 15% of the wild-type promoter upon replacement of the CDP/Cux or E2F binding site, respectively (Fig. 2, -35/-26 and -25/-16). In contrast, replacement of the -45/-36 region only had a mild effect on the stimulation by p110 CDP/Cux and E2F1. We conclude that the cooperation between p110 CDP/Cux and E2F1 requires that the DNA pol α gene promoter contain binding sites for both transcription factors.

p110 CDP/Cux interacts with E2F1 and E2F2 *in vivo*

E2F and p110 CDP/Cux may each bind to the promoter independently. Alternatively, cooperation may involve physical interaction between the two factors. To investigate this possibility, tandem affinity purification (TAP) was performed using Hs 578T cells stably carrying a retroviral vector expressing CDP 831-1336/tag[2] and transiently transfected with vectors for HA-tagged E2F proteins. As controls, transient transfections and TAP were performed in parallel using Hs 578T cells carrying an empty retroviral vector. Western blot analysis revealed weak protein-protein interactions between CDP/Cux and E2F1, and E2F2 (Fig. 3). In contrast, no band was observed for E2F5 (Fig. 3). A band for E2F3a was also observed in the purified fraction, however, since it was also present in the empty-vector control, we dismissed this result as being

evidence for interaction. Thus, in affinity chromatography CDP/Cux specifically interacted with E2F1 and E2F2.

p110 recruits E2F1 and E2F2 to the DNA pol α gene promoter.

Given the proximity of the CDP and E2F binding sites, and the observed protein-protein interactions, we next asked if co-expression of p110 and E2F would strengthen their interaction with the DNA pol α gene promoter. Chromatin immunoprecipitation assays were performed to measure the interaction *in vivo* between E2Fs or CDP/Cux and the DNA pol α gene reporter plasmid. Immunoprecipitation using E2F1 or E2F2 antibodies indicated that each factor was able to bind to the reporter plasmid *in vivo* (Fig. 4A, lanes 5 and 11). Interestingly, co-expression with p110 CDP/Cux increased the interaction of either E2F1 or E2F2 with the reporter plasmid (Fig. 4A, E2F1, compare lane 5 with 4; E2F2, compare lane 11 with 12, and Fig. 4B). Quantitative real-time PCR revealed a 5.2-fold and 2.5-fold increase in promoter binding for E2F1 and E2F2, respectively, upon co-expression of CDP (Fig. 4B). Importantly, co-expression of p110 did not increase the steady-state protein level of E2F1 and E2F2 (data not shown). In contrast, chromatin immunoprecipitation using CDP/Cux antibodies did not reveal a stronger interaction of p110 CDP/Cux with the promoter when either E2F1 or E2F2 were co-expressed (Fig. 4A, E2F1, compare lane 2 with 3; E2F2, compare lane 11 with 12, and Fig. 4B). These results demonstrate not only that E2F1 and E2F2 can bind to the DNA pol α reporter *in vivo*, but also that over-expression of p110 CDP/Cux can help recruit more of these factors to the promoter.

To ensure that the recruitment of E2F1 and E2F2 by p110 is specific for promoters activated by E2Fs, we repeated the chromatin immunoprecipitation with a CAD reporter plasmid, which had previously been shown not to be regulated by E2Fs (34). Immunoprecipitation with CDP antibodies gave 5-fold enrichment in binding, however no binding by E2F1 or E2F2 was observed, whether they were transfected alone or with p110 CDP/Cux (Fig. 4C). These results indicate that p110 CDP/Cux does not recruit E2F1 and E2F2 to every promoter to which it binds.

Cell cycle genes are overrepresented among ChIP-chip targets common to p110 CDP/Cux and E2F1.

Genome-wide location analysis was recently performed to identify transcriptional targets of p110 CDP/Cux (Harada et al., submitted for publication; please, see attached manuscript in appendix). The same 19k promoter microarray was employed in location analysis with an E2F1 antibody. 611 E2F1 targets were identified with a p-value below 0.005 (supplementary information). To this list, we added 16 genes that were identified as E2F targets in previous studies (5, 8, 18, 39, 49, 73, 77). Comparison of p110 CDP/Cux and E2F1 targets indicated that 212 targets are common to E2F1 and p110. Gene ontology analysis using EASE revealed that DNA replication and cell cycle were vastly overrepresented in the list of common p110 and E2F1 targets (Table 1). To validate these results, chromatin immunoprecipitation was performed with CDP/Cux and E2F1 antibodies and quantitative PCR was performed using primers for 16 targets. Enrichment was observed for all targets tested (Table 2).

p110 CDP/Cux and E2F cooperate in the stimulation of cell cycle-regulated gene promoters.

We have recently shown, using transient reporter assays, that p110 CDP/Cux can stimulate expression from a number of cell cycle-regulated gene promoters that were identified in ChIP-chip analysis (Harada et al., submitted for publication; please, see attached manuscript in appendix) . We asked whether E2F activity was necessary for the stimulation of cell cycle-regulated genes by p110 CDP/Cux. Cotransfection of DP1Δ103-126 with p110 caused a significant decrease in the stimulation of Cdc25A, MCM3, cyclin A2, and DHFR (Fig. 6A). However no change was observed with the CAD reporter, which has previously been shown not to be an E2F target (34). This suggested that E2F cooperates with p110 CDP/Cux in the stimulation of many, but not all CDP/Cux-regulated gene promoters. Reporter assays were repeated with p110 CDP/Cux and E2F1. Coexpression of E2F1 resulted in greater-than-additive effects on the stimulation of Cdc25A, MCM3, cyclin A2, and DHFR (Fig. 6B). No increase in the CAD promoter activity was observed, confirming that the effect of E2F1 is specific to

E2F-regulated gene promoters. Together, these results suggest that p110 CDP/Cux and E2F1 cooperate to stimulate the expression of many cell cycle-regulated gene promoters.

Discussion

CDP/Cux was originally characterized as a transcriptional repressor, but recent results indicated that its shorter isoforms could also function in transcriptional activation (20, 41, 53, 62, 63). In particular, p110 CDP/Cux was shown to stimulate expression from the DNA pol α gene promoter, whether in reporter assays or following the infection of cells with a high-titer retroviral vector (62). A direct involvement of CDP/Cux in activation was demonstrated from the correlation between the stimulation of gene expression and the binding of p110 CDP/Cux to the DNA pol α gene promoter, both *in vitro* and *in vivo* (62). A similar correlation has now been established using a number of promoters from other genes, including cyclin A2 (51), MCM3, Cdc25a, and Orc1l (Harada et al., submitted for publication; please, see attached manuscript in appendix).

The mechanism by which short isoforms of CDP/Cux function in transcriptional activation was not immediately apparent, since in the Gal4 DNA binding domain fusion assay two active repression domains were identified downstream of the Cut homeodomain, but no region of CDP/Cux was found to function as an activation domain (38). One clue, however, was suggested from the finding that CDP/Cux is the DNA binding subunit of the HiNF-D protein complex that regulates transcription of cell cycle-regulated histone genes (1, 23, 30, 65-68). These results suggested that CDP/Cux could be part of larger nucleoprotein complexes that regulate transcription. This line of reasoning led us to investigate a replacement mutation, at position –25/-16 of the DNA pol α gene promoter, that prevented transcriptional activation by p110 CDP/Cux without affecting its DNA binding site (62). In the present study, we presented evidence to show that E2F is the factor that binds to this region and cooperates with p110 CDP/Cux to trans-activate the DNA pol α gene. Briefly, in reporter assays we observed an increase in transcriptional activation upon co-expression of p110 CDP/Cux and E2F1 or E2F2 (Fig. 1C). Replacement mutations of the CDP/Cux or E2F binding sites reduced stimulation by each factor individually (Fig. 6B and 6c in (62), and Fig. 2), and also reduced cooperative stimulation when both factors were coexpressed (Fig. 2A and B). Importantly, co-

expression of a dominant negative DP1 significantly reduced the transcriptional activation mediated by p110 CDP/Cux, implying that the activity of endogenous E2F factors was necessary for the stimulatory effect of p110 (Fig. 1A). Results from tandem affinity purification and chromatin immunoprecipitation suggested a potential mechanism for the cooperation between p110 CDP/Cux and E2F factors. Firstly, E2F1 and E2F2 were found to interact with a tagged version of p110 CDP/Cux (Fig. 3). Secondly, ChIP assays had previously shown that both p110 and E2F1 could bind to the DNA pol α gene promoter ((18, 39, 62), and data not shown). In the present study, we demonstrated that co-expression with p110 CDP/Cux leads to an increase in the recruitment of E2F1 and E2F2 to this promoter (Fig. 4). In contrast, we did not observe cooperation in the recruitment to, nor the activation of, the CAD promoter, which in previous studies was shown not to be a target of E2F (Fig. 4C, and Fig. 5). Altogether, the accumulated data suggest a scenario whereby the DNA pol α gene promoter contains sub-optimal binding sites for p110 CDP/Cux and E2F. Consequently, each factor exhibits a low affinity for its binding site and, at physiological concentration, would not be expected to bind to the promoter on its own. However, the proximity of the two binding sites makes it possible for the two proteins to interact with each other as they bind to their respective sites on DNA. Thus, when present together, E2F and p110 CDP/Cux would bind to the promoter with an affinity that is equal to the sum of their protein-protein and protein-DNA interactions.

Interestingly, the cooperation between E2F and p110 CDP/Cux in the regulation of cell cycle genes was independently brought to light using a genomic approach: the location array. Gene ontology analysis of the common targets between E2F1 and p110 CDP/Cux showed a striking over-representation of genes that play a role in cell cycle progression. Indeed, a role for both E2F and p110 CDP/Cux in cell cycle regulation has previously been established using cell-based assays and transgenic models (reviewed in (10)),(4, 51). In contrast, genes involved in apoptosis were not overrepresented among the targets common to p110 CDP/Cux and E2F1. This result is also in accordance with the known cellular functions of E2F1 and p110 CDP/Cux. While overexpression of E2F1 was shown to induce quiescent cells to enter S phase and then to undergo apoptosis, p110 CDP/Cux was unable to stimulate quiescent cells to re-enter into the cell cycle. In the

presence of growth factors, however, cells over-expressing p110 CDP/Cux were able to enter S phase more rapidly, and proliferated faster than control cells, with no evidence of apoptosis. Future experiments should verify whether the induction of apoptosis by E2F1 could be circumvented by over-expressing p110 CDP/Cux.

Results for location array analyses confirmed that E2F1 and p110 CDP/Cux do not cooperate in the induction of apoptotic genes, but cooperate in the regulation of cell cycle genes. These findings confirm that the location array analysis is an unbiased method that can effectively reveal the biological functions of a transcription factor. Moreover, the comparative analysis of data obtained with several transcription factors can point out the cellular activities in which two or more transcription factors cooperate. As transcriptional regulation is a combinatorial process involving the concerted action of several factors and co-factors, a better understanding of how transcriptional programs are established will require the completion of a repository of all overlapping sets of targets for various transcription factors. The location array will be essential in the accomplishment of this task.

We presented evidence that p110 CDP/Cux cooperates with E2F1 and E2F2, but we did not observe cooperation with E2F3. The interaction with p110 CDP/Cux, therefore, appears to be specific to some E2F factors, but the significance of this specificity is not immediately apparent. Recent results using RNAi-mediated knockdown in mouse embryo fibroblasts suggested that E2F3 is the primary E2F factor responsible for the expression of genes involved in cell proliferation(28). These findings, however, do not exclude that E2F1 and E2F2 may play an essential role in promoting proliferation in distinct cell types or in specific situations. Moreover, the activator E2Fs are likely to fulfill partially redundant functions, as revealed from the various knockout mouse models (15, 25, 26, 32). One particular situation, where the stimulation of cell proliferation could be induced by any of the activator E2Fs, is cancer. While deregulation of the cyclin D/pRb pathway was most often reported, amplification and/or overexpression of E2F1 and E2F3 has been observed in erythroleukemia cell lines, primary human acute lymphoid or myeloid leukemias, gastric and colorectal carcinomas, non-small cell lung carcinomas, esophageal squamous cell carcinomas, and bladder and prostate cancer (11, 14, 16, 19, 60). On the other hand, from mRNA and immunohistochemical analyses,

CDP/Cux was found to be over-expressed in breast tumors and in malignant plasma cells, and studies addressing the specific isoforms of CDP/Cux established that p110 and p75 were over-expressed in some uterine leiomyomas and breast tumor cell lines, respectively (9, 21, 40, 42). Moreover, in transgenic mice both p110 and p75 CDP/Cux exhibited oncogenic potential ((4) and Cadieux et al., unpublisbed data). Therefore, we envision that the combined over-expression of both CDP/Cux and E2F factors in cancer cells may contribute to the aberrant stimulation of cell proliferation at the expense of differentiation.

Targeting of transcription factors to specific regulatory sites does not rely exclusively, or even primarily, on their interactions with high-affinity binding sites. Indeed, location array analysis has revealed that a sizeable fraction of targets do not include high-affinity binding sites. In these cases, targeting can be accomplished by the formation of a larger nucleo-protein complex that is stabilized by the accumulation of weak protein/DNA and protein/protein interactions. The results presented here support a model whereby p110 CDP/Cux recruits E2F to a subset of cell cycle-regulated promoters in order to stimulate gene expression.

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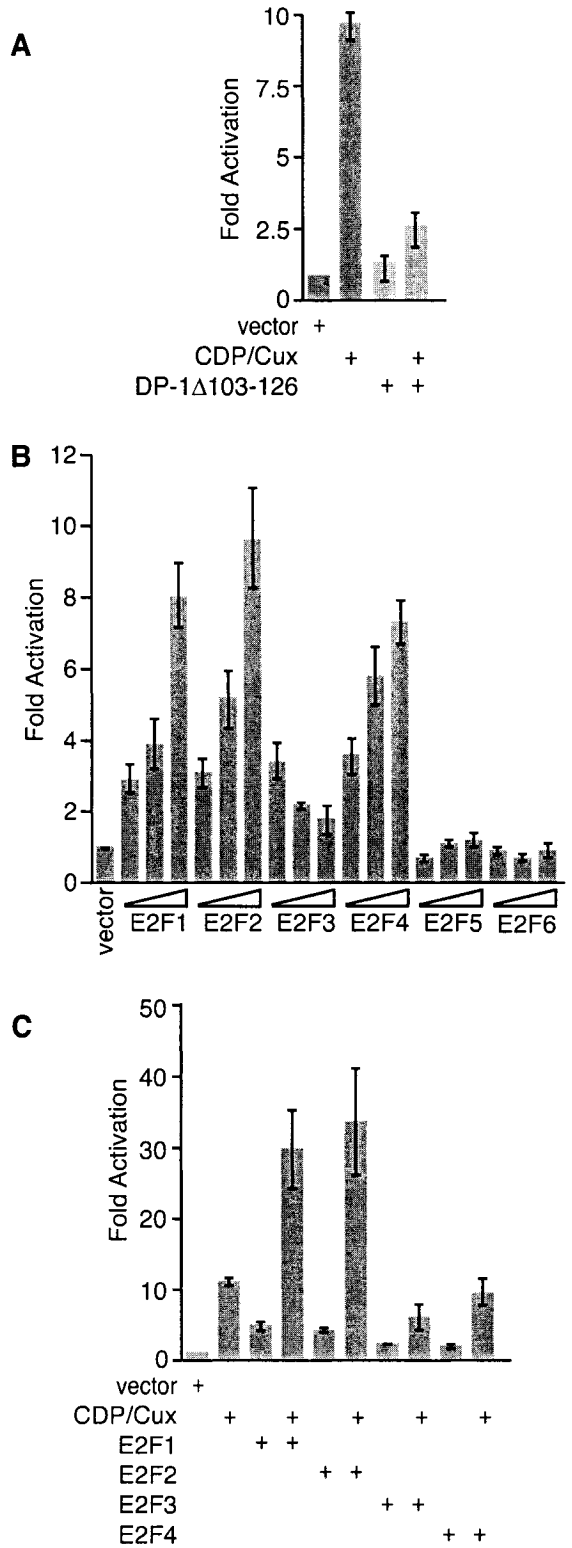
Fig. 1. p110 CDP/Cux cooperates with E2F1 and E2F2 in the stimulation of the DNA pol α reporter. Hs 578T cells were transfected with the DNA pol α (-65/+47)/luciferase reporter construct and the indicated vectors expressing CDP/Cux, DP1Δ103-126 (A), or E2F1, E2F2, E2F3, E2F4, E2F5, or E2F6 (B and C). Cytoplasmic extracts were prepared and processed to measure luciferase activity. The mean of 3 or more transfections is shown.


A
Fold Activation
10
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B
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12
10
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4
2
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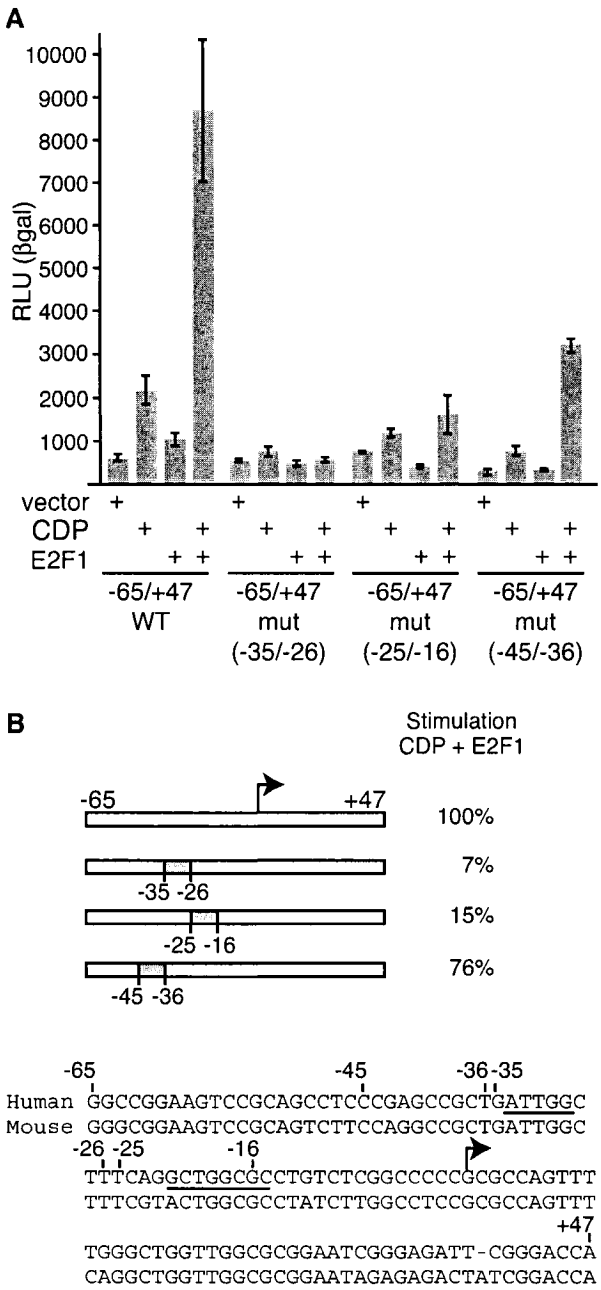
Fig. 2. p110 CDP/Cux cooperates with E2F1 in the activation of the DNA pol α gene promoter in a binding site-dependent manner. (A) Hs 578T cells were transfected with wild-type or mutant DNA pol α (-65/+47)/luciferase reporter construct and the indicated vectors expressing CDP/Cux and E2F1. Cytoplasmic extracts were prepared and processed to measure luciferase activity. Results are expressed as relative light units (RLU) normalized to β-galactosidase activity from an internal control. The mean of 3 transfections is shown and the results are expressed as fold activation over vector control. Results are representative of 3 separate experiments. (B) The diagram and DNA sequence show the position of the linker scanner mutations in the DNA pol α gene promoter. The sequences of CDP/Cux and E2F sites are underlined. The % stimulation is expressed relative to the wild type reporter construct. The transcription start site is indicated with an arrow.


A
RLU (βgal)
10000
9000
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CDP
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-65/+47
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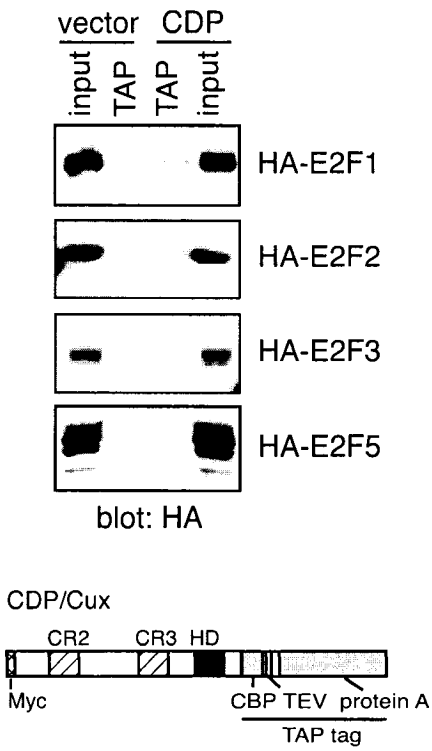
76%
-65
-45
-36 -35
Human GGCCGGAAGTCCGCAGCCTCCCGAGCCGCTGATTGGC
Mouse GGGCGGAAGTCCGCAGTCTTCCAGGCCGCTGATTGGC
-26 -25
-16
TTTCAGGCTGGCGCCTGTCTCGGCCCCCGCGCCAGTTT
TTTCGTACTGGCGCCTATCTTGGCCTCCGCGCCAGTTT
+47
TGGGCTGGTTGGCGCGGAATCGGGAGATT-CGGGACCA
CAGGCTGGTTGGCGCGGAATAGAGAGACTATCGGACCA

Fig. 3. CDP/Cux interacts with E2F1 and E2F2 *in vivo*. Hs 578T cells stably carrying an empty vector or a vector expressing CDP 831-1336/tag^2 were transfected with the indicated HA-E2F constructs. Cellular extracts were submitted to tandem-affinity purification (TAP), followed by Western blot analysis with an HA antibody. 0.2% input was loaded as a protein expression control. A schematic of CDP 831-1336/tag^2 is shown below.


vector
CDP
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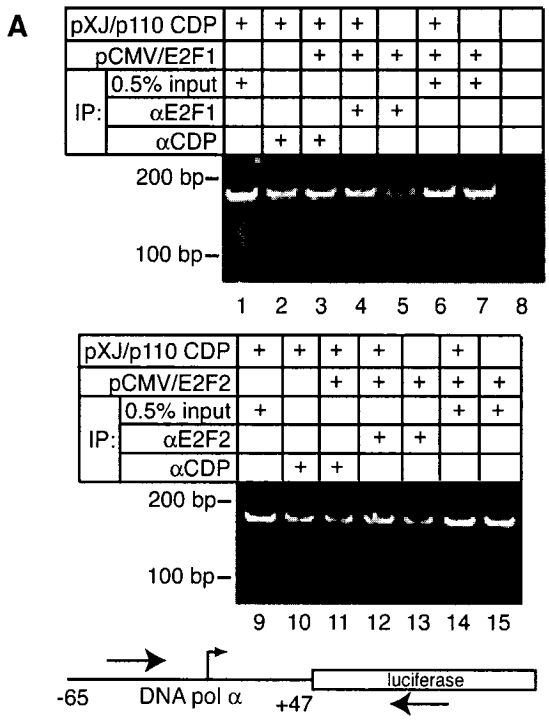
HA-E2F1
HA-E2F2
HA-E2F3
HA-E2F5
blot: HA
CDP/Cux
CR2
CR3
HD
Myc
CBP
TEV
protein A
TAP tag

Fig. 4. CDP/Cux recruits E2F1 and E2F2 to the DNA pol α gene promoter. Hs 578T cells were co-transfected with the DNA pol α reporter (A), or the CAD reporter (C), and the expression vectors indicated. The following day, ChIP assays were performed using the antibodies indicated. (A) PCR reactions were performed in parallel using the immunoprecipitated chromatin and 0.5% total chromatin (input: lanes 1, 6, 7, 9, 10, and 15). (B and C) qPCR was performed using primers recognizing the CAD or DNA pol α gene promoters, and luciferase cDNA, and were normalized to a sample transfected with reporter and empty vector DNA.


A
pXJ/p110 CDP
pCMV/E2F1
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0.5% input
αE2F1
αCDP
200 bp
100 bp
1 2 3 4 5 6 7 8
pXJ/p110 CDP
pCMV/E2F2
IP:
0.5% input
αE2F2
αCDP
200 bp
100 bp
9 10 11 12 13 14 15
-65
DNA pol α
+47
luciferase

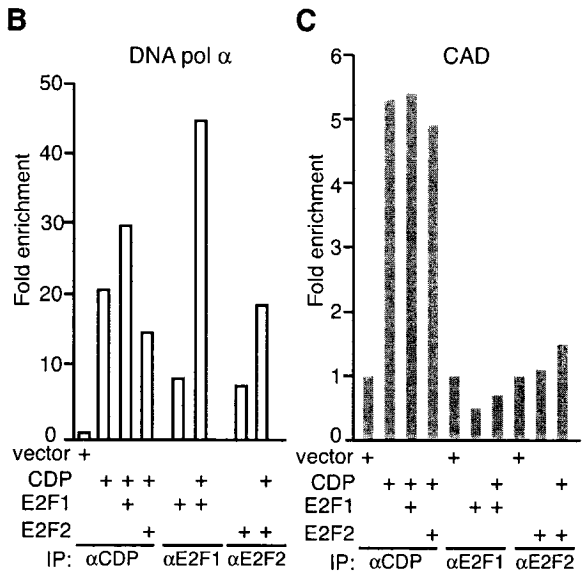

B
DNA pol α
Fold enrichment
vector
CDP
E2F1
E2F2
IP: αCDP αE2F1 αE2F2
C
CAD
Fold enrichment
vector
CDP
E2F1
E2F2
IP: αCDP αE2F1 αE2F2

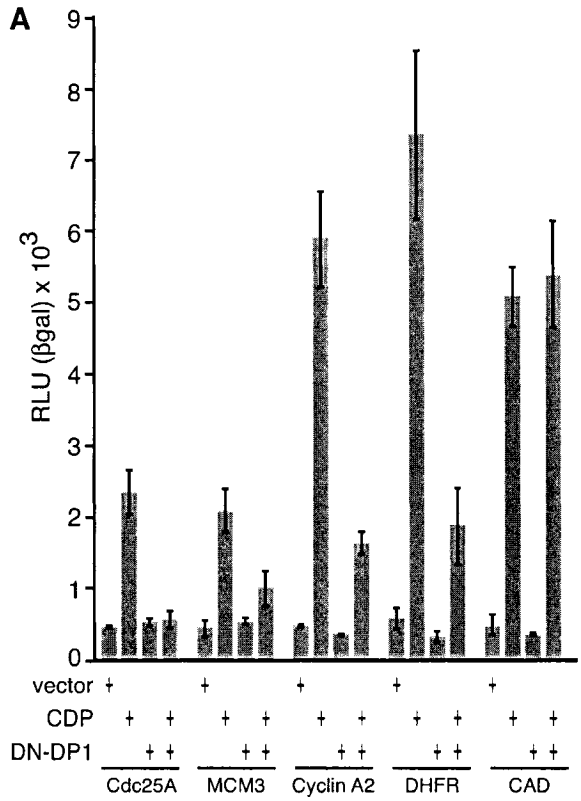
Table 1. CDP/Cux and E2F1 targets include an overrepresentation of cell cycle genes. Targets common to CDP/Cux and E2F1 from ChIP-chip analysis were analyzed for distribution among functional categories using EASE. Fisher Exact analysis represents the probability that the number of targets common to CDP/Cux and E2F1 would be found randomly found. Categories from biological level 5 with p-values of 0.02 or lower are shown.

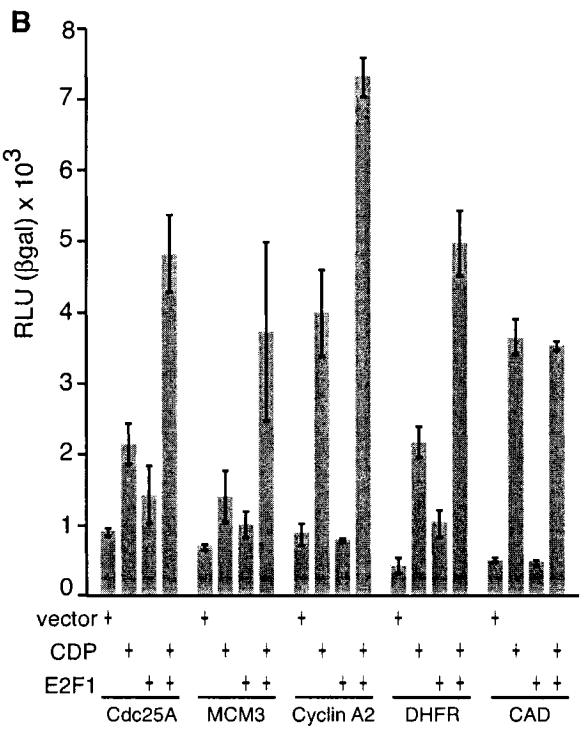
Category	Fisher Exact
cell cycle	2.15E-08
nuclear organization and biogenesis	1.10E-05
DNA packaging	2.40E-05
DNA replication	4.86E-05
mRNA metabolism	5.12E-04
cytokinesis	9.66E-04
DNA repair	1.23E-03
quinone cofactor metabolism	2.08E-03
ubiquitin cycle	1.29E-02
vitamin B6 metabolism	1.47E-02
oxidoreduction coenzyme metabolism	1.98E-02

Table 2. CDP/Cux and E2F1 bind many common cell cycle gene targets. A subset of gene targets from ChIP-chip analysis and their p-values are shown. References for E2F1 targets previously found by chromatin immunoprecipitation are shown. ChIP-quantitative PCR validation results were performed with thymidine-blocked Hs 578T cells. Results are expressed as enrichment relative to no antibody ChIP control, normalized for enrichment of G6PDH.

Function	Gene Symbol	Gene Description	location array (p-value)		ref	ChIP-PCR	
			CDP	E2F1		CDP	E2F1
Cell cycle, S	CCNA2	cyclin A2	6.0E-5		5,18,49,77	5.1	
	CDC25A	cell division cycle 25A	2.6E-4	1.2E-5	8,49	6.3	64.4
Cell cycle, G2/M	MAD2L1	MAD2 mitotic arrest deficient-like 1	2.1E-7	4.0E-5	49	3.8	16.1
Cell cycle, M	APC10	anaphase promoting complex subunit 10	2.9E-3	1.2E-3		3.6	2.7
	KNTC1	kinetochore associated 1	1.7E-3	1.7E-3		7.9	58.2
	NUMA1	nuclear mitotic apparatus protein 1	7.7E-6	2.1E-3		3.6	12.6
DNA replication	CDC7	cell division cycle 7 (S. cerevisiae)	3.0E-4	3.5E-6		5.4	14.2
	MCM3	minichromosome maintenance deficient 3	1.1E-9	3.1E-7	49	13.0	4.3
	MCM7	minichromosome maintenance deficient 7	1.9E-3			6.2	71.6
	ORC1L	origin recognition complex, subunit 1-like	5.5E-14	9.2E-5	49	7.2	2.3
	POLA	polymerase (DNA-directed), alpha, 180 kD	3.2E-3		39,49	2.5	2.1
	POLD3	polymerase (DNA-directed), delta 3	4.7E-3	9.1E-6		3.8	1.6
	RPA3	replication protein A3, 14 kDa	4.0E-3		49	2.9	9.0
Repair,	MLH1	mutL homolog 1	2.1E-4	2.4E-4	49	5.2	3.8
checkpoint	PMS1	postmeiotic segregation increased 1	3.5E-3	6.3E-4		4.8	22.3
	RAD51	RAD51 homolog (RecA homolog, E. coli)	2.5E-3	1.5E-5	49,73	2.5	2.8
	TP53	tumor protein p53 (Li-Fraumeni syndrome)	6.9E-4	3.6E-2	49		
Cell Proliferation	SCAND1	SCAN domain containing 1	9.9E-4	4.5E-5		4.7	5.3

Fig. 5. CDP/Cux and E2F1 cooperate in the activation of their common target genes. Hs 578T cells were transfected with the reporter plasmid indicated (ex. Cdc25A), and vectors expressing p110 CDP/Cux and (A) DP1Δ103-126 or (B) E2F1. Cytoplasmic extracts were prepared and processed to measure luciferase activity. Results are expressed as relative light units (RLU) normalized to β-galactosidase activity from an internal control. The mean of 3 transfections is shown and is representative of 3 separate experiments.


A
RLU (βgal) x 10^3
0
1
2
3
4
5
6
7
8
9
vector
CDP
DN-DP1
Cdc25A
MCM3
Cyclin A2
DHFR
CAD


B
RLU (βgal) x 10^3
0
1
2
3
4
5
6
7
8
vector
CDP
E2F1
Cdc25A
MCM3
Cyclin A2
DHFR
CAD

SYMBOL	GENENAME	LL	HEL	K562	U266	Jurkat	Ramos	HS578T	HeLa	293	RPMI	Average P value	# of Positive experiments (/9)
VCP	valosin-containing protein	7415	3.0E-11	1.2E-8	2.7E-7	1.8E-5	7.7E-6	2.1E-14	1.7E-11	9.3E-14	4.8E-13	3.9E-10	9
TBP	TATA box binding protein	6908	4.2E-9	4.4E-11	2.8E-7	1.1E-4	1.5E-7	1.9E-6	1.4E-5	2.4E-15	6.0E-13	6.8E-9	8
CCNH	cyclin H	902	3.8E-6	7.0E-12	4.6E-6		1.7E-9	4.6E-10	3.6E-8	3.3E-12	4.0E-15	6.8E-10	8
DUSP10	dual specificity phosphatase 10	11221	3.8E-11	2.6E-9	1.0E-7		7.1E-7	4.0E-5	1.4E-5	7.2E-13	2.0E-15	3.0E-9	8
FLJ13798	hypothetical protein FLJ13798	79831	2.0E-10	1.5E-8	1.7E-7		4.5E-3	4.5E-12	4.7E-7	1.4E-11	8.1E-13	3.9E-9	8
PSMB1	proteasome (prosome, macropain) subunit, beta type, 1	5689	6.0E-10	8.4E-9	5.1E-6		7.2E-4	4.5E-8	1.7E-5	2.7E-13	3.8E-12	1.9E-8	8
EEF2	eukaryotic translation elongation factor 2	1938	6.0E-4	2.7E-9	6.4E-6		1.8E-8	5.7E-12	2.2E-5	6.2E-11	3.5E-10	2.9E-8	8
MTBP	(mouse) binding protein, 104kDa	27085	1.3E-7	3.5E-6	1.6E-4	9.3E-4	6.2E-13	6.6E-12	1.9E-8	4.4E-10		3.5E-8	8
HMGA1L4	high mobility group AT-hook 1-like 4	83444	1.1E-7	6.2E-8	3.6E-8		4.8E-5	7.5E-9	6.9E-5	2.4E-12	4.2E-10	4.0E-8	8
ABHD2	abhydrolase domain containing 2	11057	1.1E-9	8.3E-6	1.2E-5		1.8E-7	3.1E-11	3.0E-5	4.7E-11	1.1E-6	7.4E-8	8
MGC19604	similar to RIKEN cDNA B230118G17 gene	112812	4.6E-12	8.3E-10	2.2E-5		1.4E-3	6.0E-10	2.1E-5	8.9E-8	3.8E-8	9.1E-8	8
FLJ10407	hypothetical protein FLJ10407	55706	1.2E-9	3.1E-8	3.8E-6	8.1E-4		1.1E-5	3.6E-5	5.8E-8	4.6E-11	3.2E-7	8
UBE2D2	ubiquitin-conjugating enzyme E2D 2 (UBC4/5 homolog, yeast)	7322	1.9E-5	1.4E-9	1.2E-4		1.8E-10	1.5E-10	2.3E-4	4.1E-5	3.0E-7	3.6E-7	8
BCKDHA	(maple syrup urine disease)	593	6.9E-13	2.1E-6	6.8E-8		2.4E-3	2.1E-5	1.8E-4	4.7E-8	6.4E-7	6.4E-7	8
LATS1	LATS, large tumor suppressor, homolog 1 (Drosophila)	9113	4.3E-11	3.5E-9	1.5E-3		4.0E-4	3.5E-7	1.6E-4	1.5E-8	5.7E-6	9.1E-7	8
ATP5G1	c (subunit 9), isoform 1	516	4.6E-7	2.1E-3	3.7E-7		1.5E-4	1.0E-3	1.1E-11	1.9E-11	1.2E-3	1.4E-6	8
C6orf170	chromosome 6 open reading frame 170	221322	1.8E-6	3.8E-6		1.3E-8	2.3E-3	7.6E-6	2.0E-4	8.0E-9	4.6E-8	1.8E-6	8
TLP19	endoplasmic reticulum thioredoxin superfamily member, 18 kDa	51060	3.8E-3	9.6E-9	1.3E-4		5.3E-7	1.4E-8	1.7E-4	4.5E-9	9.3E-5	2.7E-6	8
MGC23908	similar to RNA polymerase B transcription factor 3	91408	3.8E-3	9.6E-9	1.3E-4		5.3E-7	1.4E-8	1.7E-4	4.5E-9	9.3E-5	2.7E-6	8
B7	B7 gene	10233	3.0E-4	1.8E-4	5.5E-4		1.1E-4	4.1E-6	8.5E-5	1.0E-8	6.7E-8	1.3E-5	8
NDUFS7	coenzyme Q reductase)	374291	4.1E-3	2.3E-5	2.1E-4		1.0E-7	2.6E-3	2.8E-3	4.7E-6	3.2E-3	1.5E-4	8
FLJ20422	hypothetical protein FLJ20422	54929	1.1E-16	1.7E-12	3.3E-9	4.4E-7	1.1E-16	1.0E-15	5.6E-11			1.1E-12	7
PCM1	pericentriolar material 1	5108	1.9E-8	1.1E-8	1.0E-6			1.7E-11	4.2E-6	1.9E-9	1.0E-5	4.3E-8	7
DNAJC1	DnaJ (Hsp40) homolog, subfamily C, member 1	64215	2.5E-8	2.4E-9	6.3E-6			4.4E-13	1.4E-4	1.8E-7	9.8E-8	4.6E-8	7
TSG101	tumor susceptibility gene 101	7251	4.5E-10	3.9E-10	5.0E-8			5.9E-5	6.0E-6	7.8E-5	2.4E-10	9.2E-8	7
FTSJ1	FtsJ homolog 1 (E. coli)	24140	2.0E-7	1.0E-9	1.2E-5			4.0E-6	1.2E-8	1.7E-11	2.0E-3	1.7E-7	7
LOH11CR2A	loss of heterozygosity, 11, chromosomal region 2, gene A	4013	3.5E-9	1.7E-8	3.2E-4			2.0E-3	1.4E-5	2.1E-10	3.7E-11	1.7E-7	7
VAMP1	vesicle-associated membrane protein 1 (synaptobrevin 1)	6843	2.4E-14	9.4E-4	1.1E-4			1.1E-10	3.6E-4	3.9E-9	9.0E-5	2.3E-7	7
ABCB8	ATP-binding cassette, sub-family B (MDR/TAP), member 8	11194	1.6E-8	1.9E-9	8.7E-4			1.6E-5	1.8E-3	9.8E-10	3.2E-8	5.8E-7	7
ETFDH	electron-transferring-flavoprotein dehydrogenase	2110	3.8E-4	2.9E-7	3.6E-6			2.4E-8	2.0E-4	2.6E-6	5.4E-9	9.7E-7	7
ORC1L	origin recognition complex, subunit 1-like (yeast)	4998	7.6E-6	7.1E-10	5.1E-5		4.5E-5	7.0E-5		8.4E-8	1.7E-8	1.0E-6	7
DCP1B	decapping enzyme hDcp1b	196513	3.0E-7	2.2E-5	1.0E-5			7.9E-8	1.7E-4	2.5E-12	1.3E-3	1.2E-6	7
RPS11	ribosomal protein S11	6205	1.2E-5	2.8E-6	7.5E-5		7.4E-11	4.6E-6	2.2E-3	2.3E-8		1.7E-6	7
PSMA1	proteasome (prosome, macropain) subunit, alpha type, 1	5682	5.5E-5	5.0E-4	2.3E-6		3.5E-10	4.0E-6	7.5E-4	7.1E-9		2.9E-6	7
MDH1	malate dehydrogenase 1, NAD (soluble)	4190	1.1E-4	1.6E-5	3.6E-6			1.2E-9	1.0E-3	4.4E-5	9.4E-9	3.2E-6	7
SELH	selenoprotein H	280636	6.1E-6	1.9E-6	6.1E-6			3.8E-9	4.4E-5	2.1E-6	2.2E-4	3.4E-6	7
SDCCAG1	seroligically defined colon cancer antigen 1	9147	7.0E-7	2.6E-4	7.4E-5	3.7E-3		1.0E-5		6.4E-7	9.4E-10	6.1E-6	7
PREP	prolyl endopeptidase	5550	1.9E-7	1.5E-5	6.7E-4			4.2E-7	1.9E-3	2.1E-9	1.5E-3	9.1E-6	7
AMAC	acyl-malonyl condensing enzyme	83650	6.8E-6	1.6E-7			4.6E-3	6.6E-6	2.8E-4	3.5E-8	2.6E-4	1.3E-5	7
LENG1	leukocyte receptor cluster (LRC) member 1	79165	4.0E-4	2.6E-3	8.0E-5			2.7E-8	1.2E-4	2.0E-9	1.1E-3	1.8E-5	7
SLC1A3	member 3	6507	1.6E-7	2.6E-3	4.7E-3	8.5E-5		2.1E-5	7.7E-10		7.6E-4	2.1E-5	7
PEX6	peroxisomal biogenesis factor 6	5190	1.3E-3	1.9E-8	1.3E-4		4.5E-4		7.9E-4	6.4E-6	4.3E-7	2.3E-5	7
NDUFA5	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13kDa	4698		2.7E-6	2.7E-5		2.5E-6	2.0E-3	1.4E-3	4.1E-4	1.5E-6	4.4E-5	7
EIF4E	eukaryotic translation initiation factor 4E	1977	1.8E-3	1.7E-6	4.2E-5		5.2E-4	7.8E-4		2.4E-6	3.7E-6	4.6E-5	7
CSTF2T	variant	23283	3.5E-3	1.2E-8	1.0E-4			1.5E-5	1.5E-4	3.2E-5	2.0E-3	4.8E-5	7
CSTF1	Cleavage stimulation factor, 3' pre-RNA, subunit 1, 50kDa	1477	3.3E-4		5.0E-5		4.3E-5	1.6E-5	2.5E-4	5.3E-6	6.4E-4	7.2E-5	7
TRPC4AP	associated protein	26133	3.0E-4	8.8E-5	8.9E-4	6.5E-4		1.0E-6	7.8E-6	1.8E-4		8.0E-5	7
FLJ14981	hypothetical protein FLJ14981	84954	5.6E-5	4.0E-6	1.6E-5			1.8E-4	2.1E-3	6.3E-6	4.1E-3	8.6E-5	7
ACAA1	Coenzyme A synthetase)	30	1.0E-3	1.2E-5	9.3E-4	2.0E-3			2.0E-4	5.5E-4	2.8E-4	3.6E-4	7
MYD88	myeloid differentiation primary response gene (88)	4615	1.0E-3	1.2E-5	9.3E-4	2.0E-3			2.0E-4	5.5E-4	2.8E-4	3.6E-4	7
LOC165186	similar to RIKEN cDNA 4632412N22 gene	165186		1.9E-10	5.3E-4			6.5E-5	2.5E-9	6.5E-9	3.3E-9	8.4E-8	6
ZNF265	zinc finger protein 265	9406	4.0E-14	6.2E-8	4.4E-5				2.9E-4	8.6E-9	1.8E-6	8.9E-8	6
CD99L2	CD99 antigen-like 2	83692	2.1E-9	2.0E-6		6.4E-6		1.2E-3	1.6E-7		1.7E-11	2.1E-7	6
HSPCA	heat shock 90kDa protein 1, alpha	3320		2.1E-8	1.8E-5			5.6E-7	1.9E-4	1.2E-9	5.3E-8	3.7E-7	6
ILF2	interleukin enhancer binding factor 2, 45kDa	3608	4.6E-9		9.6E-6		4.2E-3	2.9E-4		5.3E-11	3.5E-9	4.6E-7	6
PC4	activated RNA polymerase II transcription cofactor 4	10923	9.9E-9	7.6E-4	1.0E-3			1.1E-8		1.7E-12	6.6E-4	6.7E-7	6
CD1A	CD1A antigen, a polypeptide	909		6.5E-5	7.9E-5			1.0E-5	3.5E-6	8.6E-12	3.0E-7	9.9E-7	6
COPB2	coatomer protein complex, subunit beta 2 (beta prime)	9276		3.1E-6	2.5E-3			4.7E-7	1.6E-6	5.7E-9	4.3E-7	1.5E-6	6
SNRPD2	small nuclear ribonucleoprotein D2 polypeptide 16.5kDa	6633	1.5E-5	1.3E-3	1.1E-5		4.2E-5			5.8E-8	1.5E-10	2.0E-6	6
FLJ20084	hypothetical protein FLJ20084	54814	1.5E-5	1.3E-3	1.1E-5		4.2E-5			5.8E-8	1.5E-10	2.0E-6	6
CBX5	chromobox homolog 5 (HP1 alpha homolog, Drosophila)	23468		1.2E-6			2.9E-3	3.1E-7	2.8E-3	2.5E-11	1.1E-5	3.0E-6	6
FLJ14936	hypothetical protein FLJ14936	84950		2.5E-8	3.1E-6		1.6E-3	2.4E-6		4.2E-5	5.0E-5	9.3E-6	6
CRADD	CASP2 and RIPK1 domain containing adaptor with death domain	8738	6.9E-4		5.5E-6			4.7E-6	5.9E-5	3.6E-6	2.2E-7	9.8E-6	6
TROAP	trophinin associated protein (tastin)	10024	2.6E-3	4.2E-4	1.9E-4			9.8E-11	2.0E-3	4.4E-8		1.1E-5	6
MGC51082	hypothetical protein MGC51082	126299	1.9E-4	5.7E-4	4.5E-6			1.4E-5		6.3E-8	2.2E-5	1.4E-5	6
RPS8	ribosomal protein S8	6202	3.3E-6	3.2E-7			1.5E-5	3.5E-3		5.7E-4	6.4E-6	2.4E-5	6
TMF1	TATA element modulatory factor 1	7110	3.6E-5	2.4E-4	1.4E-4				2.2E-5	9.9E-4	1.6E-8	2.7E-5	6
ARRDC3	arrestin domain containing 3	57561		1.9E-4	1.4E-4		5.2E-6		3.4E-3	4.2E-5	6.6E-8	3.3E-5	6
PSME3	gamma; Ki)	10197	3.8E-4	1.9E-4	1.5E-4			1.2E-7	1.3E-3		1.0E-6	3.4E-5	6
MYST2	MYST histone acetyltransferase 2	11143	3.8E-5	1.1E-3	7.0E-5			2.8E-5	3.2E-5	8.0E-7		3.6E-5	6
OS-9	amplified in osteosarcoma	10956		1.5E-3	1.2E-3			6.4E-6	5.5E-5	4.0E-7	1.4E-5	3.9E-5	6
KIAA1826	KIAA1826 protein	84437	1.3E-4	2.1E-4	7.2E-4			2.1E-6		4.1AE-6	4.4E-5	4.5E-5	6
TSPYL4	TSPY-like 4	23270		3.1E-5	2.5E-4		2.8E-5	3.2E-5		4.9E-5	3.2E-5	4.7E-5	6
PSMB7	proteasome (prosome, macropain) subunit, beta type, 7	5695		2.3E-5	1.0E-6		5.5E-4	6.5E-6	9.5E-4		3.1E-4	5.4E-5	6
PEX13	peroxisome biogenesis factor 13	5194	1.7E-3	2.1E-6	4.4E-3			9.2E-7	2.1E-5	1.1E-4		5.7E-5	6
CDC5L	CDC5 cell division cycle 5-like (S. pombe)	988	6.8E-5	4.4E-4	1.3E-3			4.6E-5		3.4E-8	6.2E-4	3.8E-5	6
MASK	multiple ankyrin repeats, single KH-domain (MASK) homolog	54582		1.0E-4	1.0E-3			2.0E-6	5.9E-5	1.9E-6	4.3E-3	6.8E-5	6
AATF	apoptosis antagonizing transcription factor	26574	8.7E-4	5.5E-5				9.5E-8	1.0E-3	2.1E-4	1.8E-4	7.5E-5	6
CHPPR	region	9650	3.1E-3	1.7E-4	1.8E-5			2.8E-6		1.6E-5	1.1E-3	8.8E-5	6
DTYMK	deoxythymidylate kinase (thymidylate kinase)	1841	1.3E-3	3.1E-6	4.2E-5			5.9E-4	3.1E-4	1.7E-5		1.1E-4	6
IMP-3	IGF-II mRNA-binding protein 3	10643	4.5E-3	7.2E-5	3.5E-3		5.8E-5			9.8E-6	1.7E-5	1.5E-4	6
QDI2	DNA-damage inducible protein 2	84301		1.5E-3			2.3E-8	1.7E-3	4.3E-3	4.6E-3	1.4E-5	1.6E-4	6
GSTA4	glutathione S-transferase A4	2941	3.4E-3	1.6E-4	7.3E-4		2.5E-6		4.0E-3	2.7E-5		2.2E-4	6
RBBP5	retinoblastoma binding protein 5	5929	1.0E-4	9.8E-4	2.8E-3			1.7E-3	8.8E-4		2.2E-6	3.1E-4	6
HAT1	histone acetyltransferase 1	8520	8.0E-4	3.7E-3	2.0E-4			2.9E-4		1.5E-3	4.1E-6	3.2E-4	6
HDAC8	histone deacetylase 8	55869		4.1E-4	7.6E-5			1.8E-3	1.6E-4	1.1E-3	4.6E-4	4.0E-4	6
DNAJB12	DnaJ (Hsp40) homolog, subfamily B, member 12	54788	2.4E-4	9.7E-5	2.2E-4			3.0E-4	2.5E-3	1.3E-3		4.1E-4	6
HNRPH2	heterogeneous nuclear ribonucleoprotein H2 (H')	3188		2.2E-3	4.8E-4	2.4E-3		4.4E-3	9.8E-4	5.8E-7		4.3E-4	6
RPC5	RNA polymerase III 80 kDa subunit RPC5	55718		2.1E-3	1.5E-3		9.9E-4	2.2E-3		2.2E-3	7.1E-5	1.0E-3	6
LOC153222	adult retina protein	153222		3.1E-3	3.5E-3			5.9E-5	1.9E-3	2.7E-3	3.4E-3	1.5E-3	6
TMG4	transmembrane gamma-carboxyglutamic acid protein 4	79056	1.4E-6		3.5E-6			4.1E-6		2.1E-13	1.2E-9	2.2E-8	5
DKFZP586A0	DKFZP586A0522 protein	25840	2.1E-4	1.9E-7				2.0E-11		4.2E-11	3.5E-4	1.0E-7	5
LOC203522	hypothetical protein LOC203522	203522	4.4E-5	3.1E-9	1.0E-5					1.0E-5	8.2E-11	2.6E-7	5
MGC33338	hypothetical protein MGC33338	126823		4.7E-3	3.8E-5				6.7E-7	1.8E-7	6.8E-13	4.3E-7	5
FLJ20073	FLJ20073 protein	54809		1.1E-7				2.2E-7	3.0E-5	8.9E-9	4.0E-6	3.8E-7	5
GLRX	glutaredoxin (thioltransferase)	2745			1.2E-4			7.3E-10	1.0E-3	1.2E-7	3.1E-8	8.0E-7	5
C9orf90	chromosome 9 open reading frame 90	203245			1.1E-4			1.3E-9	5.8E-4	2.7E-4	4.3E-11	9.9E-7	5
FLJ90024	fasting-inducible integral membrane protein TM6P1	129303	4.5E-6	9.6E-4		1.5E-4	1.1E-8				5.6E-10	1.3E-6	5
CXCL3	chemokine (C-X-C motif) ligand 3	2921	5.6E-5	2.9E-7				1.1E-9	1.8E-5	8.0E-5		1.9E-6	5
TNFSF10	tumor necrosis factor (ligand) superfamily, member 10	8743	3.5E-6	1.2E-6			4.2E-3		7.1E-4		8.4E-10	6.4E-6	5
ZNF234	zinc finger protein 234	10780	9.7E-5					2.2E-4	1.3E-4	9.5E-7	5.0E-9	6.7E-6	5
SALL4	sal-like 4 (Drosophila)	57167			2.3E-8			3.6E-5	1.0E-3	2.7E-7	3.4E-5	7.3E-6	5
GRWD1	glutamate-rich WD repeat containing 1	83743		2.9E-5		2.8E-4		3.7E-10		2.1E-4	9.4E-4	1.4E-5	5
GTPBP1	GTP binding protein 1	9567					3.9E-5	4.1E-5	2.2E-6	2.6E-5	1.5E-5	1.7E-5	5
CFL1	cofilin 1 (non-muscle)	1072		7.2E-4			2.4E-7	9.1E-4		1.1E-3	1.0E-8	1.8E-5	5
ANKRD13	ankyrin repeat domain 13	88455	3.9E-3		1.1E-3	1.3E-5	1.7E-8	5.5E-5				3.5E-5	5
TUWD12	TUWD12	282809	2.6E-4		5.0E-5			5.1E-5		1.3E-6	1.6E-3	6.8E-5	5
C10orf70	chromosome 10 open reading frame 70	55847		8.2E-5	8.6E-5			1.7E-7	1.5E-3	1.3E-3		7.5E-5	5
IMPK	inositol polyphosphate multikinase	253430		8.2E-5	8.6E-5			1.7E-7	1.5E-3	1.3E-3		7.5E-5	5
FLJ22374	hypothetical protein FLJ22374	84182	6.8E-4	1.8E-9	4.4E-4				4.1E-3	1.2E-3		7.7E-5	5
OPA1	optic atrophy 1 (autosomal dominant)	4976	8.4E-4	3.8E-3	2.9E-3			9.5E-8		2.9E-5		1.2E-4	5
FLJ12770	hypothetical protein FLJ12770	84134		4.0E-3	1.0E-4			7.9E-4		3.9E-6	3.6E-5	1.4E-4	5
MEIS1	Meis1, myeloid ecotropic viral integration site 1 homolog (mouse)	4211	4.1E-3	5.8E-5	2.4E-4					9.7E-5	3.3E-5	1.8E-4	5
FLJ11301	hypothetical protein FLJ11301	55341		9.4E-6	6.6E-5			1.7E-3	2.7E-3		1.1E-4	2.0E-4	5
FLJ40154	hypothetical protein FLJ40154	146861	5.7E-4	3.4E-5				2.3E-3		5.6E-6	1.5E-3	2.1E-4	5
PIK4CB	phosphatidylinositol 4-kinase, catalytic, beta polypeptide	5298		6.7E-4	6.1E-5			3.2E-5		1.3E-4	2.3E-3	2.1E-4	5
CASP4	cell expressed, developmentally down-regulated 2)	835	4.6E-3	1.2E-4	1.9E-5					3.9E-3	1.4E-5	2.2E-4	5
GRCC10	likely ortholog of mouse gene rich cluster, C10 gene	113246	3.4E-3	1.8E-3	4.9E-3			1.1E-6		1.8E-5		2.3E-4	5
EIF3S6	eukaryotic translation initiation factor 3, subunit 6 48kDa	3646		3.0E-5				4.5E-5	9.9E-4	7.7E-4	1.3E-3	2.6E-4	5
LOC51054	putative glycolipid transfer protein	51054	4.3E-4		1.8E-3			6.2E-4	2.7E-4	2.5E-5		3.1E-4	5
COG3	component of oligomeric golgi complex 3	83548		1.3E-3			3.0E-3	3.9E-5		6.5E-5	3.1E-4	3.2E-4	5
NXT2	nuclear transport factor 2-like export factor 2	55916	4.4E-4		7.7E-4			1.6E-3	1.3E-3	4.6E-6		3.2E-4	5
SPG4	spastic paraplegia 4 (autosomal dominant; spastin)	6683	9.7E-5	1.2E-4			8.7E-4	1.6E-4			3.7E-3	3.6E-4	5
RPL19	ribosomal protein L19	6143		4.5E-3		5.1E-5		2.2E-4		4.3E-3	4.8E-5	4.0E-4	5
DKFZP564J157	DKFZp564J157 protein	54458	7.8E-5	1.9E-3		4.2E-3				9.0E-5	9.1E-4	5.5E-4	5
LOC115704	4S gene	115704			1.4E-3			2.0E-3	9.8E-4	3.0E-5	9.2E-4	5.9E-4	5
RFX5	regulatory factor X, 5 (influences HLA class II expression)	5993	1.0E-3	1.5E-3	1.0E-3			6.3E-4		2.8E-3		1.2E-3	5
FLJ10374	hypothetical protein FLJ10374	55702		6.9E-5	3.2E-4		9.4E-14			1.3E-5		4.1E-7	4
ARL8	ADP-ribosylation factor-like 8	221079		5.7E-5	2.1E-4			2.6E-8			2.0E-10	5.0E-7	4
C6orf79	chromosome 6 open reading frame 79	63933		4.7E-3		2.3E-7	7.4E-11				1.4E-6	5.8E-7	4
AGT	(alpha-1 antiprotease, antitrypsin), member 8)	183			6.5E-7			9.8E-6	1.1E-3		8.8E-8	5.0E-6	4
ACVR1	activin A receptor, type I	90	2.3E-7	3.6E-6				6.2E-5		2.8E-5		6.2E-6	4
MTMR8	myotubularin related protein 8	55613		2.2E-3	6.1E-7					1.1E-8	8.6E-4	1.1E-5	4
DLG7	discs, large homolog 7 (Drosophila)	9787		4.2E-7				1.9E-5	1.8E-3	6.1E-6		1.7E-5	4
TESK2	testis-specific kinase 2	10420			1.0E-4			3.3E-4		6.8E-6	4.3E-7	1.8E-5	4
NKX2-3	NK2 transcription factor related, locus 3 (Drosophila)	159296		2.6E-3	7.4E-6					7.1E-8	6.1E-5	1.8E-5	4
RARB	retinoic acid receptor, beta	5915						5.6E-5	4.9E-3	3.1E-9	1.5E-4	1.9E-5	4
PCDHB3	protocadherin beta 3	56132		3.6E-6	4.7E-4			2.5E-5			5.3E-6	2.2E-5	4
KIAA1217	KIAA1217	56243			1.8E-3			3.0E-3		6.2E-4	6.9E-11	2.2E-5	4
FLJ20156	hypothetical protein FLJ20156	54839	3.8E-4		5.0E-5				1.3E-3	1.3E-8		2.4E-5	4
THAP10	THAP domain containing 10	56906	3.8E-4		5.0E-5				1.3E-3	1.3E-8		2.4E-5	4
PFTK1	PFTAIRE protein kinase 1	5218			8.0E-5			8.0E-4		1.9E-3	8.1E-9	3.1E-5	4
OGT	acetylglucosamine-polypeptide-N-acetylglucosamnyl transferase)	8473		3.6E-5				2.7E-3		1.9E-3	1.0E-6	4.5E-5	4
C14orf119	chromosome 14 open reading frame 119	55017			1.0E-4			6.9E-6	6.7E-4	2.4E-5		5.7E-5	4
SAS10	disrupter of silencing 10	57050		1.7E-3	4.8E-3			1.0E-7		2.5E-5		6.7E-5	4
DSCR9	Down syndrome critical region gene 9	257203	3.8E-7		1.3E-5		1.4E-3				3.7E-3	7.2E-5	4
ABCA7	ATP-binding cassette, sub-family A (ABC1), member 7	10347		8.4E-5	8.1E-5			1.3E-6	3.2E-3			7.4E-5	4
DCTD	dCMP deaminase	1635	3.5E-3	1.2E-6				1.3E-4			6.4E-5	7.6E-5	4
HIST1H3D	histone 1, H3d	8351		4.8E-5			1.9E-3	4.9E-3			9.6E-8	8.1E-5	4
FLJ10276	hypothetical protein FLJ10276	55108	1.7E-3	2.7E-4	9.1E-5					1.3E-6		8.6E-5	4
VDP	vesicle docking protein p115	8615	8.4E-5	7.6E-7	6.7E-4					4.0E-3		1.1E-4	4
MGC40069	hypothetical protein MGC40069	348035	3.9E-3				3.0E-3		1.7E-7	1.7E-4		1.3E-4	4
CXCL1	activity, alpha)	2919	1.9E-3	1.3E-7				1.2E-3	1.2E-3			1.4E-4	4
MRPL1	mitochondrial ribosomal protein L1	65008		5.5E-5				3.5E-4		8.1E-4	2.7E-5	1.4E-4	4
UNQ501	MBC3205	374882		1.1E-4	6.8E-4			2.6E-5		4.7E-4		1.7E-4	4

CPNE8	copine VIII	144402		5.2E-4	6.8E-4			7.1E-5		5.7E-5		1.9E-4	4
C6orf211	chromosome 6 open reading frame 211	79624		3.3E-4	1.2E-3			2.0E-4			1.9E-5	2.0E-4	4
CXXC1	CXXC finger 1 (PHD domain)	30827	2.4E-3		2.4E-3		6.9E-8		4.4E-3			2.0E-4	4
PDCD7	programmed cell death 7	10081		8.4E-5	4.6E-3			1.2E-3			6.8E-6	2.3E-4	4
CYP3A43	cytochrome P450, family 3, subfamily A, polypeptide 43	64816		1.1E-5		4.5E-3			9.8E-4		1.7E-4	3.0E-4	4
FLJ33215	hypothetical protein FLJ33215	259282		8.3E-6				4.9E-3	4.7E-4		5.0E-4	3.1E-4	4
RPC155	polymerase (RNA) III (DNA directed) (155kD)	11128		1.1E-3				2.7E-3		6.8E-6	9.2E-4	3.7E-4	4
COPS3	(Arabidopsis)	8533	4.6E-4	9.1E-4		1.4E-4				4.4E-4		4.0E-4	4
GOSR1	golgi SNAP receptor complex member 1	9527			2.3E-3				1.8E-3	6.2E-4	1.0E-5	4.0E-4	4
PSMD9	proteasome (prosome, macropain) 26S subunit, non-ATPase, 9	5715		6.6E-4	4.0E-4			1.1E-3		1.7E-4		4.7E-4	4
FLJ31875	hypothetical protein FLJ31875	197320		1.3E-3	3.2E-3			1.5E-5			9.8E-4	4.9E-4	4
CXCL2	chemokine (C-X-C motif) ligand 2	2920	1.1E-3	3.5E-5				8.2E-4		2.0E-3		5.0E-4	4
HBP1	HMG-box transcription factor 1	26959		3.9E-6	1.3E-3		3.2E-3				4.0E-3	5.0E-4	4
HSPC268	hypothetical protein HSPC268	154791		6.5E-6	2.4E-3			1.8E-3			2.4E-3	5.1E-4	4
C2F	C2f protein	10436	6.2E-6	4.6E-3				6.7E-4			4.3E-3	5.4E-4	4
BAMBI	laevis)	25805	1.1E-5	8.5E-4			3.7E-3				2.6E-3	5.6E-4	4
AF15Q14	AF15q14 protein	57082	5.7E-4	9.1E-4	1.2E-3					2.5E-4		6.3E-4	4
TIMM44	translocase of inner mitochondrial membrane 44 homolog (yeast)	10469		7.9E-5	2.7E-4		2.3E-3		3.5E-3			6.5E-4	4
MGC2705	hypothetical protein MGC2705	84787		1.5E-3	2.7E-3			1.4E-5		3.3E-3		6.5E-4	4
AP3S2	adaptor-related protein complex 3, sigma 2 subunit	10239		2.5E-3	3.3E-4					2.7E-3	1.6E-4	7.7E-4	4
CLNS1A	chloride channel, nucleotide-sensitive, 1A	1207		1.2E-4				1.7E-4	4.0E-3	4.5E-3		7.8E-4	4
MCM3	MCM3 minichromosome maintenance deficient 3 (S. cerevisiae)	4172			3.3E-4				3.8E-3	3.2E-4	1.1E-3	8.3E-4	4
SERPINI1	member 1	5274	1.0E-3	2.2E-3	1.9E-3			1.2E-4				8.5E-4	4
PDCD10	programmed cell death 10	11235	1.0E-3	2.2E-3	1.9E-3			1.2E-4				8.5E-4	4
LOC51255	hypothetical protein LOC51255	51255	1.0E-3	1.3E-4				1.5E-3	2.8E-3			8.7E-4	4
SLC36A1	solute carrier family 36 (proton/amino acid symporter), member 1	206358		4.3E-3	9.7E-4			5.6E-5		3.1E-3		9.2E-4	4
CST8	cystatin 8 (cystatin-related epididymal specific)	10047	3.6E-3	1.8E-3				3.8E-3		3.2E-5		9.6E-4	4
ZNF222	zinc finger protein 222	7673	3.5E-3		3.4E-4			5.3E-4		1.4E-3		9.7E-4	4
CYP51A1	cytochrome P450, family 51, subfamily A, polypeptide 1	1595	4.7E-3	6.2E-5	2.0E-3			2.5E-3				1.1E-3	4
ABCC1	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	4363		4.0E-3	1.4E-3					4.2E-4	8.5E-4	1.2E-3	4
SEC22L2	SEC22 vesicle trafficking protein-like 2 (S. cerevisiae)	26984	1.4E-3		7.6E-4			3.2E-3		8.2E-4		1.3E-3	4
AARSL	alanyl-tRNA synthetase like	57505		2.7E-4	1.4E-3			4.4E-3		1.9E-3		1.3E-3	4
XTP2	HBxAg transactivated protein 2	23215		2.0E-3	2.3E-3				3.6E-3		2.1E-4	1.4E-3	4
ULBP1	UL16 binding protein 1	80329		2.2E-3				2.1E-3	9.3E-4	2.4E-3		1.8E-3	4
AP1M2	adaptor-related protein complex 1, mu 2 subunit	10053		4.5E-3					1.3E-3	9.9E-4	2.3E-3	1.9E-3	4
FABP5	fatty acid binding protein 5 (psoriasis-associated)	2171	1.3E-8		3.8E-5						4.4E-8	2.8E-7	3
MINK	misshapen/NIK-related kinase	50488		3.2E-7				7.4E-7		1.2E-5		1.4E-6	3
MITF	microphthalmia-associated transcription factor	4286						2.5E-6		2.1E-7	9.7E-6	1.7E-6	3
PCNT1	pericentrin 1	79902		1.7E-6						3.5E-4	3.6E-8	2.7E-6	3
HBG1	hemoglobin, gamma A	3047	2.0E-5	2.2E-9			5.2E-4					2.9E-6	3
COMMD1	copper metabolism (Murr1) domain containing 1	150684		7.0E-5				4.3E-5		1.7E-8		3.7E-6	3
ATF7IP	activating transcription factor 7 interacting protein	55729						2.5E-7		1.2E-3	6.9E-7	5.9E-6	3
HSRG1	HSV-1 stimulation-related gene 1	27879			1.8E-4					6.3E-6	2.4E-7	6.4E-6	3
SLC9A6	solute carrier family 9 (sodium/hydrogen exchanger), isoform 6	10479		9.0E-4						5.3E-4	6.3E-10	6.7E-6	3
DUSP22	dual specificity phosphatase 22	56940			3.4E-3					7.9E-4	2.8E-10	9.1E-6	3
TTC14	tetratricopeptide repeat domain 14	151613		1.2E-7	3.9E-5					6.3E-4		1.4E-5	3
C12orf6	chromosome 12 open reading frame 6	57097	1.0E-4					1.5E-7		2.2E-4		1.5E-5	3
ASPH	aspartate beta-hydroxylase	444						1.4E-6		3.3E-5	2.8E-4	2.4E-5	3
PIP3-E	phosphoinositide-binding protein PIP3-E	26034	6.0E-5							2.6E-4	1.0E-6	2.5E-5	3
sdolf	olfactory receptor sdolf	89883		1.1E-6				1.0E-5		1.5E-3		2.5E-5	3
CSN2	casein beta	1447						1.7E-3		6.9E-9	3.9E-3	3.6E-5	3
PRKCN	protein kinase C, nu	23683			3.1E-3					6.5E-6	4.1E-6	4.4E-5	3
NEFL	neurofilament, light polypeptide 68kDa	4747			8.8E-4	5.9E-4				2.1E-7		4.8E-5	3
PJA2	praja 2, RING-H2 motif containing	9867						1.5E-3		2.5E-5	6.8E-6	6.4E-5	3
TMEM16C	transmembrane protein 16C	63982				7.5E-4	1.7E-5				2.9E-5	7.2E-5	3
ZNF317	zinc finger protein 317	57693			8.6E-5			3.8E-6		1.9E-3		8.5E-5	3
FLJ12572	hypothetical protein FLJ12572	64927						3.8E-3		4.3E-7	4.7E-4	9.2E-5	3
PPP1R3D	protein phosphatase 1, regulatory subunit 3D	5509			7.5E-4			1.1E-3			9.5E-7	9.3E-5	3
C20orf177	chromosome 20 open reading frame 177	63939			7.5E-4			1.1E-3			9.5E-7	9.3E-5	3
C6orf62	chromosome 6 open reading frame 62	81688		3.3E-3						1.2E-6	2.9E-4	1.1E-4	3
FLJ13955	hypothetical protein FLJ13955	79815					1.1E-6	4.6E-4		2.5E-3		1.1E-4	3
GPR21	G protein-coupled receptor 21	2844	1.9E-3	4.0E-3							1.8E-7	1.1E-4	3
ADSS	adenylosuccinate synthase	159		1.5E-3						2.1E-5	5.6E-5	1.2E-4	3
GAN	giant axonal neuropathy (gigaxonin)	8139		1.8E-5	2.1E-3			6.7E-5				1.4E-4	3
ROD1	ROD1 regulator of differentiation 1 (S. pombe)	9991	3.9E-6				9.2E-4				8.4E-4	1.4E-4	3
LOC57117	hypothetical nuclear factor SBBI22	57117	1.8E-3				5.4E-7	3.8E-3				1.6E-4	3
FLJ13273	hypothetical protein FLJ13273	79807	1.8E-3				5.4E-7	3.8E-3				1.6E-4	3
C6orf65	chromosome 6 open reading frame 65	221336						9.3E-4	7.2E-4	5.7E-6		1.6E-4	3
OLIG2	oligodendrocyte lineage transcription factor 2	10215		2.2E-4						3.6E-6	4.9E-3	1.6E-4	3
AGPAT4	acid acyltransferase, delta)	56895		9.5E-4				7.1E-5		7.3E-5		1.7E-4	3
PIP5K3	type III	200576	5.4E-5		3.6E-3			3.9E-5				2.0E-4	3
DKFZp586C1	hypothetical protein DKFZp586C1924	84233		4.1E-4					3.0E-4		7.2E-5	2.1E-4	3
PSAT1	phosphoserine aminotransferase 1	29968		2.5E-4	4.6E-5			7.5E-4				2.1E-4	3
UROD	uroporphyrinogen decarboxylase	7389		1.2E-3	3.6E-3			2.1E-6				2.1E-4	3
KIAA1724	selenoprotein I, 1	85465				4.3E-5	3.7E-4		9.4E-4			2.5E-4	3
BMF	Bcl2 modifying factor	90427		1.1E-4		3.7E-3					4.1E-5	2.5E-4	3
CHES1	checkpoint suppressor 1	1112			1.1E-3		1.8E-4			8.5E-5		2.6E-4	3
MTERF	transcription termination factor, mitochondrial	7978		1.8E-5				2.1E-4		4.9E-3		2.7E-4	3
RANGNRF	RAN guanine nucleotide release factor	29098		1.2E-3				5.3E-5			2.9E-4	2.7E-4	3
FLJ13912	hypothetical protein FLJ13912	64785		9.6E-5					4.4E-3	4.9E-5		2.7E-4	3
CCT8	chaperonin containing TCP1, subunit 8 (theta)	10694	3.3E-5		3.9E-4					2.6E-3		3.2E-4	3
PTPRC	protein tyrosine phosphatase, receptor type, C	5788		1.1E-3	2.4E-5		1.7E-3					3.5E-4	3
ZBTB8	zinc finger and BTB domain containing 8	127557			2.5E-3	6.2E-5				3.5E-4		3.8E-4	3
PRO1580	hypothetical protein PRO1580	55374		5.5E-4	7.7E-5					1.6E-3		4.1E-4	3
TBL1X	transducin (beta)-like 1X-linked	6907		2.4E-3				1.4E-4			2.1E-4	4.1E-4	3
EGF	epidermal growth factor (beta-urogastrone)	1950							1.8E-3	1.7E-4	2.5E-4	4.3E-4	3
YWHAZ	activation protein, zeta polypeptide	7534		2.7E-5				4.0E-3			8.0E-4	4.4E-4	3
MRPL30	mitochondrial ribosomal protein L30	51263			2.1E-3		1.3E-4				3.3E-4	4.5E-4	3
LOC129531	hypothetical protein BC018453	129531			2.1E-3		1.3E-4				3.3E-4	4.5E-4	3
SAT82	SAT8 family member 2	23314		1.9E-4				3.3E-3		1.7E-4		4.8E-4	3
AP3M2	adaptor-related protein complex 3, mu 2 subunit	10947	3.0E-3	1.4E-5				3.1E-3				5.1E-4	3
SCAND1	SCAN domain containing 1	51282		1.1E-4	1.2E-3		9.9E-4					5.1E-4	3
XAB1	XPA binding protein 1	11321				2.0E-3	4.1E-3		1.7E-3			5.2E-4	3
GLMN	glomulin, FKBP associated protein	11146	3.6E-3	1.4E-5							2.9E-3	5.2E-4	3
FLJ13150	hypothetical protein FLJ13150	79871	3.6E-3	1.4E-5							2.9E-3	5.2E-4	3
FARSLA	phenylalanine-tRNA synthetase-like, alpha subunit	2193		2.5E-4	1.4E-3						4.8E-4	5.5E-4	3
KIAA1007	KIAA1007 protein	23019			6.1E-4			1.5E-4	2.0E-3			5.6E-4	3
POMZP3	POM (POM121 homolog, rat) and ZP3 fusion	22932		5.5E-4					1.7E-3		2.0E-4	5.7E-4	3
RABL3	RAB, member of RAS oncogene family-like 3	285282	2.7E-3		1.3E-4					5.4E-4		5.7E-4	3
TOMM70A	(yeast)	9868		7.3E-5	9.1E-4						4.0E-3	6.4E-4	3
CGI-72	CGI-72 protein	51105			3.4E-3			1.3E-4			6.1E-4	6.4E-4	3
WDR33	WD repeat domain 33	55339		2.2E-3			9.8E-5	1.4E-3				6.7E-4	3
CCL3	chemokine (C-C motif) ligand 3	6348			4.2E-5	4.8E-3		1.6E-3				6.9E-4	3
GTF3C5	general transcription factor IIIC, polypeptide 5, 63kDa	9328		3.5E-4	1.1E-3			1.2E-3				7.7E-4	3
FLJ11273	hypothetical protein FLJ11273	54664						8.9E-5	3.9E-3	2.5E-3		9.6E-4	3
RAB30	RAB30, member RAS oncogene family	27314	3.5E-3	1.5E-4		1.7E-3						9.6E-4	3
C19orf13	chromosome 19 open reading frame 13	26065	2.2E-3	9.5E-4				4.9E-4				1.0E-3	3
ETV1	ets variant gene 1	2115		1.5E-3				1.3E-3		7.0E-4		1.1E-3	3
ARID4A	AT rich interactive domain 4A (RBP1-like)	5926		2.4E-3					6.8E-4	8.7E-4		1.1E-3	3
CGB	chorionic gonadotropin, beta polypeptide	1082		8.1E-4		7.4E-4	2.9E-3					1.2E-3	3
ZNF330	zinc finger protein 330	27309		9.2E-4	1.9E-3						1.1E-3	1.2E-3	3
MGC5508	hypothetical protein MGC5508	79073		2.4E-3					2.3E-4		4.3E-4	1.3E-3	3
TGFBR2	transforming growth factor, beta receptor II (70/80kDa)	7048						3.4E-4		1.8E-3	4.2E-3	1.4E-3	3
CALM2	calmodulin 2 (phosphorylase kinase, delta)	805	2.2E-4		3.5E-3				3.8E-3			1.4E-3	3
TP53TG3	TP53TG3 protein	24150	1.3E-3	1.6E-3			1.6E-3					1.5E-3	3
FLJ22833	hypothetical protein FLJ22833	64859			4.0E-3			1.9E-4			4.6E-3	1.5E-3	3
KIAA1946	KIAA1946 protein	165215			1.9E-3				5.6E-4	4.0E-3		1.6E-3	3
KIAA1117	KIAA1117	23033		2.8E-3	1.4E-3					1.5E-3		1.8E-3	3
HIST1H2AD	histone 1, H2ad	3013	2.9E-3	6.6E-4		3.8E-3						1.9E-3	3
HIST1H2BF	histone 1, H2bf	8343	2.9E-3	6.6E-4		3.8E-3						1.9E-3	3
TFEC	transcription factor EC	22797				4.8E-3	2.6E-3	5.9E-4				1.9E-3	3
NSEP1	nuclease sensitive element binding protein 1	4904	2.0E-3		1.8E-3				2.5E-3			2.1E-3	3
RNPC4	RNA-binding region (RNP1, RRM) containing 4	55147			3.0E-3					8.0E-4	4.0E-3	2.1E-3	3
FLJ30313	hypothetical protein FLJ30313	253868	4.7E-3	2.8E-3							7.6E-4	2.1E-3	3
HNRPH1	heterogeneous nuclear ribonucleoprotein H1 (H)	3187		1.5E-3	3.1E-3						2.3E-3	2.2E-3	3
CDCA3	cell division cycle associated 3	83461		1.9E-3	3.0E-3					2.2E-3		2.3E-3	3
RPS27A	ribosomal protein S27a	6233		1.9E-3	2.1E-3				3.2E-3			2.3E-3	3
FLJ31438	hypothetical protein FLJ31438	130162		1.9E-3	2.1E-3				3.2E-3			2.3E-3	3
C14orf120	chromosome 14 open reading frame 120	25983		1.9E-3				3.5E-3	3.4E-3			2.8E-3	3
PSMB5	proteasome (prosome, macropain) subunit, beta type, 5	5693			4.3E-3			1.5E-3			4.2E-3	3.0E-3	3
RPA3	replication protein A3, 14kDa	6119			3.6E-3					2.1E-3	4.0E-3	3.1E-3	3
SETDB2	SET domain, bifurcated 2	83852	3.5E-3					2.6E-3		3.5E-3		3.2E-3	3
KIAA0962	KIAA0962 protein	23341		3.2E-3				3.3E-3			3.1E-3	3.2E-3	3
KIAA1018	KIAA1018 protein	22909						4.5E-3	2.5E-3	4.3E-3		3.6E-3	3
SSR3	gamma)	6747			3.9E-3					3.3E-3	4.0E-3	3.7E-3	3
NDST4	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 4	64579							4.5E-6	4.0E-14		4.3E-10	2
TSSC1	tumor suppressing subtransferable candidate 1	7260			1.5E-3		1.8E-14					5.1E-9	2
CA1	carbonic anhydrase I	759	5.8E-12	7.7E-5								2.1E-8	2
DKFZp434B0	similar to semaF cytoplasmic domain associated protein 3	29951					8.8E-11			1.6E-5		3.8E-8	2
RHCE	Rhesus blood group, CcEe antigens	6006	1.2E-8	6.6E-7								9.0E-8	2
DNTT	deoxynucleotidyltransferase, terminal	1791					5.5E-5			1.7E-9		3.1E-7	2
ANGPT1	angiopoietin 1	284						9.5E-11		1.3E-3		3.5E-7	2
BS69	adenovirus 5 E1A binding protein	10771								1.4E-7	2.0E-6	5.2E-7	2
FLJ10260	hypothetical protein FLJ10260	55106						1.1E-7		3.8E-6		6.5E-7	2
FGF9	fibroblast growth factor 9 (glia-activating factor)	2254								1.4E-3	4.3E-10	7.9E-7	2
DKFZP667C1	hypothetical protein DKFZp667C165	83473				1.5E-5				6.0E-8		9.6E-7	2
GMDS	GDP-mannose 4,6-dehydratase	2762		2.8E-3							4.2E-9	3.4E-6	2
CSE1L	CSE1 chromosome segregation 1-like (yeast)	1434			4.8E-3						7.3E-9	5.9E-6	2
GNAI3	activity polypeptide 3	2773		3.4E-6				2.0E-5				5.3E-6	2
BHLHB2	basic helix-loop-helix domain containing, class B, 2	8553								1.5E-7	5.5E-4	9.1E-6	2
DSCR8	Down syndrome critical region gene 8	84677		9.4E-8	1.0E-3							9.8E-6	2
NT5C3	5'-nucleotidase, cytosolic III	51251		4.2E-3							2.7E-8	1.1E-5	2
SASH1	SAM and SH3 domain containing 1	23328							1.7E-5	8.7E-6		1.2E-5	2
LOC345667	metalloproteinase with thrombospondin motifs 10] (ADAM-TS 10)	345667						3.2E-8			4.9E-3	1.2E-5	2
IPLA2(GAMM	phospholipase A2 gamma	50640			3.3E-3		5.7E-8					1.4E-5	2
TRPV6	transient receptor potential cation channel, subfamily V, member 6	55503					1.1E-7		1.9E-3			1.5E-5	2
TEAD2	TEA domain family member 2	8463					1.4E-6				2.7E-4	1.9E-5	2
FLJ11848	hypothetical protein FLJ11848	80227		6.9E-4							5.2E-7	1.9E-5	2
CLK3	CDC-like kinase 3	1198				2.2E-3					3.6E-7	2.8E-5	2

FLJ20457	hypothetical protein FLJ20457	54942		1.2E-5	6.3E-5							2.8E-5	2
FLJ10874	hypothetical protein FLJ10874	55248	1.4E-5	6.3E-5								2.9E-5	2
TOMM40	translocase of outer mitochondrial membrane 40 homolog (yeast)	10452						6.5E-5	1.5E-5			3.1E-5	2
COPZ1	coatomer protein complex, subunit zeta 1	22818			4.4E-4			2.3E-6				3.2E-5	2
PCK1	phosphoenolpyruvate carboxykinase 1 (soluble)	5105		2.9E-7							4.2E-3	3.5E-5	2
CTBP1	C-terminal binding protein 1	1487					5.4E-7				2.3E-3	3.5E-5	2
AP1M1	adaptor-related protein complex 1, mu 1 subunit	8907							6.0E-6		2.2E-4	3.6E-5	2
FLJ90586	hypothetical protein FLJ90586	135932				3.5E-6			4.9E-4			4.2E-5	2
FLJ13576	hypothetical protein FLJ13576	64418								4.9E-5	8.1E-5	6.3E-5	2
EHD4	EH-domain containing 4	30844			2.5E-5			2.2E-4				7.4E-5	2
KIAA1363	KIAA1363 protein	57552						4.7E-3			1.3E-6	7.9E-5	2
CBR3	carbonyl reductase 3	874						6.3E-4			1.3E-5	9.2E-5	2
EGR2	early growth response 2 (Krox-20 homolog, Drosophila)	1959			2.9E-6						4.4E-3	1.1E-4	2
MGC29814	hypothetical protein MGC29814	283991			3.0E-5			5.4E-4				1.3E-4	2
IFRD1	interferon-related developmental regulator 1	3475		7.2E-5			2.3E-4					1.3E-4	2
LOC54499	putative membrane protein	54499		2.3E-3		7.4E-6						1.3E-4	2
IER2	immediate early response 2	9592		1.1E-4					1.8E-4			1.4E-4	2
RNASE4	ribonuclease, RNase A family, 4	6038				5.1E-6				4.0E-3		1.4E-4	2
SSX5	synovial sarcoma, X breakpoint 5	6758					1.1E-5				1.9E-3	1.5E-4	2
HIST1H3E	histone 1, H3e	8353					4.0E-5			6.0E-4		1.5E-4	2
ABT1	activator of basal transcription 1	29777		4.5E-3							5.3E-6	1.6E-4	2
SUV39H1	suppressor of variegation 3-9 homolog 1 (Drosophila)	6839		2.4E-4						1.1E-4		1.6E-4	2
CTNNA3	catenin (cadherin-associated protein), alpha 3	29119			1.6E-4					1.6E-4		1.6E-4	2
CGB5	chorionic gonadotropin, beta polypeptide 5	93659		1.3E-4		1.9E-4						1.6E-4	2
ARV1	likely ortholog of yeast ARV1	64801					7.5E-6		3.8E-3			1.7E-4	2
IGBP1	immunoglobulin (CD79A) binding protein 1	3476					8.2E-4				3.5E-5	1.7E-4	2
FLJ21144	hypothetical protein FLJ21144	64789						1.3E-5			2.3E-3	1.7E-4	2
COPS5	(Arabidopsis)	10987						2.1E-5			1.5E-3	1.8E-4	2
MYLK	myosin, light polypeptide kinase	4638	8.6E-5								3.8E-4	1.8E-4	2
PMP22	peripheral myelin protein 22	5376				4.7E-5		7.0E-4				1.8E-4	2
MGC4172	hypothetical protein MGC4172	79154				8.8E-5			4.0E-4			1.9E-4	2
FLJ20604	hypothetical protein FLJ20604	54983			1.0E-5						3.4E-3	1.9E-4	2
NUDT14	nudix (nucleoside diphosphate linked moiety X)-type motif 14	256281				1.9E-3	1.9E-5					1.9E-4	2
XTP1	HBxAg transactivated protein 1	55789		5.7E-5							6.8E-4	2.0E-4	2
SELL	selectin L (lymphocyte adhesion molecule 1)	6402					8.2E-4				5.0E-5	2.0E-4	2
NAP1L2	nucleosome assembly protein 1-like 2	4674			1.2E-4					3.8E-4		2.1E-4	2
p30	nuclear protein p30	201161		2.8E-4							1.6E-4	2.1E-4	2
RPP30	ribonuclease P (30kD)	10556						1.2E-3		3.7E-5		2.1E-4	2
XU8	jub, ajuba homolog (Xenopus laevis)	84962						8.3E-5	6.9E-4			2.4E-4	2
PDE7A	phosphodiesterase 7A	5150		6.8E-5				8.5E-4				2.4E-4	2
BFSP1	beaded filament structural protein 1, filensin	631						5.6E-4			1.0E-4	2.4E-4	2
BIRC1	baculoviral IAP repeat-containing 1	4671								1.6E-3	4.1E-5	2.5E-4	2
FLJ22419	hypothetical protein FLJ22419	79750				3.3E-4			2.0E-4			2.5E-4	2
ZNF20	zinc finger protein 20 (KOX 13)	7568			2.6E-3		2.3E-5					2.5E-4	2
MIB	ubiquitin ligase mind bomb	57534						2.9E-5		2.6E-3		2.8E-4	2
FAPP2	phosphoinositol 4-phosphate adaptor protein-2	84725		9.0E-5							8.8E-4	2.8E-4	2
LLSbeta	LLS beta	90102					1.9E-4			4.2E-4		2.8E-4	2
MLA-A	major histocompatibility complex, class I, A	3105			2.3E-3		4.0E-5					3.0E-4	2
FLJ20195	hypothetical protein FLJ20195	54853					2.0E-5		4.8E-3			3.1E-4	2
C1orf38	chromosome 1 open reading frame 38	9473								5.8E-5	1.7E-3	3.1E-4	2
GCLM	glutamate-cysteine ligase, modifier subunit	2730		1.0E-4							1.0E-3	3.2E-4	2
BRD2	bromodomain containing 2	6046		5.0E-3			2.2E-5					3.3E-4	2
CCT3	chaperonin containing TCP1, subunit 3 (gamma)	7203			7.6E-4					1.6E-4		3.5E-4	2
MARCKS	myristoylated alanine-rich protein kinase C substrate	4082						1.1E-3		1.2E-4		3.6E-4	2
C14orf126	chromosome 14 open reading frame 126	112487								3.0E-4	4.3E-4	3.6E-4	2
C21orf59	chromosome 21 open reading frame 59	56683				7.3E-4		2.4E-4				4.2E-4	2
SMARCA2	chromatin, subfamily a, member 2	6595	5.4E-4		3.3E-4							4.2E-4	2
TP53	tumor protein p53 (Li-Fraumeni syndrome)	7157								1.2E-3	1.6E-4	4.4E-4	2
SIAT10	sialyltransferase 10 (alpha-2,3-sialyltransferase VI)	10402			2.6E-3					7.7E-5		4.5E-4	2
LOC55580	hypothetical protein LOC55580	55580		1.0E-4	2.1E-3							4.6E-4	2
DKFZp434C0	hypothetical protein DKFZp434C0328	54762								3.2E-4	7.1E-4	4.7E-4	2
TRIP10	thyroid hormone receptor interactor 10	9322		1.7E-3						1.4E-4		4.7E-4	2
FLJ32844	hypothetical protein FLJ32844	199221						1.6E-4	1.4E-3			4.8E-4	2
CD226	CD226 antigen	10666			4.3E-3				5.4E-5			4.8E-4	2
FLJ40125	hypothetical protein FLJ40125	147699		5.3E-5						4.6E-3		4.9E-4	2
PARG	poly (ADP-ribose) glycohydrolase	8505		8.2E-4				3.0E-4				5.0E-4	2
COPS7A	(Arabidopsis)	50813						1.4E-3			1.8E-4	5.0E-4	2
B29	B29 protein	83876				6.0E-4	4.3E-4					5.1E-4	2
FXYD6	FXYD domain containing ion transport regulator 6	53826				4.1E-3	6.7E-5					5.2E-4	2
PTK9	PTK9 protein tyrosine kinase 9	5756								6.5E-4	4.6E-4	5.5E-4	2
DEFA4	defensin, alpha 4, corticostatin	1669				1.1E-4	2.8E-3					5.5E-4	2
FLJ22688	hypothetical protein FLJ22688	80199		6.2E-4	5.2E-4							5.7E-4	2
OAT	ornithine aminotransferase (gyrate atrophy)	4942		1.3E-3	2.5E-4							5.7E-4	2
C22orf5	chromosome 22 open reading frame 5	25829								8.3E-5	4.0E-3	5.8E-4	2
MGC26856	hypothetical protein MGC26856	256710				4.8E-3			7.9E-5			6.1E-4	2
LOC51058	hypothetical protein LOC51058	51058		1.8E-4					2.4E-3			6.6E-4	2
LTAH4	leukotriene A4 hydrolase	4048		3.4E-3	1.3E-4							6.7E-4	2
CHAF1A	chromatin assembly factor 1, subunit A (p150)	10036		5.5E-4			8.6E-4					6.9E-4	2
VN1R2	vomeronasal 1 receptor 2	317701		9.5E-5				5.0E-3				6.9E-4	2
SPP2	secreted phosphoprotein 2, 24kDa	6694							4.9E-4		9.8E-4	6.9E-4	2
BXDC1	brix domain containing 1	84154		8.5E-4			5.7E-4					7.0E-4	2
FLJ27099	FLJ27099 protein	374574		2.2E-3		2.2E-4						7.1E-4	2
AQP11	aquaporin 11	282679		1.6E-3							3.2E-4	7.2E-4	2
FLJ14299	hypothetical protein FLJ14299	80139				1.6E-3					3.5E-4	7.4E-4	2
SND1	staphylococcal nuclease domain containing 1	27044		9.9E-4							5.6E-4	7.5E-4	2
XPO6	exportin 6	23214			1.5E-3		4.2E-4					7.9E-4	2
ACYP2	acylphosphatase 2, muscle type	98					1.6E-4	3.9E-3				7.9E-4	2
URP	urotensin II-related peptide	257313							2.2E-4	3.0E-3		8.0E-4	2
RPL27	ribosomal protein L27	6155		2.2E-3							2.9E-4	8.1E-4	2
LAPTM4B	lysosomal associated protein transmembrane 4 beta	55353	1.3E-3	5.3E-4								8.2E-4	2
C16orf7	chromosome 16 open reading frame 7	9605				1.9E-4	3.6E-3					8.2E-4	2
BDKRB1	bradykinin receptor B1	623						4.6E-3	1.5E-4			8.3E-4	2
RPL17	ribosomal protein L17	6139						9.6E-4			7.9E-4	8.7E-4	2
RPS18	ribosomal protein S18	6222					2.3E-4		3.3E-3			8.8E-4	2
VPS52	vacuolar protein sorting 52 (yeast)	6293					2.3E-4		3.3E-3			8.8E-4	2
C4orf15	chromosome 4 open reading frame 15	55787			6.6E-4				1.2E-3			8.9E-4	2
RPL15	ribosomal protein L15	6138		1.3E-3					6.2E-4			9.0E-4	2
KBRAS1	I-kappa-B-interacting Ras-like protein 1	28512		1.3E-3					6.2E-4			9.0E-4	2
KLHL6	kelch-like 6 (Drosophila)	89857	8.6E-4								9.5E-4	9.1E-4	2
CDC42	cell division cycle 42 (GTP binding protein, 25kDa)	998				1.3E-3				6.7E-4		9.2E-4	2
TRAD	serine/threonine kinase with Dbl- and pleckstrin homology domains	11139	4.4E-3								1.9E-4	9.2E-4	2
CRA	cisplatin resistance associated	10903			2.2E-3			3.9E-4				9.2E-4	2
CCNG2	cyclin G2	901						4.9E-3		1.7E-4		9.2E-4	2
ATF6	activating transcription factor 6	22926						2.7E-3		3.4E-4		9.5E-4	2
PRRG2	proline-rich Gla (G-carboxyglutamic acid) polypeptide 2	5639						1.6E-3		5.9E-4		9.7E-4	2
NOSIP	nitric oxide synthase interacting protein	51070						1.6E-3		5.9E-4		9.7E-4	2
C6orf82	chromosome 6 open reading frame 82	51596							2.3E-4		4.5E-3	1.0E-3	2
CSRP3	cysteine and glycine-rich protein 3 (cardiac LIM protein)	8048				6.4E-4			1.7E-3			1.0E-3	2
FLJ22347	hypothetical protein FLJ22347	64852		5.0E-4					2.3E-3			1.1E-3	2
KPNA6	karyopherin alpha 6 (importin alpha 7)	23633		3.8E-3			3.0E-4					1.1E-3	2
F2RL2	coagulation factor II (thrombin) receptor-like 2	2151				1.7E-3	6.7E-4					1.1E-3	2
RPL28	ribosomal protein L28	6158			4.1E-3			2.9E-4				1.1E-3	2
CAPN1	calpain 1, (mu/I) large subunit	823				4.3E-3			2.9E-4			1.1E-3	2
COVA1	cytosolic ovarian carcinoma antigen 1	10495					1.7E-3		7.4E-4			1.1E-3	2
C1Rorf1	chromosome 1R open reading frame 1	753				4.0E-4	3.7E-3					1.2E-3	2
ERAL1	Era G-protein-like 1 (E. coli)	26284			7.4E-4				2.0E-3			1.2E-3	2
MGC33864	ADP-ribosylation-like factor 6-interacting protein 6	151188			6.4E-4			2.6E-3				1.3E-3	2
ACCN2	amiloride-sensitive cation channel 2, neuronal	41	4.9E-3							3.4E-4		1.3E-3	2
SLC16A1	member 1	6566					1.3E-3		1.3E-3			1.3E-3	2
FLJ25555	hypothetical protein FLJ25555	124930					1.4E-3				1.2E-3	1.3E-3	2
MGC40170	hypothetical protein MGC40170	253128				6.2E-4			2.7E-3			1.3E-3	2
SRPR	signal recognition particle receptor ('docking protein')	6734	4.5E-3	4.1E-4								1.4E-3	2
H17	hypothetical protein H17	55572	4.5E-3	4.1E-4								1.4E-3	2
TNFRSF21	tumor necrosis factor receptor superfamily, member 21	27242					4.2E-4			4.6E-3		1.4E-3	2
MGST3	microsomal glutathione S-transferase 3	4259		3.9E-3	4.9E-4							1.4E-3	2
C15orf12	chromosome 15 open reading frame 12	55272		6.3E-4	3.1E-3							1.4E-3	2
P2RY8	purinergic receptor P2Y, G-protein coupled, 8	286530			4.0E-3				5.4E-4			1.5E-3	2
DAD1	defender against cell death 1	1603		4.7E-3	4.6E-4							1.5E-3	2
RIF1	receptor-interacting factor 1	55791			8.7E-4		2.6E-3					1.5E-3	2
ATR	ataxia telangiectasia and Rad3 related	545						3.4E-3	6.6E-4			1.5E-3	2
RPL23A	ribosomal protein L23a	6147		5.9E-4				3.9E-3				1.5E-3	2
PDLIM3	PDZ and LIM domain 3	27295				1.2E-3				1.9E-3		1.5E-3	2
NUMA1	nuclear mitotic apparatus protein 1	4926	4.3E-3	5.6E-4								1.6E-3	2
CCT6B	chaperonin containing TCP1, subunit 6B (zeta 2)	10693	1.7E-3					1.5E-3				1.6E-3	2
MGC20398	hypothetical protein MGC20398	91603	1.7E-3					1.5E-3				1.6E-3	2
LSM5	LSM5 homolog, U6 small nuclear RNA associated (S. cerevisiae)	23658			6.9E-4					4.0E-3		1.7E-3	2
C20orf7	chromosome 20 open reading frame 7	79133		1.3E-3	2.2E-3							1.7E-3	2
MTMR4	myotubularin related protein 4	9110			3.1E-3		9.1E-4					1.7E-3	2
C21orf4	chromosome 21 open reading frame 4	757				6.9E-4	4.2E-3					1.7E-3	2
FNBP4	formin binding protein 4	23360					1.4E-3	2.1E-3				1.7E-3	2
GOLGB1	transmembrane signal), 1	2804		1.4E-3					2.1E-3			1.7E-3	2
FLJ10103	hypothetical protein FLJ10103	55070			3.2E-3					9.1E-4		1.7E-3	2
ZNF575	zinc finger protein 575	284346		3.1E-3	1.0E-3							1.8E-3	2
MGC12466	hypothetical protein MGC12466	93474		1.4E-3							2.2E-3	1.8E-3	2
RPC2	RNA polymerase III subunit RPC2	55703		2.2E-3						1.5E-3		1.8E-3	2
LDLR	low density lipoprotein receptor (familial hypercholesterolemia)	3949		9.3E-4				3.5E-3				1.8E-3	2
AP2A1	adaptor-related protein complex 2, alpha 1 subunit	160		7.8E-4				4.2E-3				1.8E-3	2
GAS8	growth arrest-specific 8	2622		1.0E-3				3.1E-3				1.8E-3	2
PSCDBP	pleckstrin homology, Sec7 and coiled-coil domains, binding protein	9595					2.6E-3				1.3E-3	1.8E-3	2
HIST1H2AE	histone 1, H2ae	3012		1.9E-3				1.9E-3				1.9E-3	2
ZNF23	zinc finger protein 23 (KOX 16)	7571		7.4E-4				4.8E-3				1.9E-3	2
PLIN	palilidin homolog (mouse)	26258			1.5E-3				2.4E-3			1.9E-3	2
ZNF286	zinc finger protein 286	57335	4.8E-3					8.0E-4				1.9E-3	2
RAB5B	RAB5B, member RAS oncogene family	5869						3.4E-3		1.2E-3		2.0E-3	2
ZNF505	zinc finger protein 505	81931						3.9E-3			1.1E-3	2.1E-3	2
AKAP1	A kinase (PRKA) anchor protein 1	8165			1.3E-3						3.7E-3	2.2E-3	2
AUTS2	autism susceptibility candidate 2	26053				4.0E-3			1.2E-3			2.2E-3	2
ACTR1A	ARP1 actin-related protein 1 homolog A, centractin alpha (yeast)	10121								2.7E-3	1.8E-3	2.2E-3	2
SUFU	suppressor of fused homolog (Drosophila)	51684								2.7E-3	1.8E-3	2.2E-3	2

FLJ23311	FLJ23311 protein	79733	2.7E-3					1.8E-3			2.2E-3	2
MGC20460	hypothetical protein MGC20460	92454		2.6E-3				1.9E-3			2.2E-3	2
LOC149830	M8 protein	149830			3.4E-3			1.5E-3			2.2E-3	2
PPM1L	protein phosphatase 1 (formerly 2C)-like	151742						3.0E-3		1.6E-3	2.2E-3	2
RAD51	RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae)	5888		2.5E-3	2.0E-3						2.2E-3	2
PRICKLE1	prickle-like 1 (Drosophila)	144165				4.9E-3				1.0E-3	2.3E-3	2
ZNF297	zinc finger protein 297	9278				4.5E-3				1.2E-3	2.3E-3	2
FBXO4	F-box only protein 4	26272					3.5E-3			1.5E-3	2.3E-3	2
MGC14425	hypothetical protein MGC14425	84989							1.6E-3	3.5E-3	2.3E-3	2
CDC25C	cell division cycle 25C	995				1.3E-3			4.6E-3		2.4E-3	2
STK24	serine/threonine kinase 24 (STE20 homolog, yeast)	8428				2.5E-3				2.5E-3	2.5E-3	2
CDC25A	cell division cycle 25A	993		2.2E-3	2.8E-3						2.5E-3	2
HSD17B7	hydroxysteroid (17-beta) dehydrogenase 7	51478				3.2E-3	2.0E-3				2.5E-3	2
MYNN	myoneurin	55892				2.2E-3		2.8E-3			2.5E-3	2
FLJ32704	hypothetical protein FLJ32704	158067				3.8E-3	1.6E-3				2.5E-3	2
ATP6AP2	ATPase, H+ transporting, lysosomal accessory protein 2	10159		2.8E-3			2.3E-3				2.5E-3	2
ZMPSTE24	zinc metalloproteinase (STE24 homolog, yeast)	10269				4.5E-3				1.5E-3	2.6E-3	2
SKB1	SKB1 homolog (S. pombe)	10419			2.0E-3		3.6E-3				2.7E-3	2
PSMC4	proteasome (prosome, macropain) 26S subunit, ATPase, 4	5704	2.8E-3	2.6E-3							2.7E-3	2
TRADD	TNFRSF1A-associated via death domain	8717		2.8E-3		2.7E-3					2.8E-3	2
FBXL8	F-box and leucine-rich repeat protein 8	55336		2.8E-3		2.7E-3					2.8E-3	2
GRID1	glutamate receptor, ionotropic, delta 1	2894				3.4E-3				2.3E-3	2.8E-3	2
SLC25A1	member 1	6576				2.3E-3				3.5E-3	2.8E-3	2
FBXO16	F-box only protein 16	157574					2.5E-3	3.2E-3			2.8E-3	2
PPIL3	peptidylprolyl isomerase (cyclophilin)-like 3	53938		4.2E-3		2.0E-3					2.9E-3	2
NIF3L1	NIF3 NGG1 interacting factor 3-like 1 (S. pombe)	60491		4.2E-3		2.0E-3					2.9E-3	2
RPL18	ribosomal protein L18	6141		3.3E-3		2.6E-3					2.9E-3	2
PGBD2	piggyBac transposable element derived 2	267002		3.0E-3	3.0E-3						3.0E-3	2
RICS	Rho GTPase-activating protein	9743						3.8E-3	2.5E-3		3.1E-3	2
RASGRP4	RAS guanyl releasing protein 4	115727				2.2E-3				4.4E-3	3.1E-3	2
PPIL5	peptidylprolyl isomerase (cyclophilin) like 5	122769					3.2E-3	3.1E-3			3.1E-3	2
TEGT	testis enhanced gene transcript (BAX inhibitor 1)	7009			2.9E-3				3.6E-3		3.2E-3	2
MGC34648	hypothetical protein MGC34648	199870				4.3E-3		2.4E-3			3.2E-3	2
PPOX	protoporphyrinogen oxidase	5498					4.2E-3		2.6E-3		3.3E-3	2
HRB2	HIV-1 rev binding protein 2	11103		2.9E-3					4.1E-3		3.5E-3	2
ARHGL1	ras homolog gene family, member G like 1	57460				4.1E-3				2.9E-3	3.5E-3	2
VIM	vimentin	7431				4.0E-3				3.2E-3	3.5E-3	2
PSMD13	proteasome (prosome, macropain) 26S subunit, non-ATPase, 13	5719	3.0E-3							4.2E-3	3.6E-3	2
CAPNS1	calpain, small subunit 1	826		3.0E-3				4.6E-3			3.7E-3	2
RNF13	ring finger protein 13	11342			3.4E-3		4.1E-3				3.8E-3	2
MLH1	mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli)	4292					4.4E-3		3.6E-3		4.0E-3	2
HIST1H3H	histone 1, H3h	8357				4.1E-3	4.1E-3				4.1E-3	2
LOC152687	hypothetical protein LOC152687	152687				3.8E-3	4.7E-3				4.3E-3	2
FLJ11331	hypothetical protein FLJ11331	55345		4.8E-3				3.8E-3			4.3E-3	2
GPR39	G protein-coupled receptor 39	2863						4.5E-3	4.9E-3		4.7E-3	2
ZNF363	zinc finger protein 363	25898					1.1E-15				1.1E-15	1
THAP6	THAP domain containing 6	152815					1.1E-15				1.1E-15	1
PSMB3	proteasome (prosome, macropain) subunit, beta type, 3	5691					2.1E-14				2.1E-14	1
KIAA1244	KIAA1244	57221					2.4E-13				2.4E-13	1
FLJ10706	hypothetical protein FLJ10706	55732					2.5E-13				2.5E-13	1
FLJ11752	NTKL-binding protein 1	92344					2.5E-12				2.5E-12	1
MGC5309	hypothetical protein MGC5309	84246					5.8E-12				5.8E-12	1
NCALD	neurocalcin delta	83988					8.7E-11				8.7E-11	1
LOC90333	hypothetical protein LOC90333	90333					1.4E-10				1.4E-10	1
FLJ25224	hypothetical protein FLJ25224	120406						2.3E-10			2.3E-10	1
OSGEPL1	O-sialoglycoprotein endopeptidase-like 1	64172					2.4E-10				2.4E-10	1
TCL6	T-cell leukemia/lymphoma 6	27004					2.4E-10				2.4E-10	1
DEF6	differentially expressed in FDCP 6 homolog (mouse)	50619					3.7E-10				3.7E-10	1
SPIN2	spindlin family, member 2	54466					4.3E-10				4.3E-10	1
LOCS1334	mesenchymal stem cell protein DSC54	51334						4.7E-10			4.7E-10	1
WNT8B	wingless-type MMTV integration site family, member 8B	7479					6.5E-10				6.5E-10	1
HOXA2	homeo box A2	3199						8.7E-10			8.7E-10	1
MGAT4A	acetylglucosaminyltransferase, isoenzyme A	11320							2.3E-9		2.3E-9	1
C20orf40	chromosome 20 open reading frame 40	149986					2.9E-9				2.9E-9	1
TTC3	tetratricopeptide repeat domain 3	7267					3.3E-9				3.3E-9	1
ZFP106	zinc finger protein 106 homolog (mouse)	64397					4.6E-9				4.6E-9	1
MUT	methylmalonyl Coenzyme A mutase	4594					4.7E-9				4.7E-9	1
C6orf139	chromosome 6 open reading frame 139	55166					4.7E-9				4.7E-9	1
KIAA0792	KIAA0792 gene product	9725					5.3E-9				5.3E-9	1
GPR151	G protein-coupled receptor 151	134391					1.1E-8				1.1E-8	1
FLJ12750	hypothetical protein FLJ12750	79720					2.3E-8				2.3E-8	1
LOC284680	hypothetical protein LOC284680	284680					3.1E-8				3.1E-8	1
JCG3	olfactory receptor-like protein JCG3	120065							3.2E-8		3.2E-8	1
C14orf54	chromosome 14 open reading frame 54	161142								3.4E-8	3.4E-8	1
OIP5	Opa-interacting protein 5	11339					3.6E-8				3.6E-8	1
NUSAP1	nucleolar and spindle associated protein 1	51203					3.6E-8				3.6E-8	1
AQP5	aquaporin 5	362				4.6E-8					4.6E-8	1
TXK	TXK tyrosine kinase	7294					5.0E-8				5.0E-8	1
DNAJC3	DnaJ (Hsp40) homolog, subfamily C, member 3	5611					5.7E-8				5.7E-8	1
LOC13030	ELEJ0030	375387					7.4E-8				7.4E-8	1
IRTA2	immunoglobulin superfamily receptor translocation associated 2	83416								7.5E-8	7.5E-8	1
CHRNA6	cholinergic receptor, nicotinic, alpha polypeptide 6	8973								8.5E-8	8.5E-8	1
MGC3121	hypothetical protein MGC3121	78994					1.1E-7				1.1E-7	1
CARD11	caspase recruitment domain family, member 11	84433					1.2E-7				1.2E-7	1
FCRH1	Fc receptor-like protein 1	115350				1.2E-7					1.2E-7	1
CXorf9	chromosome X open reading frame 9	54440					1.3E-7				1.3E-7	1
TBX2	T-box 2	6909							1.3E-7		1.3E-7	1
SMAC3L	spinal muscular atrophy candidate gene 3-like	375513						1.4E-7			1.4E-7	1
MAC30	hypothetical protein MAC30	27346					1.6E-7				1.6E-7	1
TAS2R7	taste receptor, type 2, member 7	50837					2.0E-7				2.0E-7	1
HTPAP	HTPAP protein	84513					2.0E-7				2.0E-7	1
LRRTM3	leucine rich repeat transmembrane neuronal 3	347731							2.0E-7		2.0E-7	1
FLJ31795	hypothetical protein FLJ31795	124808		2.0E-7							2.0E-7	1
MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	4085					2.1E-7				2.1E-7	1
ROBO4	roundabout homolog 4, magic roundabout (Drosophila)	54538					2.1E-7				2.1E-7	1
KIF11	kinesin family member 11	3832					2.2E-7				2.2E-7	1
MNS1	meiosis-specific nuclear structural protein 1	55329				3.1E-7					3.1E-7	1
PKP2	plakophilin 2	5318	3.5E-7								3.5E-7	1
DDX27	DEAD (Asp-Glu-Ala-Asp) box polypeptide 27	55661					3.6E-7				3.6E-7	1
RNF128	ring finger protein 128	79589							3.7E-7		3.7E-7	1
TCEAL1	transcription elongation factor A (SII)-like 1	9338				3.9E-7					3.9E-7	1
MSL3L1	male-specific lethal 3-like 1 (Drosophila)	10943							4.0E-7		4.0E-7	1
FLJ37357	hypothetical protein FLJ37357	284944					5.0E-7				5.0E-7	1
APBA2	(X11-like)	321					5.2E-7				5.2E-7	1
MGC3036	hypothetical protein MGC3036	65999		5.4E-7							5.4E-7	1
COX6C	cytochrome c oxidase subunit VIc	1345					5.9E-7				5.9E-7	1
FLJ36600	hypothetical protein FLJ36600	125115								6.0E-7	6.0E-7	1
GRIA3	glutamate receptor, ionotrophic, AMPA 3	2892							7.5E-7		7.5E-7	1
FLJ13171	hypothetical protein FLJ13171	65979								8.3E-7	8.3E-7	1
BRF1	initiation factor IIIB (S. cerevisiae)	2972				8.8E-7					8.8E-7	1
ABCB7	ATP-binding cassette, sub-family B (MDR/TAP), member 7	22					1.1E-6				1.1E-6	1
HIPK2	homeodomain interacting protein kinase 2	28996		1.3E-6							1.3E-6	1
GNAQ	guanine nucleotide binding protein (G protein), q polypeptide	2776								1.4E-6	1.4E-6	1
MGC10067	hypothetical protein MGC10067	134510					1.5E-6				1.5E-6	1
FLJ23790	hypothetical protein FLJ23790	157769						1.5E-6			1.5E-6	1
LRP3	low density lipoprotein receptor-related protein 3	4037							1.6E-6		1.6E-6	1
ARMET	arginine-rich, mutated in early stage tumors	7873					1.6E-6				1.6E-6	1
UPK1A	uroplakin 1A	11045					1.7E-6				1.7E-6	1
MRPS33	mitochondrial ribosomal protein S33	51650					1.8E-6				1.8E-6	1
RBM7	RNA binding motif protein 7	10179					1.9E-6				1.9E-6	1
PHTF1	putative homeodomain transcription factor 1	10745	1.9E-6								1.9E-6	1
		403314				1.9E-6					1.9E-6	1
LCK	lymphocyte-specific protein tyrosine kinase	3932				2.0E-6					2.0E-6	1
FLJ25989	FLJ25989 protein	347516	2.0E-6								2.0E-6	1
FLJ21963	FLJ21963 protein	79611								2.1E-6	2.1E-6	1
BNIP2	BCL2/adenovirus E1B 19kDa interacting protein 2	663								2.2E-6	2.2E-6	1
PNN	pinin, desmosome associated protein	5411					2.2E-6				2.2E-6	1
KIAA1446	brain-enriched guanylate kinase-associated protein	57596				2.2E-6					2.2E-6	1
FLJ20366	hypothetical protein FLJ20366	55638		2.3E-6							2.3E-6	1
POLA2	polymerase (DNA-directed), alpha (70kD)	23649					2.7E-6				2.7E-6	1
GRIN2B	glutamate receptor, ionotropic, N-methyl D-aspartate 2B	2904		2.7E-6							2.7E-6	1
OR2H1	olfactory receptor, family 2, subfamily H, member 1	26716					2.8E-6				2.8E-6	1
SUPT16H	suppressor of Ty 16 homolog (S. cerevisiae)	11198					3.1E-6				3.1E-6	1
NAALAD2	N-acetylated alpha-linked acidic dipeptidase 2	10003					3.2E-6				3.2E-6	1
DNAJB4	DnaJ (Hsp40) homolog, subfamily B, member 4	11080						3.3E-6			3.3E-6	1
EGR1	early growth response 1	1958					3.3E-6				3.3E-6	1
C20orf18	chromosome 20 open reading frame 18	10616							3.4E-6		3.4E-6	1
USP54	ubiquitin specific protease 54	159195					3.5E-6				3.5E-6	1
CRTAC1	cartilage acidic protein 1	55118					3.6E-6				3.6E-6	1
PCDHA5	protocadherin alpha 5	56143							3.8E-6		3.8E-6	1
SAH	SA hypertension-associated homolog (rat)	6296					3.9E-6				3.9E-6	1
KCNIP4	Kv channel interacting protein 4	80333	4.0E-6								4.0E-6	1
GRM1	glutamate receptor, metabotropic 1	2911								4.1E-6	4.1E-6	1
AMY1A	amylase, alpha 1A; salivary	276					4.2E-6				4.2E-6	1
PILRB	paired immunoglobin-like type 2 receptor beta	29990					4.4E-6				4.4E-6	1
PTPRO	protein tyrosine phosphatase, receptor type, O	5800								4.7E-6	4.7E-6	1
MFAP3	microfibrillar-associated protein 3	4238		4.8E-6							4.8E-6	1
C5orf3	chromosome 5 open reading frame 3	10827		4.8E-6							4.8E-6	1
ZNF548	zinc finger protein 548	147694					5.3E-6				5.3E-6	1
LOCS8486	transposon-derived Buster1 transposase-like protein	58486					5.6E-6				5.6E-6	1
DSIPI	delta sleep inducing peptide, immunoreactor	1831								5.8E-6	5.8E-6	1
C9orf42	chromosome 9 open reading frame 42	116224					6.2E-6				6.2E-6	1
RRAGA	Ras-related GTP binding A	10670					6.7E-6				6.7E-6	1
FURIN	furin (paired basic amino acid cleaving enzyme)	5045					6.9E-6				6.9E-6	1
LAPTM5	lysosomal-associated multispanning membrane protein-5	7805							7.0E-6		7.0E-6	1
CAPZB	capping protein (actin filament) muscle Z-line, beta	832				7.4E-6					7.4E-6	1
SLC39A9	solute carrier family 39 (zinc transporter), member 9	55334								7.5E-6	7.5E-6	1
SLC7A10	member 10	56301			7.5E-6						7.5E-6	1
SLC9A9	solute carrier family 9 (sodium/hydrogen exchanger), isoform 9	285195								8.4E-6	8.4E-6	1

KEAP1	kelch-like ECH-associated protein 1	9817					9.3E-6					9.3E-6	1
UFD1L	ubiquitin fusion degradation 1-like	7352				9.4E-6						9.4E-6	1
PDE4D	homolog, Drosophila)	5144							9.6E-6			9.6E-6	1
MS4A8B	membrane-spanning 4-domains, subfamily A, member 8B	83661							9.7E-6			9.7E-6	1
LOC286046	hypothetical protein LOC286046	286046				1.0E-5						1.0E-5	1
QDPR	quinoid dihydropteridine reductase	5860				1.0E-5						1.0E-5	1
TMEM18	transmembrane protein 18	129787				1.0E-5						1.0E-5	1
ALDH1A2	aldehyde dehydrogenase 1 family, member A2	8854	1.1E-5									1.1E-5	1
FLOT2	flotillin 2	2319	1.1E-5									1.1E-5	1
UBAP2	ubiquitin associated protein 2	55833				1.1E-5						1.1E-5	1
TNFSF4	transcriptionally activated glycoprotein 1, 34kDa)	7292									1.2E-5	1.2E-5	1
WFDC11	WAP four-disulfide core domain 11	259239								1.2E-5		1.2E-5	1
TOPK	T-LAK cell-originated protein kinase	55872				1.2E-5						1.2E-5	1
ITIH4	glycoprotein)	3700				1.3E-5						1.3E-5	1
SULF1	sulfatase 1	23213								1.3E-5		1.3E-5	1
MGC39372	hypothetical protein MGC39372	221756						1.3E-5				1.3E-5	1
CRLF1	cytokine receptor-like factor 1	9244									1.3E-5	1.3E-5	1
GPR84	G protein-coupled receptor 84	53831									1.3E-5	1.3E-5	1
PMCH	pre-melanin-concentrating hormone	5367								1.4E-5		1.4E-5	1
CKLF	chemokine-like factor	51192		1.4E-5								1.4E-5	1
ATP11B	ATPase, Class VI, type 11B	23200									1.5E-5	1.5E-5	1
POLR1B	polymerase (RNA) I polypeptide B, 128kDa	84172	1.7E-5									1.7E-5	1
CSNK2B	casein kinase 2, beta polypeptide	1460				1.8E-5						1.8E-5	1
BAT4	HLA-B associated transcript 4	7918				1.8E-5						1.8E-5	1
DAZAP2	DAZ associated protein 2	9802				1.8E-5						1.8E-5	1
PEN2	presenilin enhancer 2	55851				1.8E-5						1.8E-5	1
U2AF1L3	U2(RNU2) small nuclear RNA auxiliary factor 1-like 3	199745				1.8E-5						1.8E-5	1
MGC20785	hypothetical protein MGC20785	220164									1.9E-5	1.9E-5	1
ZNF569	zinc finger protein 569	148266				1.9E-5						1.9E-5	1
NINJ1	ninjurin 1	4814				1.9E-5						1.9E-5	1
CIB1	calcium and integrin binding 1 (calmyrin)	10519						2.0E-5				2.0E-5	1
WBP2	WW domain binding protein 2	23558				2.0E-5						2.0E-5	1
XPNPEP1	X-prolyl aminopeptidase (aminopeptidase P) 1, soluble	7511						2.1E-5				2.1E-5	1
PTBP1	polypyrimidine tract binding protein 1	5725						2.1E-5				2.1E-5	1
GATA6	GATA binding protein 6	2627				2.3E-5						2.3E-5	1
GPC4	glypican 4	2239						2.3E-5				2.3E-5	1
METTL2	methyltransferase like 2	55798						2.8E-5				2.8E-5	1
CAP350	centrosome-associated protein 350	9857				2.8E-5						2.8E-5	1
APOC1	apolipoprotein C-I	341	2.9E-5									2.9E-5	1
FLJ25952	hypothetical protein FLJ25952	253832		2.9E-5								2.9E-5	1
BPAG1	bullous pemphigoid antigen 1, 230/240kDa	667					3.0E-5					3.0E-5	1
AMD1	adenosylmethionine decarboxylase 1	262				3.1E-5						3.1E-5	1
RBBP7	retinoblastoma binding protein 7	5931						3.1E-5				3.1E-5	1
NICAL	domains	64780				3.1E-5						3.1E-5	1
CHI3L1	chitinase 3-like 1 (cartilage glycoprotein-39)	1116									3.2E-5	3.2E-5	1
LRAP	leukocyte-derived arginine aminopeptidase	64167							3.2E-5			3.2E-5	1
CALCB	calcitonin-related polypeptide, beta	797						3.3E-5				3.3E-5	1
WNT4	wingless-type MMTV integration site family, member 4	54361						3.3E-5				3.3E-5	1
	similar to hypothetical protein	388506		3.4E-5								3.4E-5	1
NMNAT3	nicotinamide nucleotide adenylyltransferase 3	349565							3.7E-5			3.7E-5	1
C10orf13	chromosome 10 open reading frame 13	143282							3.8E-5			3.8E-5	1
UGCGL1	UDP-glucose ceramide glucosyltransferase-like 1	56886						3.9E-5				3.9E-5	1
RP2	retinitis pigmentosa 2 (X-linked recessive)	6102				3.9E-5						3.9E-5	1
BCAS1	breast carcinoma amplified sequence 1	8537		4.0E-5								4.0E-5	1
USF1	upstream transcription factor 1	7391					4.0E-5					4.0E-5	1
CD84	CD84 antigen (leukocyte antigen)	8832	4.3E-5									4.3E-5	1
LEPRE1	leucine proline-enriched proteoglycan (leprecan) 1	64175				4.4E-5						4.4E-5	1
FLJ10546	hypothetical protein FLJ10546	55167						4.6E-5				4.6E-5	1
RAVER1	RAVER1	125950		4.6E-5								4.6E-5	1
MOCS1	molybdenum cofactor synthesis 1	4337				4.6E-5						4.6E-5	1
FKSG2	apoptosis inhibitor	59347					4.7E-5					4.7E-5	1
AGPP-21	cyclic AMP-regulated phosphoprotein, 21 kD	10977									4.8E-5	4.8E-5	1
TG1019	G-protein coupled receptor TG1019	165140		4.9E-5								4.9E-5	1
PMS2L5	postmeiotic segregation increased 2-like 5	5383				4.9E-5						4.9E-5	1
PFDN1	prefoldin 1	5201					5.0E-5					5.0E-5	1
FLJ20244	hypothetical protein FLJ20244	55621	5.0E-5									5.0E-5	1
FGF21	fibroblast growth factor 21	26291						5.2E-5				5.2E-5	1
C20orf31	chromosome 20 open reading frame 31	55741	5.3E-5									5.3E-5	1
FCER1A	Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide	2205				5.3E-5						5.3E-5	1
CGI-32	CGI-32 protein	51076				5.4E-5						5.4E-5	1
FLJ20313	hypothetical protein FLJ20313	54893									5.7E-5	5.7E-5	1
KIAA1228	KIAA1228 protein	57488	5.7E-5									5.7E-5	1
PDXP	pyridoxal (pyridoxine, vitamin B6) phosphatase	57026		5.8E-5								5.8E-5	1
MGC11349	hypothetical protein MGC11349	79364				5.9E-5						5.9E-5	1
CCNA2	cyclin A2	890						6.0E-5				6.0E-5	1
LMNB1	lamin B1	4001				6.4E-5						6.4E-5	1
TMEFF2	domains 2	23671							6.5E-5			6.5E-5	1
KR-ZNF1	zinc finger protein KR-ZNF1	162966		6.7E-5								6.7E-5	1
TLR1	toll-like receptor 1	7096									6.7E-5	6.7E-5	1
C10orf42	chromosome 10 open reading frame 42	90550	6.7E-5									6.7E-5	1
RIS1	Ras-induced senescence 1	25907							6.9E-5			6.9E-5	1
BZW1	basic leucine zipper and W2 domains 1	9689						7.0E-5				7.0E-5	1
SELPLG	selectin P ligand	6404		7.2E-5								7.2E-5	1
ARF1	ADP-ribosylation factor 1	375						7.5E-5				7.5E-5	1
GPC1	glypican 1	2817				7.5E-5						7.5E-5	1
C20orf54	chromosome 20 open reading frame 54	113278				7.6E-5						7.6E-5	1
TIC	SEC7 homolog	23550				7.6E-5						7.6E-5	1
NOS3	nitric oxide synthase 3 (endothelial cell)	4846				7.7E-5						7.7E-5	1
FLJ20254	hypothetical protein FLJ20254	54867									7.7E-5	7.7E-5	1
CRELD1	cysteine-rich with EGF-like domains 1	78987		7.9E-5								7.9E-5	1
PON1	paraoxonase 1	5444	8.0E-5									8.0E-5	1
PKIB	protein kinase (cAMP-dependent, catalytic) inhibitor beta	5570	8.0E-5									8.0E-5	1
TDE2	tumor differentially expressed 2	57515	8.0E-5									8.0E-5	1
CBFA2T1	1; cyclin D-related	862							8.8E-5			8.8E-5	1
KCNN3	channel, subfamily N, member 3	3782				9.4E-5						9.4E-5	1
DESC1	DESC1 protein	28983			9.7E-5							9.7E-5	1
MEST	mesoderm specific transcript homolog (mouse)	4232			9.8E-5							9.8E-5	1
PLA2R1	phospholipase A2 receptor 1, 180kDa	22925			1.0E-4							1.0E-4	1
ZNF439	zinc finger protein 439	90594			1.0E-4							1.0E-4	1
FLJ22624	FLJ22624 protein	79866						1.1E-4				1.1E-4	1
CLU	glycoprotein 2, testosterone-repressed prostate message 2,	1191				1.1E-4						1.1E-4	1
TM4SF1	transmembrane 4 superfamily member 1	4071				1.1E-4						1.1E-4	1
KIAA1463	KIAA1463 protein	57609			1.1E-4							1.1E-4	1
SCOC	short coiled-coil protein	60592						1.1E-4				1.1E-4	1
MGC4308	hypothetical protein MGC4308	84319				1.1E-4						1.1E-4	1
WBP11	WW domain binding protein 11	51729					1.2E-4					1.2E-4	1
MGC47869	hypothetical protein MGC47869	144608					1.2E-4					1.2E-4	1
7h3	hypothetical protein FLJ13511	85360									1.2E-4	1.2E-4	1
ARS2	arsenate resistance protein ARS2	51593				1.2E-4						1.2E-4	1
H1ST1H2BM	histone 1, H2bm	8342				1.2E-4						1.2E-4	1
HIVEP3	human immunodeficiency virus type I enhancer binding protein 3	59269			1.2E-4							1.2E-4	1
TSGA10	testis specific, 10	80705								1.2E-4		1.2E-4	1
RANBP2L1	RAN binding protein 2-like 1	84220									1.3E-4	1.3E-4	1
KIAA0379	KIAA0379 protein	23243					1.3E-4					1.3E-4	1
C1orf36	chromosome 1 open reading frame 36	343035			1.3E-4							1.3E-4	1
HOZFP	ovarian zinc finger protein	152518								1.3E-4		1.3E-4	1
HBP17	heparin-binding growth factor binding protein	9982				1.4E-4						1.4E-4	1
ACTG2	actin, gamma 2, smooth muscle, enteric	72				1.4E-4						1.4E-4	1
TAF12	associated factor, 20kDa	6883		1.4E-4								1.4E-4	1
ARL5	ADP-ribosylation factor-like 5	26225				1.4E-4						1.4E-4	1
VTI1B	(yeast)	10490				1.4E-4						1.4E-4	1
IF	I factor (complement)	3426	1.4E-4									1.4E-4	1
DAOA	D-amino acid oxidase activator	267012						1.4E-4				1.4E-4	1
MGC17299	hypothetical protein MGC17299	126218				1.4E-4						1.4E-4	1
IL1A	interleukin 1, alpha	3552				1.4E-4						1.4E-4	1
CYP2A7	cytochrome P450, family 2, subfamily A, polypeptide 7	1549				1.5E-4						1.5E-4	1
HMGN1	high-mobility group nucleosome binding domain 1	3150						1.5E-4				1.5E-4	1
HGRG8	high-glucose-regulated protein 8	51441			1.5E-4							1.5E-4	1
P2RX3	purinergic receptor P2X, ligand-gated ion channel, 3	5024				1.5E-4						1.5E-4	1
PRAME	preferentially expressed antigen in melanoma	23532	1.5E-4									1.5E-4	1
TA-LRRP	T-cell activation leucine repeat-rich protein	23507				1.5E-4						1.5E-4	1
SF3B3	splicing factor 3b, subunit 3, 130kDa	23450			1.6E-4							1.6E-4	1
RBAF600	retinoblastoma-associated factor 600	23352	1.6E-4									1.6E-4	1
LBR	lamin B receptor	3930									1.6E-4	1.6E-4	1
MGC12972	hypothetical protein MGC12972	84769			1.7E-4							1.7E-4	1
ZNF192	zinc finger protein 192	7745							1.7E-4			1.7E-4	1
SUHW1	suppressor of hairy wing homolog 1 (Drosophila)	129025	1.7E-4									1.7E-4	1
KIAA1219	KIAA1219 protein	57148				1.8E-4						1.8E-4	1
GIPR	gastric inhibitory polypeptide receptor	2696				1.8E-4						1.8E-4	1
GADD45G	growth arrest and DNA-damage-inducible, gamma	10912									1.8E-4	1.8E-4	1
PIGS	phosphatidylinositol glycan, class S	94005				1.8E-4						1.8E-4	1
C6orf134	chromosome 6 open reading frame 134	79969				1.8E-4						1.8E-4	1
RPS29	ribosomal protein S29	6235				1.8E-4						1.8E-4	1
FLJ11000	hypothetical protein FLJ11000	55281									1.8E-4	1.8E-4	1
MGC15730	hypothetical protein MGC15730	84966						1.8E-4				1.8E-4	1
FLJ25476	FLJ25476 protein	149076				1.8E-4						1.8E-4	1
TAS2R60	taste receptor, type 2, member 60	338398				1.9E-4						1.9E-4	1
CAPS2	calcyphosphine 2	84698			1.9E-4							1.9E-4	1
KIAA0423	KIAA0423	23116				1.9E-4						1.9E-4	1
RPIA	ribose 5-phosphate isomerase A (ribose 5-phosphate epimerase)	22934					1.9E-4					1.9E-4	1
PHF15	PHD finger protein 15	23338						1.9E-4				1.9E-4	1
KIAA0205	KIAA0205 gene product	9926									2.0E-4	2.0E-4	1
RAB37	RAB37, member of RAS oncogene family	326624									2.0E-4	2.0E-4	1
FLJ35171	FLJ35171 protein	374649									2.0E-4	2.0E-4	1
CNR1	cannabinoid receptor 1 (brain)	1268				2.0E-4						2.0E-4	1
FLJ36525	hypothetical protein LOC259173	259173						2.0E-4				2.0E-4	1
SLC38A6	solute carrier family 38, member 6	145389				2.1E-4						2.1E-4	1
RPL22	ribosomal protein L22	6146				2.1E-4						2.1E-4	1
HMG20A	high-mobility group 20A	10363	2.1E-4									2.1E-4	1

MYH10	myosin, heavy polypeptide 10, non-muscle	4628					2.1E-4						2.1E-4	1
IKBKB	kinase beta	3551		2.2E-4									2.2E-4	1
MGC9651	hypothetical protein MGC9651	114932					2.2E-4						2.2E-4	1
SRF	transcription factor)	6722		2.3E-4									2.3E-4	1
IMAP1	immunity associated protein 1	170575				2.3E-4							2.3E-4	1
ZSWIM3	zinc finger, SWIM domain containing 3	140831					2.3E-4						2.3E-4	1
MCCC2	methylcrotonoyl-Coenzyme A carboxylase 2 (beta)	64087						2.3E-4					2.3E-4	1
CRSP2	150kDa	9282										2.4E-4	2.4E-4	1
LOC349114	hypothetical protein LOC349114	349114		2.4E-4									2.4E-4	1
FGL1	fibrinogen-like 1	2267								2.4E-4			2.4E-4	1
LRRFIP2	leucine rich repeat (in FLII) interacting protein 2	9209						2.4E-4					2.4E-4	1
MGAT1	acetylglucosaminyltransferase	4245										2.4E-4	2.4E-4	1
HTR4	5-hydroxytryptamine (serotonin) receptor 4	3360										2.5E-4	2.5E-4	1
HK2	hexokinase 2	3099		2.5E-4									2.5E-4	1
BLK	B lymphoid tyrosine kinase	640					2.5E-4						2.5E-4	1
VAMP4	vesicle-associated membrane protein 4	8674					2.5E-4						2.5E-4	1
ABCA8	ATP-binding cassette, sub-family A (ABC1), member 8	10351		2.6E-4									2.6E-4	1
C20orf179	chromosome 20 open reading frame 179	140836					2.6E-4						2.6E-4	1
PTPN2	protein tyrosine phosphatase, non-receptor type 2	5771				2.6E-4							2.6E-4	1
SLC17A6	cotransporter), member 6	57084				2.6E-4							2.6E-4	1
RPS6KL1	ribosomal protein S6 kinase-like 1	83694					2.6E-4						2.6E-4	1
XPMC2H	XPMC2 prevents mitotic catastrophe 2 homolog (Xenopus laevis)	57109		2.6E-4									2.6E-4	1
HRG	histidine-rich glycoprotein	3273										2.6E-4	2.6E-4	1
USP1	ubiquitin specific protease 1	7398			2.6E-4								2.6E-4	1
ZNF322A	zinc finger protein 322A	79692					2.6E-4						2.6E-4	1
PAK2	p21 (CDKN1A)-activated kinase 2	5062										2.7E-4	2.7E-4	1
KIFC3	kinesin family member C3	3801								2.7E-4			2.7E-4	1
STX8P3	syntaxin binding protein 3	6814						2.7E-4					2.7E-4	1
LOC51204	clone HQ0477 PRO0477p	51204					2.7E-4						2.7E-4	1
FLJ10359	protein BAP28	55127		2.8E-4									2.8E-4	1
MGC70924	hypothetical LOC284338	284338				2.8E-4							2.8E-4	1
FLJ12118	hypothetical protein FLJ12118	79587				2.8E-4							2.8E-4	1
CRSP8	34kDa	9442										2.8E-4	2.8E-4	1
POSTN	periostin, osteoblast specific factor	10631						2.8E-4					2.8E-4	1
EDNRB	endothelin receptor type B	1910		2.8E-4									2.8E-4	1
CDC45L	CDC45 cell division cycle 45-like (S. cerevisiae)	8318					2.8E-4						2.8E-4	1
FLJ23825	hypothetical protein FLJ23825	284004										2.8E-4	2.8E-4	1
DACH	dachshund homolog (Drosophila)	1602	2.9E-4										2.9E-4	1
PRKCI	protein kinase C, iota	5584				2.9E-4							2.9E-4	1
EZI	likely ortholog of mouse zinc finger protein EZI	168544								2.9E-4			2.9E-4	1
FLJ23654	hypothetical protein FLJ23654	153769						2.9E-4					2.9E-4	1
MVP	major vault protein	9961										3.0E-4	3.0E-4	1
CEPT1	choline/ethanolaminephosphotransferase	10390					3.0E-4						3.0E-4	1
CXCL12	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	6387				3.0E-4							3.0E-4	1
PDCD4	programmed cell death 4 (neoplastic transformation inhibitor)	27250					3.0E-4						3.0E-4	1
ATP1A3	ATPase, Na+/K+ transporting, alpha 3 polypeptide	478				3.1E-4							3.1E-4	1
SSX1	synovial sarcoma, X breakpoint 1	6756					3.1E-4						3.1E-4	1
FALZ	fetal Alzheimer antigen	2186										3.1E-4	3.1E-4	1
IGLJ3	immunoglobulin lambda joining 3	28831										3.1E-4	3.1E-4	1
ARL2BP	ADP-ribosylation factor-like 2 binding protein	23568								3.1E-4			3.1E-4	1
SCA1	dominant, ataxin 1)	6310				3.1E-4							3.1E-4	1
PACSIN2	protein kinase C and casein kinase substrate in neurons 2	11252								3.2E-4			3.2E-4	1
TNFRSF19	tumor necrosis factor receptor superfamily, member 19	55504					3.3E-4						3.3E-4	1
FLJ35119	hypothetical protein FLJ35119	126074				3.3E-4							3.3E-4	1
MLA-B	major histocompatibility complex, class I, B	3106										3.3E-4	3.3E-4	1
ASNA1	arsA arsenite transporter, ATP-binding, homolog 1 (bacterial)	439						3.3E-4					3.3E-4	1
APOL1	apolipoprotein L, 1	8542				3.3E-4							3.3E-4	1
ING3	inhibitor of growth family, member 3	54556		3.4E-4									3.4E-4	1
SGKL	serum/glucocorticoid regulated kinase-like	23678										3.4E-4	3.4E-4	1
LOC220074	Hypothetical 55.1 kDa protein F09G8.5 in chromosome III	220074		3.4E-4									3.4E-4	1
CP	ceruloplasmin (ferroxidase)	1356						3.5E-4					3.5E-4	1
KIR2DL4	cytoplasmic tail, 4	3805					3.5E-4						3.5E-4	1
SNW1	SKI-interacting protein	22938								3.5E-4			3.5E-4	1
DCK	deoxycytidine kinase	1633					3.5E-4						3.5E-4	1
ITGB3BP	integrin beta 3 binding protein (beta3-endonexin)	23421						3.6E-4					3.6E-4	1
KIAA1799	KIAA1799 protein	84455						3.6E-4					3.6E-4	1
LOC148523	hypothetical protein BC017397	148523								3.6E-4			3.6E-4	1
MRPS15	mitochondrial ribosomal protein S15	64960		3.6E-4									3.6E-4	1
CDC25B	cell division cycle 25B	994					3.6E-4						3.6E-4	1
PSMB2	proteasome (prosome, macropain) subunit, beta type, 2	5690					3.6E-4						3.6E-4	1
JTB	jumping translocation breakpoint	10899					3.6E-4						3.6E-4	1
MGC40053	hypothetical protein MGC40053	158947								3.7E-4			3.7E-4	1
PRC1	protein regulator of cytokinesis 1	9055		3.7E-4									3.7E-4	1
TNFRSF6	tumor necrosis factor receptor superfamily, member 6	355						3.7E-4					3.7E-4	1
SFRS1	splicing factor)	6426			3.7E-4								3.7E-4	1
SSH1	slingshot 1	54434				3.8E-4							3.8E-4	1
STX8P4	syntaxin binding protein 4	252983					3.8E-4						3.8E-4	1
BCL9L	B-cell CLL/lymphoma 9-like	283149										3.8E-4	3.8E-4	1
RPL3	ribosomal protein L3	6122								3.9E-4			3.9E-4	1
ARD1	ARD1 homolog, N-acetyltransferase (S. cerevisiae)	8260										3.9E-4	3.9E-4	1
ECG2	esophagus cancer related gene-2	84651					3.9E-4						3.9E-4	1
FLJ35721	hypothetical protein FLJ35721	286051						3.9E-4					3.9E-4	1
LSM11	U7 snRNA-associated Sm-like protein	134353				4.1E-4							4.1E-4	1
KIAA0179	KIAA0179	23076			4.1E-4								4.1E-4	1
MRPL9	mitochondrial ribosomal protein L9	65005								4.1E-4			4.1E-4	1
P2RX1	purinergic receptor P2X, ligand-gated ion channel, 1	5023				4.1E-4							4.1E-4	1
ZNF410	zinc finger protein 410	57862					4.1E-4						4.1E-4	1
FLJ10120	hypothetical protein FLJ10120	55073				4.2E-4							4.2E-4	1
FLJ37970	hypothetical protein FLJ37970	283234					4.3E-4						4.3E-4	1
XDH	xanthine dehydrogenase	7498					4.3E-4						4.3E-4	1
SMARCA4	chromatin, subfamily a, member 4	6597								4.5E-4			4.5E-4	1
ZNF451	zinc finger protein 451	26036		4.5E-4									4.5E-4	1
RABL2A	RAB, member of RAS oncogene family-like 2A	11159				4.5E-4							4.5E-4	1
CCNB1IP1	cyclin B1 interacting protein 1	57820				4.5E-4							4.5E-4	1
FLJ33710	FLJ33710 protein	284370									4.5E-4		4.5E-4	1
PPIL1	peptidylprolyl isomerase (cyclophilin)-like 1	51645		4.6E-4									4.6E-4	1
MGC46496	hypothetical protein MGC46496	285555	4.6E-4										4.6E-4	1
		403323										4.6E-4	4.6E-4	1
GBA	glucosidase, beta; acid (includes glucosylceramidase)	2629								4.7E-4			4.7E-4	1
SRP46	Splicing factor, arginine/serine-rich, 46kD	10929										4.7E-4	4.7E-4	1
TRIM43	tripartite motif-containing 43	129868									4.8E-4		4.8E-4	1
GNMT	glycine N-methyltransferase	27232				4.8E-4							4.8E-4	1
HARS2	histidyl-tRNA synthetase 2	92675		4.8E-4									4.8E-4	1
STAG2	stromal antigen 2	10735				4.8E-4							4.8E-4	1
BTBD4	BTB (POZ) domain containing 4	140685				4.9E-4							4.9E-4	1
CAST	CAZ-associated structural protein	26059										4.9E-4	4.9E-4	1
TPP2	tripeptidyl peptidase II	7174								4.9E-4			4.9E-4	1
SLC39A14	solute carrier family 39 (zinc transporter), member 14	23516					5.1E-4						5.1E-4	1
USP51	ubiquitin specific protease 51	158880					5.1E-4						5.1E-4	1
PGLYRP	peptidoglycan recognition protein	8993								5.2E-4			5.2E-4	1
PURA	purine-rich element binding protein A	5813				5.2E-4							5.2E-4	1
TNNI3	troponin I, cardiac	7137								5.2E-4			5.2E-4	1
NFIL3	nuclear factor, interleukin 3 regulated	4783					5.3E-4						5.3E-4	1
C16orf5	chromosome 16 open reading frame 5	29965										5.4E-4	5.4E-4	1
FLJ31461	hypothetical protein FLJ31461	145978										5.4E-4	5.4E-4	1
QP-C	low molecular mass ubiquinone-binding protein (9.5kD)	27089		5.4E-4									5.4E-4	1
CGI-115	CGI-115 protein	51018				5.4E-4							5.4E-4	1
PCDHGA1	protocadherin gamma subfamily A, 1	56114					5.4E-4						5.4E-4	1
NR1I2	nuclear receptor subfamily 1, group I, member 2	8856					5.4E-4						5.4E-4	1
TPX2	TPX2, microtubule-associated protein homolog (Xenopus laevis)	22974					5.5E-4						5.5E-4	1
		400590										5.5E-4	5.5E-4	1
EBP	emopamil binding protein (sterol isomerase)	10682									5.5E-4		5.5E-4	1
MHC2TA	MHC class II transactivator	4261										5.6E-4	5.6E-4	1
CD200R	cell surface glycoprotein receptor CD200	131450	5.7E-4										5.7E-4	1
EIF2S2	eukaryotic translation initiation factor 2, subunit 2 beta, 38kDa	8894				5.7E-4							5.7E-4	1
C1orf34	chromosome 1 open reading frame 34	22996				5.7E-4							5.7E-4	1
ZNF291	zinc finger protein 291	49855					5.7E-4						5.7E-4	1
PIK3R1	alpha)	5295		5.8E-4									5.8E-4	1
SLC39A11	solute carrier family 39 (metal ion transporter), member 11	201266								5.8E-4			5.8E-4	1
RWDD1	RWD domain containing 1	51389					5.8E-4						5.8E-4	1
FLJ20171	hypothetical protein FLJ20171	54845					5.8E-4						5.8E-4	1
ITGB1BP2	integrin beta 1 binding protein (melusin) 2	26548									6.0E-4		6.0E-4	1
SELB	elongation factor for selenoprotein translation	60678										6.0E-4	6.0E-4	1
KIAA0795	KIAA0795 protein	23276			6.0E-4								6.0E-4	1
KIF9	kinesin family member 9	64147			6.0E-4								6.0E-4	1
LRIG1	leucine-rich repeats and immunoglobulin-like domains 1	26018				6.1E-4							6.1E-4	1
CAP2A2	capping protein (actin filament) muscle Z-line, alpha 2	830				6.1E-4							6.1E-4	1
NKTR	natural killer-tumor recognition sequence	4820								6.1E-4			6.1E-4	1
MGC33382	hypothetical protein MGC33382	162681					6.2E-4						6.2E-4	1
KIS	kinase interacting with leukemia-associated gene (stathmin)	127933								6.3E-4			6.3E-4	1
SORT1	sortilin 1	6272								6.3E-4			6.3E-4	1
PPP1R2	protein phosphatase 1, regulatory (inhibitor) subunit 2	5504					6.4E-4						6.4E-4	1
SNTB2	component 2)	6645										6.4E-4	6.4E-4	1
LOC57146	prometrin	57146					6.4E-4						6.4E-4	1
SF1	splicing factor 1	7536			6.4E-4								6.4E-4	1
DUSP18	dual specificity phosphatase 18	150290					6.5E-4						6.5E-4	1
SLC35C2	solute carrier family 35, member C2	51006								6.5E-4			6.5E-4	1
FLJ22761	hypothetical protein FLJ22761	80201									6.5E-4		6.5E-4	1
RAP1A	RAP1A, member of RAS oncogene family	5906								6.5E-4			6.5E-4	1
FLJ10618	hypothetical protein FLJ10618	55186								6.5E-4			6.5E-4	1
LTBP1	latent transforming growth factor beta binding protein 1	4052						6.5E-4					6.5E-4	1
FLJ25378	hypothetical protein FLJ25378	255055									6.6E-4		6.6E-4	1
CDK11	cyclin-dependent kinase (CDC2-like) 11	23097					6.6E-4						6.6E-4	1
PRDM13	PR domain containing 13	59336									6.6E-4		6.6E-4	1
ARHGAP11A	similar to human GTPase-activating protein	9824								6.6E-4			6.6E-4	1
MANEA	mannosidase, endo-alpha	79694										6.8E-4	6.8E-4	1
C14orf108	chromosome 14 open reading frame 108	55745										6.8E-4	6.8E-4	1
NUDT2	nudix (nucleoside diphosphate linked moiety X)-type motif 2	318			6.9E-4								6.9E-4	1
ARL10C	ADP-ribosylation factor-like 10C	55207									7.0E-4		7.0E-4	1

FRAP1	FK506 binding protein 12-rapamycin associated protein 1	2475		7.0E-4										7.0E-4	1
FLJ14800	hypothetical protein FLJ14800	84926								7.1E-4				7.1E-4	1
PLEKHB1	member 1	58473								7.2E-4				7.2E-4	1
ANGPT2	angiopoietin 2	285									7.2E-4			7.2E-4	1
PKHD1	polycystic kidney and hepatic disease 1 (autosomal recessive)	5314				7.2E-4								7.2E-4	1
RBM8A	RNA binding motif protein 8A	9939		7.3E-4										7.3E-4	1
NAP1L4	nucleosome assembly protein 1-like 4	4676				7.3E-4								7.3E-4	1
CGI-01	CGI-01 protein	51603						7.4E-4						7.4E-4	1
SIN3A	SIN3 homolog A, transcriptional regulator (yeast)	25942					7.4E-4							7.4E-4	1
STMN2	stathmin-like 2	11075									7.4E-4			7.4E-4	1
DKFZP564B1	DKFZP564B147 protein	26071		7.5E-4										7.5E-4	1
KIAA0907	KIAA0907 protein	22889		7.5E-4										7.5E-4	1
SLITRK5	slit and trk like gene 5	26050											7.5E-4	7.5E-4	1
TPST1	tyrosylprotein sulfotransferase 1	8460				7.6E-4								7.6E-4	1
ITGB2	associated antigen 1; macrophage antigen 1 (mac-1) beta subunit)	3689				7.7E-4								7.7E-4	1
FLJ32416	JP-45	126306					7.8E-4							7.8E-4	1
FLJ20534	hypothetical protein FLJ20534	54969					7.8E-4							7.8E-4	1
ZFPM1	zinc finger protein, multitype 1	161882								7.8E-4				7.8E-4	1
IL1RL1LG	interleukin 1 receptor-like 1 ligand	11018								7.8E-4				7.8E-4	1
MAGEA3	melanoma antigen, family A, 3	4102				7.9E-4								7.9E-4	1
FETUB	fetuin B	26998					7.9E-4							7.9E-4	1
C6orf157	chromosome 6 open reading frame 157	90025								8.0E-4				8.0E-4	1
DKFZP434Q0	DKFZP434Q0047 protein	26083		8.0E-4										8.0E-4	1
BGALT1S	beta galactosyltransferase BGALT1S	374907					8.0E-4							8.0E-4	1
AIP	aryl hydrocarbon receptor interacting protein	9049											8.0E-4	8.0E-4	1
RCL1	RNA terminal phosphate cyclase-like 1	10171									8.2E-4			8.2E-4	1
OK/SW-cl.56	beta 5-tubulin	203068			8.2E-4									8.2E-4	1
P2RY5	purinergic receptor P2Y, G-protein coupled, 5	10161				8.3E-4								8.3E-4	1
ZDHHC7	zinc finger, DHHC domain containing 7	55625					8.3E-4							8.3E-4	1
CRYGA	crystallin, gamma A	1418											8.3E-4	8.3E-4	1
MAML2	mastermind-like 2 (Drosophila)	84441				8.3E-4								8.3E-4	1
IFIT5	interferon-induced protein with tetratricopeptide repeats 5	24138					8.3E-4							8.3E-4	1
C22orf1	chromosome 22 open reading frame 1	758									8.3E-4			8.3E-4	1
CCT7	chaperonin containing TCP1, subunit 7 (eta)	10574		8.4E-4										8.4E-4	1
C2orf7	chromosome 2 open reading frame 7	84279		8.4E-4										8.4E-4	1
ALDH3B2	aldehyde dehydrogenase 3 family, member B2	222					8.4E-4							8.4E-4	1
FREB	Fc receptor homolog expressed in B cells	84824						8.5E-4						8.5E-4	1
DKFZP434A1	hypothetical protein DKFZp434A1319	84080				8.6E-4								8.6E-4	1
FLJ10871	hypothetical protein FLJ10871	55756	8.6E-4											8.6E-4	1
C20orf24	chromosome 20 open reading frame 24	55969					8.6E-4							8.6E-4	1
PLAC8	placenta-specific 8	51316					8.7E-4							8.7E-4	1
LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	4067		8.8E-4										8.8E-4	1
KIAA0592	KIAA0592 protein	253725											8.8E-4	8.8E-4	1
ADCY5	adenylate cyclase 5	111											9.0E-4	9.0E-4	1
SAP18	sin3-associated polypeptide, 18kDa	10284						9.0E-4						9.0E-4	1
SURF2	surfeit 2	6835						9.0E-4						9.0E-4	1
FLJ12541	stimulated by retinoic acid gene 6	84220					9.2E-4							9.2E-4	1
LOC51244	hypothetical protein LOC51244	51244	9.2E-4											9.2E-4	1
CNIH	cornichon homolog (Drosophila)	10175		9.2E-4										9.2E-4	1
LOC286075	hypothetical protein LOC286075	286075		9.3E-4										9.3E-4	1
TJP4	tight junction protein 4 (peripheral)	93643					9.4E-4							9.4E-4	1
FBXW2	F-box and WD-40 domain protein 2	26190								9.4E-4				9.4E-4	1
LOC151648	hypothetical protein BC001339	151648		9.4E-4										9.4E-4	1
FIGNL1	fidgetin-like 1	63979				9.4E-4								9.4E-4	1
ZNF547	zinc finger protein 547	284306					9.5E-4							9.5E-4	1
LOC113444	hypothetical protein BC011880	113444								9.6E-4				9.6E-4	1
MUS81	MUS81 endonuclease homolog (yeast)	80198					9.7E-4							9.7E-4	1
EGFL6	EGF-like-domain, multiple 6	25975				9.7E-4								9.7E-4	1
LOC283687	hypothetical protein LOC283687	283687		9.7E-4										9.7E-4	1
POLS	polymerase (DNA directed) sigma	11044											9.7E-4	9.7E-4	1
NNMT	histamine N-methyltransferase	3176											9.7E-4	9.7E-4	1
MGC13159	hypothetical protein MGC13159	85013					9.7E-4							9.7E-4	1
CASC1	cancer susceptibility candidate 1	55259					9.8E-4							9.8E-4	1
VAMP5	vesicle-associated membrane protein 5 (myobrevin)	10791					9.8E-4							9.8E-4	1
DCAL1	dendritic cell-associated lectin-1	160365			9.9E-4									9.9E-4	1
FLJ20047	hypothetical protein FLJ20047	54798		9.9E-4										9.9E-4	1
PABPN1	poly(A) binding protein, nuclear 1	8106					1.0E-3							1.0E-3	1
ATP2A2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	488				1.0E-3								1.0E-3	1
SPAG5	sperm associated antigen 5	10615		1.0E-3										1.0E-3	1
LOC283537	hypothetical protein LOC283537	283537								1.0E-3				1.0E-3	1
EPLIN	epithelial protein lost in neoplasm beta	51474								1.0E-3				1.0E-3	1
SEC13L	sec13-like protein	81929				1.1E-3								1.1E-3	1
PDE6D	phosphodiesterase 6D, cGMP-specific, rod, delta	5147						1.1E-3						1.1E-3	1
ARHGEF10	Rho guanine nucleotide exchange factor (GEF) 10	9639				1.1E-3								1.1E-3	1
LOC158160	hypothetical protein LOC158160	158160					1.1E-3							1.1E-3	1
DKFZp566C0	putative MAPK activating protein PM20,PM21	26099				1.1E-3								1.1E-3	1
FLJ25735	hypothetical protein FLJ25735	158506					1.1E-3							1.1E-3	1
JTV1	JTV1 gene	7965					1.1E-3							1.1E-3	1
GPR10	G protein-coupled receptor 10	2834					1.1E-3							1.1E-3	1
FLJ37562	hypothetical protein FLJ37562	134553					1.1E-3							1.1E-3	1
SOX11	SRY (sex determining region Y)-box 11	6664					1.1E-3							1.1E-3	1
STAT5B	signal transducer and activator of transcription 5B	6777								1.1E-3				1.1E-3	1
TNFRSF8	tumor necrosis factor receptor superfamily, member 8	943									1.1E-3			1.1E-3	1
FLJ33979	hypothetical protein FLJ33979	196074		1.1E-3										1.1E-3	1
LOXL4	lysyl oxidase-like 4	84171				1.2E-3								1.2E-3	1
C10orf4	chromosome 10 open reading frame 4	118924						1.2E-3						1.2E-3	1
CES2	carboxylesterase 2 (intestine, liver)	8824				1.2E-3								1.2E-3	1
GABBR1	gamma-aminobutyric acid (GABA) B receptor, 1	2550				1.2E-3								1.2E-3	1
CYP21A2	cytochrome P450, family 21, subfamily A, polypeptide 2	1589						1.2E-3						1.2E-3	1
TPRA40	seven transmembrane domain orphan receptor	131601											1.2E-3	1.2E-3	1
C6orf66	chromosome 6 open reading frame 66	29078		1.2E-3										1.2E-3	1
LIMS1	LIM and senescent cell antigen-like domains 1	3987						1.2E-3						1.2E-3	1
NPAS3	neuronal PAS domain protein 3	64067				1.2E-3								1.2E-3	1
ARTS-1	regulator	51752											1.2E-3	1.2E-3	1
EML4	echinoderm microtubule associated protein like 4	27436					1.2E-3							1.2E-3	1
ZNF579	zinc finger protein 579	163033								1.2E-3				1.2E-3	1
ZNF499	zinc finger protein 499	84878						1.2E-3						1.2E-3	1
FKSG42	FKSG42	83956								1.2E-3				1.2E-3	1
FLJ12760	hypothetical protein FLJ12760	339175								1.2E-3				1.2E-3	1
KIAA0643	KIAA0643 protein	23059				1.2E-3								1.2E-3	1
INPP5A	inositol polyphosphate-5-phosphatase, 40kDa	3632					1.2E-3							1.2E-3	1
C6orf80	chromosome 6 open reading frame 80	25901					1.2E-3							1.2E-3	1
KIAA1458	KIAA1458 protein	57606						1.2E-3						1.2E-3	1
SLC16A6	member 6	9120				1.2E-3								1.2E-3	1
DBCCR1L	DBCCR1-like	339479									1.2E-3			1.2E-3	1
SNRPB	small nuclear ribonucleoprotein polypeptides B and B1	6628						1.2E-3						1.2E-3	1
DPCK	CoA kinase	80347						1.2E-3						1.2E-3	1
C8G	complement component 8, gamma polypeptide	733		1.2E-3										1.2E-3	1
FBXW5	F-box and WD-40 domain protein 5	54461		1.2E-3										1.2E-3	1
NRM	nurim (nuclear envelope membrane protein)	11270					1.3E-3							1.3E-3	1
VANGL1	vang-like 1 (van gogh, Drosophila)	81839		1.3E-3										1.3E-3	1
POLR2L	polymerase (RNA) II (DNA directed) polypeptide L, 7.6kDa	5441				1.3E-3								1.3E-3	1
TARDBP	TAR DNA binding protein	23435				1.3E-3								1.3E-3	1
FLJ30678	hypothetical protein FLJ30678	139324									1.3E-3			1.3E-3	1
KIAA0922	KIAA0922 protein	23240											1.3E-3	1.3E-3	1
ATRX	homolog, S. cerevisiae)	546											1.3E-3	1.3E-3	1
ANXA5	annexin A5	308				1.3E-3								1.3E-3	1
C9orf23	chromosome 9 open reading frame 23	138716		1.3E-3										1.3E-3	1
DNAJB1	DnaJ (Hsp40) homolog, subfamily B, member 1	3337								1.3E-3				1.3E-3	1
LOC283887	hypothetical protein LOC283887	283887					1.3E-3							1.3E-3	1
SFPQ	protein associated)	6421					1.3E-3							1.3E-3	1
ERAS	ES cell expressed Ras	3266					1.3E-3							1.3E-3	1
STK11IP	serine/threonine kinase 11 interacting protein	114790						1.3E-3						1.3E-3	1
HS6ST1	heparan sulfate 6-O-sulfotransferase 1	9394				1.3E-3								1.3E-3	1
KIAA1924	hypothetical protein KIAA1924	197335				1.4E-3								1.4E-3	1
	similar to RIKEN cDNA 1810056O20	388962	1.4E-3											1.4E-3	1
ABCC5	ATP-binding cassette, sub-family C (CFTR/MRP), member 5	10057		1.4E-3										1.4E-3	1
DOLPP1	dolichyl pyrophosphate phosphatase 1	57171								1.4E-3				1.4E-3	1
MRTF-B	myocardin-related transcription factor B	57496				1.4E-3								1.4E-3	1
DEPDC6	DEP domain containing 6	64798								1.4E-3				1.4E-3	1
CEBPZ	CCAAT/enhancer binding protein zeta	10153					1.4E-3							1.4E-3	1
PRO1853	hypothetical protein PRO1853	55471					1.4E-3							1.4E-3	1
NDUFA2	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2, 8kDa	4695		1.4E-3										1.4E-3	1
TXN	thioredoxin	7295			1.4E-3									1.4E-3	1
WDR23	WD repeat domain 23	80344		1.4E-3										1.4E-3	1
FHL5	four and a half LIM domains 5	9457				1.4E-3								1.4E-3	1
RBBP6	retinoblastoma binding protein 6	5930								1.4E-3				1.4E-3	1
HADHA	thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha	3030					1.4E-3							1.4E-3	1
HADHB	thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta	3032					1.4E-3							1.4E-3	1
RAB11A	RAB11A, member RAS oncogene family	8766						1.4E-3						1.4E-3	1
ZNF384	zinc finger protein 384	171017				1.4E-3								1.4E-3	1
MRPL37	mitochondrial ribosomal protein L37	51253					1.4E-3							1.4E-3	1
LOC200008	hypothetical protein LOC200008	200008					1.4E-3							1.4E-3	1
FRBZ1	FRBZ1 protein	360023		1.4E-3										1.4E-3	1
SLC26A10	solute carrier family 26, member 10	65012											1.4E-3	1.4E-3	1
USP25	ubiquitin specific protease 25	29761								1.4E-3				1.4E-3	1
SYT7	synaptotagmin VII	9066											1.4E-3	1.4E-3	1
RAIN	Ras-interacting protein	54922		1.4E-3										1.4E-3	1
CGI-141	CGI-141 protein	51026						1.5E-3						1.5E-3	1
EVI2B	ecotropic viral integration site 2B	2124						1.5E-3						1.5E-3	1
ATP6V1F	ATPase, H+ transporting, lysosomal 14kDa, V1 subunit F	9296								1.5E-3				1.5E-3	1
FLJ38705	hypothetical protein FLJ38705	286128					1.5E-3							1.5E-3	1
STXBP5	syntaxin binding protein 5 (tomosyn)	134957								1.5E-3				1.5E-3	1
VPS33B	vacuolar protein sorting 33B (yeast)	26276											1.5E-3	1.5E-3	1
SLC39A1	solute carrier family 39 (zinc transporter), member 1	27173		1.5E-3										1.5E-3	1
CREB3L4	cAMP responsive element binding protein 3-like 4	148327		1.5E-3										1.5E-3	1
GLG1	golgi apparatus protein 1	2734						1.5E-3						1.5E-3	1
MGC35048	hypothetical protein MGC35048	124152						1.5E-3						1.5E-3	1
KIAA0853	KIAA0853	23091						1.5E-3						1.5E-3	1

CRI1	CREBBP/EP300 inhibitory protein 1	23741			1.5E-3							1.5E-3	1
HCG4	HLA complex group 4	54435					1.5E-3					1.5E-3	1
C9orf87	chromosome 9 open reading frame 87	55151		1.5E-3								1.5E-3	1
FLJ21945	hypothetical protein FLJ21945	80304						1.5E-3				1.5E-3	1
TM4SF8	transmembrane 4 superfamily member 8	10099							1.5E-3			1.5E-3	1
LY86	lymphocyte antigen 86	9450					1.5E-3					1.5E-3	1
SLC33A1	solute carrier family 33 (acetyl-CoA transporter), member 1	9197			1.5E-3							1.5E-3	1
MRPS16	mitochondrial ribosomal protein S16	51021							1.5E-3			1.5E-3	1
ANXA7	annexin A7	310									1.5E-3	1.5E-3	1
FLJ90709	hypothetical protein FLJ90709	153129							1.5E-3			1.5E-3	1
C6orf210	chromosome 6 open reading frame 210	57107							1.5E-3			1.5E-3	1
ACK1	activated Cdc42-associated kinase 1	10188					1.5E-3					1.5E-3	1
GUSB	glucuronidase, beta	2990						1.5E-3				1.5E-3	1
PRPSAP2	phosphoribosyl pyrophosphate synthetase-associated protein 2	5636				1.5E-3						1.5E-3	1
FLJ11526	hypothetical protein FLJ11526	79685							1.6E-3			1.6E-3	1
ZNRF1	zinc and ring finger protein 1	84937						1.6E-3				1.6E-3	1
ZNF574	zinc finger protein 574	64763		1.6E-3								1.6E-3	1
PACE-1	ezrin-binding partner PACE-1	57147				1.6E-3						1.6E-3	1
VN1R3	vomeronasal 1 receptor 3	317702				1.6E-3						1.6E-3	1
POLR3K	polymerase (RNA) III (DNA directed) polypeptide K, 12.3 kDa	51728					1.6E-3					1.6E-3	1
HLA-C	major histocompatibility complex, class I, C	3107									1.6E-3	1.6E-3	1
PRDM8	PR domain containing 8	56978					1.6E-3					1.6E-3	1
RTKN	rhotekin	6242							1.6E-3			1.6E-3	1
PANK1	pantothenate kinase 1	53354					1.6E-3					1.6E-3	1
KIAA0586	KIAA0586	9786						1.6E-3				1.6E-3	1
TIMM9	translocase of inner mitochondrial membrane 9 homolog (yeast)	26520						1.6E-3				1.6E-3	1
MGC2474	hypothetical protein MGC2474	65988							1.6E-3			1.6E-3	1
ZNF258	zinc finger protein 258	9204		1.6E-3								1.6E-3	1
GNAI1	activity polypeptide 1	2770							1.6E-3			1.6E-3	1
TBXA2R	thromboxane A2 receptor	6915									1.6E-3	1.6E-3	1
LDHL	lactate dehydrogenase A -like	92483					1.6E-3					1.6E-3	1
SOX5	SRY (sex determining region Y)-box 5	6660								1.6E-3		1.6E-3	1
ATP6V1G2	ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G isoform 2	534							1.6E-3			1.6E-3	1
ATP6V0D1	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d isoform 1	9114				1.6E-3						1.6E-3	1
DKFZP564O0823	DKFZP564O0823 protein	25849					1.6E-3					1.6E-3	1
MIPOL1	mirror-image polarity 1	145282		1.6E-3								1.6E-3	1
FLJ25660	hypothetical protein FLJ25660	148109					1.6E-3					1.6E-3	1
ANAPC4	anaphase promoting complex subunit 4	29945				1.6E-3						1.6E-3	1
SLC7A6	system), member 6	9057							1.7E-3			1.7E-3	1
DKFZp45110	tasilin	20008			1.7E-3							1.7E-3	1
KNTC1	kinetochore associated 1	9735							1.7E-3			1.7E-3	1
FLJ11021	similar to splicing factor, arginine/serine-rich 4	65117							1.7E-3			1.7E-3	1
C5orf5	chromosome 5 open reading frame 5	51306									1.7E-3	1.7E-3	1
ERK8	extracellular signal-regulated kinase 8	225689									1.7E-3	1.7E-3	1
EPB41L1	erythrocyte membrane protein band 4.1-like 1	2036							1.7E-3			1.7E-3	1
HOXB5	homeo box B5	3215							1.7E-3			1.7E-3	1
BTBD3	BTB (POZ) domain containing 3	22903									1.7E-3	1.7E-3	1
MRPS27	mitochondrial ribosomal protein S27	23107							1.7E-3			1.7E-3	1
FLJ12598	hypothetical protein FLJ12598	79810							1.7E-3			1.7E-3	1
ELF3	)	1999							1.7E-3			1.7E-3	1
MGC13040	hypothetical protein MGC13040	85016						1.7E-3				1.7E-3	1
NFKBIA	inhibitor, alpha	4792			1.7E-3							1.7E-3	1
FOXG1B	forkhead box G1B	2290		1.7E-3								1.7E-3	1
JARID1D	Jumonji, AT rich interactive domain 1D (RBP2-like)	8284			1.7E-3							1.7E-3	1
PLEKHA3	binding specific) member 3	65977		1.7E-3								1.7E-3	1
SCAP2	src family associated phosphoprotein 2	8935							1.7E-3			1.7E-3	1
FLJ22671	hypothetical protein FLJ22671	79919							1.8E-3			1.8E-3	1
OPRL1	opiate receptor-like 1	4987							1.8E-3			1.8E-3	1
RGS19	regulator of G-protein signaling 19	10287							1.8E-3			1.8E-3	1
PAM	peptidylglycine alpha-amidating monooxygenase	5066									1.8E-3	1.8E-3	1
PGM2	phosphoglucomutase 2	55276		1.8E-3								1.8E-3	1
ZFP28	zinc finger protein 28 homolog (mouse)	140612		1.8E-3								1.8E-3	1
DKFZp586M1	putative lysophosphatidic acid acyltransferase	137964				1.8E-3						1.8E-3	1
NIPSNAP3A	nipsnap homolog 3A (C. elegans)	25934									1.8E-3	1.8E-3	1
CSNK2A2	casein kinase 2, alpha prime polypeptide	1459									1.8E-3	1.8E-3	1
FLJ10287	hypothetical protein FLJ10287	54482	1.8E-3									1.8E-3	1
MB	myoglobin	4151							1.8E-3			1.8E-3	1
HSPA1B	heat shock 70kDa protein 1B	3304		1.8E-3								1.8E-3	1
HSPC132	hypothetical protein HSPC132	51499		1.8E-3								1.8E-3	1
MGC2641	hepatoma-derived growth factor-related protein 2	84717			1.8E-3							1.8E-3	1
IFIT2	interferon-induced protein with tetratricopeptide repeats 2	3433				1.8E-3						1.8E-3	1
GRK5	G protein-coupled receptor kinase 5	2869									1.8E-3	1.8E-3	1
RNPC2	RNA-binding region (RNP1, RRM) containing 2	9584							1.8E-3			1.8E-3	1
NT5C1B	5'-nucleotidase, cytosolic IB	93034									1.8E-3	1.8E-3	1
MAGEA2	melanoma antigen, family A, 2	4101									1.8E-3	1.8E-3	1
PRDX3	peroxiredoxin 3	10935				1.8E-3						1.8E-3	1
XRCC4	cells 4	7518		1.8E-3								1.8E-3	1
MGC23909	hypothetical protein MGC23909	153339		1.8E-3								1.8E-3	1
RUNX3	runt-related transcription factor 3	864				1.8E-3						1.8E-3	1
GCLC	glutamate-cysteine ligase, catalytic subunit	2729									1.9E-3	1.9E-3	1
NPY	neuropeptide Y	4852				1.9E-3						1.9E-3	1
ODAG	ocular development-associated gene	57798					1.9E-3					1.9E-3	1
SCN4B	sodium channel, voltage-gated, type IV, beta	6330				1.9E-3						1.9E-3	1
SNIP1	Smad nuclear interacting protein	79753					1.9E-3					1.9E-3	1
MCM7	MCM7 minichromosome maintenance deficient 7 (S. cerevisiae)	4176					1.9E-3					1.9E-3	1
	LOC388152	388152		1.9E-3								1.9E-3	1
PIP5K2B	phosphatidylinositol-4-phosphate 5-kinase, type II, beta	8396									1.9E-3	1.9E-3	1
GNAT2	activity polypeptide 2	2780					1.9E-3					1.9E-3	1
FLJ10312	hypothetical protein FLJ10312	79822				1.9E-3						1.9E-3	1
LOC65121	melanoma	65121									1.9E-3	1.9E-3	1
SH3GL1	SH3-domain GRB2-like 1	6455									1.9E-3	1.9E-3	1
SPAP1	SH2 domain containing phosphatase anchor protein 1	79368									1.9E-3	1.9E-3	1
MGC15763	hypothetical protein BC008322	92106			2.0E-3							2.0E-3	1
LOC285381	hypothetical protein LOC285381	285381			2.0E-3							2.0E-3	1
EYA4	eyes absent homolog 4 (Drosophila)	2070									2.0E-3	2.0E-3	1
KNSL7	kinesin-like 7	56992						2.0E-3				2.0E-3	1
KIAA1143	KIAA1143 protein	57456						2.0E-3				2.0E-3	1
ZFP67	zinc finger protein 67 homolog (mouse)	51043							2.0E-3			2.0E-3	1
LAPTM4A	lysosomal-associated protein transmembrane 4 alpha	9741			2.0E-3							2.0E-3	1
WTAP	Wilms tumor 1 associated protein	9589					2.0E-3					2.0E-3	1
MAP4K5	mitogen-activated protein kinase kinase kinase kinase 5	11183									2.0E-3	2.0E-3	1
MGAT4B	acetylglucosaminyltransferase, isoenzyme B	11282									2.0E-3	2.0E-3	1
CDC7	CDC7 cell division cycle 7 (S. cerevisiae)	8317						2.0E-3				2.0E-3	1
RPS6KA3	ribosomal protein S6 kinase, 90kDa, polypeptide 3	6197				2.0E-3						2.0E-3	1
KIFAP3	kinesin-associated protein 3	22920		2.0E-3								2.0E-3	1
ELL2	elongation factor, RNA polymerase II, 2	22936		2.0E-3								2.0E-3	1
FLJ10201	hypothetical protein FLJ10201	55689				2.0E-3						2.0E-3	1
GJA10	gap junction protein, alpha 10, 59kDa	81025									2.0E-3	2.0E-3	1
DCN	decorin	1634						2.0E-3				2.0E-3	1
MDS027	MDS027	55845					2.0E-3					2.0E-3	1
ATP5B	polypeptide	506								2.0E-3		2.0E-3	1
CGI-96	CGI-96 protein	27341		2.0E-3								2.0E-3	1
ALG3	mannosyltransferase)	10195				2.0E-3						2.0E-3	1
MGC2408	hypothetical protein MGC2408	84291				2.0E-3						2.0E-3	1
DKFZp686L1	hypothetical protein DKFZp686L1814	132660									2.0E-3	2.0E-3	1
UBXD3	UBX domain containing 3	127733								2.0E-3		2.0E-3	1
KBTBD6	kelch repeat and BTB (POZ) domain containing 6	89890									2.0E-3	2.0E-3	1
GPR144	G protein-coupled receptor 144	347088							2.0E-3			2.0E-3	1
FLJ25222	CXYorf1-related protein	374666		2.1E-3								2.1E-3	1
MTR	5-methyltetrahydrofolate-homocysteine methyltransferase	4548								2.1E-3		2.1E-3	1
FBLN5	fibulin 5	10516				2.1E-3						2.1E-3	1
PRO2000	PRO2000 protein	29028	2.1E-3									2.1E-3	1
NSFL1C	NSFL1 (p97) cofactor (p47)	55968		2.1E-3								2.1E-3	1
GTF2A1	general transcription factor IIA, 1, 19/37kDa	2957							2.1E-3			2.1E-3	1
C21orf59	chromosome 21 open reading frame 59	84537									2.1E-3	2.1E-3	1
C21orf70	chromosome 21 open reading frame 70	85395									2.1E-3	2.1E-3	1
IAN4L1	immune associated nucleotide 4 like 1 (mouse)	55340									2.1E-3	2.1E-3	1
GH1	growth hormone 1	2688				2.1E-3						2.1E-3	1
FLJ12331	hypothetical protein FLJ12331	80052				2.1E-3						2.1E-3	1
LRRN5	leucine rich repeat neuronal 5	10446				2.1E-3						2.1E-3	1
AMY2A	amylase, alpha 2A; pancreatic	279								2.1E-3		2.1E-3	1
FCGR2B	Fc fragment of IgG, low affinity IIb, receptor for (CD32)	2213				2.1E-3						2.1E-3	1
RCBTB1	domain containing protein 1	55213				2.1E-3						2.1E-3	1
MDS028	MDS028	55846							2.1E-3			2.1E-3	1
RPS6KB1	ribosomal protein S6 kinase, 70kDa, polypeptide 1	6198						2.1E-3				2.1E-3	1
TUBD1	likely ortholog of mouse tubulin, delta 1	51174						2.1E-3				2.1E-3	1
MRPS30	mitochondrial ribosomal protein S30	10884						2.1E-3				2.1E-3	1
HNRPR	heterogeneous nuclear ribonucleoprotein R	10236	2.1E-3									2.1E-3	1
SLC9A8	solute carrier family 9 (sodium/hydrogen exchanger), isoform 8	23315		2.1E-3								2.1E-3	1
MGC22265	hypothetical protein MGC22265	349035								2.1E-3		2.1E-3	1
C20orf172	chromosome 20 open reading frame 172	79980	2.1E-3									2.1E-3	1
FLJ30473	hypothetical protein FLJ30473	150209									2.1E-3	2.1E-3	1
MRGX3	G protein-coupled receptor MRGX3	117195					2.2E-3					2.2E-3	1
FLJ21172	hypothetical protein FLJ21172	79863					2.2E-3					2.2E-3	1
RAP2C	RAP2C, member of RAS oncogene family	57826				2.2E-3						2.2E-3	1
CYP3A5	cytochrome P450, family 3, subfamily A, polypeptide 5	1577		2.2E-3								2.2E-3	1
LOC286148	hypothetical protein LOC286148	286148				2.2E-3						2.2E-3	1
ACTR8	ARP8 actin-related protein 8 homolog (yeast)	93973				2.2E-3						2.2E-3	1
PDE10A	phosphodiesterase 10A	10846				2.2E-3						2.2E-3	1
DNMT2	DNA (cytosine-5-)-methyltransferase 2	1787		2.2E-3								2.2E-3	1
LOC285601	seven transmembrane helix receptor LOC285601	285601								2.2E-3		2.2E-3	1
EIF2B5	eukaryotic translation initiation factor 2B, subunit 5 epsilon, 82kDa	8893						2.2E-3				2.2E-3	1
C8orf4	chromosome 8 open reading frame 4	56892								2.2E-3		2.2E-3	1
PTOV1	prostate tumor overexpressed gene 1	53635				2.2E-3						2.2E-3	1
TGM7	transglutaminase 7	116179					2.2E-3					2.2E-3	1
CHRM2	cholinergic receptor, muscarinic 2	1129					2.2E-3					2.2E-3	1
FLJ13490	hypothetical protein FLJ13490	80007							2.2E-3			2.2E-3	1
FLJ12969	hypothetical protein FLJ12969	64860								2.2E-3		2.2E-3	1
EPS8L1	EPS8-like 1	54869					2.3E-3					2.3E-3	1

FLJ23058	hypothetical protein FLJ23058	79749									2.3E-3			2.3E-3	1
RC3	rabconnectin-3	23312											2.3E-3	2.3E-3	1
TUBB4Q	tubulin, beta polypeptide 4, member Q	56604											2.3E-3	2.3E-3	1
SRP14	protein)	6727									2.3E-3			2.3E-3	1
HOXD3	homeo box D3	3232					2.3E-3							2.3E-3	1
ZNF513	zinc finger protein 513	130557					2.3E-3							2.3E-3	1
C7orf25	chromosome 7 open reading frame 25	79020									2.3E-3			2.3E-3	1
ANKRD10	ankyrin repeat domain 10	55608			2.3E-3									2.3E-3	1
IRF2BP2	interferon regulatory factor 2-binding protein 2	359948											2.3E-3	2.3E-3	1
TM4SF10	transmembrane 4 superfamily member 10	83604		2.3E-3										2.3E-3	1
OPN3	opsin 3 (encephalopsin, panopsin)	23596			2.3E-3									2.3E-3	1
PABPC1	poly(A) binding protein, cytoplasmic 1	26986			2.3E-3									2.3E-3	1
AXIN2	axin 2 (conductin, axil)	8313			2.3E-3									2.3E-3	1
IL24	interleukin 24	11009									2.3E-3			2.3E-3	1
FLJ32658	hypothetical protein FLJ32658	147872									2.4E-3			2.4E-3	1
C6orf142	chromosome 6 open reading frame 142	90523	2.4E-3											2.4E-3	1
RNPEP	arginyl aminopeptidase (aminopeptidase B)	6051									2.4E-3			2.4E-3	1
EMP1	epithelial membrane protein 1	2012									2.4E-3			2.4E-3	1
DOC1	downregulated in ovarian cancer 1	11259						2.4E-3						2.4E-3	1
DTNA	dystrobrevin, alpha	1837						2.4E-3						2.4E-3	1
FLJ14166	hypothetical protein FLJ14166	79616										2.4E-3		2.4E-3	1
LAK	lymphocyte alpha-kinase	80216										2.4E-3		2.4E-3	1
MGC61550	hypothetical protein MGC61550	348110			2.4E-3									2.4E-3	1
D85298E	reproduction 8	7993		2.4E-3										2.4E-3	1
ZNF28	zinc finger protein 28 (KOX 24)	7576					2.4E-3							2.4E-3	1
IFNA16	interferon, alpha 16	3449					2.4E-3							2.4E-3	1
SP2	Sp2 transcription factor	6668	2.5E-3											2.5E-3	1
SMG1	PI-3-kinase-related kinase SMG-1	23049										2.5E-3		2.5E-3	1
EDN3	endothelin 3	1908			2.5E-3									2.5E-3	1
KIAA0323	KIAA0323	23351			2.5E-3									2.5E-3	1
WASPIP	Wiskott-Aldrich syndrome protein interacting protein	7456		2.5E-3										2.5E-3	1
BPIL2	bactericidal/permeability-increasing protein-like 2	254240										2.5E-3		2.5E-3	1
TCOF1	Treacher Collins-Franceschetti syndrome 1	6949						2.5E-3						2.5E-3	1
LNX	ligand of numb-protein X	84708			2.5E-3									2.5E-3	1
FLJ46072	FLJ46072 protein	286077			2.5E-3									2.5E-3	1
ADPRHL1	ADP-ribosylhydrolase like 1	113622									2.5E-3			2.5E-3	1
TRIM38	tripartite motif-containing 38	10475						2.5E-3						2.5E-3	1
FLJ26056	hypothetical protein LOC375127	375127											2.6E-3	2.6E-3	1
C9orf89	chromosome 9 open reading frame 89	84270											2.6E-3	2.6E-3	1
C6orf145	chromosome 6 open reading frame 145	221749									2.6E-3			2.6E-3	1
FLJ32332	likely ortholog of mouse protein phosphatase 2C eta	132160			2.6E-3									2.6E-3	1
TFIP11	tuftelin interacting protein 11	24144			2.6E-3									2.6E-3	1
HNF4A	hepatocyte nuclear factor 4, alpha	3172			2.6E-3									2.6E-3	1
SCRN1	secernin 1	9805										2.6E-3		2.6E-3	1
DKFZP564O0	hypothetical protein DKFZp564O0523	84060			2.6E-3									2.6E-3	1
VNN2	vanin 2	8875											2.6E-3	2.6E-3	1
PCDHB15	protocadherin beta 15	56121				2.6E-3								2.6E-3	1
ELMO2	engulfment and cell motility 2 (ced-12 homolog, C. elegans)	63916											2.6E-3	2.6E-3	1
CFDP1	craniofacial development protein 1	10428						2.6E-3						2.6E-3	1
MGC34695	hypothetical protein MGC34695	219743						2.6E-3						2.6E-3	1
G3BP2	Ras-GTPase activating protein SH3 domain-binding protein 2	9908			2.6E-3									2.6E-3	1
NYD-SP12	NYD-SP12 protein	83893									2.6E-3			2.6E-3	1
CGI-07	CGI-07 protein	51068											2.6E-3	2.6E-3	1
ZNF161	zinc finger protein 161	7716									2.6E-3			2.6E-3	1
PKD1	polycystic kidney disease 1 (autosomal dominant)	5310									2.6E-3			2.6E-3	1
ITSN2	intersectin 2	50618									2.7E-3			2.7E-3	1
DKK4	dickkopf homolog 4 (Xenopus laevis)	27121										2.7E-3		2.7E-3	1
FLJ10462	hypothetical protein FLJ10462	55711						2.7E-3						2.7E-3	1
TPR	translocated promoter region (to activated MET oncogene)	7175						2.7E-3						2.7E-3	1
CSH1	chorionic somatomammotropin hormone 1 (placental lactogen)	1442			2.7E-3									2.7E-3	1
RNF121	ring finger protein 121	55298					2.7E-3							2.7E-3	1
CD1C	CD1C antigen, c polypeptide	911			2.7E-3									2.7E-3	1
PRAM-1	PRAM-1 protein	84106			2.7E-3									2.7E-3	1
TXNDC4	thioredoxin domain containing 4 (endoplasmic reticulum)	23071					2.7E-3							2.7E-3	1
INVS	inversin	27130					2.7E-3							2.7E-3	1
HIST1H4F	histone 1, H4f	8361											2.7E-3	2.7E-3	1
VPS45A	vacuolar protein sorting 45A (yeast)	11311						2.7E-3						2.7E-3	1
DKFZP566M1	hypothetical protein DKFZp566M114	84068											2.7E-3	2.7E-3	1
PPP2R5E	protein phosphatase 2, regulatory subunit B (B56), epsilon isoform	5529		2.7E-3										2.7E-3	1
BBP	beta-amyloid binding protein precursor	83941		2.7E-3										2.7E-3	1
FMO1	flavin containing monooxygenase 1	2326										2.7E-3		2.7E-3	1
FBXL11	F-box and leucine-rich repeat protein 11	22992			2.7E-3									2.7E-3	1
MGC10701	hypothetical protein MGC10701	84744			2.7E-3									2.7E-3	1
C14orf45	chromosome 14 open reading frame 45	80127			2.7E-3									2.7E-3	1
RSU1	Ras suppressor protein 1	6251		2.8E-3										2.8E-3	1
RDH11	retinol dehydrogenase 11 (all-trans and 9-cis)	51109									2.8E-3			2.8E-3	1
APOL5	apolipoprotein L, 5	80831			2.8E-3									2.8E-3	1
MPZ	myelin protein zero (Charcot-Marie-Tooth neuropathy 1B)	4359			2.8E-3									2.8E-3	1
SPY1	speedy	245711			2.8E-3									2.8E-3	1
FLJ12975	hypothetical protein FLJ12975	79867											2.8E-3	2.8E-3	1
APC10	anaphase-promoting complex subunit 10	10393										2.8E-3		2.8E-3	1
76P	gamma tubulin ring complex protein (76p gene)	27229										2.8E-3		2.8E-3	1
ASB3	ankyrin repeat and SOCS box-containing 3	51130		2.8E-3										2.8E-3	1
TNKS2	2	80351						2.8E-3						2.8E-3	1
FPGS	folylpolyglutamate synthase	2356			2.8E-3									2.8E-3	1
TAT	tyrosine aminotransferase	6898											2.8E-3	2.8E-3	1
FLJ40217	similar to 1810012H11Rik	399512									2.8E-3			2.8E-3	1
MYL5	myosin, light polypeptide 5, regulatory	4636			2.8E-3									2.8E-3	1
GPC6	glypican 6	10082					2.8E-3							2.8E-3	1
FLJ14639	hypothetical protein FLJ14639	84901					2.8E-3							2.8E-3	1
GPR30	G protein-coupled receptor 30	2852			2.8E-3									2.8E-3	1
HIST1H3C	histone 1, H3c	8352						2.8E-3						2.8E-3	1
RFX3	regulatory factor X, 3 (influences HLA class II expression)	5991			2.8E-3									2.8E-3	1
FILIP1	filamin A interacting protein 1	27145			2.8E-3									2.8E-3	1
KIAA1627	KIAA1627 protein	57721						2.8E-3						2.8E-3	1
SLC23A1	solute carrier family 23 (nucleobase transporters), member 1	9963									2.9E-3			2.9E-3	1
ARGBP2	Arg/Abl-interacting protein ArgBP2	8470									2.9E-3			2.9E-3	1
CGI-77	CGI-77 protein	51633		2.9E-3										2.9E-3	1
IPO13	importin 13	9670					2.9E-3							2.9E-3	1
ZCCHC10	zinc finger, CCHC domain containing 10	54819			2.9E-3									2.9E-3	1
FUT3	blood group included)	2525		2.9E-3										2.9E-3	1
FLJ90637	FLJ90637 protein	374987						2.9E-3						2.9E-3	1
C14orf141	chromosome 14 open reading frame 141	83981									2.9E-3			2.9E-3	1
KIAA1354	KIAA1354 protein	55958			2.9E-3									2.9E-3	1
APOL4	apolipoprotein L, 4	80832			2.9E-3									2.9E-3	1
XPO1	exportin 1 (CRM1 homolog, yeast)	7514			2.9E-3									2.9E-3	1
ABCA12	ATP-binding cassette, sub-family A (ABC1), member 12	26154			2.9E-3									2.9E-3	1
TMEM15	transmembrane protein 15	22845		2.9E-3										2.9E-3	1
FLJ13195	hypothetical protein FLJ13195 similar to stromal antigen 3	64940					2.9E-3							2.9E-3	1
MBNL2	muscleblind-like 2 (Drosophila)	10150						3.0E-3						3.0E-3	1
FLJ21839	hypothetical protein FLJ21839	60509										3.0E-3		3.0E-3	1
COL23A1	collagen, type XXIII, alpha 1	91522											3.0E-3	3.0E-3	1
LOC91137	hypothetical protein BC017169	91137											3.0E-3	3.0E-3	1
APOF	apolipoprotein F	319			3.0E-3									3.0E-3	1
TCERG1	transcription elongation regulator 1 (CA150)	10915					3.0E-3							3.0E-3	1
ZFP36	zinc finger protein 36, C3H type, homolog (mouse)	7538											3.0E-3	3.0E-3	1
LOC51252	hypothetical protein LOC51252	51252					3.0E-3							3.0E-3	1
FLJ13710	hypothetical protein FLJ13710	79875			3.0E-3									3.0E-3	1
FH	fumarate hydratase	2271						3.0E-3						3.0E-3	1
CD68	CD68 antigen	968						3.0E-3						3.0E-3	1
FLJ13448	hypothetical protein FLJ13448	80219		3.0E-3										3.0E-3	1
FLJ45645	FLJ45645 protein	375287			3.0E-3									3.0E-3	1
FLJ90022	hypothetical protein FLJ90022	131583									3.0E-3			3.0E-3	1
KIAA0515	KIAA0515	84726			3.0E-3									3.0E-3	1
CCL28	chemokine (C-C motif) ligand 28	56477									3.0E-3			3.0E-3	1
CHCHD7	coiled-coil-helix-coiled-coil-helix domain containing 7	79145									3.0E-3			3.0E-3	1
C14orf80	chromosome 14 open reading frame 80	283643				3.0E-3								3.0E-3	1
ZNF587	zinc finger protein 587	84914		3.0E-3										3.0E-3	1
	similar to AVLV472	389336			3.0E-3									3.0E-3	1
CCM1	cerebral cavernous malformations 1	889									3.0E-3			3.0E-3	1
NDUFB9	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9, 22kDa	4715			3.0E-3									3.0E-3	1
CDA11	CDA11 protein	83940			3.0E-3									3.0E-3	1
MGP	matrix Gla protein	4256						3.1E-3						3.1E-3	1
MSF	MLL septin-like fusion	10801											3.1E-3	3.1E-3	1
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	4609			3.1E-3									3.1E-3	1
MOV10	Mov10, Moloney leukemia virus 10, homolog (mouse)	4343									3.1E-3			3.1E-3	1
ID4	protein	3400					3.1E-3							3.1E-3	1
MCSP	mitochondrial capsule selenoprotein	4184										3.1E-3		3.1E-3	1
GPSM3	G-protein signalling modulator 3 (AGS3-like, C. elegans)	63940									3.1E-3			3.1E-3	1
L1CAM	Sylvius 1, MASA (mental retardation, aphasia, shuffling gait and	3897			3.1E-3									3.1E-3	1
NDUFS2	coenzyme Q reductase)	4720		3.1E-3										3.1E-3	1
PCYT1A	phosphate cytidylyltransferase 1, choline, alpha isoform	5130		3.1E-3										3.1E-3	1
KIAA0052	KIAA0052 protein	23517		3.1E-3										3.1E-3	1
ALS2CR2	candidate 2	55437		3.1E-3										3.1E-3	1
ALS2CR3	candidate 3	66008		3.1E-3										3.1E-3	1
PPFIA1	interacting protein (liprin), alpha 1	8500											3.1E-3	3.1E-3	1
MGC21518	hypothetical protein MGC21518	147184									3.1E-3			3.1E-3	1
MGC39520	hypothetical protein MGC39520	196472									3.1E-3			3.1E-3	1
HOXA11	homeo box A11	3207					3.1E-3							3.1E-3	1
ZNF336	zinc finger protein 336	64412											3.2E-3	3.2E-3	1
NUP43	nucleoporin 43kDa	348995		3.2E-3										3.2E-3	1
LOC56965	hypothetical protein from EUROIMAGE 1977056	56965			3.2E-3									3.2E-3	1
KRTAP1-5	keratin associated protein 1-5	83895											3.2E-3	3.2E-3	1
POLA	polymerase (DNA directed), alpha	5422						3.2E-3						3.2E-3	1
C15orf20	chromosome 15 open reading frame 20	80119						3.2E-3						3.2E-3	1
KIAA1944	KIAA1944 protein	121256			3.2E-3									3.2E-3	1
FLJ21901	hypothetical protein FLJ21901	79675											3.2E-3	3.2E-3	1
IL21R	interleukin 21 receptor	50615						3.2E-3						3.2E-3	1
FLJ25461	hypothetical protein FLJ25461	158326									3.2E-3			3.2E-3	1

PRDX5	peroxiredoxin 5	25824					3.2E-3						3.2E-3	1
TGFBR1	(-like kinase, 53kDa)	7046							3.3E-3				3.3E-3	1
KIAA1279	KIAA1279	26128		3.3E-3									3.3E-3	1
FGF12	fibroblast growth factor 12	2257				3.3E-3							3.3E-3	1
MRPL42	mitochondrial ribosomal protein L42	28977		3.3E-3									3.3E-3	1
FLJ21062	hypothetical protein FLJ21062	79846							3.3E-3				3.3E-3	1
TPCN1	two pore segment channel 1	53373					3.3E-3						3.3E-3	1
LOC115811	hypothetical protein BC013151	115811					3.3E-3						3.3E-3	1
DOK5	docking protein 5	55816										3.3E-3	3.3E-3	1
AP4M1	adaptor-related protein complex 4, mu 1 subunit	9179					3.3E-3						3.3E-3	1
CDH12	cadherin 12, type 2 (N-cadherin 2)	1010									3.3E-3		3.3E-3	1
NPTX2	neuronal pentraxin II	4885										3.4E-3	3.4E-3	1
G1P3	interferon, alpha-inducible protein (clone IFI-6-16)	2537									3.4E-3		3.4E-3	1
TEAD4	TEA domain family member 4	7004										3.4E-3	3.4E-3	1
NEK6	NIMA (never in mitosis gene a)-related kinase 6	10783							3.4E-3				3.4E-3	1
FLJ12610	hypothetical protein FLJ12610	79840				3.4E-3							3.4E-3	1
MGC39571	hypothetical protein MGC39571	221241							3.4E-3				3.4E-3	1
STAM	1	8027					3.4E-3						3.4E-3	1
ARF3	ADP-ribosylation factor 3	377					3.4E-3						3.4E-3	1
CML2	putative N-acetyltransferase Camello 2	51471					3.5E-3						3.5E-3	1
ELOVL5	(FEN1/Elo2, SUR4/Elo3-like, yeast)	60481						3.5E-3					3.5E-3	1
CCL17	chemokine (C-C motif) ligand 17	6361				3.5E-3							3.5E-3	1
ZNF585B	zinc finger protein 585B	92285		3.5E-3									3.5E-3	1
HIST1H2BB	histone 1, H2bb	3018							3.5E-3				3.5E-3	1
CPT1B	carnitine palmitoyltransferase 1B (muscle)	1375							3.5E-3				3.5E-3	1
HCRTR1	hypocretin (orexin) receptor 1	3061							3.5E-3				3.5E-3	1
SYNJ2BP	synaptojanin 2 binding protein	55333									3.5E-3		3.5E-3	1
ATP13A	ATPase type 13A	57130				3.5E-3							3.5E-3	1
PMS1	PMS1 postmeiotic segregation increased 1 (S. cerevisiae)	5378	3.5E-3										3.5E-3	1
LOC51240	hypothetical protein LOC51240	51240	3.5E-3										3.5E-3	1
KNTC2	kinetochore associated 2	10403						3.5E-3					3.5E-3	1
BDNF	brain-derived neurotrophic factor	627										3.5E-3	3.5E-3	1
EHD3	EH-domain containing 3	30845			3.6E-3								3.6E-3	1
XRN2	5'-3' exoribonuclease 2	22803							3.6E-3				3.6E-3	1
KIAA0090	KIAA0090 protein	23065									3.6E-3		3.6E-3	1
C1orf33	chromosome 1 open reading frame 33	51154									3.6E-3		3.6E-3	1
ZNF558	zinc finger protein 558	148156				3.6E-3							3.6E-3	1
PRDM9	PR domain containing 9	56979							3.6E-3				3.6E-3	1
TOPBP1	toposomerase (DNA) II binding protein	11073						3.6E-3					3.6E-3	1
LOC159091	hypothetical protein BC017868	159091							3.6E-3				3.6E-3	1
DKFZp667B1	hypothetical protein DKFZp667B1218	201626										3.6E-3	3.6E-3	1
BRI3	brain protein 13	25798				3.6E-3							3.6E-3	1
NTNG1	netrin G1	22854				3.6E-3							3.6E-3	1
SEMA3A	secreted, (semaphorin) 3A	10371					3.7E-3						3.7E-3	1
HIST1H4H	histone 1, H4h	8365		3.7E-3									3.7E-3	1
CTMP	C-terminal modulator protein	117145	3.7E-3										3.7E-3	1
TUBA4	tubulin, alpha 4	80086		3.7E-3									3.7E-3	1
C2orf13	chromosome 2 open reading frame 13	200558							3.7E-3				3.7E-3	1
SIL1	endoplasmic reticulum chaperone SIL1, homolog of yeast	64374				3.7E-3							3.7E-3	1
SLC16A14	member 14	151473									3.7E-3		3.7E-3	1
ZP4	zona pellucida glycoprotein 4	57829						3.7E-3					3.7E-3	1
PRPF4B	PRP4 pre-mRNA processing factor 4 homolog B (yeast)	8899									3.7E-3		3.7E-3	1
RPS24	ribosomal protein S24	6229		3.7E-3									3.7E-3	1
DKFZp56600	DKFZP566O084 protein	25979		3.7E-3									3.7E-3	1
FOXC1	forkhead box C1	2296				3.7E-3							3.7E-3	1
CCKAR	cholecystokinin A receptor	886					3.7E-3						3.7E-3	1
SDCCAG16	serologically defined colon cancer antigen 16	10813							3.7E-3				3.7E-3	1
GLRA3	glycine receptor, alpha 3	8001							3.7E-3				3.7E-3	1
KIAA0391	KIAA0391	9692							3.7E-3				3.7E-3	1
KIAA1295	KIAA1295 protein	57517				3.7E-3							3.7E-3	1
KIAA1443	KIAA1443	57594		3.7E-3									3.7E-3	1
FLJ33860	hypothetical protein FLJ33860	284756	3.8E-3										3.8E-3	1
PDHX	pyruvate dehydrogenase complex, component X	8050				3.8E-3							3.8E-3	1
MMRP19	likely ortholog of mouse monocyte macrophage 19	51074				3.8E-3							3.8E-3	1
HS3ST3B1	heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1	9953				3.8E-3							3.8E-3	1
ELAC2	elaC homolog 2 (E. coli)	60528		3.8E-3									3.8E-3	1
THBS1	thrombospondin 1	7057				3.8E-3							3.8E-3	1
ZCCHC9	zinc finger, CCHC domain containing 9	84240							3.8E-3				3.8E-3	1
C20orf140	chromosome 20 open reading frame 140	128637										3.8E-3	3.8E-3	1
PART1	prostate androgen-regulated transcript 1	25859						3.8E-3					3.8E-3	1
MLYCD	malonyl-CoA decarboxylase	23417				3.9E-3							3.9E-3	1
LOC285513	hypothetical protein LOC285513	285513				3.9E-3							3.9E-3	1
MGMT	O-6-methylguanine-DNA methyltransferase	4255										3.9E-3	3.9E-3	1
ASAH2	N-acylsphingosine amidohydrolase (non-lysosomal ceramidase) 2	56624									3.9E-3		3.9E-3	1
LOC201191	hypothetical protein LOC201191	201191				3.9E-3							3.9E-3	1
FLJ20719	hypothetical protein FLJ20719	55672		3.9E-3									3.9E-3	1
GZMA	serine esterase 3)	3001										3.9E-3	3.9E-3	1
CKLFSF8	chemokine-like factor super family 8	152189				3.9E-3							3.9E-3	1
ZNF445	zinc finger protein 445	353274										3.9E-3	3.9E-3	1
CGB2	chorionic gonadotropin, beta polypeptide 2	114336		3.9E-3									3.9E-3	1
KIAA1160	KIAA1160 protein	57461						3.9E-3					3.9E-3	1
DNAJB8	DnaJ (Hsp40) homolog, subfamily B, member 8	165721										3.9E-3	3.9E-3	1
LOC144233	hypothetical protein LOC144233	144233							4.0E-3				4.0E-3	1
DUSP19	dual specificity phosphatase 19	142679										4.0E-3	4.0E-3	1
DUSP11	dual specificity phosphatase 11 (RNA/RNP complex 1-interacting)	8446						4.0E-3					4.0E-3	1
RYDS	ligand binding protein RYDS	147199										4.0E-3	4.0E-3	1
SLC31A1	solute carrier family 31 (copper transporters), member 1	1317						4.0E-3					4.0E-3	1
FZR1	Fzr1 protein	51343		4.0E-3									4.0E-3	1
DEC1	deleted in esophageal cancer 1	50514							4.0E-3				4.0E-3	1
PMEPA1	polyamine modulated factor 1 binding protein 1	83449						4.0E-3					4.0E-3	1
PPAP2A	phosphatidic acid phosphatase type 2A	8611										4.0E-3	4.0E-3	1
hIAN6	human immune associated nucleotide 6	155038				4.0E-3							4.0E-3	1
PPARG	peroxisome proliferative activated receptor, gamma	5468						4.0E-3					4.0E-3	1
EHF	ets homologous factor	26298							4.0E-3				4.0E-3	1
FLJ10006	hypothetical protein FLJ10006	55677							4.0E-3				4.0E-3	1
DRD3	dopamine receptor D3	1814						4.1E-3					4.1E-3	1
HIMAP4	immunity associated protein 4	55303					4.1E-3						4.1E-3	1
CPSF2	cleavage and polyadenylation specific factor 2, 100kDa	53981					4.1E-3						4.1E-3	1
SEMA4C	domain (TM) and short cytoplasmic domain, (semaphorin) 4C	54910				4.1E-3							4.1E-3	1
PCDHA11	protocadherin alpha 11	56138				4.1E-3							4.1E-3	1
DKFZP566E1	small fragment nuclease	25996					4.1E-3						4.1E-3	1
LMO2	LIM domain only 2 (rhombotin-like 1)	4005					4.1E-3						4.1E-3	1
PRKRIR	dependent inhibitor, repressor of (P58 repressor)	5612							4.1E-3				4.1E-3	1
KCNE4	potassium voltage-gated channel, isk-related family, member 4	23704										4.1E-3	4.1E-3	1
MYOHD1	myosin head domain containing 1	80179					4.1E-3						4.1E-3	1
ORC3L	origin recognition complex, subunit 3-like (yeast)	23595							4.1E-3				4.1E-3	1
RARSL	arginyl-tRNA synthetase-like	57038							4.1E-3				4.1E-3	1
DKFZP564I1	DKFZP564I122 protein	25974							4.1E-3				4.1E-3	1
TMPRSS3	transmembrane protease, serine 3	64699							4.1E-3				4.1E-3	1
SCOTIN	scotin	51246							4.1E-3				4.1E-3	1
BF	B-factor, properdin	629						4.1E-3					4.1E-3	1
DHX35	DEAH (Asp-Glu-Ala-His) box polypeptide 35	60625							4.1E-3				4.1E-3	1
POLD2	polymerase (DNA directed), delta 2, regulatory subunit 50kDa	5425				4.1E-3							4.1E-3	1
DACT1	dapper homolog 1, antagonist of beta-catenin (xenopus)	51339				4.2E-3							4.2E-3	1
APM1	adipose most abundant gene transcript 1	9370				4.2E-3							4.2E-3	1
COXVIB2	cytochrome c oxidase subunit VIb, testes specific	125965				4.2E-3							4.2E-3	1
TCF4	transcription factor 4	6925									4.2E-3		4.2E-3	1
HHLA1	MERV-H LTR-associating 1	10086					4.2E-3						4.2E-3	1
DEK	DEK oncogene (DNA binding)	7913				4.2E-3							4.2E-3	1
PEMT	phosphatidylethanolamine N-methyltransferase	10400					4.2E-3						4.2E-3	1
RING1	ring finger protein 1	6015							4.2E-3				4.2E-3	1
SERTAD1	SERTA domain containing 1	29950							4.2E-3				4.2E-3	1
TAPBP	TAP binding protein (tapasin)	6892										4.2E-3	4.2E-3	1
EPIM	epimorphin	2054				4.2E-3							4.2E-3	1
SCN10A	sodium channel, voltage-gated, type X, alpha	6336					4.2E-3						4.2E-3	1
UBE2D3	ubiquitin-conjugating enzyme E2D 3 (UBC4/5 homolog, yeast)	7323			4.2E-3								4.2E-3	1
NP	nucleoside phosphorylase	4860			4.2E-3								4.2E-3	1
OSBP	oxysterol binding protein	5007						4.3E-3					4.3E-3	1
COX17	COX17 homolog, cytochrome c oxidase assembly protein (yeast)	10063							4.3E-3				4.3E-3	1
CUTL1	cut-like 1, CCAAT displacement protein (Drosophila)	1523				4.3E-3							4.3E-3	1
HSD17B4	hydroxysteroid (17-beta) dehydrogenase 4	3295						4.3E-3					4.3E-3	1
CPSF5	cleavage and polyadenylation specific factor 5, 25 kDa	11051							4.3E-3				4.3E-3	1
FLJ10826	hypothetical protein FLJ10826	55239							4.3E-3				4.3E-3	1
SOX4	SRY (sex determining region Y)-box 4	6659									4.3E-3		4.3E-3	1
PUM1	pumilio homolog 1 (Drosophila)	9698		4.3E-3									4.3E-3	1
H2AFZ	H2A histone family, member Z	3015										4.3E-3	4.3E-3	1
SOAT	sodium-dependent organic anion transporter	345274									4.3E-3		4.3E-3	1
KCTD15	potassium channel tetramerisation domain containing 15	79047									4.3E-3		4.3E-3	1
HMG2L1	high-mobility group protein 2-like 1	10042						4.3E-3					4.3E-3	1
TUBE1	tubulin, epsilon 1	51175									4.3E-3		4.3E-3	1
AK3	adenylate kinase 3	205						4.3E-3					4.3E-3	1
PFDN4	prefoldin 4	5203		4.4E-3									4.4E-3	1
FEM1B	fem-1 homolog b (C. elegans)	10116				4.4E-3							4.4E-3	1
WDR21	WD repeat domain 21	26094							4.4E-3				4.4E-3	1
ITGB1	CD29 includes MDF2, MSK12)	3688	4.4E-3										4.4E-3	1
ARFD1	ADP-ribosylation factor domain protein 1, 64kDa	373					4.4E-3						4.4E-3	1
FLJ13611	hypothetical protein FLJ13611	80006					4.4E-3						4.4E-3	1
SMC4L1	SMC4 structural maintenance of chromosomes 4-like 1 (yeast)	10051					4.4E-3						4.4E-3	1
MCOLN1	mucolipin 1	57192					4.4E-3						4.4E-3	1
GTF2IRD1	GTF2I repeat domain containing 1	9569				4.4E-3							4.4E-3	1
TIMP3	pseudoinflammatory)	7078							4.4E-3				4.4E-3	1
caspr5	caspr5 protein	129684							4.4E-3				4.4E-3	1
RBM3	RNA binding motif protein 3	5935							4.5E-3				4.5E-3	1
FLJ20125	hypothetical protein FLJ20125	54826		4.5E-3									4.5E-3	1
RTN1	reticulon 1	6252							4.5E-3				4.5E-3	1
UNC93B1	unc-93 homolog B1 (C. elegans)	81622										4.5E-3	4.5E-3	1
G3BP	Ras-GTPase-activating protein SH3-domain-binding protein	10146			4.5E-3								4.5E-3	1
MGC11266	hypothetical protein MGC11266	79172							4.5E-3				4.5E-3	1
MSH6	mutS homolog 6 (E. coli)	2956			4.5E-3								4.5E-3	1

LIAS	lipoic acid synthetase	11019				4.5E-3						4.5E-3	1
KIAA0053	KIAA0053 gene product	9938		4.5E-3								4.5E-3	1
		400120									4.5E-3	4.5E-3	1
NXPH3	neurexophilin 3	11248					4.5E-3					4.5E-3	1
DBT	branched chain keto acid dehydrogenase complex; maple syrup	1629		4.5E-3								4.5E-3	1
AMOTL2	angiomotin like 2	51421			4.5E-3							4.5E-3	1
SIAT7C	1,3)-N-acetyl galactosaminide alpha-2,6-sialyltransferase] C	256435		4.5E-3								4.5E-3	1
BAT1	HLA-B associated transcript 1	7919				4.6E-3						4.6E-3	1
FLJ35775	hypothetical protein FLJ35775	138009			4.6E-3							4.6E-3	1
TRUB2	TruB pseudouridine (psi) synthase homolog 2 (E. coli)	26995		4.6E-3								4.6E-3	1
COQ4	coenzyme Q4 homolog (yeast)	51117		4.6E-3								4.6E-3	1
C14orf50	chromosome 14 open reading frame 50	145376									4.6E-3	4.6E-3	1
FLJ20359	hypothetical protein FLJ20359	54905			4.6E-3							4.6E-3	1
COQ6	coenzyme Q6 homolog (yeast)	51004		4.6E-3								4.6E-3	1
UGT2B7	UDP glycosyltransferase 2 family, polypeptide B7	7364					4.6E-3					4.6E-3	1
EPHA3	EphA3	2042					4.6E-3					4.6E-3	1
DLEU2	deleted in lymphocytic leukemia, 2	8847							4.6E-3			4.6E-3	1
DLEU1	deleted in lymphocytic leukemia, 1	10301							4.6E-3			4.6E-3	1
RNF17	ring finger protein 17	56163				4.6E-3						4.6E-3	1
FLJ36576	hypothetical protein FLJ36576	158801					4.6E-3					4.6E-3	1
CAD	and dihydroorotase	790							4.7E-3			4.7E-3	1
MRPS18A	mitochondrial ribosomal protein S18A	55168					4.7E-3					4.7E-3	1
FLJ90798	hypothetical protein FLJ90798	219654						4.7E-3				4.7E-3	1
IL22RA1	interleukin 22 receptor, alpha 1	58985			4.7E-3							4.7E-3	1
POLD3	polymerase (DNA-directed), delta 3, accessory subunit	10714						4.7E-3				4.7E-3	1
C13orf18	chromosome 13 open reading frame 18	80183			4.7E-3							4.7E-3	1
NMNAT2	nicotinamide nucleotide adenylyltransferase 2	23057	4.7E-3									4.7E-3	1
FECH	ferrochelatase (protoporphyria)	2235		4.7E-3								4.7E-3	1
MCRS1	microspherule protein 1	10445					4.7E-3					4.7E-3	1
COL8A2	collagen, type VIII, alpha 2	1296			4.7E-3							4.7E-3	1
MAX	MAX protein	4149			4.7E-3							4.7E-3	1
ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta	397									4.7E-3	4.7E-3	1
ZNF223	zinc finger protein 223	7766					4.7E-3					4.7E-3	1
FLJ36175	hypothetical protein FLJ36175	151525				4.7E-3						4.7E-3	1
GNB5	guanine nucleotide binding protein (G protein), beta 5	10681			4.7E-3							4.7E-3	1
FLJ10747	hypothetical protein FLJ10747	55219									4.7E-3	4.7E-3	1
LASS5	LAG1 longevity assurance homolog 5 (S. cerevisiae)	91012							4.7E-3			4.7E-3	1
Nup37	nucleoporin Nup37	79023				4.7E-3						4.7E-3	1
EPB41L2	erythrocyte membrane protein band 4.1-like 2	2037				4.7E-3						4.7E-3	1
ZDHHC5	zinc finger, DHHC domain containing 5	25921		4.7E-3								4.7E-3	1
CNOT3	CCR4-NOT transcription complex, subunit 3	4849		4.7E-3								4.7E-3	1
MAP3K7	mitogen-activated protein kinase kinase kinase 7	6885					4.8E-3					4.8E-3	1
LOC339287	hypothetical protein LOC339287	339287			4.8E-3							4.8E-3	1
FLJ10846	hypothetical protein FLJ10846	55751				4.8E-3						4.8E-3	1
C20orf102	chromosome 20 open reading frame 102	128434									4.8E-3	4.8E-3	1
ZNF462	zinc finger protein 462	58499				4.8E-3						4.8E-3	1
SCAMP1	secretory carrier membrane protein 1	9522						4.8E-3				4.8E-3	1
MRPS21	mitochondrial ribosomal protein S21	54460									4.8E-3	4.8E-3	1
BACH2	2	60468			4.8E-3							4.8E-3	1
NNT	nicotinamide nucleotide transhydrogenase	23530									4.8E-3	4.8E-3	1
PNLDC1	poly(A)-specific ribonuclease (PARN)-like domain containing 1	154197									4.9E-3	4.9E-3	1
GOLGA2	golgi autoantigen, golgin subfamily a, 2	2801		4.9E-3								4.9E-3	1
NY-REN-7	NY-REN-7 antigen	285596			4.9E-3							4.9E-3	1
DDHD1	DDHD domain containing 1	80821						4.9E-3				4.9E-3	1
MEF2D	enhancer factor 2D	4209			4.9E-3							4.9E-3	1
NRBP	nuclear receptor binding protein	29959						4.9E-3				4.9E-3	1
MORF4	mortality factor 4	10934							5.0E-3			5.0E-3	1
GRB2	growth factor receptor-bound protein 2	2885									5.0E-3	5.0E-3	1
NEB	nebulin	4703			5.0E-3							5.0E-3	1
LY64	lymphocyte antigen 64 homolog, radioprotective 105kDa (mouse)	4064						5.0E-3				5.0E-3	1
SIVA	CD27-binding (Siva) protein	10572						5.0E-3				5.0E-3	1
DUSP21	dual specificity phosphatase 21	63906						5.0E-3				5.0E-3	1
RRP4	exoribonuclease	23404									5.0E-3	5.0E-3	1
PEO1	progressive external ophthalmoplegia 1	56652			5.0E-3							5.0E-3	1
MRPL43	mitochondrial ribosomal protein L43	84545			5.0E-3							5.0E-3	1
ZNF556	zinc finger protein 556	80032						5.0E-3				5.0E-3	1
TACR3	tachykinin receptor 3	6870			5.0E-3							5.0E-3	1
ZF	HCF-binding transcription factor Zhangfei	58487		5.0E-3								5.0E-3	1

SYMBOL	GENENAME	LL	Hs 578T E2F1 ChIP #1	Hs 578T E2F1 ChIP #2	Hs 578T E2F1 ChIP #3	Hs 578T E2F1 ChIP #4 (1k array)
SFRS1	splicing factor, arginine	6426	2.87E-03	4.79E-04	2.11E-04	4.39E-02
FBXO5	F-box only protein 5	26271	2.84E-03	6.45E-05	1.90E-06	2.46E-02
HnRNP A2	na	na	2.08E-04	8.93E-05	2.95E-04	4.34E-02
HnRNP A1	na	na	1.08E-04	3.55E-04	1.05E-06	4.02E-03
RAD21	RAD21 homolog (S. pombe)	5885	4.72E-03		2.83E-03	4.75E-02
GTF2H1	general transcription factor IIH, polypeptide 1, 62kDa	2965	3.94E-03	2.80E-04		1.32E-02
USP1	ubiquitin specific protease 1	7398	3.94E-03	7.18E-04	1.18E-11	
FLJ41131	Homo sapiens cDNA FLJ41131 fis, clone BRACE2024627 6626	284325	3.25E-03	4.48E-04	3.39E-05	
SNRPA	small nuclear ribonucleoprotein polypeptide A 6626	6626	3.25E-03	4.48E-04	3.39E-05	
GTF2H2	general transcription factor IIH, polypeptide 2, 44kDa	2966	3.20E-03	1.87E-04		2.03E-03
C20orf172	chromosome 20 open reading frame 172	79980	2.98E-03	1.43E-04	2.45E-04	
KLIP1	KSHV latent nuclear antigen interacting protein 1	79682	2.97E-03	4.56E-04	2.76E-04	
SF3B3	splicing factor 3b, subunit 3, 130kDa	23450	2.91E-03	5.35E-04	7.37E-04	
HNRPH3	heterogeneous nuclear ribonucleoprotein H3 (2H9)	3189	2.77E-03	1.71E-05	2.58E-03	
TAF12	TAF12 RNA polymerase II, TATA box binding protein (TBP)-associated fact	6883	2.45E-03	1.22E-04		2.69E-02
LOC220074	hypothetical 55.1 kDa protein F09G8.5 in chromosome III	220074	2.44E-03	2.80E-03	3.27E-03	
MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	4085	2.42E-03		4.05E-05	9.05E-03
ILF2	interleukin enhancer binding factor 2, 45kDa	3608	2.22E-03	4.21E-04	3.22E-09	
FLJ25409	hypothetical protein FLJ25409	137994	1.83E-03	1.03E-04	2.81E-03	
SF3A2	splicing factor 3a, subunit 2, 66kDa	8175	1.82E-03	4.68E-04	4.27E-04	
ARCH	archease 339487	339487	1.73E-03	2.21E-03	1.65E-03	
RBBP4	retinoblastoma binding protein 4 339487	5928	1.73E-03	2.21E-03	1.65E-03	
HSPC150	HSPC150 protein similar to ubiquitin-conjugating enzyme	29089	1.72E-03	1.05E-06	9.78E-09	
MGC13170	MGC13170 gene	84798	1.68E-03	6.53E-04	5.81E-04	
TULP3	tubby like protein 3	7289	1.20E-03	1.23E-06	1.25E-03	
HNRPAB	heterogeneous nuclear ribonucleoprotein A	3182	1.01E-03	3.38E-04	2.89E-03	
SFRS6	splicing factor, arginine	6431	9.03E-04	1.03E-03	1.63E-03	
KIAA0073	KIAA0073 protein	23398	8.38E-04	2.53E-04	8.19E-05	
GBA	glucosidase, beta; acid (includes glucosylceramidase)	2629	8.09E-04	2.55E-03	5.27E-04	
E2O1	exonuclease 1	9156	5.32E-04	7.17E-04	2.16E-09	
FANCD2	Fanconi anemia, complementation group D2	2177	4.59E-04	9.78E-04	3.20E-05	
DKFZP564J0123	nuclear protein E3-3 55152	25915	4.53E-04	6.98E-04	2.92E-04	
FLJ10496	hypothetical protein FLJ10496 55152	55152	4.53E-04	6.98E-04	2.92E-04	
SRRP40	na	na	4.12E-04		4.15E-10	6.51E-04
LUC7L2	LUC7-like 2 (S. cerevisiae)	51631	3.84E-04	3.32E-06	1.06E-03	
XRCC5	X-ray repair complementing defective repair in Chinese hamster cells 5 (d	7520	3.24E-04	2.73E-04	8.36E-07	
ATF5	activating transcription factor 5	22809	2.21E-04	3.13E-05	1.10E-04	
ATF4	activating transcription factor 4 (ta2-responsive enhancer element B67)	468	2.01E-04	1.07E-04	2.20E-03	
HMGB2	high-mobility group box 2	3148	1.99E-04	4.09E-04	8.04E-12	
MRPL51	mitochondrial ribosomal protein L51	51258	1.30E-04	4.47E-07	7.63E-06	
POLR2A	polymerase (RNA) II (DNA directed) polypeptide A, 220kDa	5430	1.02E-04	1.32E-06	1.43E-03	
NPM1	nucleophosmin (nucleolar phosphoprotein B23, numatrin)	4869	9.45E-05	4.29E-04	4.53E-09	
HIST2H4	histone 2, H4	8370	5.95E-05	1.12E-03	2.63E-04	
MCM3	MCM3 minichromosome maintenance deficient 3 (S. cerevisiae)	4172	5.72E-05	1.74E-05	3.08E-07	
RPS16	ribosomal protein S16	6217	5.21E-05	8.64E-05	1.40E-08	
ATP5G1	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subu	516	3.04E-05	1.06E-03	4.79E-04	
HIST2H3C	histone 2, H3c	126961	2.35E-05	3.19E-04	6.97E-07	
HIST1H2AG	histone 1, H2ag	8969	1.82E-05	5.50E-04	6.64E-04	
CDKN2D	cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4)	1032	7.31E-06	2.64E-06	1.19E-11	
RAD54B	RAD54B homolog	25788		3.58E-03	4.38E-03	4.63E-03
KIF2C	kinesin family member 2C	11004		2.99E-03	4.21E-05	5.81E-03
CDC23	CDC23 (cell division cycle 23, yeast, homolog)	8697		1.38E-03	4.30E-04	2.02E-02
HNRPK	heterogeneous nuclear ribonucleoprotein K	3190		5.64E-04	2.29E-03	8.76E-03
CDCA3	cell division cycle associated 3	83461		2.60E-04	2.86E-04	6.28E-03
PRIM2A	primase, polypeptide 2A, 58kDa	5558		2.40E-05	3.66E-04	3.01E-02
CDC7	CDC7 cell division cycle 7 (S. cerevisiae)	8317		3.50E-06	4.16E-03	2.64E-03
PA2G4	proliferation-associated 2G4, 38kDa	5036	4.91E-03	1.31E-03		
ZNF431	zinc finger protein 431	170959	4.91E-03	2.95E-04		
THEM2	thioesterase superfamily member 2	55856	4.90E-03	1.02E-04		
MGC9718	zinc finger protein (clone 647)	58500	4.77E-03	6.35E-04		
OK	beta 5-tubulin	203068	4.70E-03	6.03E-04		
CSTF1	cleavage stimulation factor, 3' pre-RNA, subunit 1, 50kDa	1477	4.65E-03	4.55E-06		
IFRD1	interferon-related developmental regulator 1	3475	4.62E-03	3.91E-05		
E2F3	E2F transcription factor 3	1871	4.56E-03		3.30E-03	
WBP4	WW domain binding protein 4 (formin binding protein 21)	11193	4.50E-03	2.13E-03		
TM4SF1	transmembrane 4 superfamily member 1	4071	4.49E-03	4.67E-03		
NUSAP1	nucleolar and spindle associated protein 1	51203	4.47E-03		3.52E-10	
C6orf18	chromosome 6 open reading frame 18	54535	4.16E-03		3.68E-04	
DR1	down-regulator of transcription 1, TBP-binding (negative cofactor 2)	1810	4.08E-03	1.06E-03		
182-FIP	82-kD FMRP Interacting Protein	57532	4.07E-03	9.76E-04		
DKFZp434C1714	hypothetical protein DKFZp434C1714	129787	4.07E-03	6.67E-04		
TMSB42	thymosin, beta 4, 2-linked	7114	4.04E-03	6.37E-04		
LOC200008	hypothetical protein LOC200008 51253	200008	3.97E-03		5.12E-05	
MRPL37	mitochondrial ribosomal protein L37 51253	51253	3.97E-03		5.12E-05	
FLJ14451	hypothetical protein FLJ14451	84872	3.92E-03	7.67E-05		
QP-C	low molecular mass ubiquinone-binding protein (9.5kD)	27089	3.90E-03	2.83E-04		
FLJ14346	hypothetical protein FLJ14346	80097	3.88E-03		1.26E-07	
FLJ10640	hypothetical protein FLJ10640	54496	3.84E-03	1.74E-04		
KIAA1007	KIAA1007 protein	23019	3.83E-03	6.79E-05		
LOC220988	heterogeneous nuclear ribonucleoprotein A3	220988	3.82E-03	2.10E-03		
BANF1	barrier to autointegration factor 1 8815	8815	3.76E-03	6.60E-04		
MGC11102	hypothetical protein MGC11102 8815	84285	3.76E-03	6.60E-04		
CARHSP1	calcium regulated heat stable protein 1, 24kDa	23589	3.72E-03	2.69E-05		
RPS12	ribosomal protein S12	6206	3.72E-03	2.47E-04		
MGC5178	hypothetical protein MGC5178	79008	3.71E-03		5.98E-05	
DLEU1	deleted in lymphocytic leukemia, 1 10301	10301	3.61E-03	8.07E-04		
DLEU2	deleted in lymphocytic leukemia, 2 10301	8847	3.61E-03	8.07E-04		
CCT3	chaperonin containing TCP1, subunit 3 (gamma)	7203	3.58E-03	2.42E-04		
MOCS3	molybdenum cofactor synthesis 3	27304	3.44E-03	7.77E-05		
FLJ10706	hypothetical protein FLJ10706	55732	3.44E-03	2.04E-04		
RPL10A	ribosomal protein L10a	4736	3.34E-03	3.08E-04		
CBWD2	COBW domain containing 2	150472	3.26E-03	2.49E-05		
PSMC4	proteasome (prosome, macropain) 26S subunit, ATPase, 4	5704	3.26E-03	6.63E-04		
GMRP-1	0	0	3.13E-03	2.06E-03		
EIF4A2	eukaryotic translation initiation factor 4A, isoform 2	1974	3.09E-03	9.36E-04		
FLJ12998	hypothetical protein FLJ12998	0	3.08E-03	1.98E-05		

TOB3	AAA-ATPase TOB3	83858	3.01E-03		2.14E-03	
RPS18	ribosomal protein S18	6222	3.00E-03	1.50E-04		
RPS8	ribosomal protein S8	6202	3.00E-03	1.01E-03		
CDCA8	cell division cycle associated 8 55143	55143	2.97E-03		2.00E-03	
FLJ20508	hypothetical protein FLJ20508 55143	54955	2.97E-03		2.00E-03	
MGC26717	hypothetical protein MGC26717	285237	2.94E-03	3.19E-03		
DCLRE1B	DNA cross-link repair 1B (PSO2 homolog, S. cerevisiae)	64858	2.82E-03	1.51E-03		
FLJ20241	putative NFkB activating protein 54862	54862	2.78E-03		1.83E-09	
MGC11271	hypothetical protein MGC11271 54862	79173	2.78E-03		1.83E-09	
HARSL	histidyl-tRNA synthetase-like	23438	2.74E-03	3.15E-03		
PRD21	peroxiredoxin 1	5052	2.71E-03	3.73E-04		
MLH1	mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli)	4292	2.68E-03	2.35E-04		
ERCC1	excision repair cross-complementing rodent repair deficienc3, complement	2067	2.68E-03	1.70E-06		
TMSL3	thymosin-like 3	7117	2.56E-03	1.12E-04		
PAI-RBP1	PAI-1 mRNA-binding protein	26135	2.53E-03	1.55E-04		
MTBP	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse	27085	2.41E-03		8.28E-06	
ZNF9	zinc finger protein 9 (a cellular retroviral nucleic acid binding protein)	7555	2.40E-03	2.40E-03		
GRCC10	likely ortholog of mouse gene rich cluster, C10 gene	113246	2.10E-03	6.15E-04		
ARHGAP6	Rho GTPase activating protein 6	395	2.06E-03	1.08E-04		
TMLS	thymosin-like 6	7120	2.02E-03	4.97E-03		
DNAJA3	DnaJ (Hsp40) homolog, subfamily A, member 3	9093	2.00E-03	5.18E-04		
FLJ10986	hypothetical protein FLJ10986	55277	1.98E-03	1.29E-03		
H2AFZ	H2A histone family, member Z	3015	1.96E-03	1.38E-03		
UNRIP	unr-interacting protein	11171	1.95E-03	1.25E-03		
TFB1M	transcription factor B1, mitochondrial	51106	1.94E-03		2.32E-03	
RQCD1	RCD1 required for cell differentiation1 homolog (S. pombe)	9125	1.81E-03		7.82E-05	
C12orf8	chromosome 12 open reading frame 8	10961	1.80E-03		1.17E-06	
BET1L	blocked early in transport 1 homolog (S. cerevisiae) like	51272	1.79E-03	1.46E-03		
POP4	POP4 (processing of precursor , S. cerevisiae) homolog	10775	1.71E-03	9.33E-05		
MNAT1	menage a trois 1 (CAK assembl3 factor)	4331	1.67E-03	5.53E-06		
ETR101	immediate early protein	9592	1.64E-03	6.82E-04		
HIST1H4E	histone 1, H4e	8367	1.55E-03	1.61E-04		
MGC3121	hypothetical protein MGC3121	78994	1.51E-03		4.25E-03	
BRD2	bromodomain containing 2	6046	1.42E-03	9.74E-04		
HIST1H1D	histone 1, H1d	3007	1.32E-03	3.19E-05		
MYNN	myoneurin	55892	1.31E-03	6.88E-05		
NSEP1	nuclease sensitive element binding protein 1	4904	1.29E-03	1.26E-04		
LOC51236	brain protein 16	51236	1.28E-03	1.69E-04		
DKFZP564O0463	DKFZP564O0463 protein	25879	1.27E-03	2.85E-05		
RPS9	ribosomal protein S9	6203	1.23E-03	1.04E-06		
EIF4G2	eukaryotic translation initiation factor 4 gamma, 2	1982	1.22E-03	1.12E-04		
METTL2	methyltransferase like 2	55798	1.22E-03	4.71E-03		
HNRPA0	heterogeneous nuclear ribonucleoprotein A0	10949	1.20E-03	2.72E-03		
NEK11	NIMA (never in mitosis gene a)- related kinase 11	79858	1.14E-03	1.60E-06		
HIST2H2AA	histone 2, H2aa	8337	9.48E-04	2.96E-04		
KPNB1	karyopherin (importin) beta 1	3837	8.83E-04	2.22E-04		
SLC3A2	solute carrier family 3 (activators of dibasic and neutral amino acid transp	6520	8.48E-04			1.51E-04
RRM2	ribonucleotide reductase M2 polypeptide	6241	8.32E-04	7.04E-05		
RAB30	RAB30, member RAS oncogene family	27314	7.52E-04	7.83E-06		
FGF5	fibroblast growth factor 5	2250	7.50E-04	2.37E-04		
KIAA0907	KIAA0907 protein	22889	6.75E-04	1.90E-04		
LIP8	L3ST-interacting protein LIP8	116840	6.25E-04	2.69E-07		
TRIP11	thyroid hormone receptor interactor 11	9321	5.97E-04	7.16E-04		
RPL27A	ribosomal protein L27a	6157	5.40E-04	8.44E-05		
TOMM70A	translocase of outer mitochondrial membrane 70 homolog A (yeast)	9868	5.37E-04	2.43E-05		
NKTR	natural killer-tumor recognition sequence	4820	5.34E-04	3.91E-04		
NDUFB9	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9, 22kDa 4715	4715	5.28E-04	7.53E-04		
RARB	retinoic acid receptor, beta	5915	5.24E-04	1.02E-03		
UQCR	ubiquinol-cytochrome c reductase (6.4kD) subunit	10975	5.07E-04	4.20E-06		
MGC4618	hypothetical protein MGC4618	84286	4.99E-04	3.37E-04		
POLR2A	polymerase (RNA) II (DNA directed) polypeptide A, 220kDa	5430	4.77E-04	2.36E-03		
PRO2000	PRO2000 protein	29028	4.77E-04		1.68E-12	
RPL37A	ribosomal protein L37a	6168	4.72E-04	2.42E-04		
UBE2M	ubiquitin-conjugating enzyme E2M (UBC12 homolog, yeast)	9040	4.62E-04	1.04E-03		
TRAPPC4	trafficking protein particle complex 4	51399	4.21E-04	6.02E-05		
FLJ22833	hypothetical protein FLJ22833	64859	3.61E-04	1.05E-07		
KIAA1404	KIAA1404 protein	57169	3.53E-04	4.98E-04		
ATP6V1C1	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C, isoform 1	528	3.16E-04	7.24E-04		
RPS28	ribosomal protein S28	6234	3.05E-04	2.40E-04		
HIST3H2BB	histone 3, H2bb	128312	2.66E-04	3.24E-04		
PTD012	PTD012 protein	28970	2.11E-04	6.02E-04		
PILRB	paired immunoglobin-like type 2 receptor beta	29990	2.08E-04	1.48E-03		
RPL10	ribosomal protein L10	6134	1.87E-04	2.18E-04		
KBRAS1	I-kappa-B-interacting Ras-like protein 1 6138	28512	1.86E-04	4.33E-03		
RPL15	ribosomal protein L15 6138	6138	1.86E-04	4.33E-03		
LOC51240	hypothetical protein LOC51240 5378	51240	1.66E-04	6.31E-04		
PMS1	PMS1 postmeiotic segregation increased 1 (S. cerevisiae) 5378	5378	1.66E-04	6.31E-04		
HIST1H3B	histone 1, H3b	8358	1.64E-04	1.68E-04		
HIST1H4B	histone 1, H4b	8366	1.59E-04	1.33E-03		
LMNB1	lamin B1	4001	1.33E-04	1.54E-04		
HSPA8	heat shock 70kDa protein 8	3312	1.24E-04	9.88E-04		
FLJ22624	hypothetical protein FLJ22624	79866	1.14E-04	2.00E-03		
PMS2L5	postmeiotic segregation increased 2-like 5	5383	9.22E-05	2.78E-04		
HIST2H2AA	histone 2, H2aa	8337	6.82E-05	3.10E-04		
HIST1H2AC	histone 1, H2ac	8334	4.88E-05	2.84E-03		
CALM2	calmodulin 2 (phosphorylase kinase, delta)	805	4.88E-05	3.30E-03		
RPL3	ribosomal protein L3	6122	4.58E-05	2.56E-03		
HIST1H2AE	histone 1, H2ae	3012	4.12E-05	4.43E-03		
RPL28	ribosomal protein L28	6158	4.03E-05	5.04E-04		
HIST1H2BD	histone 1, H2bd	3017	3.23E-05	1.45E-03		
HIST1H2AH	histone 1, H2ah 85235	85235	2.65E-05	4.89E-04		
HIST1H2BK	histone 1, H2bk 85235	85236	2.65E-05	4.89E-04		
HIST1H2BM	histone 1, H2bm	8342	1.19E-05	1.18E-03		
HIST1H2BF	histone 1, H2bf 8343	8343	1.06E-05	5.19E-04		
HIST1H3D	histone 1, H3d 8343	3013	1.06E-05	5.19E-04		
HIST1H2BN	histone 1, H2bn	8341	7.43E-06	5.39E-05		
HIST1H2AI	histone 1, H2ai	8329	3.56E-06	3.53E-04		
ZW10	ZW10 homolog, centromere	9183		4.81E-03		3.22E-02
IDE	insulin-degrading enzyme	3416		4.31E-03		3.34E-02
CDCA1	cell division cycle associated 1	na		4.23E-03		9.64E-03

JUN	v-jun sarcoma virus 17 oncogene homolog (avian)	3725		3.71E-03		3.18E-03
PARK7	Parkinson disease (autosomal recessive, early onset) 7	11315		3.42E-03	4.50E-03	
FLJ10292	hypothetical protein FLJ10292	55110		2.98E-03	1.22E-04	
SFPQ	splicing factor proline	6421		2.65E-03		1.19E-02
DD211	DEAD	1663		1.85E-03	4.55E-04	
SMC2L1	SMC2 structural maintenance of chromosomes 2-like 1 (yeast)	10592		1.20E-03		1.95E-02
APC10	anaphase-promoting complex subunit 10	10393		1.17E-03		1.46E-02
DNAJC9	DnaJ (Hsp40) homolog, subfamily C, member 9	23234		1.03E-03	7.39E-06	
TEBP	unactive progesterone receptor, 23 kD	10728		9.56E-04	4.50E-03	
KIAA0092	translokin 143684	9702		5.87E-04	3.54E-04	
MGC33371	hypothetical protein MGC33371 143684	143684		5.87E-04	3.54E-04	
C3CS	cytochrome c, somatic	54205		3.62E-04		3.33E-02
CSPG6	chondroitin sulfate proteoglycan 6 (bamacan)	9126		2.44E-04	5.62E-07	
C20orf77	chromosome 20 open reading frame 77	58490		1.35E-04	1.52E-03	
DCLRE1C	DNA cross-link repair 1C (PSO2 homolog, S. cerevisiae)	64421		1.25E-04	4.07E-03	
IPLA2(GAMMA)	intracellular membrane-associated calcium-independent phospholipase A2	50640		1.21E-04	4.33E-05	
ASF1B	ASF1 anti-silencing function 1 homolog B (S. cerevisiae)	55723		1.16E-04	4.47E-03	
SIVA	CD27-binding (Siva) protein	10572		3.93E-05	3.80E-05	
MSH2	mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)	4436		3.44E-05	1.63E-04	
FLJ12760	hypothetical protein FLJ12760	339175		3.23E-05	3.66E-07	
HSPC157	HSPC157 protein	29092		1.26E-05	2.36E-03	
HAT1	histone acetyltransferase 1	8520		6.91E-07	1.16E-04	
ORC1L	origin recognition complex, subunit 1-like (yeast)	4998			9.20E-05	3.80E-02
THOC1	THO complex 1	9984	4.68E-03	2.32E-05		
MGC47869	hypothetical protein MGC47869 144608	144608	1.73E-03		1.94E-04	
WBP11	WW domain binding protein 11 51729	51729	1.73E-03		1.94E-04	
GNAI3	guanine nucleotide binding protein (G protein), alpha inhibiting activity po	2773		3.03E-03	2.03E-03	
EEF1E1	eukaryotic translation elongation factor 1 epsilon 1	9521		5.86E-04	1.37E-03	
VAMP1	vesicle-associated membrane protein 1 (synaptobrevin 1)	6843	4.91E-03			
LOC54499	putative membrane protein	54499	4.84E-03			
API5	apoptosis inhibitor 5	8539	4.60E-03			
DLAT	dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydro	1737	4.46E-03			
FEN1	flap structure-specific endonuclease 1	2237	4.44E-03			
PDZGEF2	PDZ domain containing guanine nucleotide exchange factor (GEF) 2	51735	4.43E-03			
ZNF192	zinc finger protein 192	7745	4.43E-03			
HNRPD	heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding	3184	4.41E-03			
TOMM40	translocase of outer mitochondrial membrane 40 homolog (yeast)	10452	4.32E-03			
IARS	isoleucine-tRNA synthetase	3376	4.27E-03			
MGC5309	hypothetical protein MGC5309	84246	4.24E-03			
APG-1	heat shock protein (hsp110 family)	22824	4.23E-03			
PE212	peroxisomal biogenesis factor 12	5193	4.09E-03			
DDT	D-dopachrome tautomerase	1652	4.04E-03			
FLJ90024	fasting-inducible integral membrane protein TM6P1	129303	4.04E-03			
MGC2655	hypothetical protein MGC2655	79228	3.81E-03			
LOC55580	hypothetical protein LOC55580	55580	3.34E-03			
RSU1	Ras suppressor protein 1	6251	3.32E-03			
MPHOSPH1	M-phase phosphoprotein 1	9585	3.27E-03			
HERC1	hect (homologous to the E6-AP (UBE3A) carboxyl terminus) domain and R	8925	3.26E-03			
RPL31	ribosomal protein L31	6160	3.16E-03			
FLJ20364	hypothetical protein FLJ20364	54908	3.11E-03			
LOC148523	hypothetical protein BC017397	148523	3.11E-03			
TERF2IP	telomeric repeat binding factor 2, interacting protein	54386	2.99E-03			
HMGA1	high mobility group AT-hook 1	3159	2.98E-03			
RAB5A	RAB5A, member RAS oncogene family	5868	2.83E-03			
NEUGRIN	mesenchymal stem cell protein DSC92	51335	2.81E-03			
CCT6B	chaperonin containing TCP1, subunit 6B (zeta 2) 91603	10693	2.81E-03			
MGC20398	hypothetical protein MGC20398 91603	91603	2.81E-03			
C14orf100	chromosome 14 open reading frame 100 51528	51528	2.55E-03			
FLJ25436	hypothetical protein FLJ25436 51528	112849	2.55E-03			
SEPW1	selenoprotein W, 1	6415	2.49E-03			
IER5L	immediate early response 5-like	389792	2.39E-03			
C7orf24	chromosome 7 open reading frame 24	79017	2.30E-03			
ABT1	activator of basal transcription 1	29777	2.27E-03			
PHLDA1	pleckstrin homology-like domain, family A, member 1	22822	2.26E-03			
MRPS16	mitochondrial ribosomal protein S16	51021	2.07E-03			
STAG1	stromal antigen 1	10274	2.02E-03			
HRMT1L2	HMT1 hnRNP methyltransferase-like 2 (S. cerevisiae)	3276	1.93E-03			
LOC113251	c-Mpl binding protein	113251	1.85E-03			
JTV1	JTV1 gene	7965	1.78E-03			
FLJ21103	hypothetical protein FLJ21103	79607	1.72E-03			
L3K5	protein kinase L3K5	92335	1.70E-03			
PCNT2	pericentrin 2 (kendrin)	5116	1.67E-03			
HRD1	HRD1 protein	84447	1.61E-03			
ACO21	acyl-Coenzyme A oxidase 1, palmitoyl 1018	51	1.55E-03			
CDK3	cyclin-dependent kinase 3 1018	1018	1.55E-03			
BCAT1	branched chain aminotransferase 1, cytosolic	na	1.54E-03			
NCL	nucleolin	4691	1.50E-03			
HSPA4	heat shock 70kDa protein 4	3308	1.47E-03			
C2orf9	chromosome 2 open reading frame 9	84269	1.36E-03			
C20orf147	chromosome 20 open reading frame 147	140838	1.16E-03			
SLC23A3	solute carrier family 23 (nucleobase transporters), member 3	151295	1.10E-03			
UBQLN1	ubiquilin 1	29979	9.31E-04			
MGC2734	hypothetical protein MGC2734 92399	92400	8.66E-04			
MRRF	mitochondrial ribosome recycling factor 92399	92399	8.66E-04			
Lrp2bp	low density lipoprotein receptor-related protein binding protein	353322	7.88E-04			
FBL	fibrillarin	2091	1.31E-04			
HIST2H2AC	histone 2, H2ac	8338	5.71E-05			
AP4E1	adaptor-related protein complex 4, epsilon 1 subunit	23431	7.68E-06			
C1orf33	chromosome 1 open reading frame 33 51154	51154		4.95E-03		
KIAA0090	KIAA0090 protein 51154	23065		4.95E-03		
ETFDH	electron-transferring-flavoprotein dehydrogenase	2110		4.94E-03		
NDUFA11	NADH-ubiquinone oxidoreductase subunit B14.7	126328		4.93E-03		
ANAPC5	anaphase promoting complex subunit 5	51433		4.92E-03		
RNPS1	RNA binding protein S1, serine-rich domain	10921		4.92E-03		
BLCAP	bladder cancer associated protein	10904		4.91E-03		
FLJ20582	hypothetical protein FLJ20582	54989		4.85E-03		
FLJ14779	hypothetical protein FLJ14779	84924		4.85E-03		
GTF2B	general transcription factor IIB	2959		4.85E-03		
GTPBG3	mitochondrial GTP binding protein	84705		4.75E-03		
PRO0971	hypothetical protein PRO0971	55435		4.73E-03		

R31	putative nucleic acid binding protein R3-1	11017		4.73E-03		
FLJ00166	FLJ00166 protein	151613		4.72E-03		
RPS42	ribosomal protein S4, 2-linked	6191		4.70E-03		
CPSF5	cleavage and polyadenylation specific factor 5, 25 kDa 55239	11051		4.68E-03		
FLJ10826	hypothetical protein FLJ10826 55239	55239		4.68E-03		
ZNF43	zinc finger protein 43 (HTF6)	7594		4.67E-03		
C6orf152	chromosome 6 open reading frame 152	167691		4.63E-03		
LDHA	lactate dehydrogenase A	3939		4.60E-03		
TAF13	TAF13 RNA polymerase II, TATA box binding protein (TBP)-associated fact	na		4.42E-03		
DH235	DEAH (Asp-Glu-Ala-His) box polypeptide 35	60625		4.41E-03		
RPS17	ribosomal protein S17	6218		4.39E-03		
IPO11	importin 11	51194		4.37E-03		
POLD2	polymerase (DNA directed), delta 2, regulatory subunit 50kDa	5425		4.37E-03		
FLJ11331	hypothetical protein FLJ11331	55345		4.36E-03		
CQ27A2	cytochrome c oxidase subunit VIIa polypeptide 2 (liver)	1347		4.36E-03		
ZWINT	ZW10 interactor	11130		4.29E-03		
POLD4	polymerase (DNA-directed), delta 4	57804		4.22E-03		
MRPL43	mitochondrial ribosomal protein L43 56652	84545		4.20E-03		
PEO1	progressive external ophthalmoplegia 1 56652	56652		4.20E-03		
NCK1	NCK adaptor protein 1	4690		4.16E-03		
STC2	stanniocalcin 2	8614		4.16E-03		
FDFT1	farnesyl-diphosphate farnesyltransferase 1	2222		4.15E-03		
HSPA1B	heat shock 70kDa protein 1B	3304		4.12E-03		
RP3-366L4.2	J-type co-chaperone HSC20 150274	150274		4.06E-03		
CHEK2	CHK2 checkpoint homolog (S. pombe) 150274	11200		4.06E-03		
RPL8	ribosomal protein L8	6132		4.04E-03		
FLJ12598	hypothetical protein FLJ12598 79810	79810		4.04E-03		
MRPS27	mitochondrial ribosomal protein S27 79810	23107		4.04E-03		
MGC2404	hypothetical protein MGC2404	84320		4.04E-03		
DR1	down-regulator of transcription 1, TBP-binding (negative cofactor 2)	1810		4.01E-03		
ARHGAP11A	KIAA0013 gene product	9824		3.96E-03		
SMAP	small acidic protein	10944		3.96E-03		
PSMD12	proteasome (prosome, macropain) 26S subunit, non-ATPase, 12	5718		3.95E-03		
FLJ22002	hypothetical protein FLJ22002 79896	79896		3.87E-03		
MGC26778	hypothetical protein MGC26778 79896	219670		3.87E-03		
MGC39558	hypothetical protein MGC39558	148789		3.81E-03		
LZTR1	leucine-zipper-like transcriptional regulator, 1	8216		3.79E-03		
EAF2	ELL associated factor 2	55840		3.78E-03		
ARLTS1	ADP-ribosylation factor-like tumor suppressor protein 1	115761		3.78E-03		
FLJ22059	hypothetical protein FLJ22059	64763		3.76E-03		
SLC39A9	solute carrier family 39 (zinc transporter), member 9	55334		3.76E-03		
PMSCL1	polymyositis	5393		3.74E-03		
GAN	giant axonal neuropathy (gigaxonin)	8139		3.71E-03		
RTP801	HIF-1 responsive RTP801	54541		3.68E-03		
RBAF600	retinoblastoma-associated factor 600	23352		3.66E-03		
FLJ10803	hypothetical protein FLJ10803	55744		3.64E-03		
ZNF302	zinc finger protein 302	55900		3.54E-03		
RPS24	ribosomal protein S24	6229		3.47E-03		
POLR3D	polymerase (RNA) III (DNA directed) polypeptide D, 44kDa	661		3.47E-03		
MGC2198	hypothetical protein MGC2198	192286		3.41E-03		
FLJ39485	hypothetical protein FLJ39485	285603		3.35E-03		
HIST1H4F	histone 1, H4f	8361		3.34E-03		
WDR3	WD repeat domain 3	10885		3.31E-03		
0	0	0		3.31E-03		
IPP	intracisternal A particle-promoted polypeptide	3652		3.27E-03		
C2orf7	chromosome 2 open reading frame 7 84279	84279		3.23E-03		
CCT7	chaperonin containing TCP1, subunit 7 (eta) 84279	10574		3.23E-03		
ARMET	arginine-rich, mutated in early stage tumors	7873		3.19E-03		
FLJ39582	hypothetical protein FLJ39582 353117	353117		3.05E-03		
THAP7	THAP domain containing 7 353117	80764		3.05E-03		
SELH	selenoprotein H	280636		3.02E-03		
FLJ14346	hypothetical protein FLJ14346	80097		3.02E-03		
APEH	N-acylaminoacyl-peptide h3drolase	327		2.99E-03		
HIST1H2BE	histone 1, H2be	8344		2.97E-03		
NDRG1	N-myc downstream regulated gene 1	10397		2.95E-03		
HIS1	HMBA-inducible	10614		2.85E-03		
MGC14421	hypothetical protein MGC14421	84993		2.85E-03		
RBM8A	RNA binding motif protein 8A	9939		2.84E-03		
IDH3G	isocitrate dehydrogenase 3 (NAD+) gamma 6748	3421		2.84E-03		
SSR4	signal sequence receptor, delta (translocon-associated protein delta) 6748	6748		2.84E-03		
BCL2L12	BCL2-like 12 (proline rich)	83596		2.83E-03		
AGA	aspartylglucosaminidase	175		2.82E-03		
MAP3K4	mitogen-activated protein kinase kinase kinase 4	4216		2.82E-03		
NR1H3	nuclear receptor subfamily 1, group H, member 3	10062		2.81E-03		
VMP1	likely ortholog of rat vacuole membrane protein 1	81671		2.78E-03		
DKFZp686L20145	similar to rab11-binding protein	54521		2.78E-03		
THAP8	THAP domain containing 8	199745		2.76E-03		
CGI-18	CGI-18 protein 119504	51008		2.74E-03		
LOC119504	hypothetical protein LOC119504 119504	119504		2.74E-03		
ILK	integrin-linked kinase 3611	3611		2.74E-03		
KIAA0409	KIAA0409 protein 3611	23378		2.74E-03		
H326	H326	50717		2.62E-03		
TAGLN2	transgelin 2	8407		2.61E-03		
SUGT1	SGT1, suppressor of G2 allele of SKP1 (S. cerevisiae)	10910		2.60E-03		
TRAP150	thyroid hormone receptor-associated protein, 150 kDa subunit	9967		2.55E-03		
HSU79266	protein predicted by clone 23627	29901		2.54E-03		
LPAAT-e	acid acyltransferase-epsilon	55326		2.53E-03		
CENPA	centromere protein A, 17kDa	1058		2.51E-03		
NRD1	nardil3sin (N-arginine dibasic convertase)	4898		2.51E-03		
NDUFA5	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13kDa	4698		2.47E-03		
TAZ	transcriptional co-activator with PDZ-binding motif (TAZ)	25937		2.47E-03		
NNMT	nicotinamide N-methyltransferase	4837		2.47E-03		
MAP2K5	mitogen-activated protein kinase kinase 5	5607		2.46E-03		
G3BP	Ras-GTPase-activating protein SH3-domain-binding protein	10146		2.37E-03		
CDC6	CDC6 cell division cycle 6 homolog (S. cerevisiae)	990		2.36E-03		
FLJ25078	hypothetical protein FLJ25078	148304		2.35E-03		
CCT2	chaperonin containing TCP1, subunit 2 (beta)	10576		2.35E-03		
RABL2A	RAB, member of RAS oncogene family-like 2A	11159		2.34E-03		
PIK3R3	phosphoinositide-3-kinase, regulatory subunit, polypeptide 3 (p55, gamma	8503		2.34E-03		
MGC33864	ADP-ribosylation-like factor 6-interacting protein 6	151188		2.28E-03		

SMARCA3	SWI	6596		2.24E-03		
AP2A1	adaptor-related protein complex 2, alpha 1 subunit	160		2.22E-03		
GTF2I	general transcription factor II, i	2969		2.22E-03		
FLJ10359	hypothetical protein FLJ10359	55127		2.19E-03		
RPC62	polymerase (RNA) III (DNA directed) (62kD) 27246	10623		2.16E-03		
ZNF364	zinc finger protein 364 27246	27246		2.16E-03		
UBE2D3	ubiquitin-conjugating enzyme E2D 3 (UBC4	7323		2.15E-03		
FLJ12886	hypothetical protein FLJ12886	56006		2.11E-03		
H2AF2	H2A histone family, member 2	3014		2.10E-03		
CG005	hypothetical protein from BCRA2 region	10443		2.05E-03		
ALCAM	activated leukocyte cell adhesion molecule	214		2.04E-03		
C3orf31	chromosome 3 open reading frame 31	132001		2.04E-03		
STK18	serine	10733		2.04E-03		
PRKACB	protein kinase, cAMP-dependent, catalytic, beta	5567		2.00E-03		
MPV17	MpV17 transgene, murine homolog, glomerulosclerosis	4358		1.99E-03		
NDUFB5	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5, 16kDa	4711		1.99E-03		
RPS5	ribosomal protein S5	6193		1.95E-03		
TOB2	transducer of ERBB2, 2	10766		1.89E-03		
CTAG3	cancer	285782		1.88E-03		
RIOK1	RIO kinase 1 (yeast)	83732		1.88E-03		
RBBP1	retinoblastoma binding protein 1	5926		1.86E-03		
PLCD1	phospholipase C, delta 1	5333		1.86E-03		
EIF3S10	eukaryotic translation initiation factor 3, subunit 10 theta, 150	8661		1.76E-03		
CLCN3	chloride channel 3	1182		1.74E-03		
ZFP91	zinc finger protein 91 homolog (mouse)	80829		1.70E-03		
P2MP3	peroxisomal membrane protein 3, 35kDa (Zellweger syndrome)	5828		1.70E-03		
GTF2A1	general transcription factor IIA, 1, 19	2957		1.64E-03		
DKFZP434D1335	DKFZP434D1335 protein	26065		1.61E-03		
ARF6	ADP-ribosylation factor 6	382		1.58E-03		
DD25	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5 1655	1655		1.56E-03		
LOC90799	hypothetical protein BC009518 1655	90799		1.56E-03		
C14orf87	chromosome 14 open reading frame 87	51218		1.55E-03		
RAF1	v-raf-1 murine leukemia viral oncogene homolog 1	5894		1.54E-03		
RNGTT	RNA guan3l3ltransferase and 5'-phosphatase	8732		1.54E-03		
MCCC2	methylcrotonoyl-Coenzyme A carboxylase 2 (beta)	64087		1.53E-03		
C6orf53	chromosome 6 open reading frame 53	51522		1.53E-03		
C15orf12	chromosome 15 open reading frame 12	55272		1.45E-03		
C14orf9	chromosome 14 open reading frame 9	90809		1.44E-03		
JAZ	double-stranded RNA-binding zinc finger protein JAZ	23567		1.44E-03		
SEC23A	Sec23 homolog A (S. cerevisiae)	10484		1.42E-03		
C6orf37	chromosome 6 open reading frame 37	55603		1.40E-03		
CGI-143	CGI-143 protein	51027		1.34E-03		
H17	hypothetical protein H17 55572	55572		1.32E-03		
SRPR	signal recognition particle receptor ('docking protein') 55572	6734		1.32E-03		
ZNF23	zinc finger protein 23 (KO2 16)	7571		1.31E-03		
CGI-30	CGI-30 protein	51611		1.29E-03		
RPS14	ribosomal protein S14	6208		1.21E-03		
CML66	chronic m3elogenous leukemia tumor antigen 66 56943	84955		1.20E-03		
DC6	DC6 protein 56943	56943		1.20E-03		
ZNF258	zinc finger protein 258	9204		1.19E-03		
SSBP1	single-stranded DNA binding protein	6742		1.19E-03		
MGC9515	hypothetical protein MGC9515	348162		1.18E-03		
NAKAP95	neighbor of A-kinase anchoring protein 95	26993		1.18E-03		
HCCR1	cervical cancer 1 protooncogene	25875		1.18E-03		
CCNL1	cyclin L1	57018		1.17E-03		
DDOST	dolichyl-diphosphooligosaccharide-protein glycosyltransferase	1650		1.17E-03		
RTN4	reticulon 4	57142		1.15E-03		
MGC40157	hypothetical protein MGC40157	125144		1.14E-03		
RPL37	ribosomal protein L37	6167		1.13E-03		
HIRA	HIR histone cell cycle regulation defective homolog A (S. cerevisiae) 7290	7290		1.12E-03		
MRPL40	mitochondrial ribosomal protein L40 7290	64976		1.12E-03		
DKFZp761J139	hypothetical protein DKFZp761J139	84240		1.09E-03		
GRWD1	glutamate-rich WD repeat containing 1	83743		1.08E-03		
LOC81691	exonuclease NEF-sp 81691	81691		1.01E-03		
MGC16943	similar to RIKEN cDNA 4933424N09 gene 81691	112479		1.01E-03		
E2-EPF	ubiquitin carrier protein	27338		9.90E-04		
na	0	0		9.45E-04		
WDR23	WD repeat domain 23	80344		9.39E-04		
LMO4	LIM domain only 4	8543		9.07E-04		
DPY19L3	dpy-19-like 3 (C. elegans)	147991		9.06E-04		
CENPB	centromere protein B, 80kDa	1059		8.88E-04		
SFRS2	splicing factor, arginine	6427		8.61E-04		
CENPF	centromere protein F, 350	1063		8.59E-04		
UBC	ubiquitin C	7316		8.42E-04		
CLONE24922	hypothetical protein CLONE24922 51117	26995		8.36E-04		
COQ4	coenzyme Q4 homolog (yeast) 51117	51117		8.36E-04		
MGC4093	hypothetical protein MGC4093	80776		8.24E-04		
FLJ14936	hypothetical protein FLJ14936	84950		8.22E-04		
GNB1L	guanine nucleotide binding protein (G protein), beta polypeptide 1-like	54584		8.08E-04		
SAP18	sin3-associated polypeptide, 18kDa	10284		7.98E-04		
C13orf3	chromosome 13 open reading frame 3 78988	221150		7.90E-04		
MRP63	mitochondrial ribosomal protein 63 78988	78988		7.90E-04		
RBM14	RNA binding motif protein 14	10432		7.83E-04		
ATR	ataxia telangiectasia and Rad3 related	545		7.74E-04		
CCT5	chaperonin containing TCP1, subunit 5 (epsilon) 22948	22948		7.62E-04		
LOC134145	hypothetical protein LOC134145 22948	134145		7.62E-04		
DKFZP564O123	DKFZP564O123 protein	25978		7.56E-04		
GALE	galactose-4-epimerase, UDP-	2582		6.94E-04		
MGC43690	hypothetical protein MGC43690	253769		6.82E-04		
C21orf119	chromosome 21 open reading frame 119	84996		6.68E-04		
ARHGAP18	Rho GTPase activating protein 18	93663		6.16E-04		
HMGB1	high-mobility group box 1	3146		6.15E-04		
AMD1	adenos3lmethionine decarboxylase 1	262		6.06E-04		
MGC10067	hypothetical protein MGC10067	134510		6.01E-04		
SFRS10	splicing factor, arginine	6434		5.94E-04		
DNAJB4	DnaJ (Hsp40) homolog, subfamily B, member 4	11080		5.70E-04		
MRPS15	mitochondrial ribosomal protein S15	64960		5.57E-04		
DJ465N24.2.1	hypothetical protein dJ465N24.2.1	57035		5.36E-04		
SFRS3	splicing factor, arginine	6428		4.92E-04		
RNF121	ring finger protein 121	55298		4.91E-04		

BRI2	BRI2 55299	55299		4.86E-04		
RAD1	RAD1 homolog (S. pombe) 55299	5810		4.86E-04		
RFC4	replication factor C (activator 1) 4, 37kDa	5984		4.82E-04		
TPM3	tropomyosin 3	7170		4.82E-04		
CSNK1A1	casein kinase 1, alpha 1	1452		4.13E-04		
HIST1H2BB	histone 1, H2bb	3018		3.78E-04		
NUP88	nucleoporin 88kDa	4927		3.67E-04		
ARL6IP	ADP-ribosylation factor-like 6 interacting protein	23204		3.48E-04		
FLJ10006	hypothetical protein FLJ10006	55677		3.46E-04		
KIAA1724	KIAA1724 protein	85465		2.80E-04		
RNF34	ring finger protein 34	80196		2.75E-04		
FLJ10504	misato	55154		2.40E-04		
C3B5-M	cytochrome b5 outer mitochondrial membrane precursor	80777		2.36E-04		
C10orf7	chromosome 10 open reading frame 7	8872		2.33E-04		
HIST1H3D	histone 1, H3d	8351		2.32E-04		
ZNF79	zinc finger protein 79 (pT7)	7633		2.18E-04		
STIP1	stress-induced-phosphoprotein 1 (Hsp70	10963		1.48E-04		
C2F	C2f protein	10436		1.41E-04		
RPL3	ribosomal protein L3	6122		1.37E-04		
BRD8	na	na		1.36E-04		
LOC90678	hypothetical protein BC009239	90678		1.36E-04		
PDIP46	polymerase delta interacting protein 46	84271		1.27E-04		
TSG101	tumor susceptibilit3 gene 101	7251		1.24E-04		
MGC15763	hypothetical protein BC008322	92106		1.15E-04		
RPS23	ribosomal protein S23	6228		1.13E-04		
HMGCR	3-h3dro23-3-meth3iglutar3l-Coenzyme A reductase	3156		7.47E-05		
HnRNP U	na	na		7.20E-05		
CLTA	clathrin, light polypeptide (Lca)	1211		7.20E-05		
RPS13	ribosomal protein S13	6207		6.99E-05		
FLJ10514	hypothetical protein FLJ10514	55157		6.81E-05		
PARG	poly (ADP-ribose) glycohydrolase	8505		5.78E-05		
FLJ22347	hypothetical protein FLJ22347	64852		5.78E-05		
MUS81	MUS81 endonuclease	80198		5.62E-05		
FLJ35775	hypothetical protein FLJ35775	138009		5.52E-05		
LOC55871	COBW-like protein	55871		4.71E-05		
C11orf1	chromosome 11 open reading frame 1	64776		4.58E-05		
M96	likely ortholog of mouse metal response element binding transcription fact	22823		4.52E-05		
SCAND1	SCAN domain containing 1	51282		4.50E-05		
GTF2H1	general transcription factor IIH, polypeptide 1, 62kDa	2965		4.46E-05		
HIST1H4H	histone 1, H4h	8365		4.43E-05		
CHC1	chromosome condensation 1	1104		4.40E-05		
COPS5	COP9 constitutive photomorphogenic homolog subunit 5 (Arabidopsis)	10987		4.30E-05		
C19orf6	chromosome 19 open reading frame 6	91304		3.89E-05		
KIAA0186	KIAA0186 gene product	9837		3.85E-05		
PAK1IP1	PAK1 interacting protein 1	55003		3.38E-05		
DD246	DEAD (Asp-Glu-Ala-Asp) box polypeptide 46	9879		3.13E-05		
ST210	syntaxin 10	8677		2.90E-05		
CKLF	chemokine-like factor	51192		2.47E-05		
KITLG	KIT ligand	4254		2.38E-05		
HNLF	putative NFkB activating protein HNLF	222068		2.24E-05		
DKFZP434H0115	hypothetical protein DKFZp434H0115	83538		2.03E-05		
BTD	biotinidase	686		1.91E-05		
FLJ31364	homolog of yeast EME1 endonuclease	146956		1.89E-05		
CD44	CD44 antigen (homing function and Indian blood group s3stem)	960		1.76E-05		
RAD51	RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae)	5888		1.49E-05		
CENPH	centromere protein H	64946		1.05E-05		
TRIP	TRAF interacting protein	10293		5.20E-06		
EIF2AK3	eukaryotic translation initiation factor 2-alpha kinase 3	9451		5.13E-06		
RPL21	ribosomal protein L21	6144		1.58E-07		
3WHAQ	tyrosine 3-monooxygenase	10971			9.92E-04	
AATF	apoptosis antagonizing transcription factor	26574			6.16E-05	
AD024	AD024 protein	57405			5.62E-05	
ADSS	aden3losuccinate synthase	159			2.81E-04	
ALG8	asparagine-linked gl3cos3lation 8 homolog (yeast, alpha-1,3-glucos3ltrans	79053			6.12E-04	
ANKTM1	ankyrin-like with transmembrane domains 1	8989			2.44E-03	
APA1	likely ortholog of mouse another partner for ARF 1	57862			7.91E-05	
BCKDHA	branched chain keto acid dehydrogenase E1, alpha polypeptide (maple s3r	593			6.73E-04	
BRIP1	BRCA1 interacting protein C-terminal helicase 1	83990				4.80E-02
C14orf80	chromosome 14 open reading frame 80	283643			5.62E-05	
C20orf30	chromosome 20 open reading frame 30	29058			8.01E-04	
C20orf72	chromosome 20 open reading frame 72	92667			2.09E-03	
C21orf66	chromosome 21 open reading frame 66	94104			2.66E-07	
C3P2F1	cytochrome P450, family 2, subfamily F, polypeptide 1	1572			4.40E-03	
CALU	calumenin	813			3.89E-03	
CASP8	caspase 8, apoptosis-related cysteine peptidase	841				4.12E-02
CASP8AP2	CASP8 associated protein 2	9994			1.39E-03	
CDC25A	cell division cycle 25A	993			2.69E-04	
CDC25A	cell division cycle 25A	993			1.18E-05	
CDC5L	CDC5 cell division cycle 5-like (S. pombe)	988				3.00E-02
CDK5RAP2	CDK5 regulatory subunit associated protein 2	55755			4.86E-03	
CGI-141	CGI-141 protein	51026			1.51E-07	
CHEK1	CHK1 checkpoint homolog (S. pombe)	1111			3.24E-05	
CLCF1	cardiotrophin-like cytokine factor 1	23592				2.05E-02
COPS3	COP9 constitutive photomorphogenic homolog subunit 3 (Arabidopsis)	8533			2.18E-06	
COQ6	coenzyme Q6 homolog (yeast)	51004			4.59E-06	
CPSF2	cleavage and polyadenylation specific factor 2, 100kDa	53981			2.09E-04	
CSTF3	cleavage stimulation factor, 3' pre-RNA, subunit 3, 77kDa	1479			2.62E-03	
CTGF	connective tissue growth factor	1490				4.98E-02
DH240	DEAH (Asp-Glu-Ala-His) box polypeptide 40	79665			7.60E-05	
E1B-AP5	E1B-55kDa-associated protein 5	11100			1.40E-03	
FAM111B	family with sequence similarity 111, member B	374393			1.18E-03	
FHOD1	formin homology 2 domain containing 1	29109			1.17E-03	
FIGNL1	fidgetin-like 1	63979			1.19E-03	
FLJ00005	FLJ00005 protein	57184			1.80E-03	
FLJ10520	hypothetical protein FLJ10520	55159			1.14E-03	
FLJ10579	hypothetical protein FLJ10579	55177			1.98E-03	
FLJ11021	similar to splicing factor, arginine	65117			1.70E-03	
FLJ13912	hypothetical protein FLJ13912	64785			1.50E-09	
FLJ14639	hypothetical protein FLJ14639	84901			3.43E-05	
FLJ14640	hypothetical protein FLJ14640 91442	84902			2.22E-04	

FLJ22301	hypothetical protein FLJ22301	79894			2.16E-03	
FLJ30574	hypothetical protein FLJ30574	130132			9.52E-04	
FLJ31100	hypothetical protein FLJ31100	284306			1.62E-05	
FLJ32783	hypothetical protein FLJ32783	158787			7.83E-04	
FLJ40137	hypothetical protein FLJ40137	124817			1.38E-03	
FUT2	fucosyltransferase 2 (secretor status included)	2524			4.85E-04	
GRIA3	glutamate receptor, ionotrophic, AMPA 3	2892			2.88E-03	
HCAP-G	chromosome condensation protein G	64151			4.14E-03	
HEC	highl3 e2pressed in cancer, rich in leucine heptad repeats	10403			5.28E-05	
HHLA3	HERV-H LTR-associating 3	11147			3.36E-03	
HSPC129	hypothetical protein HSPC129	51496			8.92E-04	
KCNIP4	potassium channel-interacting protein 4	80333			2.53E-09	
KHDRBS1	KH domain containing, RNA binding, signal transduction associated 1	10657			7.65E-06	
KIAA0152	KIAA0152 gene product	9761			8.93E-05	
KIAA1799	KIAA1799 protein	84455			9.32E-06	
KNSL7	kinesin-like 7	56992			1.50E-04	
KNTC1	kinetochore associated 1 9735	9735			1.70E-03	
LIMD1	LIM domains containing 1	8994			4.43E-03	
LOC151648	hypothetical protein BC001339	151648			1.56E-03	
LOC57149	hypothetical protein A-211C6.1 123879	57149			1.76E-03	
MAP2	microtubule-associated protein 2	4133			2.13E-03	
MASA	E-1 enzyme	58478			7.95E-07	
MATR3	matrin 3	9782			2.16E-03	
MGC16733	hypothetical gene MGC16733 similar to CG12113	92105			3.03E-03	
MGC17358	hypothetical protein MGC17358	257169			3.56E-03	
MGC32020	hypothetical protein MGC32020 91442	91442			2.22E-04	
MGC35361	hypothetical protein MGC35361	222234			2.56E-03	
MGC48972	hypothetical protein MGC48972 123879	123879			1.76E-03	
MLH1	mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli)	4292			4.57E-03	
MTHFD1	methylenetetrahydrofolate dehydrogenase (NADP+ dependent), methenyl	4522			1.18E-03	
NASP	nuclear autoantigenic sperm protein (histone-binding)	4678				3.92E-02
NCOA5	nuclear receptor coactivator 5	57727				2.58E-02
NDUFS7	NADH dehydrogenase (ubiquinone) Fe-S protein 7, 20kDa (NADH-coenzyn	374291			5.60E-04	
NUMA1	nuclear mitotic apparatus protein 1	4926			2.07E-03	
OSRF	osmosis responsive factor	23548			3.09E-03	
PABPC5	poly(A) binding protein, cytoplasmic 5	140886			2.35E-05	
PCNA	proliferating cell nuclear antigen	5111				4.02E-02
PCNT1	pericentrin 1	79902			4.65E-06	
PIR51	RAD51-interacting protein	10635			2.32E-03	
PJA2	praja 2, RING-H2 motif containing	9867			1.76E-03	
POLD3	polymerase (DNA directed), delta 3	10714			9.12E-06	
POLR2I	polymerase (RNA) II (DNA directed) polypeptide I, 14.5kDa	5438			1.57E-04	
POLR2K	polymerase (RNA) II (DNA directed) polypeptide K, 7.0kDa [Homo sapiens	5440				3.99E-02
PPARG	peroxisome proliferative activated receptor, gamma	5468				3.95E-02
PPP6C	protein phosphatase 6, catalytic subunit	5537				3.19E-02
PRKCSH	protein kinase C substrate 80K-H	5589			1.62E-03	
PSMD9	proteasome (prosome, macropain) 26S subunit, non-ATPase, 9	5715				4.14E-02
RAB11A	RAB11A, member RAS oncogene family	8766				1.79E-02
RAD9B	RAD9 homolog B (S. cerevisiae) 144715	144715			1.09E-04	
RAVER1	RAVER1	125950			3.23E-04	
RBL1	retinoblastoma-like 1 (p107)	5933			4.21E-03	
RHEB	Ras homolog enriched in brain	6009			9.43E-04	
RHEBL1	Ras homolog enriched in brain like 1	121268			3.31E-03	
RNASE4	ribonuclease, RNase A family, 4	6038			1.58E-04	
RTTN	rotatin	25914			2.24E-03	
S3NGR4	synaptogyrin 4	23546			3.39E-03	
SACM1L	SAC1 suppressor of actin mutations 1-like (yeast)	22908			3.98E-05	
SERPINI2	serine (or cysteine) proteinase inhibitor, clade I (neuroserpin), member 2	5276			3.25E-03	
SFRS5	splicing factor, arginine	6430				1.20E-02
SLBP	stem-loop (histone) binding protein	7884			5.08E-05	
SPAG5	sperm associated antigen 5	10615			4.31E-03	
TOP2A	topoisomerase (DNA) II alpha 170kDa	7153			2.05E-03	
TRAP25	TRAP	90390			1.94E-03	
ZF	HCF-binding transcription factor Zhangfei	58487			1.93E-03	
ST26	syntaxin 6	10228			1.68E-03	
ZNF317	zinc finger protein 317	57693			1.27E-04	
VPS29	vacuolar protein sorting 29 (yeast) 144715	51699			1.09E-04	
TRIM26	tripartite motif-containing 26	7726			8.51E-05	
SSR3	signal sequence receptor, gamma (translocon-associated protein gamma)	6747			4.08E-07	
TIAM1	T-cell lymphoma invasion and metastasis 1	7074			1.65E-07	
T3R	tyrosinase (oculocutaneous albinism IA)	7299			3.00E-08	
TPX2	TPX2, microtubule-associated, homolog (Xenopus laevis)	22974				4.82E-02
TP53	tumor protein p53 (Li-Fraumeni syndrome)	7157				3.66E-02
STIL	SCL	6491				3.23E-02
SRP72	signal recognition particle 72kDa	6731				2.34E-02

Note that for the ChIp-chip performed on the 1k array, the cut-off for the p-value was raised to compensate for the lack of sensitivitly due to the loss of signal caused by high ozone level

Chapter IV - Carboxy-terminal proteolytic processing of CDP/Cux by a Caspase Enables Transcriptional Activation in Proliferating Cells

Preface

This part of my project came from an observation made by Dr. Nam Sung Moon. He had recently shown that p200 CDP/Cux was subject to limited proteolysis, in a cell cycle-regulated manner. In his identification and characterization of p110 CDP/Cux, Nam Sung noticed another isoform in his Western blots that was consistent with proteolytic processing near the carboxy-terminus. He could prevent the appearance of this band with a broad-spectrum caspase inhibitor. Mapping of the processing site revealed that last DNA binding domain, the Cut homeodomain was not cleaved. I reasoned that this C-terminal processing could influence transcriptional regulation by CDP/Cux.

In this chapter, I describe caspase-mediated processing of CDP/Cux in proliferating cells. This proteolytic processing enabled transcriptional activation of cell cycle-regulated promoters by CDP/Cux. This new isoform, p85 CDP/Cux, accelerated entry into S phase following serum starvation. I demonstrated that CDP/Cux is a substrate for caspases in proliferating cells.

ABSTRACT

Proteolytic processing at the end of the G1 phase generates a CDP/Cux isoform, p110, which functions either as a transcriptional activator or repressor and can accelerate entry into S phase. Here we describe a second proteolytic event that generates an isoform lacking two active repression domains in the C-terminus. This processing event was inhibited by treatment of cells with Z-VAD-FMK and by co-transfection of CrmA. *In vitro*, several caspases generated a processed isoform that co-migrated with the *in vivo* generated product. In cells, recombinant CDP/Cux proteins in which the region of cleavage was deleted, or in which D residues were mutated to A, were not proteolytically processed. Importantly, this processing event was not associated with apoptosis, as assessed by TUNEL assay, cytochrome c localization, PARP cleavage, and FACS. Moreover, processing was observed in S phase but not in early G1, suggesting that processing indeed occurs in proliferating cells. The functional importance of this processing event was revealed in reporter and cell cycle assays. A recombinant, processed, CDP/Cux protein was a more potent transcriptional activator of the DNA pol alpha promoter and was able to accelerate entry of Kit225 T cells into S phase, whereas mutants that could not be processed were inactive in either assay. Together our results identify a substrate of caspases in proliferating cells and suggest a mechanism by which caspases may accelerate cell cycle progression.

INTRODUCTION

The caspase family of cysteine proteases contains 14 known members. Caspase substrates have aspartate in the P1 position, and the specificity of recognition by individual caspases is determined by the amino acids in the P2, P3, and P4 positions. Their roles in cytokine maturation and apoptosis have been thoroughly documented (for a detailed review, see (29, 39)). Caspases are expressed as inactive zymogens, and their activation proceeds by one of two mechanisms. Caspase-2, -8, -9, and -10, the initiator caspases, are activated by dimerization. They contain CARD (caspase recruitment domain) or DED (death effector domain) domains, and are brought together via homotypic interactions with upstream molecules. However, caspase-3, -6, and -7, the effector caspases, are present as inactive dimers that are activated by proteolysis (6).

Caspase activity plays a role in the differentiation of a number of tissues, including red blood cells and lens fiber cells. This is often accompanied by some of the morphological changes associated with apoptosis, such as chromatin condensation, and nuclear destruction (15, 23, 45).

An inherited mutation in humans, causing defects in the activation of T, B, and NK (natural killer) cells, was mapped to caspase 8 (9). While targeted disruption of caspase 8 in mice is embryonic lethal, insight into its function in T cells was gained by targeted deletion of caspase 8 in the T cell lineage. This resulted in fewer than normal peripheral T cells, and an inability to mount an immune response following infection with choriomeningitis virus. In addition, ex vivo, the ability of T cells to respond to activation stimuli was impaired (28).

Results from a number of studies suggested that caspases were activated following T cell stimulation with PHA (21), or with IL-2 and mitogens (43), where no evidence of cell death was detected. Levels of cleavage of procaspase 3 into its active form were higher than what is normally observed upon induction of apoptosis (21). This suggested that caspase activity could be regulated following activation. It was later shown that granzyme B, or another aspartate-specific protease, was activated post-lysis, and was responsible for the cleavage of procaspase 3 (44). However, following this study, another group showed that caspase 3 was activated following PHA-stimulation of T cells, even in

the presence of granzyme B inhibitors. Furthermore, IL-2 release from activated T cells was blocked in the presence of peptide-based caspase inhibitors (26).

A non-apoptotic role for FADD in T cell proliferation has been supported by recent work from a number of groups. In mice that lack FADD function, either due to gene ablation, or expression of a dominant negative, T cells are defective in activation-induced proliferation (25, 46). T cells expressing dominant negative FADD arrest at G0/G1 of the cell cycle (25). Mice expressing a FADD mutant, in which serine 191 was mutated to aspartate, were smaller, anemic and presented splenomegaly. No apoptotic defects were found, however, their T cells were defective in cell cycle progression. Interestingly, FADD is differentially phosphorylated throughout the cell cycle, at serine 194, which is equivalent to serine 191 in mice, further suggesting that post-translational modification could modulate its function (33).

Limited proteolysis regulates the activities of many transcription factors. Cleavage of Notch, SREBP, and others results in their translocation to the nucleus (1, 7). Alternatively, limited proteolysis of IRF2, Stats 3, 5, and 6, and C/EBP removes their activation domains (13).

CDP/Cux has recently been identified as a substrate for the cathepsin L cysteine protease in the S phase of the cell cycle. The product of this proteolysis, p110, lacks the amino-terminal inhibitory domain and one cut repeat (CR1) and binds more stably to DNA. p110 is predicted to repress or activate the transcription of a number of G1 or S phase-specific genes, respectively. Putative or confirmed cell cycle targets include p21WAF1, p27, histone H1, H2A, H2B, H3, and H4, DNA polymerase alpha, DHFR, cyclin A2, and CAD (insert refs). Two active repression domains have been mapped downstream of the homeodomain and were shown to be dispensable for the stimulation of the DNA polymerase alpha gene promoter.

Immunoblotting experiments with an antibody that recognizes the N-terminus have revealed a protein of 175 kDa. Interestingly, upon expression of a recombinant CDP/Cux protein corresponding to p110, a shorter isoform was also detected by Western blot with an N-terminal antibody ((40) and Fig 3B, lane 2). These two results suggested that CDP/Cux is also processed near the C-terminus.

In the present study, we show that CDP/Cux is a substrate for another protease. Treatment of cells with a panel of protease inhibitors suggested a caspase was responsible. Using site-directed mutagenesis, we mapped the region of cleavage to one or more aspartate residues downstream of the Cut homeodomain. This cleavage was observed in proliferating cells and was confirmed to occur in the absence of apoptosis. *In vitro* processing assays suggested that CDP/Cux is a substrate for multiple caspases. Interestingly, a truncated recombinant protein was a more potent activator in reporter assays and was also able to accelerate entry into S phase. These results identify a novel caspase substrate that plays a role in cell proliferation.

MATERIALS AND METHODS

Plasmid construction.

Sequences and/or maps will be provided upon request for CDP/Cux 831-1505 Δ1320-1351, Myc-CDP/Cux 878-1505 D1320,36,39A-HA, Myc-CDP/Cux 878-1336, pTriEx/his/Myc1062-1505/TAPtag constructs. All other CDP/Cux constructs have been described in our previous studies (22, 31).

Expression and purification of CDP/Cux fusion proteins.

The pTriEx/his/Myc1062-1505/TAPtag expression plasmid was introduced into the BL21 (DE3) strain of *E. coli* and induced with 1mM IPTG for 1.5 hours. Cleared lysates were resuspended in IPP-100 buffer (10 mM Tris (pH 8.0)/ 100 mM NaCl, 1 mM Imidazole, 1 mM Mg-Acetate, 4.5 mM $CaCl_2$, 10 mM β-mercatoethanol. 10% glycerol, 0.1% NP40) and incubated with calmodulin affinity resin (Stratagene). EGTA was used to elute the purified CDP/Cux containing complexes from the calmodulin column.

Recombinant caspase expression, purification and titration.

Recombinant caspases were expressed in E. coli as C-terminal His-tagged fusion proteins using the pET expression system (Novagen, Madison, WI). Proteins were expressed in the BL21(DE3) E. coli strain (Novagen) and purified by Ni2+-affinity chromatography as previously described (36).

Cell culture and transfection and synchronization. Hs578T cells were grown in DMEM medium supplemented with 5% fetal bovine serum (FBS). NIH3T3 and MCF-7 cells were grown in DMEM medium supplemented with 10% FBS. Kit225 T cells stably expressing CDP/Cux proteins were generated as in (30). Cells were grown in RPMI supplemented with 10% FBS and 75ng/ml human IL-2. Transient transfections were performed with GeneJuice (Novagen) according to the manufacturer's instructions.

Cell cycle synchronization.

Synchronization of NIH3T3 cells was performed by two methods. Serum starvation/stimulation: post-transfection, cells were maintained in DMEM for 72h, followed by 5.5h or 10h or 16h in DMEM plus 10% FBS. Thymidine block: Post-transfection, cells were cultured overnight in DMEM plus 10% FBS supplemented with 2 mM thymidine and harvested. Stably infected Kit225 cells were deprived of IL-2 for 48 hours, followed by readdition for 18 or 25 hours. Cell cycle distribution was monitored by fluorescence-

activated cell sorting following ethanol fixation and propidium iodide staining (30)

Luciferase assay.

Luciferase assays were performed as previously described (22). Because the internal control plasmid is itself often repressed by CDP/Cux, as a control for transfection efficiency the purified β-galactosidase protein (Sigma) was included in the transfection mix, as previously described (14). The luciferase activity was then normalized based on β-galactosidase activity.

CDP/Cux antibodies.

Antibodies 510, 861, 1061, and 1300 have been described previously (12, 22) (Goulet 2002). Antibody c-20 is a goat polyclonal antibody raised against the last 20 amino acids of CDP (sc-6327).

Preparation of nuclear extracts and Western blot analysis.

Nuclear extracts were prepared according to the procedure of Lee et al., except that nuclei were obtained by submitting cells to 3 freeze/thaw cycles in buffer A (10 mM Hepes, pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM DTT) (16). Nuclei were then resuspended in Buffer C (20 mM Hepes, pH 7.9, 25 % glycerol 1.5mM $MgCl_2$, 420 mM $NaCl_2$, 0.2 mM EDTA.) and incubated at 4 °C for 30 min. After 15 min. of centrifugation, the supernatant was collected. Buffers A and C were supplemented with protease inhibitor mix tablet purchased from Roche. Total extracts were prepared by applying buffer X (50 mM Hepes, pH 7.9, 0.4 M KCl, 4 mM NaF, 4 mM Na_3VO_4, 0.2 mM EGTA, 0.2 mM EDTA, 0.1% NP-40, 10 % glycerol, 0.5 mM DTT, Protease inhibitor mix tablet from Roche) to a monolayer plate. After 10 min. incubation on ice, the resulting slurry was centrifuged for 15 min at 4 °C and the supernatant was collected.

After electrophoretic transfer to PVDF, membranes were washed in Tris-buffered saline-0.1% Tween-20 (TBS 0.1%T) followed by incubation in TBS 0.1%T, 5% milk, for 1 hour. Antibodies were diluted in TBS 0.1%T and incubated for 1.5 hours followed by 4 X 10 minute washes in TBS 0.1%T. Horseradish peroxidase (HRP) conjugated α-rabbit (1/4000) or α-mouse secondary antibody (1/10 000) was diluted in a solution of TBS 0.1% T blots. Immuno-reactive proteins were visualized by chemiluminescence with ECL Western Blotting Detection Kit (Amersham Pharmacia Biotech).

Protease inhibitors.

Protease inhibitors were purchased from Calbiochem and used at the following working concentrations: 20 μM EST, 40 μM MG132, 100 μM Z-VAD-FMK, 100 μM DEVD-CHO, 100 μM Pepstatin A.

Electrophoretic Mobility Shift Assay (EMSA).

EMSA was performed with the indicated quantity of purified protein. Samples were incubated at room temperature for 20 minutes in a final volume of 30 μl of 25 mM NaCl, 10 mM Tris, pH 7.5, 1 mM MgCl2, 5 mM EDTA, pH 8.0, 5% glycerol, 1 mM DTT, 3 μg BSA with 0.2 pmol of radiolabeled oligonucleotides. Samples were loaded on a 5% polyacrylamide (29:1), 0.5X TBE gel and separated by electrophoresis at 8V/cm in 0.5X TBE. Gels were dried and visualized by autoradiography.

In vitro proteolytic processing assay.

In vitro cleavage reactions were performed using 50mM Hepes pH 7.2, 50mM NaCl, 0.015% CHAPS, 1M sodium citrate, 10mM DTT (for caspase-2,3,8,9,10) or 20mM PIPES pH 7.2, 100mM NaCl, 10mM DTT, 1mM EDTA, 0.1% CHAPS, 10% sucrose (for caspase-6 and –7). Briefly, caspases were activated at 37C for 10 minutes prior to the addition of 65 nM purified CDP protein. Reactions were carried out at 37C for 20 minutes, after which loading buffer was added and samples were boiled.

TUNEL assay.

TUNEL assay for free DNA 3'OH ends was performed as per manufacturers directions - ApopTag kit #S7110 (Serologicals).

Immunofluorescence

NIH3T3 cells were plated on cover slips and transfected or not (see figure legends). Cells were fixed with 2% paraformaldehyde then solubilized (95% PBS + 5% FBS + 0.5% Triton-X-100) and incubated with primary antibodies αMyc9e10 (1/3000), Falloidin, αcytochrome c, or αTom-20. Secondary antibody (anti-mouse alexa-488 1:1000 or anti-rabbit alexa-594) was added for 30 minutes. Cells were visualized using a Zeiss AxioVert 135 microscope with a 63X objective or using a Zeiss LSM 510 confocal microscope

RESULTS

CDP/Cux is proteolytically processed downstream of the homeodomain in proliferating cells.

CDP/Cux proteins with different N-terminal truncations were expressed in Hs578T cells. Western blot analysis of nuclear extracts using an antibody against the N-terminal Myc epitope tag revealed isoforms, shorter than the parental molecule by 20-30 kDa, suggesting that CDP/Cux was proteolytically processed at its C-terminus (Fig 1A). We next asked whether this proteolytic event was cell cycle regulated. NIH3T3 cells were transfected with a vector expressing Myc-CDP/Cux 878-1505 and were then synchronized in early G1, mid-G1 and S phases by serum deprivation and restimulation or thymidine block (see Materials and Methods). Nuclear extracts were prepared and analyzed by Western blot with the Myc antibody (Fig. 1B). The C-terminally cleaved protein was barely visible in early G1 and mid-G1, but was strongly expressed in unsynchronized cells and in S phase using either method of synchronization. Importantly, the leftmost portion of the FACS profile did not reveal the presence of apoptotic cells at those time points where processing was observed (Fig. 1B; also see below, Fig. 3B). These results showed that CDP/Cux is C-terminally truncated in proliferating cells.

Evidence for the existence of an amino- and carboxy-terminally truncated CDP/Cux isoform.

We then verified whether an endogenous CDP/Cux protein lacking both the N- and C-terminus could be detected. Hs578T cells were labeled overnight with ^{35}S Met and Cys. Whole cell extracts were immunoprecipitated with 861 and 1300 antibodies or, as a control, an HA antibody (Fig. 1C). Following autoradiography, the p200 and p110 CDP/Cux isoforms were detected, as well as an 85 kDa isoform that could be the product of processing events at the N and C-termini. In order to address this possibility and to attempt to map the epitopes present in the 85 kDa isoform, nuclear extracts were immunoprecipitated with a series of CDP/Cux antibodies, followed by Western blotting with the 1300 antibody (Fig. 1D). As expected, p200 was immunoprecipitated by all of the antibodies (Fig. 1D, lanes 1-5). In contrast, p110 was detected following immunoprecipitation with antibodies 861, 1061 and 1300, but not with 510 and only weakly with C-20

(Fig. 1D, lanes 1-5). The 80-85 kDa species was immunoprecipitated with antibodies 861, 1061 and 1300, but not with 510 or C-20 (Fig. 1D, lanes 1-5). These results are consistent with the notion that an endogenous 85 kDa CDP/Cux species is truncated at both its C and N-termini. Hereafter in the text, this species will be called p80.

A caspase cleaves CDP/Cux in the C-terminal region.

To determine which protease(s) cleaves the CDP/Cux C-terminus, NIH3T3 cells were transfected with a vector expressing Myc-CDP 878-1505-HA and, following treatment with a series of protease inhibitors, nuclear extracts were analyzed by Western blot with the Myc antibody (Fig. 2A). Proteolytic processing was partially inhibited by treatment with Z-VAD-FMK, a broad-spectrum caspase inhibitor, but not by any of the other inhibitors tested (Fig. 2A, compare lane 5 with the others). Inhibition by Z-VAD-FMK was also observed in transfected MCF-7 and Kit225 T cells stably expressing Myc-CDP 747-1505-HA (Fig. 2A, compare lanes 9 and 13 with 10-12 and 14). Inhibition of processing by Z-VAD-FMK and the absence of inhibition by E-64d and MG132 implicated a cysteine protease and discriminated against cathepsins B and H. Indeed, co-transfection of a vector expressing a viral caspase inhibitor variant with broad-range caspase inhibition, $CrmA^{DQMD}$, inhibited C-terminal processing (Fig. 2B). Expression of a recombinant CDP/Cux 831-1505 protein generated a processed species that migrated close to the recombinant CDP/Cux 831-1336 protein (Fig. 2C, lanes 1-2) Examination of the amino acid sequence in this region revealed the presence of four Asp residue: D1320, D1336, D1339, D1351 (Fig. 2E). Deletion of amino acids 1320 to 1351 prevented proteolytic processing (Fig. 2C, compare lanes 2 and 3). Moreover, replacement of 1, 2 or 3 Asp residues with alanine reduced or eliminated proteolytic processing (Fig. 2D). Altogether, these results suggest that CDP/Cux is processed by a caspase in the region downstream of the Cut homeodomain.

CDP/Cux is processed in non-apoptotic conditions.

To ensure that processing was not occurring post-lysis (11), Z-VAD-FMK was added to the cells 5 minutes prior to lysis and was included in all buffers used in subsequent steps. The processed isoform was still observed in Western blot analysis (data not

shown). We next asked whether there was any evidence of apoptosis in cells in which processing is observed. No evidence of membrane blebbing or abnormal cellular morphology was observed upon actin staining (Fig. 3A, and data not shown). No apoptotic population was observed in flow cytometry profiles of cells stained with propidium iodide (Fig. 3B). Using the TUNEL assay, we examined 250 Myc-positive cells and found that none of these transfected cells was TdT-positive (Fig. 3C). In contrast, TdT-positive cells were easily detected following five hours of treatment of NIH3T3 cells with TNFα and cycloheximide (Fig. 3C). Protein extracts from transfected cells (see Fig. 2B) were immunoblotted for the caspase substrate, PARP, but cleavage products were not detected (Fig. 3D). Another indicator of apoptosis, the release of cytochrome c from the mitochondria, was only observed upon induction of apoptosis with TNFα and cycloheximide (Fig. 3E). Altogether, these results confirmed that proteolytic processing of CDP/Cux was occurring in non-apoptotic cells.

CDP/Cux is a substrate for caspases *in vitro*.

A C-terminally epitope-tagged CDP/Cux protein was produced in bacteria and affinity purified on calmodulin beads. The approximate concentration of substrate was determined by comparing Coommassie staining to BSA standards (data not shown). 66 nM of substrate, which is below the predicted Km of 100 nM (36) and therefore allows the observation of first-order kinetics, was incubated with increasing amounts of a panel of activated titrated recombinant caspases for 30 minutes at 37°C. Western blot analysis with a Myc antibody revealed that CDP/Cux was a substrate for caspases-2, -3, -7, -8, -9, and –10, but not for caspase-6 (Fig. 4). A recombinant protein truncated at 1336 co-migrated with the caspase cleavage product (Fig. 4), confirming that cleavage happens at or near Asp1336, like it does *in vivo* (Fig. 4, 2C and 2D). C-terminal processing was observed in MCF-7 cells, which lack caspase-3, and caspase-8-null Jurkat cells (data not shown). These findings indicate that other caspases must cleave CDP/Cux in these cells, but the involvement of caspases -3 and -8 in other cells cannot be excluded. Altogether, these results confirm that CDP/Cux is a substrate for one or more caspase(s).

A CTD-truncated CDP/Cux isoform stimulates the DNA pol α gene promoter.

Functional assays were performed to investigate the consequence of C-terminal processing. The Myc-CDP 878-1505-HA construct was introduced into Hs578T cells and nuclear extracts were prepared from cells treated or not with Z-VAD. As predicted, this treatment inhibited the production of the processed isoform (Fig. 5A). Electrophoretic mobility shift assays with a consensus CDP/Cux binding site demonstrated that the processed isoform was able to bind to DNA (Fig. 5B, lane 3, p80). Note that while the p110-retarded complex was supershifted by both the Myc and HA antibodies, the p80-complex was shifted by the Myc antibodies, but not by the C-terminal HA antibodies (Fig. 5B, lanes 1 and 2). In previous studies, the p110 isoform was shown to function as a transcriptional activator of the DNA polymerase alpha gene promoter (22, 32, 40, 41). However, in retrospect, we realized that the p110 isoform was always expressed together with its C-terminally processed isoform (see for example Fig. 3B in (40)). The question arose, therefore, as to which of the two isoforms was able to transactivate this promoter, especially that proteolytic processing in the C-terminal region results in the removal of two active repression domains (17, 18). The availability of mutants that are defective in this processing event enabled us to compare the transactivation potential of both the processed and unprocessed p110 isoforms. Whereas both the recombinant CDP/Cux 878-1505 and 878-1336 proteins strongly stimulated the DNA pol alpha reporter, the two mutants that are not C-terminally processed, 878-1505$^{\Delta 1320\text{-}1351}$ and 878-1505^{D1336,1339A}, were unable to transactivate this reporter (Fig. 5C-F). While 878-1336 strongly stimulated the cyclin A2, CAD, DHFR, and B-myb reporters, the 878-1505^{D1336,1339A} had little effect, if any (Fig. 5G). Importantly, the mutant 878-1505$^{\Delta 1320\text{-}1351}$ was able to repress the p21$^{WAF1/CKI}$ gene reporter, a finding that confirms that the protein was able to bind to DNA and carry transcriptional regulation (Fig. 5C). We have recently shown that CDP/Cux cooperates with E2F transcription factors in the transcriptional activation of cell cycle-regulated genes (Truscott et al., manuscript submitted). 878-1336 cooperated with E2F1 in the transactivation of the DNA pol alpha reporter. However, no cooperation was observed between 878-1505$^{\Delta 1320\text{-}1351}$ and E2F1 (Fig. 5H), suggesting that it is p80 and not p110, which forms a complex with E2F1 on the DNA pol alpha promoter, and mediates transcriptional

activation. Altogether, these results demonstrate that only the C-terminally processed isoform is able to transactivate the DNA polymerase alpha reporter.

Overexpression of a truncated CDP/Cux protein accelerates entry into S phase.

In cell-based assays, the p110 isoform was previously shown to stimulate cell cycle progression by accelerating entry into S phase (30). Again, the availability of mutants that are less efficiently processed enabled us to investigate the cell cycle activities of the distinct isoforms. We used the IL-2-dependent Kit 225 T cell line and generated populations of cells stably carrying a retroviral vector expressing either nothing, or the CDP/Cux 747-1505, 747-1336 or 747-1505^{D1320,1336,1339A} proteins. Cell cycle progression assays were performed three times with similar results. A representative example is shown in Fig. 6A. Following IL-2-starvation and restimulation, the control Kit 225 cells started to enter into S phase after 18 hours (Fig. 6A). Expression of CDP/Cux 747-1505 stimulated proportionally more cells were in S phase at 18 hours (Fig. 6A). These results are consistent with those from a previous study (30). In contrast, cells expressing the 747-1505^{D1320,1336,1339A} protein progressed just like the control cells (Fig. 6A). These results suggest that it is the truncated isoform of CDP/Cux, generated from p110, that stimulates entry into S phase.

DISCUSSION

The DNA polymerase alpha promoter, as well as other S phase promoters, was efficiently activated by a C-terminally truncated p80 CDP/Cux isoform, but not significantly by a mutant p110 CDP/Cux protein that was not C-terminally processed (Fig. 5). Furthermore, C-terminal processing occurs as cells re-enter the cell cycle following serum deprivation-induced quiescence (Fig. 1B), which correlates with timing of transcriptional activation of the DNA pol alpha promoter (ref Wang). Moreover, p80 accelerated the entry of quiescent T cells into S phase, while cells expressing $p110^{D1320,36,39A}$ entered S phase at a rate similar to vector control cells (Fig. 6). These results are consistent with a model whereby C-terminal processing converts CDP/Cux from a transcriptional repressor to an activator of cell cycle genes in proliferating cells. The mechanism by which CDP/Cux activates transcription has not yet been fully elucidated. Transcriptional activation of S phase genes requires cooperation with E2F transcription factors (Truscott et al., manuscript submitted). E2F1 cooperated with p80, but not with $p110^{\Delta 1320\text{-}1351}$, in the activation of the DNA polymerase alpha reporter (Fig. 5H). Indeed, proteolytic processing downstream of the homeodomain results in the removal of two active repression domains, which would not be expected to hinder its role as a transcriptional activator. The loss of ability to recruit histone deacetylase, or methyltransferase activity would be consistent with the loss of repression. While, derepression is not synonymous with true activation, the C-terminal active repression domains could impose steric hindrance on the recruitment to a subset of gene promoters, and the formation of a transcriptional activation complex.

In opposition to this model, an uncleavable p110 CDP/Cux protein was able to stimulate expression from the CAD and B-Myb promoters, albeit to a lesser extent than p80 CDP/Cux. This would suggest that p110 and p80 can both function as transcriptional activators. However, the observed stimulation of transcription by $p110^{D1320,36,39A}$ could actually be mediated by the small portion of the p110 molecules that is still proteolytically processed even with the mutation of three of the four D residues to A (see Fig. 2D). Alternatively, forced expression of a CDP/Cux protein that contains the same DNA binding domains, and overall is similar to p80 could function as a mild activator in this

context. We have detected transcriptional activation of an N-Myc reporter construct by $p110^{D1320,36,39A}$ (Leduy and Nepveu, unpublished observations), which suggests that p110 could function as a transcriptional activator of a different subset of promoters. Therefore, transcriptional activation by different CDP/Cux isoforms could be context-dependent.

Chromatin immunoprecipitation (ChIP) in combination with expression analysis is required to determine conclusively whether a transcription factor regulates the expression of a target gene. In order to discriminate between which isoform(s) regulates a gene promoter, antibodies specific for each isoform are required. Attempts to make an antibody that recognizes the neo-epitope generated as a result of caspase-mediated processing were unsuccessful. However, it is possible that both p110 and p80 interact with cell cycle-regulated gene promoters in different contexts, therefore it would be difficult to assign a particular role to each factor. As such, there is not one assay, but rather a combination of different techniques, that will determine the transcriptional activity of the p80 and p110 isoforms of CDP/Cux.

Caspase activity plays a role in the differentiation of a number of tissues. These processes are often accompanied by some of the morphological changes associated with apoptosis, such as chromatin condensation, and nuclear destruction (15, 23, 45). Proteolytic processing of interleukin-1B by caspase-1 occurs in inflammation, and does not result in apoptosis. Similarly, caspase activity is necessary for proper spermatid differentiation in drosophila (ref Huh, and other papers). Finally caspases play roles in the activation and proliferation of lymphocytes (insert refs).

A role for caspase 8 in the proliferation of immune cells has been well documented. Humans with mutations in the caspase 8 gene have impaired proliferation of T, B, and natural killer (NK) cells. While gene ablation of caspase 8 in mice produced an embryonic lethal phenotype, conditional ablation in the T cell lineage resulted in normal thymocyte development, yet fewer peripheral T cells, and impaired activation-induced proliferation. However, the mechanism by which caspase 8 affects immune cell proliferation is not fully understood. Caspase 8 was shown to induce the nuclear translocation of

NF-κB, in a manner dependent on its catalytic activity. In this same study, low levels of caspase activity were detected in stimulated human T cells. However, substrates cleaved by caspase 8 under these conditions were not identified (37).

How caspases can cleave some substrates, and yet not others, is also an important question. The subcellular localization of the caspase and/or its substrate could determine whether or not cleavage could occur. An alternative explanation would be via a post-translational modification of the caspase, its substrate, or a regulator of caspase activity.

While autocatalytic cleavage, which results in the removal of the prodomain, is necessary to sustain catalytic activity, procaspase 8 dimers can be catalytically competent (5). One could then speculate that the activity of caspase 8, and possibly other initiator caspases, could be modulated at the level of dimerization. Non-apoptotic activity could be mediated by dimerized proforms, which could then be subsequently shut off by disrupting dimerization, which would also prevent the cleavage of targets that play roles apoptosis. Interestingly, FADD, which facilitates the dimerization of caspase 8 and caspase 10, was shown to be required for cell cycle entry, and its activity is regulated in a cell cycle-dependent manner (ref 25 and ref 32 from 1.6b). In addition, the activity of effector caspases is commonly measured by Western blot detection of cleaved, activated forms. However, once activated, caspases are difficult to detect, as they are rapidly degraded (38). Therefore, while there are standard methods used to detect caspase activity in apoptotic conditions, these methods may not detect lower levels of caspase activity in other conditions.

In this study, we characterized the carboxy-terminal proteolytic processing of the CDP/Cux transcription factor. Treatment of cells with Z-VAD-FMK and co-transfection of CrmA prevented C-terminal processing, as did the removal or replacement of aspartate residues in the region of proteolysis (Fig. 2). The *in vitro* proteolysis observed for caspases-2, -3, -7, –8, –9, and –10 proceeded with relative efficiency (k_{cat}/K_m in the 10^{-7} M^{-1} sec^{-1} range) and corresponded to that of known caspase substrates, such as PARP

whose k_{cat}/K_m is in the 10^{-6} M^{-1} sec^{-1} range) (Fig. 4, data not shown, and (27)). That cleavage was significantly reduced but not entirely prevented by mutation of D residues near the site of proteolysis suggests that the sequence around the P_1 Asp is not overly specific and that when one Asp is mutated, another Asp becomes the P_1, as has been shown for PARP and Huntingtin (27).

None of our results point to one caspase in particular, as being the caspase that cleaves CDP/Cux. *In vitro*, all caspases tested but one, caspase 6, processed CDP/Cux. However, C-terminal processing was observed in caspase 3-deficient MCF-7 cells and caspase 8-null Jurkat cells. Therefore, *in vivo*, processing of CDP/Cux is not performed exclusively by caspase 3 or caspase 8. However, we cannot rule out the possibility that these caspases process CDP/Cux in some cells and not in others. *In vitro*, caspases are not subject to the effects of post-translational modification and subcellular localization, therefore it is possible that not caspases 1,2,3,7,8,9, and 10 don't all process CDP/Cux *in vivo*. As CDP/Cux is a nuclear transcription factor, we believe that proteolytic processing occurs in the nucleus. Evidence exists for the presence of caspases 1, 2, 3, 6, 7, 8, and 9 in the nucleus (3) (8, 19, 24, 34, 35, 47) (4, 10). In some of these cases, nuclear localization was observed in apoptotic cells with high levels of caspase activity. However, as discussed above, traditional methods are likely to miss the detection of active caspases, so it is likely that lower levels of active caspases are located in the nucleus in conditions beyond those tested to date. At this time, it is not clear which caspase(s) cleave CDP/Cux. As this cleavage event would give cells an advantage, whether it is at the level of survival or proliferation, we predict that CDP/Cux is a substrate for more than one caspase.

In addition, caspases have been implicated in the regulation of cell motility. Treatment of NIH3T3 cells with a caspase inhibitor prevented cell spreading and migration (42). It was also shown that stimulation of the CD95/Fas death receptor in apoptosis-resistant tumour cells induced increased motility and invasiveness. This was shown to involve activation of caspase-8 (2). Interestingly, the stimulation of CDP/Cux activity downstream of TGFβ was associated with increased migration and invasion. Furthermore, CDP/Cux regulated the expression of genes with functions in cell motility, and in-

vasion (20). One could speculate that caspase-mediated processing of CDP/Cux would stimulate its function in transcriptional activation, allowing upregulation of genes involved not only in cell cycle progression but also in cell migration and invasion.

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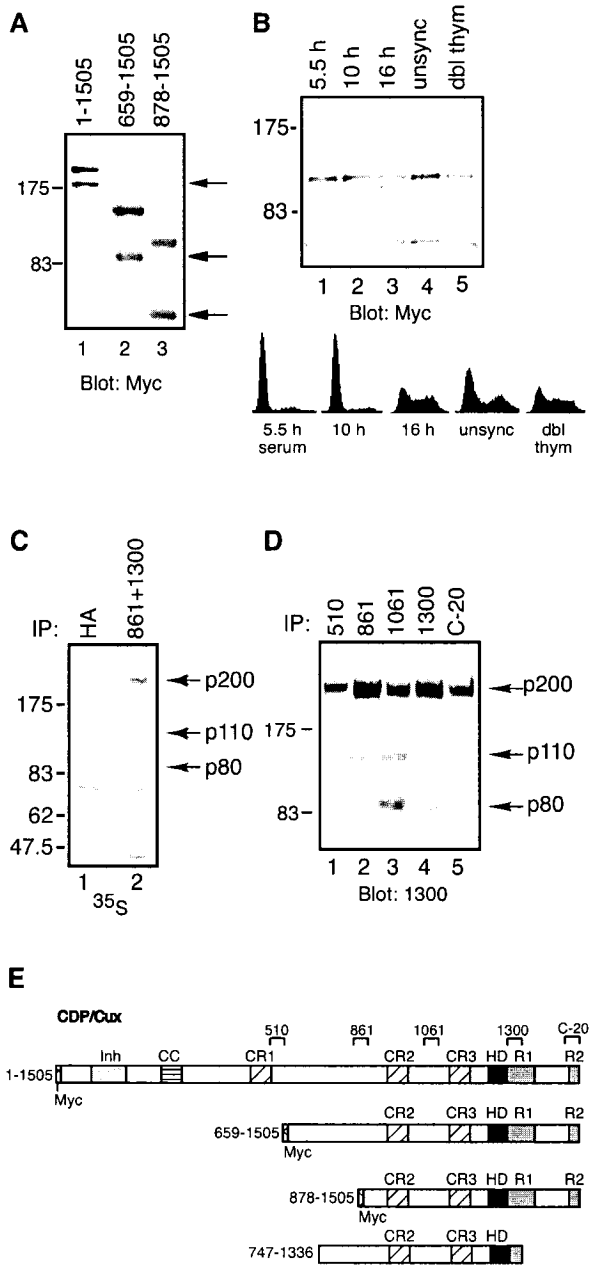
FIGURE LEGENDS

Fig. 1. CDP/Cux is proteolytically processed downstream of the homeodomain in proliferating cells. (A) Hs578T cells were transfected with vectors expressing recombinant CDP/Cux proteins with a Myc epitope tag at their N-terminus. Nuclear extracts were prepared and analyzed by Western blot with a Myc antibody. Arrows indicate cleaved isoforms. (B) NIH3T3 cells were transfected with pXJ/MCH878-1505 and synchronized by serum starvation and restimulation or thymidine block. Cells were harvested and cell cycle distribution was monitored by fluorescence-activated cell sorting (FACS) analysis after staining of the DNA with propidium iodide. Nuclear extracts were analyzed by Western blot with the Myc antibody. (C) Hs578T cells were grown overnight in medium containing ^{35}S-labeled Met and Cys. Whole cell extracts were immunoprecipitated with the indicated antibodies, run on SDS-PAGE and exposed to film overnight. (D) Nuclear extracts from Hs578T cells were immunoprecipitated with the antibodies indicated, and analyzed by Western blot with the 1300 antibody. (E) A schematic representation of CDP/Cux proteins.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1-1505
659-1505
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175-
83-
1 2 3
Blot: Myc
B
5.5 h
10 h
16 h
unsync
dbl thym
175-
83-
1 2 3 4 5
Blot: Myc
5.5 h serum
10 h
16 h
unsync
dbl thym
C
IP:
HA
861+1300
p200
p110
p80
175-
83-
62-
47.5-
1 2
35S
D
IP:
510
861
1061
1300
C-20
p200
p110
p80
175-
83-
1 2 3 4 5
Blot: 1300
E
CDP/Cux
510
861
1061
1300
C-20
Inh
CC
CR1
CR2
CR3
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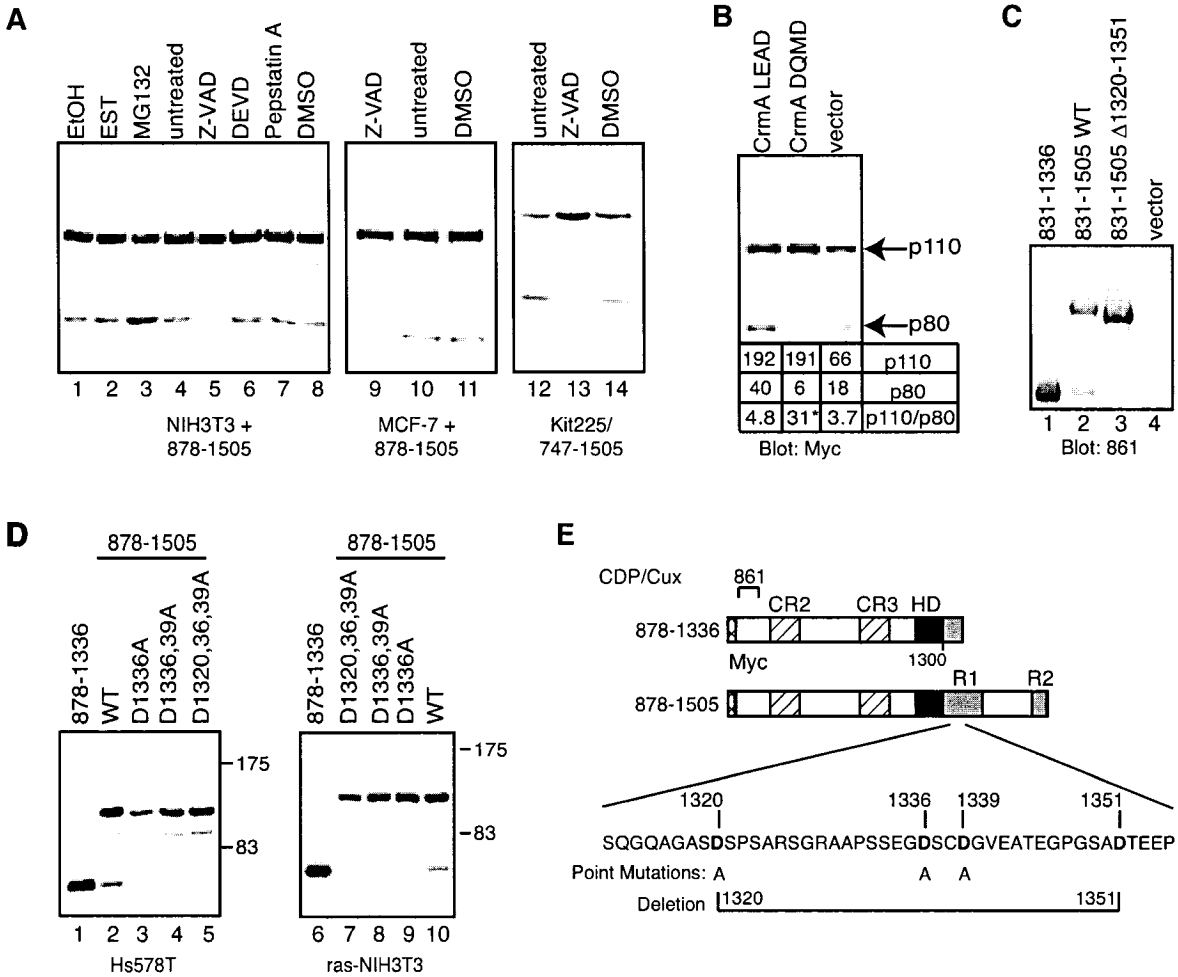
R1
R2
1-1505
Myc
659-1505
878-1505
747-1336

Fig. 2 C-terminal proteolytic processing can be inhibited by caspase inhibitors. (A) NIH3T3 and MCF-7 cells transiently transfected with a vector expressing Myc-CDP878-1505, or Kit225 cells stably expressing Myc-CDP747-1505 were treated with protease inhibitors as indicated. Nuclear extracts were analyzed by Western blot with a Myc antibody. (B) Hs578T cells were co-transfected with a pXJ plasmid expressing a Myc-tagged CDP/Cux protein and a plasmid expressing either nothing or the indicated CrmA mutant. Western blot analysis was performed on nuclear extracts 48h post-transfection. (C and D) Hs578T cells were transfected with vectors expressing the 878-1336 or 878-1505 CDP/Cux proteins, either wild type or with the indicated mutations: deletion of residues 1320-1351, replacement of Asp 1320, 1336 and/or 1339 with alanine. Nuclear extracts were analyzed by Western blot with a Myc antibody. (E) A schematic representation of recombinant CDP/Cux proteins CDP/Cux proteins in which the indicated Asp residues were mutated to Ala were expressed and analyzed by Western blot as indicated.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EST
MG132
untreated
Z-VAD
DEVD
Pepstatin A
DMSO
Z-VAD
untreated
DMSO
untreated
Z-VAD
DMSO
1 2 3 4 5 6 7 8
9 10 11
12 13 14
NIH3T3 + 878-1505
MCF-7 + 878-1505
Kit225/ 747-1505
B
CrmA LEAD
CrmA DQMD
vector
p110
p80
192 191 66 p110
40 6 18 p80
4.8 31* 3.7 p110/p80
Blot: Myc
C
831-1336
831-1505 WT
831-1505 Δ1320-1351
vector
1 2 3 4
Blot: 861
D
878-1505
878-1505
878-1336
WT
D1336A
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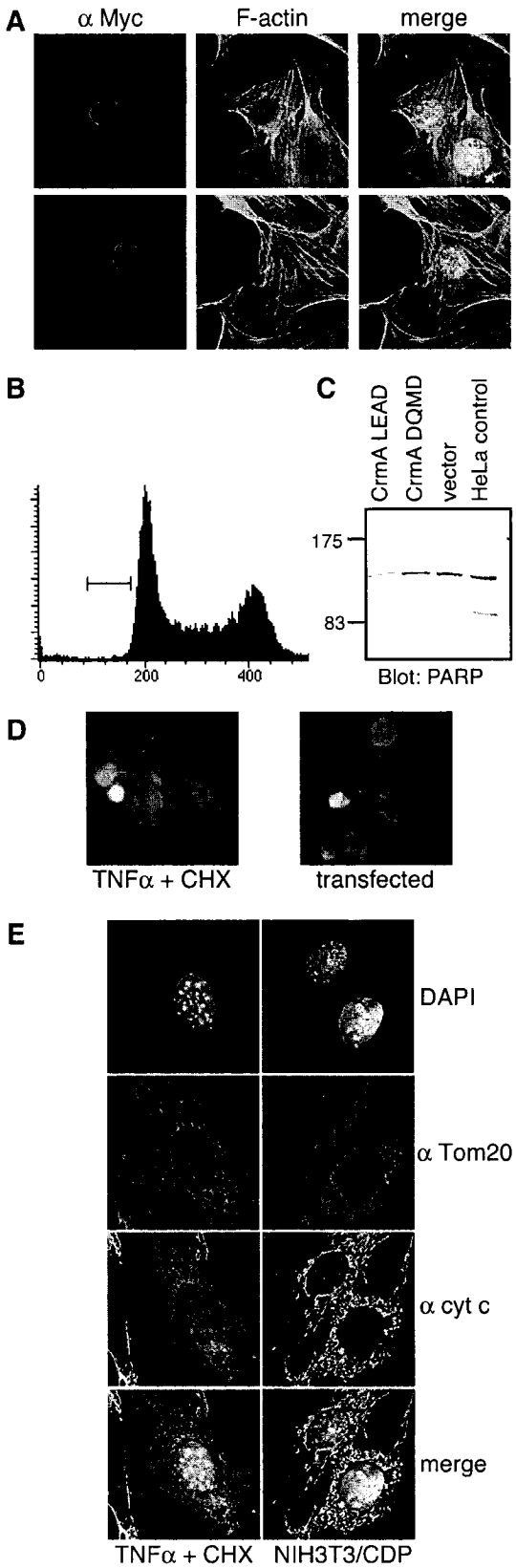
ras-NIH3T3
E
CDP/Cux
861
CR2
CR3
HD
878-1336
Myc
1300
R1
R2
878-1505
1320
1336
1339
1351
SQGQAGASDSPSARSGRAAPSSEGDSCDGVEATEGPGSADTEEP
Point Mutations: A A A
Deletion 1320 1351

Fig. 3 CTD processing occurs in non-apoptotic conditions. (A) NIH3T3 cells were transfected with a construct expressing Myc-CDP878-1505. Immunofluorescence was performed with antibodies that recognize transfected CDP (Myc) and F-actin (Phalloidin). (B) Hs578T cells were transfected with Myc-CDP 878-1505. The following day, cells were fixed and stained with propidium idodide and the DNA content was analyzed. The bar indicates where cells with sub-2N content would be found. (C) NIH3T3 cells were plated on coverslips and half were transfected with a construct expressing Myc-CDP878-1505. The following day, the untransfected sample was treated with TNFalpha and cycloheximide for 5 hours. Cells were fixed and stained with DAPI (blue), Myc (red), or free 3'OH (green). (D) Extracts from Fig. 2B were analyzed by Western blot with an anti-PARP antibody. (E) NIH3T3 cells were plated on coverslips and transfected or not with a construct expressing Myc-CDP878-1505. The following day, the untransfected sample was treated with TNFalpha and cycloheximide for 5 hours. Cells were fixed and stained with DAPI (blue), for mitochondrial marker (Tom20, red) or for cytochrome c (green). In parallel, coverslips with transfected cells were stained with a Myc antibody (data not shown). In the transfected, untreated sample, 3 of 155 cells (1.9%) had no mitochondrial cytochrome c staining.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α Myc
F-actin
merge
B
0
200
400
C
CrmA LEAD
CrmA DQMD
vector
HeLa control
175
83
Blot: PARP
D
TNFα + CHX
transfected
E
DAPI
α Tom20
α cyt c
merge
TNFα + CHX
NIH3T3/CDP

Fig. 4 CDP/Cux is cleaved by caspases-2,3,7,8,9,10 *in vitro*. Titrated activated caspases were incubated with 66 nM Myc-CDP 1062-1505/CBP/protA that had been affinity purified from bacteria with calmodulin beads. Western blot analysis was performed with a Myc antibody. Extracts from Hs578T cells transfected with CDP/Cux 1062-1336 were run to compare to *in vitro* cleavage products.

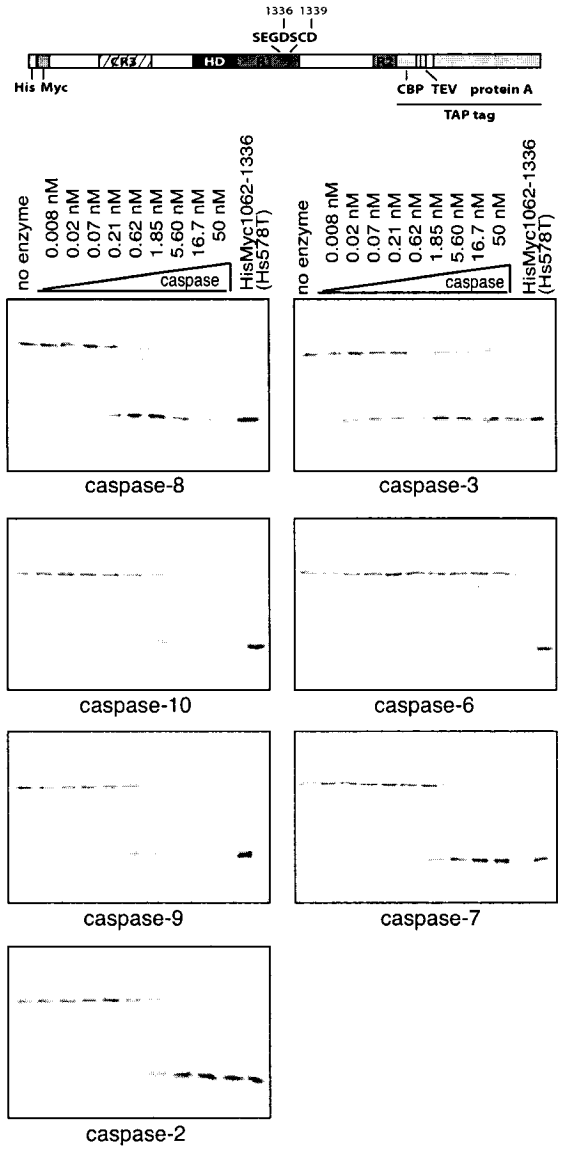
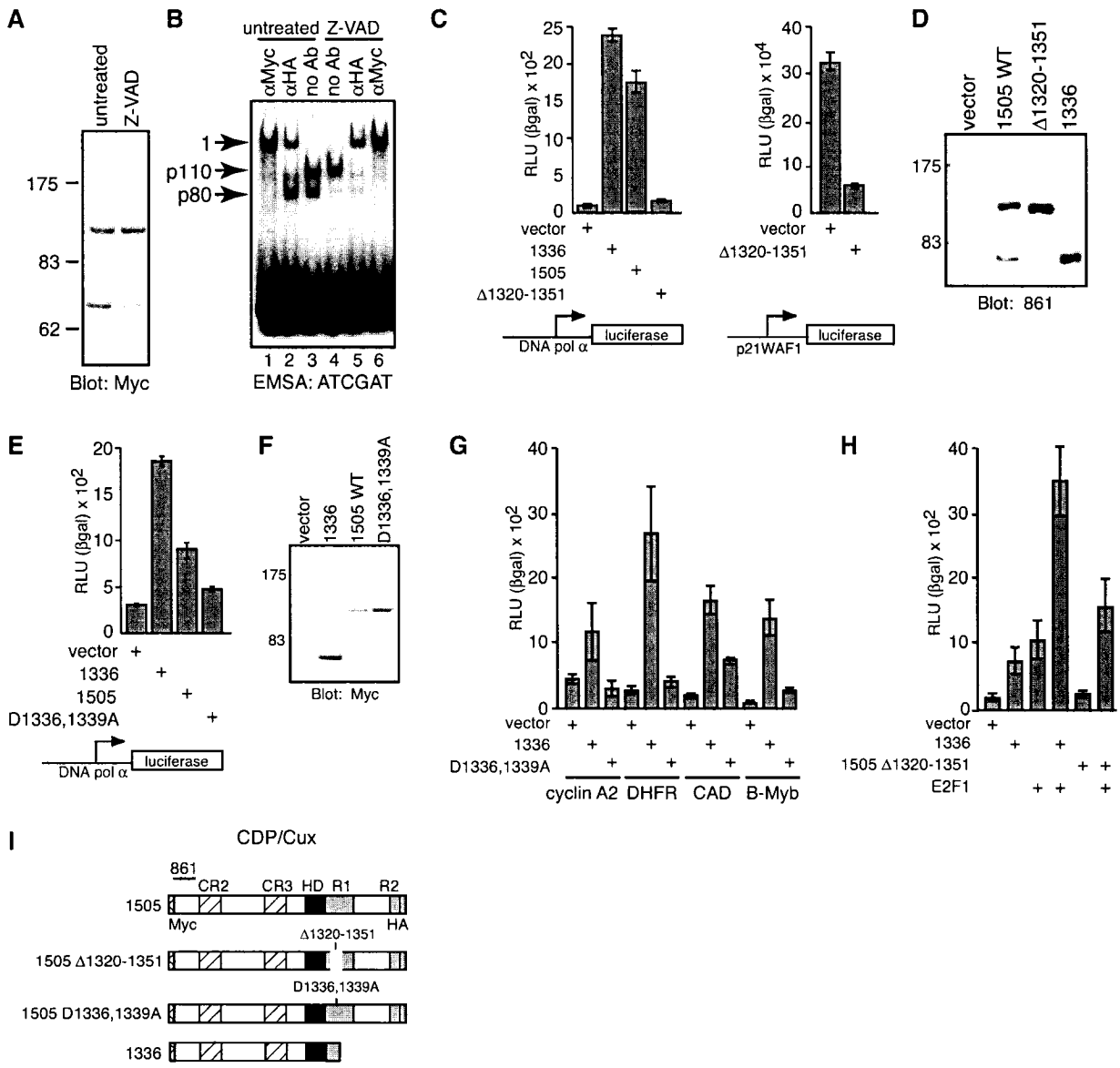

1336 1339
SEGDSCD
HD
His Myc
CBP TEV protein A
TAP tag
no enzyme
0.008 nM
0.02 nM
0.07 nM
0.21 nM
0.62 nM
1.85 nM
5.60 nM
16.7 nM
50 nM
caspase
HisMyc1062-1336 (Hs578T)
caspase-8
caspase-3
caspase-10
caspase-6
caspase-9
caspase-7
caspase-2

Fig. 5 C-terminal proteolytic processing is necessary for transcriptional activation of the DNA polymerase alpha gene promoter. (A) Hs578T cells were transfected with Myc-CDP 878-1505-HA, followed by treatment with Z-VAD for 6 hours prior to harvest. Nuclear extracts were analyzed by Western blot with a Myc antibody. (B) Nuclear extracts from (A) were used in EMSA. Supershift analysis was performed with the indicated antibodies. (C and D) Hs578T cells were cotransfected with the DNA polymerase alpha/luciferase reporter construct or a p21 reporter, and the indicated vectors expressing CDP/Cux. Cytoplasmic extracts were prepared and processed to measure luciferase activity. The mean of 3 transfections is shown and the results are expressed as relative light units (RLU) normalized to β-galactosidase activity from an internal control. Western blot analysis was performed with nuclear extracts made from parallel transfections. (E) An illustration of the CDP/Cux constructs used.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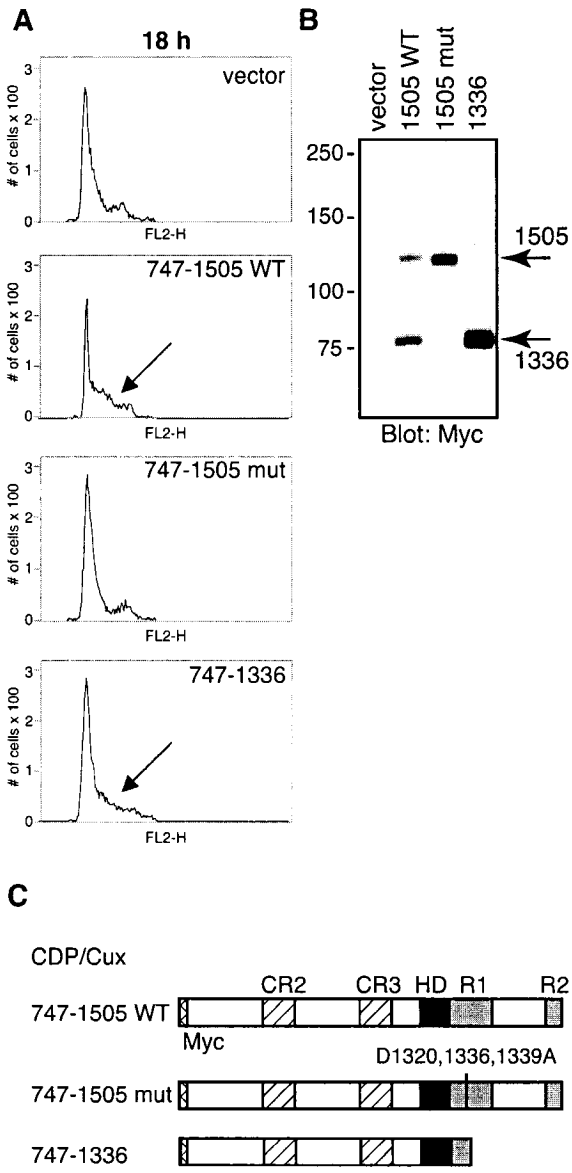
untreated
Z-VAD
175
83
62
Blot: Myc
B
untreated
Z-VAD
αMyc
αHA
no Ab
no Ab
αHA
αMyc
1
p110
p80
1 2 3 4 5 6
EMSA: ATCGAT
C
RLU (βgal) x 10^2
vector
1336
1505
Δ1320-1351
DNA pol α
luciferase
RLU (βgal) x 10^4
vector
Δ1320-1351
p21WAF1
luciferase
D
vector
1505 WT
Δ1320-1351
1336
175
83
Blot: 861
E
RLU (βgal) x 10^2
vector
1336
1505
D1336,1339A
DNA pol α
luciferase
F
vector
1336
1505 WT
D1336,1339A
175
83
Blot: Myc
G
RLU (βgal) x 10^2
vector
1336
D1336,1339A
cyclin A2
DHFR
CAD
B-Myb
H
RLU (βgal) x 10^2
vector
1336
1505 Δ1320-1351
E2F1
I
CDP/Cux
861
CR2
CR3
HD
R1
R2
1505
Myc
HA
Δ1320-1351
1505 Δ1320-1351
D1336,1339A
1505 D1336,1339A
1336

Fig. 6. A C-terminally truncated CDP protein accelerates entry into S phase

(A) IL-2-dependent Kit225 T cells were infected with retroviruses expressing the indicated CDP isoforms. 48h later, cells were deprived of IL-2 for 48 hours, after which IL-2 was reintroduced for 18h or 25h. Cells were then fixed and stained with propidium iodide. Cell cycle distribution was analyzed by FACS. (B) Western blot analysis was performed with nuclear extracts from the cells used in (A).


A
18 h
vector
747-1505 WT
747-1505 mut
747-1336
# of cells x 100
FL2-H
B
vector
1505 WT
1505 mut
1336
250-
150-
100-
75-
1505
1336
Blot: Myc
C
CDP/Cux
CR2
CR3
HD
R1
R2
747-1505 WT
Myc
D1320,1336,1339A
747-1505 mut
747-1336

Chapter V – General Discussion

Introduction

In this thesis, I have begun to define the molecular basis for transcriptional activation by the CDP/Cux transcription factor. I showed a correlation between DNA binding by p110 CDP/Cux, and activation of the DNA polymerase alpha gene promoter. I also demonstrated that p110 CDP/Cux recruits E2F1 and E2F2 to the DNA pol alpha gene promoter. These E2Fs cooperate with p110 CDP/Cux in activation. In fact, E2F activity was necessary for p110 CDP/Cux to activate cell cycle-regulated promoters. Finally, I showed that CDP/Cux is a substrate for caspases in proliferating cells. The truncated isoform was a more potent transcriptional activator, and accelerated entry into S phase. I will discuss these findings, and their significance, as well as some of the questions that remain to be answered.

When I started this project, there was already some evidence supporting a role for CDP/Cux in activation. I will start by reviewing this. Studies published from two labs showed that CDP/Cux could function as part of a transcriptional activation complex (reword). The group of Gary S. Stein and Janet L. Stein had identified CDP/Cux as the DNA binding subunit of the HiNF-D complex that bound to cell cycle-regulated histone gene promoters in S phase, when they were transcriptionally active (10, 25-28, 31). Another group showed binding by CDP/Cux to Xenopus histone gene promoters (4). However, CDP/Cux repressed the histone H4 promoter in reporter assays. van Wijnen et al. proposed that HiNF-D may both activate and repress transcription, and this would depend on the availability of other proteins (27). It was also shown that the rat homolog, CDP2 cooperated with the ITF2 transcription factor in the activation of the tyrosine hydroxylase enhancer (32). Results from the Nepveu lab also suggested that CDP/Cux could function in activation. CDP/Cux weakly, but consistently, stimulated expression from the CMV promoter in reporter assays (Ginette Berube and Alain Nepveu, unpublished observations). However, no activation domain had been identified in Gal4-fusion reporter assays (18). Altogether, these results showed that CDP/Cux could stimulate gene expression. But it was not clear whether CDP/Cux could function as a transcriptional activator, or if it repressed a repressor. The latter explanation could be

supported by many publications showing that CDP/Cux repressed transcription by competition for binding site occupancy (12, 16, 17, 20, 24)

After having recently published a paper showing that CDP/Cux could repress the transcription of the $p21^{WAF1}$ cyclin-dependent kinase inhibitor (2), Dr. Nepveu looked for other cell cycle genes that had CDP/Cux binding sites in their promoters. The lab had recently shown that the preferred binding site for CDP/Cux was ATC(G/A)AT (6). The DNA polymerase alpha promoter had seven ATCGAT-like sites. However, it is activated at the G1/S transition. But perhaps given the proper binding sites, CDP/Cux could function as a transcriptional activator. Around this same time, Nam Sung Moon had found that CDP/Cux DNA binding activity was upregulated at the G1/S transition. In addition p200 CDP/Cux could be proteolytically processed between CR1 and CR2, generating p110 CDP/Cux, which bound DNA more stably.

DNA binding by CDP/Cux correlates with transcriptional activation

I found that p110 CDP/Cux, but not p200, could stimulate expression from a DNA polymerase alpha gene promoter in transient reporter assays (Chapter II). Promoter-mapping revealed that -65/+47 was sufficient for stimulation by p110. Linker-scanning analysis studies showed that three mutants could not be stimulated by p110. One of them, -35/-26 contained an inverted CCAAT site, which was a known CDP- sequence. p110 bound this mutant site with 2.8-fold lower affinity in EMSA. Chromatin immunoprecipitation studies of binding to the DNA pol alpha reporter in cells confirmed the decrease in recruitment of p110 upon mutation of -35/-26. In chapter II, I also showed that CDP/Cux bound, and stimulated, the endogenous DNA polymerase alpha gene promoter. This was the first evidence showing a correlation between DNA binding and transcriptional activation by p110 CDP/Cux. We have since shown that this is a general theme. p110 bound to, and activated many cell cycle-regulated gene promoters ((7), (21)see also Chapter III).

We predicted that p110 CDP/Cux was the DNA binding subunit of HiNF-D. Indeed, Brigitte Goulet confirmed that p110 bound the Site II element of the histone H4

gene promoter in EMSA analysis (Brigitte Goulet, unpublished observations). Gupta et al. showed that p110 CDP/Cux could not repress a histone H4 reporter, while p200 could. This experiment was performed in unsynchronized NIH 3T3 cells (5). I had shown in Chapter II that, while p110 could activate the DNA pol alpha reporter in many unsynchronized transformed cell lines, activation was observed in NIH 3T3 cells only following synchronization in S phase. To my knowledge, a reporter assay with the histone H4 reporter and p110 has not been performed in S phase-synchronized NIH 3T3 cells, or in another cell line that does not require synchronizing. I predict that p110 would stimulate the histone H4 promoter under these conditions. Even more informative, would be to show that the endogenous gene promoter could be activated by retroviral infection with a virus expressing p110. We observed activation of the endogenous DNA pol alpha gene by p110, but not p200, in this manner (Chapter II).

Cooperation with other transcription factors

Results from linker-scanning analysis (see Chapter II, and Mary Truscott and Alain Nepveu, unpublished observations) showed that two mutants, in addition to –35/-26, were not stimulated by p110 in reporter assays. A transcription factor-binding site lies within each of these two mutants.

E2F

E2F1 and E2F2 cooperated with p110 in the activation of the DNA pol alpha, Cdc25A, MCM3, Cyclin A2, and DHFR gene promoters in transient reporter assays. Moreover, inhibition of E2F DNA binding by a dominant negative DP1 protein also prevented activation of these promoters by p110. These results suggest that E2F1 or E2F2 is necessary for activation of these promoters by p110. In vivo DNA binding studies suggested that p110 recruits E2F1 and E2F2 to the DNA polymerase alpha. Location array analysis showed many targets common to p110 CDP/Cux and E2F1, and an overrepresentation of cell cycle targets (Chapter II and (7)).

GA-binding protein (GABP)

A –65/-55 linker-scanning mutant was not activated by p110 CDP/Cux in reporter assasys. This mutation resulted in the removal of an Ets-like binding site. The specificity of DNA binding by different Ets family members is similar, and it is therefore difficult to predict which family member(s) will be recruited (9). However, another group had shown that GA-binding protein (GABP), an Ets transcription factor family member, bound to this site in the mouse DNA polymerase alpha promoter (11). GABP protein cooperated with both E2F1 and p110 in reporter assays. Another group has also shown that GABP interacted with E2F1, but not with other E2F family members (8). Interestingly, this interaction also prevented E2F1-mediated apoptosis.

Cyclin A

Results published from the Nepveu laboratory showed that CDP/Cux interacted with cyclin/Cdk complexes ((22, 23), and Marianne Santaguida, unpublished observations). While cyclin A/cdk1 prevented DNA binding by p110 CDP/Cux in the G2 phase of the cell cycle. Cyclin A/cdk2 did not prevent DNA binding or activation by p110. On the contrary, it stimulated activation of DNA polymerase alpha by p110 in reporter assays.

Chromatin immunoprecipitation should be performed with cyclin A and/or Cdk2 antibodies. I predict that these immunoprecipitated complexes will be enriched for the DNA polymerase alpha, and other cell cycle-regulated gene promoters. Functional studies could then be performed to look at regulation of these endogenous gene promoters. There is evidence in support of this hypothesis - the HiNF-D complex contains cyclin A/Cdk (27, 28)

B-myb

B-myb had previously been shown to regulate the expression of DNA polymerase alpha (30). I have preliminary results showing cooperation between p110 and B-myb in the activation of the DNA polymerase alpha gene promoter. B-myb has also been shown to interact with Cyclin A/Cdk2 (27, 28).

An enhanceosome for the regulation of cell cycle gene promoters

These results support a model whereby p110 binds a cell cycle-regulated promoter, and recruits an activator E2F, thereby activating transcription. Preliminary observations suggest that GABP could also be part of a complex with p110 and E2F. This raises a number of questions. What recruits p110 to the promoter? Or is CDP/Cux already at the promoter, inactive, awaiting a post-translational modification of some sort? Results from Chapter II showed that there is a cluster of high affinity CDP/Cux binding sites (ATCGAT-like) approximately 1kb upstream of the transcription start site. ChIP experiments showed significantly more enrichment of these sequences than the core promoter. It is likely that the low affinity CCAAT site at –34/-30 is sufficient for activation in reporter assays, but that the higher affinity binding sites upstream are required for stimulation of the endogenous promoter. Alternatively, since binding to high affinity binding sites is less easily modulated, they may not be involved in the formation of a nucleoprotein complex that functions in activation. Instead, they could serve as parking spots for CDP molecules to wait. However, such clusters were not generally found upstream of the cell cycle-regulated CDP/Cux targets identified in location microarray analysis. CDP/Cux has been reporter to interact with DNA organized in nucleosomes (14). CDP/Cux may facilitate chromatin remodeling, which would favour the recruitment of other transcription factors to form activation complexes.

Are there different mechanisms of recruitment of CDP/Cux to different nucleoprotein complexes? Is there really more than one nucleoprotein complex that contains CDP/Cux, which binds to cell cycle-regulated gene promoters? High affinity binding sites for E2F transcription factors were not found in histone gene promoters (27). However, enhanceosomes are formed with weak protein-protein and protein-DNA interactions, which enables modulation (1). Perhaps high affinity E2F binding sites are used for transcriptional repression, by the recruitment of pocket protein complexes. Lower affinity binding sites, which would not be identified using searches for a consensus site, are not easily identified, as they would not conform to a strict consensus site. Interestingly, from my location microarray analysis, I found that E2F1 bound a number of histone gene promoters (Mary Truscott and Alain Nepveu, unpublished observations).

What other transcription factors and/or coactivators are recruited? By analyzing the promoter sequences targeted by both p110 and E2F1, one can identify DNA binding motifs that are overrepresented. Indeed this has been done with p110 promoter targets. Overrepresentation of binding sites for different transcription factors was found. One, Pax2, is currently being investigated in the laboratory as a potential CDP/Cux binding partner and coactivator. The p/CAF acetyltransferase has been shown to interact with CDP/Cux ((15), (19), and unpublished observations). However, Li, et al. showed that p/CAF inhibited binding by CDP/Cux, while results from the Nepveu lab suggest that it stimulates DNA binding.

The tandem affinity purification (TAP-tag) protocol has allowed the biochemical purification of complexes within which components have few interaction partners. For example, transcriptional elongation complexes (13) and histone acetyltransferase complexes (3). However, our evidence suggests that CDP/Cux is part of many different nucleoprotein complexes, and has different binding partners. In addition to interacting with E2Fs and the HiNF-D complex, I predict that CDP/Cux would interact with different proteins on promoters that it represses. In addition, CDP/Cux would cooperate with other factors to regulate genes that are not cell cycle-regulated, such as those involved in cell migration. CDP/Cux also interacts with matrix attachment regions, and represses cell type-specific genes.

Carboxy-terminal processing of CDP/Cux

I showed that CDP/Cux is a caspase substrate in proliferating cells (see chapter IV). This proteolytic processing removed two carboxy-terminal active repression domains. A recombinant, processed CDP/Cux protein activated the DNA polymerase alpha gene promoter, while a mutant that could not be processed did not. This recombinant protein accelerated entry of Kit225 cells into S phase. While CDP/Cux is similarly processed in apoptotic conditions (Mary Truscott, unpublished observations), I observed processing in cells for which there was no sign of apoptosis.

Evidence for a role for caspases in proliferation is mounting (see Chapter I). However a mechanism must ensure that substrate processing is selective. Subcellular compartmentalization would be one way to regulate processing of substrates. This could

serve to limit the access of the caspase(s) to substrates, or to bring endogenous caspase inhibitors into close proximity of the active caspase. Alternatively, the cleavage site could be masked by regulating the conformation of the substrate, and/or by post-translational modification.

Due to this requirement for lower levels of caspase activity, it has been difficult to detect active caspases in non-apoptotic cells. Reagents are needed to identify conditions in which low levels of caspase activity occur, and in what subcellular compartment(s) this could be happening.

Interestingly, the activity of CDP/Cux was associated with increased migration and invasion. In addition, CDP/Cux regulated the expression of genes with functions in cell motility, and invasion. Similarly, caspases have been implicated in the regulation of cell migration (29). CDP/Cux could be a substrate for caspases in cellular proliferation, and also migration and invasion. Rather than inactivating its substrate, as is observed in apoptosis (see Chapter I), this caspase activity could serve to activate CDP/Cux and its cell cycle, and cell migration targets.

Conclusion

In summary, I have shown that CDP/Cux can function as a transcriptional activator. p110 CDP/Cux binds cell cycle-regulated gene promoters and stimulates their expression. This is accomplished in cooperation with E2F, and likely other transcriptional activators and coactivators. This explains, at the molecular level, how p110 accelerates entry into S phase. Finally, I showed that CDP/Cux is a substrate for caspases in proliferating cells. This suggests a mechanism by which caspases may accelerate cell cycle progression.

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Chapter VI – Contribution to Original Research

1. I demonstrated that p110 CDP/Cux, but not p200 CDP/Cux could stimulate expression from the DNA polymerase alpha gene promoter. This was dependent on DNA binding, and occurred in cells synchronized at the G1/S cell cycle transition. The endogenous DNA polymerase alpha gene promoter was bound, and stimulated by CDP/Cux. This was the first evidence that CDP/Cux could stimulate the expression of S phase promoters.

2. I demonstrated that p110 CDP/Cux could cooperate with E2F1 and E2F2 in the activation of the DNA polymerase alpha gene promoter. In fact, E2F activity was necessary for activation by p110. E2F1 and E2F2 were recruited to the DNA polymerase alpha gene promoter by p110. In fact, p110 and E2F1 were recruited to many common targets, among which cell cycle targets were overrepresented. My results suggested that p110 recruits E2F1 and E2F2 to cell cycle-regulated gene promoters, and that this results in cooperative activation of the expression of these genes.

3. I demonstrated that CDP/Cux is a substrate for caspases. This proteolytic processing occurred in non-apoptotic proliferating cells. To my knowledge, this is the first demonstration of the regulation of a transcription factor by non-apoptotic caspases. This processed isoform was a more potent activator of cell cycle-regulated gene promoters. Furthermore, this isoform accelerated the entry of cells into S phase.

Abbreviations:

ACTR	(a.k.a. AIB1, RAC3, p/CIP, TRAM-1, SRC-3)
APAF-1	apoptotic protease-activating factor-1
APP	amyloid precursor protein
ASC-2	activating signal co-integrator-2
ATM	Ataxia Telangiectasia mutant
ATR	ATM and Rad3-related
Bcl-2	B-cell CLL/lymphoma 2
BIR	baculoviral IAP repeat
bp	base pair
CAD	carbamoyl-phosphate synthase (glutamine-hydrolyzing)/aspartate carbamoyltransferase/dihydroorotase
CASP	cut alternatively spliced product
CBP	CREB-binding protein
CC	coiled-coil
cdc	cell division cycle
Cdk	cyclin-dependent kinase
CDP	CCAAT Displacement Protein
ChIP	chromatin immunoprecipitation
CHR	cell cycle homology region
CKII	casein kinase II
CLL	chronic lymphocytic leukemia
CR	Cut Repeat
CREB	cAMP receptor element binding protein
CrmA	Cytokine Response Modifier A
CUTL1	Cut-like 1
DP-1	DRTF1 protein-1
DRTF1	differentiation regulated transcription factor 1
E2	Early gene 2
E2F	E2 Factor
ER	endoplasmic reticulum
FADD	Fas-associated death domain
FHL2	four-and-a-half LIM domain-2
G1	Gap 1
G2	Gap 2
GFP	green fluorescence protein
GTF	general transcription factor
HCF-1	host cell factor-1
HD	homeodomain
HiNF-D	histone nuclear factor D
HSV	herpes simplex virus
IAP	inhibitor of apoptosis protein
ICE	interleukin 1β converting enzyme
IFN	interferon
IGHM	immunoglobulin heavy constant mu
IHC	immunohistochemistry

IRF	IFN response factor
ITF2	immunoglobulin transcription factor 2
kb	kilobase
MAR	matrix attachment region
MEF	mouse embryonic fibroblast
MMTV	mouse mammary tumour virus
NALP-1	NACHT-LRR-PYD-containing protein
NCAM	neural cell adhesion molecule
NF-Y	nuclear factor Y
NLS	nuclear localization signal
$p14^{ARF}$	p14 Alternative Reading Fram
p200	200 kDa, full-length CDP/Cux protein
p/CAF	p300/CREB-binding protein-associated factor
PHA	phytohemagglutinin
PKA	protein kinase A
PKC	protein kinase C
Rb	retinoblastoma
RNA	ribonucleic acid
RNAi	ribonucleic acid interference
RNAP	RNA polymerase
rRNA	ribosomal RNA
RYBP	Ring1- and YY1-binding protein
S phase	Synthesis phase
SATB1	special AT-rich DNA-binding protein 1
SMAR1	scaffold/matrix associated region 1
Sp1	specificity protein 1
STAT	signal transducer and activator of transcription
TFE3	transcription factor binding to IGHM enhancer 3
TH	tyrosine hydroxylase
TNF	tumour necrosis factor
tRNA	transfer RNA
YY1	yin yang 1