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Perspective

New Modes of Translational Control in Development, Behavior, and Disease

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Over the last 10 years, the field of translational control has been enriched by atomic resolution structures of ribosomal complexes and factors in different functional states, and increased in sophistication by wedding genetics, reconstituted systems, and structural biology to elucidate basic reactions and mRNA-specific control mechanisms. New regulatory principles have emerged, including repression by micro-RNAs (miRNAs) and mRNA sequestration in cytoplasmic granules, and the field has extended its reach into development, brain function, and human disease. Here we seek to highlight some of the exciting developments of the last decade from the perspectives of our own approaches and expertise; accordingly, many highly noteworthy achievements could not be mentioned and we refer interested readers to the numerous excellent reviews cited below.

Mechanism of Translation

The last decade has witnessed stunning progress on the structures of ribosomes and translation factors, providing molecular insights into the fundamental reactions of protein synthesis. Although attempts to crystallize the ribosome began in the late 1970s by Yonath's group (Yonath et al., 1980), it was not until 2000 that an explosion of information from X-ray crystallography on ribosome structures was generated by the groups of Yonath, Ramakrishnan, Noller, and Steitz/Moore (reviewed in Noller, 2007). These studies revealed atomic details of the path of mRNA, binding of tRNAs to the aminoacyl (A), peptidyl (P), and exit (E) decoding sites, and the peptidyl transferase center. They showed that the decoding center is predominantly RNA—indicating that ribosomes are undoubtedly ribozymes—and illustrated how rRNA-tRNA interactions promote the accuracy of decoding.

In parallel, cryo-EM reconstructions of increasing resolution were augmented by fitting the models with crystal structures of ribosomal components or translation factors to obtain detailed views of ribosomes trapped in different stages of translation and conformational states (reviewed in Mitra and Frank, 2006). A dramatic ratcheting of the large and small subunits occurs as elongation factor EF-G promotes translocation, and intersubunit motion also underlies the ability of the GTPase RF3 to stimulate dissociation of release factors RF1/RF2 to complete polypeptide termination (Gao et al., 2007). The final step of translation, where deacylated tRNA is released and ribosomal subunits dissociate, requires ribosome recycling factor RRF and EF-G, and cryo-EM studies showed that RRF binds across the decoding sites, disrupting intersubunit bridges, and after being nudged into the proper position by EF-G, displaces the tRNA (Barat et al., 2007).

Termination has emerged as a key step for several regulatory mechanisms in eukaryotes. For the upstream open reading frame (uORF) that inhibits translation of the cytomegalovirus *UL4* gene, the uORF-encoded peptidyl-tRNA interacts with eRF1 to block peptide hydrolysis and stall the ribosome at termination, creating a roadblock to other ribosomes scanning from upstream (Janzen et al., 2002). Another class of short uORFs is thought to permit reinitiation by allowing ribosomes to remain attached and to resume scanning after terminating at the uORF stop codon, and it appears that initiation factor eIF4G must be retained during translation of the uORF for scanning to resume (Poyry et al., 2004). Termination factor eRF3 is a target for the factors UPF1, UPF2, and UPF3 that mediate rapid degradation of mRNAs bearing premature termination codons in the process of nonsense mediated decay (NMD). This fate seems to be prevented at authentic stop codons by their proximity to the poly(A) tail and interaction of poly(A) binding protein (PABP) with the termination complex (Amrani et al., 2004).

As most regulation occurs at initiation, this stage has been examined most intensively over the last decade. Initiation is more complicated in eukaryotes, requiring the >30 polypeptides that comprise 13 eukaryotic initiation factors (eIFs) (Figure 1), compared to only three IFs in bacteria. However, the simplifying fact emerged that bacterial IFs have recognizable counterparts in eukaryotes (and archaea): IF1/eIF1A, IF2/eIF5B, and IF3/eIF1 (reviewed in Pestova et al., 2007). eIF1A shares with IF1 a conserved globular domain that likely fills the A site in the manner described in the 30S•IF1 crystal structure (reviewed in Noller, 2007). eIF1A has additional domains, however, required for eukaryotic-specific functions of recruiting the eIF2-GTP-tRNAi ternary complex (TC), to

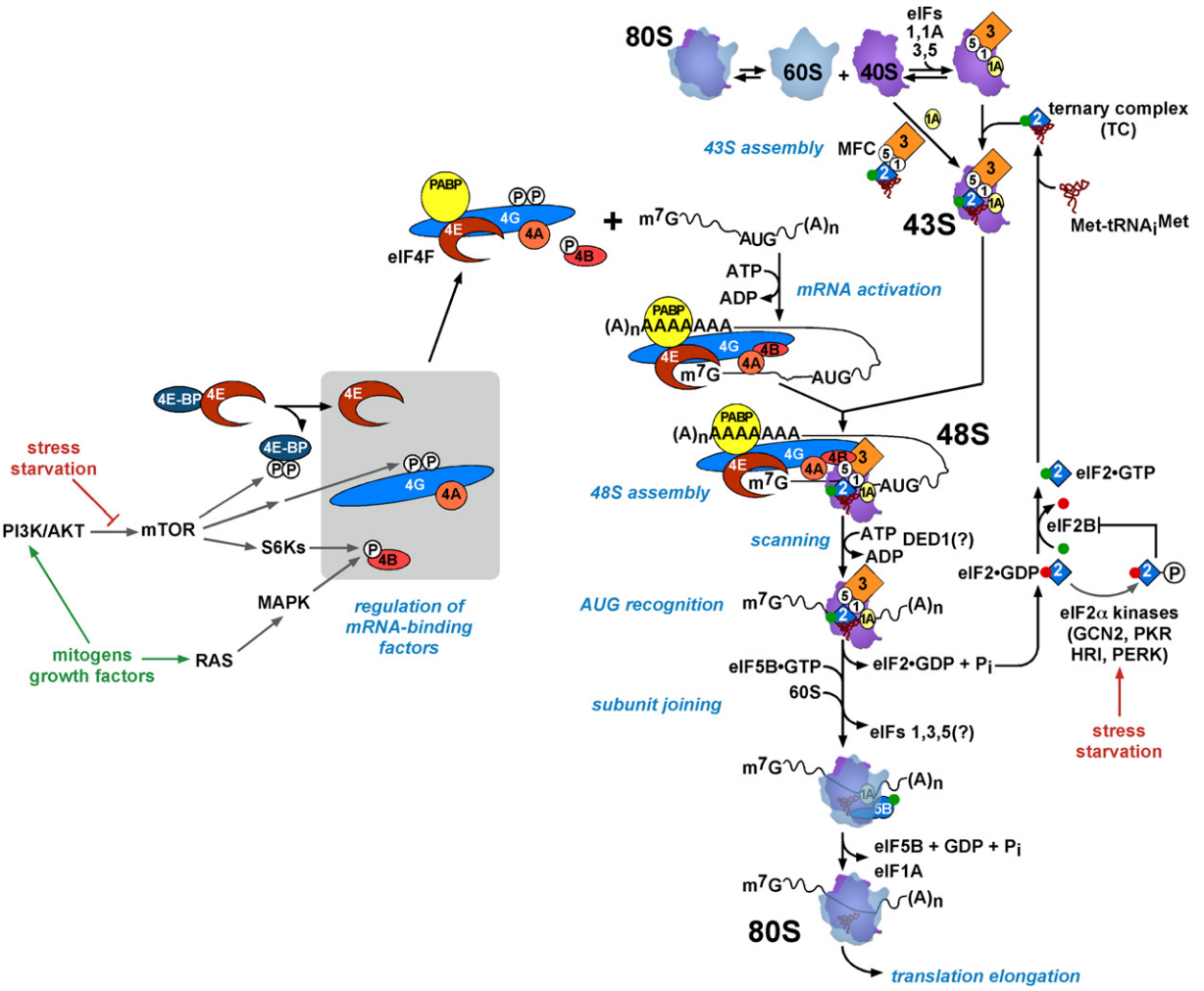

Figure 1. Eukaryotic Cap-Dependent Translation Initiation and Its Regulation by eIF2α Kinases and Other Signaling Pathways
eIFs 1, 1A, and 3 promote dissociation of 80S ribosomes and, together with eIF5 and TC (eIF2•GTP•Met-tRNA$_i$), assemble the 43S PIC. In yeast, these eIFs form a multifactor complex (MFC), which could bind 40S subunits as a unit. mRNA is activated by binding of eIF4F (eIF4E•eIF4G•eIF4A) to the cap and PABP to the poly(A) tail, circularizing the mRNA. The 43S PIC binds near the cap, facilitated by eIF3/eIF5 interactions with eIF4G/eIF4B, and scans the leader for AUG in an ATP-dependent (and possibly DED1-stimulated) reaction, with partial hydrolysis of the eIF2-bound GTP in the TC to eIF2•GDP•Pi. AUG recognition triggers eIF1 dissociation from the 40S platform (not depicted), allowing release of Pi and eIF2•GDP. Joining of the 60S subunits, with release of other eIFs, is catalyzed by eIF5B-GTP, and GTP hydrolysis triggers release of eIF5B•GDP and eIF1A, to yield the final 80S initiation complex. Under stress or starvation conditions, TC formation is reduced by eIF2α phosphorylation and eIF4F assembly is blocked by 4E-BP binding to eIF4E. Phosphorylation by mTOR dissociates 4E-BP from eIF4E. mTOR also promotes eIF4G and eIF4B phosphorylation either directly or via S6Ks. Mitogens and growth factors promote these phosphorylation events by activating mTOR via PI3K/Akt signaling or RAS/MAPK signaling. Not depicted here, MAPK signaling also engenders phosphorylation of eIF4E by kinases Mnk1/Mnk2.

produce a 43S preinitiation complex (PIC), in scanning, and in AUG selection (Fekete et al., 2007; Pestova et al., 1998a). IF2 and eIF5B are structurally similar GTPases that catalyze the last step of initiation—joining of the large subunit—and both depend on GTP hydrolysis for final release from the initiation complex (Pestova et al., 2000; Shin et al., 2002). Although eIF1 and IF3 are not related, they both bind the small subunit near the P site and function interchangeably in rejecting noninitiator tRNAs or non-AUGs in reconstituted systems (Lomakin et al., 2006).

eIF1 also collaborates with eIF1A to promote scanning past non-AUG triplets (Pestova and Kolupaeva, 2002), possibly by stabilizing an open conformation of the mRNA channel of the 40S subunit (Passmore et al., 2007), and it inhibits the release of Pi from the partially hydrolyzed eIF2-GDP-Pi in the TC until an AUG fills the P site. This "gate-keeper" function of eIF1 is neutralized at AUGs by its dissociation from the 40S (Algire et al., 2005). eIF1 also interacts directly with TC, eIF5 (the GTPase-activating protein for eIF2), and the eIF3 complex, functionally coordinating these factors in AUG selection and enabling a concerted mechanism of PIC assembly (Asano et al., 2000) rather than the stepwise binding of factors envisioned previously.

The 43S PIC is directed to the 5′ cap by the eIF4F complex, comprising the cap-binding protein eIF4E, DEAD box helicase eIF4A, and scaffold subunit eIF4G. Over the last decade, high-resolution structures have emerged for eIF4E bound to cap, eIF4A, and the eIF4E- and eIF4A-binding domains of eIF4G. Association with eIF4G increases eIF4E's affinity for the cap (Gross et al., 2003) and triggers an activating conformational change in eIF4A (Oberer et al., 2005). In addition to recruiting and activating eIF4A, eIF4G also makes a protein bridge with eIF3 (Korneeva et al., 2000), which should serve to recruit the 43S PIC to the mRNA 5′ end. A cryo-EM model of the eIF3-eIF4G-40S complex places the bulk of eIF3 on the "backside" of the 40S and, surprisingly, puts eIF4F near the mRNA exit channel (Siridechadilok et al., 2005), where it would have to "pull" mRNA through the 40S subunit. Perhaps other helicases, like yeast DED1 (Chuang et al., 1997), unwind mRNA structure at the leading edge of the ribosome.

PABP binding to eIF4G mediates the circularization of the mRNA (Tarun and Sachs, 1996) and is thought to underlie PABP's ability to stimulate mRNA binding to the 43S PIC by stabilizing eIF4F binding to the cap (Kahvejian et al., 2005). Consistent with this, the inhibition of translation by poly(A)-binding protein interacting protein 2 (Paip2) involves its competition with eIF4G for binding to PABP (Karim et al., 2006).

It has been ~20 years since the discovery of internal ribosome entry sites (IRESs) in picornaviruses, and it was known that these IRESs (with the exception of hepatitis A virus) do not require eIF4E but need all other eIFs to recruit the 40S ribosomal subunit (reviewed by Doudna and Sarnow, 2007). Over the past decade, it was realized that the hepatitis C virus (HCV) IRES dispenses with eIF4F entirely, binding directly to the 40S, and requires only eIF3 and eIF2 to pair $tRNA_i$ with the IRES start codon (Pestova et al., 1998b). Structural analysis of this element culminated in a cryo-EM model of the 40S-eIF3-IRES complex (Spahn et al., 2001). Even more remarkable, the IRES of insect virus cricket paralysis virus (CrPV) dispenses with all eIFs and $tRNA_i$ (Wilson et al., 2000), using different pseudoknot domains to contact 40S and 60S components or occupy the decoding center and place a GCU triplet in the A site, where translation begins (Schuler et al., 2006). Clearly, HCV and CrPV are exemplars of viral IRESs that can hijack host ribosomes without competing for limiting eIFs in infected cells. In the same vein, several IRESs were described in cellular mRNAs that are active during mitosis (Cornelis et al., 2000; Pyronnet et al., 2000), or under stress conditions in mammalian cells and yeast, when cap-dependent translation is impaired (Elroy-Stein and Merrick, 2007; Gilbert et al., 2007).

Regulation of Translation

Diverse mechanisms control translation. One of the key mechanisms, particularly during stress, is phosphorylation of eIF2 on Ser51 of its α subunit, converting eIF2-GDP into a competitive inhibitor of eIF2B, the five-subunit guanine nucleotide exchange factor (GEF), thus decreasing TC assembly (Figure 1). Remarkably, only a small portion of the ε subunit of eIF2B is sufficient for GEF function (Gomez et al., 2002), and three of the remaining subunits provide a binding site for phosphorylated eIF2α (eIF2[αP]) needed to inhibit GEF function. In addition to reducing general initiation, eIF2(αP) paradoxically induces translation of yeast transcriptional activator *GCN4* by overcoming the inhibitory effects of uORFs on reinitiation at the *GCN4* ORF (reviewed in Hinnebusch, 2005). Translation of the transcription factor *ATF4* mRNA is upregulated by eIF2(αP) in mammalian cells by essentially the same reinitiation mechanism (Harding et al., 2000; Vattem and Wek, 2004), leading to transcriptional activation of stress response genes, which include the regulatory subunit of an eIF2(αP) phosphatase (GADD34), to provide negative feedback (Novoa et al., 2001). There are four different eIF2α kinases in mammals activated by different stresses, including PKR, PERK, HRI, and GCN2. The crystal structure of human PKR bound to eIF2α revealed a novel interaction of the kinase domain G helix with a segment of eIF2α remote from Ser51, explaining the exquisite substrate specificity of eIF2α kinases (Dar et al., 2005).

Another frequently regulated step in translation is the mRNA 5′ cap recognition process by eIF4F. Assembly of the eIF4F complex is suppressed by a family of related eIF4E-binding proteins (4E-BPs) (Figure 1) (reviewed in Raught and Gingras, 2007). The 4E-BPs share a common eIF4E-binding sequence with eIF4G, and structural analysis showed that these eIF4E-binding segments undergo an induced-fit transformation to α helical structures to achieve high affinity binding to eIF4E (Gross et al., 2003; Marcotrigiano et al., 1999). Phosphorylation of 4E-BPs disrupts their interactions with eIF4E, and four of the seven known phosphorylation sites on 4E-BP1 are phosphorylated in a hierarchical manner to effect the release of eIF4E.

The elucidation of signaling pathways that control the phosphorylation and activity of translation factors has led to better understanding of the importance of translational control in cell growth, proliferation, development, survival, learning and memory, metabolism, etc. The two major pathways that signal to the translation machinery are the PI3K/Akt/mTOR and the Ras-MAPK signaling cascades (Raught and Gingras, 2007) (Figure 1). mTOR is responsible directly for the phosphorylation of 4E-BPs and S6 kinases (S6Ks), and indirectly for eIF4B and eIF4G phosphorylation. The Ras-MAPK pathway engenders the phosphorylation of eIF4E and eIF4B.

The phosphorylation of many translation factors correlates with increased translation rates and cell growth/proliferation. The best understood cases are the 4E-BPs (see above) and eIF4B where, for the latter, phosphorylation at Ser422 results in better interaction with eIF3 (Holz et al., 2005). Contrary to the long-held belief that ribosomal protein S6 is a functionally important substrate of S6K in stimulating translation, mutation of all the phosphorylation sites on S6 stimulated rather than inhibited translation

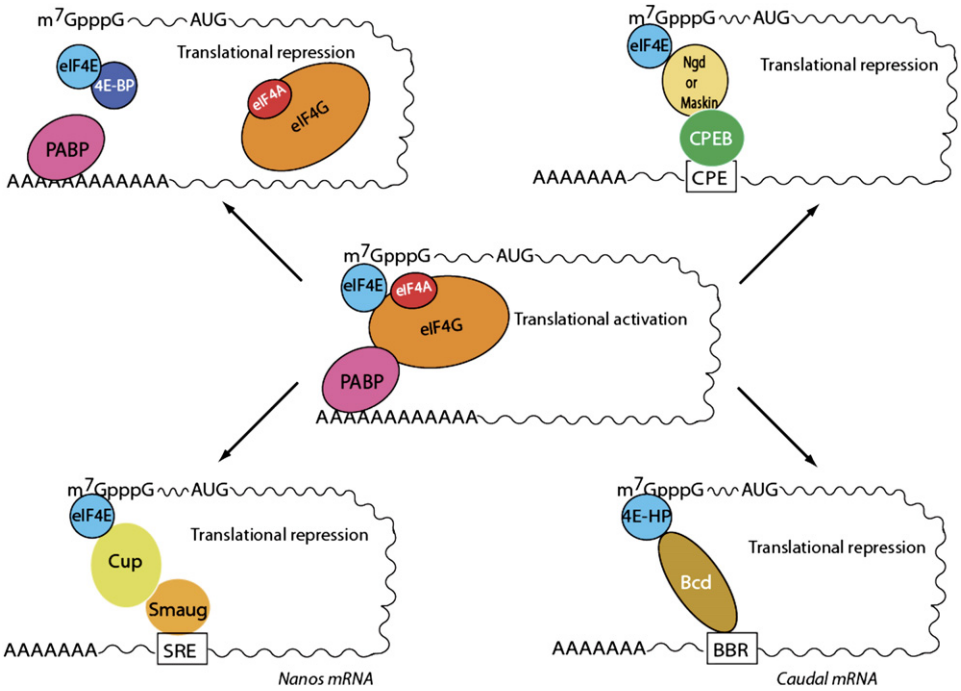

Figure 2. Models for Cap-Dependent Translational Repression

Cap-dependent translation initiation requires the interaction of the cap-binding protein eIF4E with the mRNA 5′ cap structure. eIF4E is a subunit of the eIF4F complex, which also includes the RNA helicase, eIF4A, and eIF4G. The latter binds to PABP, which brings about the circularization of the mRNA. Translational repression could occur by several different mechanisms. The more general mechanism is engendered by members of a family of small proteins, called 4E-binding proteins (4E-BPs), which compete with eIF4G for the interaction with eIF4E. Other repression mechanisms are more mRNA specific. Translation of mRNAs that contain a cytoplasmic polyadenylation element (CPE) is repressed by the displacement of eIF4G by Neuroguidin (Ngd)/Maskin, which are recruited to the mRNA through their interaction with the CPE-binding protein (CPEB). The latter paradigm functions for specific mRNAs using different modules. For example, the translation of *Drosophila nanos* mRNA is inhibited by tethering eIF4E to the Smaug response element (SRE) via Smaug and Cup. A variation on the theme is presented by Bicoid, which inhibits specifically the *Drosophila caudal* mRNA translation by binding simultaneously to the 3′UTR Bicoid-binding region (BBR) and the eIF4E-homologous protein, 4E-HP.

(Ruvinsky et al., 2005). Other S6K substrates have been described that could mediate some of S6K's stimulatory effects on translation, including eIF4B, S6K1 Aly/REF-like target (SKAR), and eukaryotic elongation factor-2 kinase (eEF2K). Pdcd4, a tumor suppressor and inhibitor of eIF4A (Yang et al., 2003), is ubiquitinated and degraded upon being phosphorylated by S6K (Dorrello et al., 2006).

Translational Control in Development and Differentiation

Translational control is paramount during early embryogenesis, where it controls embryonic axis, body pattern, and cell fate, as transcription is largely quiescent at this stage. In *Drosophila,* mRNAs encoding Bicoid and Nanos are targeted to the two poles of the embryo to establish posterior-to-anterior or anterior-to-posterior concentration gradients. These proteins inhibit the translation of uniformly distributed mRNAs encoding other morphogens to establish secondary gradients. An attractive paradigm for the mechanism of this inhibition was first provided in *Xenopus* oocytes. The translational inhibition of maternal mRNAs with short poly(A) tails is mediated by Maskin, which binds simultaneously to the cytoplasmic polyadenylation-element binding protein (CPEB), bound to the 3′UTR, and to eIF4E bound to the cap. It is thought that Maskin acts like a tethered 4E-BP to displace eIF4G from eIF4E and produce an inactive circular mRNA incapable of recruiting the 43S PIC (Stebbins-Boaz et al., 1999) (Figure 2). A similar mechanism mediates repression of *nanos* mRNA by the 3′UTR-binding protein Smaug and the eIF4E-binding protein, Cup (Nelson et al., 2004). An interesting variation on this theme is the repression of *caudal* mRNA translation in early fly embryogenesis, where the homeodomain transcription factor Bicoid binds to the *caudal* mRNA 3′UTR and recruits an eIF4E-related protein (d4E-HP) that cannot interact with eIF4G to assemble eIF4F (Cho et al., 2005).

3′UTR-binding proteins can inhibit steps other than eIF4F assembly at the cap. The sex-lethal (SXL) protein regulates dosage compensation in *Drosophila* by repressing translation of male-specific-lethal 2 (*msl-2*) mRNA by a two-pronged mechanism demonstrated in cell-free extracts. Binding in the 3′UTR, SXL inhibits recruitment of the 43S PIC to the cap, while SXL binding to the 5′UTR blocks scanning (Beckmann et al., 2005). Fox and colleagues discovered that repression of ceruloplasmin mRNA by interferon-γ treatment involves inhibition of 43S PIC recruitment by a mechanism that seems to target

the eIF4G-eIF3 protein bridge. Interestingly, the repressor here consists of a multisubunit complex (GAIT) that includes L13a and glutamyl, prolyl-tRNA synthetase. These proteins are liberated from the 60S subunit and the tRNA synthetase complex, respectively, by phosphorylation (Kapasi et al., 2007). Binding of hnRNPs K and E to the 3′UTR of 15-lipoxygenase (LOX) mRNA appears to silence translation of this transcript in erythroid precursor cells by inhibiting a step after AUG recognition by the scanning PIC (Ostareck et al., 2001).

Translational Control of Synaptic Plasticity, Learning, and Memory

A fundamental cellular process that is thought to underlie learning and memory is synaptic plasticity, which describes the changes to synaptic strength in response to experience. Long-term potentiation (LTP) and long-term depression (LTD) are electrophysiological models for the synaptic changes that strengthen or weaken, respectively, learning and memory processes. It has been known for many years that dendrites contain ribosomes, mRNA, and the translation machinery. Local translation of mRNAs at synapses, caused by activation of signaling pathways such as the PI3K and Erk, play important roles in eliciting and maintaining both LTP and LTD (Sutton and Schuman, 2006). For example, LTD is caused by the removal of the receptors for the neurotransmitter glutamate. The products of translation are required for the persistent decrease in receptors and thus maintain reduced synaptic strength for a longer period of time (Pfeiffer and Huber, 2006). Local translation also plays a role in stabilizing more permanent forms of memory. In *Aplysia*, ongoing local synthesis, mediated in part by local activation of CPEB, is required for a late increase in synaptic strength that depends on the stabilization of new synapses (Si et al., 2003).

Strong genetic evidence that regulating translation initiation (although not necessarily in a manner localized at synapses) controls learning and memory was provided by studying mice lacking the eIF2α kinase GCN2 and *eIF2*$\alpha^{Ser51Ala/+}$ heterozygote "knockin" mice. These mutants exhibit enhanced memory using different training protocols. This could be explained by augmented synaptic plasticity, as evidenced by a lower threshold of stimulation to achieve long-lasting LTP (Costa-Mattioli et al., 2007). Activated GCN2 suppresses memory formation by phosphorylating eIF2, which in turn results in increased translation of *ATF4* mRNA in the brain. ATF4 is a memory suppressor because it inhibits the transcription factor cyclic AMP response element binding protein (CREB)-mediated gene expression of early-immediate gene targets that are critical for long-term synaptic plasticity and memory (reviewed in Kandel, 2001). The role of mouse GCN2 as translational regulator of transcription in learning and memory parallels its role in translational control of amino acid biosynthesis gene transcription in yeast. Strikingly, GCN2 in mouse also mediates aversion to amino acid-deficient diets in response to the cognate uncharged tRNAs in neurons of the anterior piriform cortex (Hao et al., 2005; Maurin et al., 2005). It is remarkable that an ancient translational control mechanism that senses amino acid availability in microorganisms was adapted in higher animals to control learning and behavior.

Translational Control in Disease

The major cellular signaling pathways that control cell growth and proliferation also upregulate translation activity. Consistent with this finding, upregulation of the amount and activity of initiation factors results in diseases, including cancer and heart disease. The most convincing causal relationship was established for eIF4E, whose overexpression causes malignant transformation of human and mouse cells in tissue culture and tumors in mice (reviewed in Schneider and Sonenberg, 2007). Moreover, when eIF4E abundance is reduced by siRNA or its activity is repressed by 4E-BPs, the transformed phenotype is reverted in cells transformed by oncogenes, such as Ras and Src. Strikingly, tumor growth in mice is severely retarded by administering antisense oligonucleotide against eIF4E with no significant side effects (Graff et al., 2007), and this DNA compound is now in clinical trials in humans. Several studies described small molecule drugs that interfere with eIF4E-eIF4G interaction or eIF4A function and could be developed into anticancer drugs (Bordeleau et al., 2006; Moerke et al., 2007). Interestingly, loss of a specific eIF4E isoform reduces protein synthesis, confers protection from oxidative stress, and extends lifespan in *C. elegans* (Syntichaki et al., 2007).

Several key translational regulatory proteins, such as eIF2α kinases, 4E-BPs, and S6Ks, play major roles in controlling metabolism, and their malfunction results in metabolic diseases, most notably altered glycemic control and obesity. eIF2α phosphorylation is important for ER homeostasis by controlling the flux of newly synthesized secretory proteins that enter the ER. Mice that are homozygous for the *eIF2*^{Ser51Ala} mutation die shortly after birth because of severe hypoglycemia. Mice heterozygous for the *eIF2*^{Ser51Ala} mutation survive, but when put on a high-fat diet display glucose intolerance, increased body weight, and hyperlipidemia (relative to the wild-type). These preclinical type 2 diabetic manifestations are likely due to impairment in pancreatic β cell function and lipid metabolism. Consistent with these findings, PERK inactivation in mice and humans results in early postnatal β cell destruction and diabetes mellitus (reviewed in Ron and Harding, 2007).

4E-BPs and S6Ks control metabolism by acting as downstream effectors of the evolutionarily conserved PI3K/Akt/mTOR signaling pathway, which couples nutrient and mitogen (such as insulin) availability with the metabolic state at the cellular and organismal level. When nutrients and mitogens are replete, 4E-BPs and S6Ks are phosphorylated, activating translation and inducing anabolic processes. Single or double "knockout" mice for 4E-BPs and S6Ks exhibit changes in metabolism. For example, the *S6K1*$^{-/-}$ mice are protected from age- and diet-induced obesity while expressing enhanced

insulin sensitivity (Um et al., 2004). Double knockout mice for 4E-BP1 and 4E-BP2 become obese when fed a high-fat diet, and are insulin insensitive and glucose intolerant (Le Bacquer et al., 2007). Although the mechanisms underlying these changes are not well understood, these findings underscore the importance of translational control in metabolic homeostasis.

Translational control is also relevant to the fragile X mental retardation (FMR) syndrome, in which the responsible protein, FMRP, is produced in smaller amounts. FMRP suppresses the translation of a subset of mRNAs, but the exact mechanism has not been elucidated, as studies have shown association of FMRP with polysomes or nonpolysomal mRNPs (reviewed in Klann and Richter, 2007). It is of great interest, however, that FMRP is associated with the microRNA (miRNA) RISC complex (see below) (Ishizuka et al., 2002; Jin et al., 2004), possibly suggesting an miRNA-associated mechanism.

Viruses, without exception, use the cellular machinery to synthesize their proteins and, accordingly, have evolved sophisticated mechanisms to compete with cellular mRNAs for the host translation machinery. Studies that began more than five decades ago revealed that viruses shut off host protein synthesis, and the phenomenon is particularly dramatic for picornaviruses, such as poliovirus, that use IRESs to recruit ribosomes. Viral IRESs are typically much stronger than their cellular counterparts, a feature that viruses exploit to selectively inhibit cap-dependent translation of host mRNAs, and thus take over the host translational machinery. A recent striking example of the ability of viruses to inhibit cellular mRNA translation is that some DNA viruses, such as SV40, herpes simplex virus (HSV), and cytomegalovirus (CMV), encode miRNAs that suppress the translation of a subset of cellular mRNAs as a way to inhibit apoptosis (reviewed in Sullivan and Ganem, 2005).

miRNA and P Bodies

One of the newest areas of research on translational control involves the inhibition of protein synthesis by miRNAs—short (~21 nt) oligonucleotides that may regulate about a third of the mammalian genome, as there are ~1000 miRNAs and each could control ~10 mRNAs. miRNAs form nonperfect duplexes on the target mRNA (usually in the 3′UTR) and recruit the repressing complex termed *R*NA *i*nduced *s*ilencing *c*omplex (RISC). From studies in cells, diverse mechanisms were proposed to account for repression by RISC, including translational repression at the level of initiation or elongation, mRNA degradation, or even degradation of the nascent protein. The case for translation initiation as a key target of miRNA action was bolstered by recent studies in which miRNA-mediated repression was recapitulated in cell-free extracts and shown to affect cap-dependent, but not IRES-mediated, translation (Mathonnet et al., 2007; Thermann and Hentze, 2007). The suggestion that Argonaute, a core component of RISC, is a cap-binding protein (Kiriakidou et al., 2007) provides an attractive model by which the assembly of RISC at the mRNA 3′UTR promotes Argonaute binding to the cap and prevents eIF4F binding and attendant 43S PIC recruitment. This would resemble the repression of *caudal* by Bicoid and d4E-HP (Figure 2).

miRNAs, repressed mRNAs, and components of RISC concentrate in P bodies, which Parker and colleagues identified in budding yeast as foci containing translationally repressed mRNAs. P bodies also contain mRNA decapping and 5′ to 3′ degradation proteins (reviewed in Parker and Sheth, 2007). It appears that global translational repression in starved yeast cells requires exclusion of ribosomes from mRNAs by the formation of mRNP complexes with DEAD box helicase DHH1 and the PAT1 protein, which then aggregate in P bodies. mRNAs in P bodies are not necessarily decapped and degraded and can return to the translated pool when nutrients are resupplied. Interestingly, miRNA-induced repression involves DHH1 homologs (p54/Rck/Me31B) and decapping enzymes that are also required for translational repression of maternal mRNAs in flies, nematodes, and frogs, suggesting that this general mechanism of repression is widespread in eukaryotes. Thus, for example, translational repression of *oskar* mRNA in *Drosophila* requires the fly DHH1 homolog, Me31B, in early oogenesis (Nakamura et al., 2004).

Future Prospects

As crystallography continues to provide atomic details of the structure of translation intermediates, single-particle analysis of ribosomal complexes will identify new conformational transitions and interactions occurring in the course of protein synthesis. This will be coupled with in-depth kinetic analysis of fundamental reactions in reconstituted systems. It is very important to show that mutations in ribosomes or factors have phenotypes in living cells consistent with their effects in reconstituted systems. For example, the physiological importance of PABP-eIF4G interactions in circularizing mRNA, and of the protein bridge between eIF4G and eIF3 in recruiting the PIC to activated mRNA, has not been thoroughly demonstrated in vivo.

Reconstituting the functions of viral IRESs in vitro, as well as structural analysis of IRES-ribosome complexes, has proven that these elements provide an alternative initiation mechanism that bypasses scanning. This level of validation is needed for cellular IRESs, including the involvement of noncanonical *trans*-acting factors (ITAFs), and both the importance of ITAFs and the eIF independence of IRESs must be established in vivo.

A major undertaking in the future will be the study of translational control at the systems biology level. Existing microarray technologies, so valuable in genome-wide descriptions of transcription, are cumbersome and prone to artifacts because of the need for gradient fractionation of polysomal mRNAs. However, some studies have begun to address translational control networks and have demonstrated the existence of "RNA operons" whereby the expression of discrete subsets of mRNAs is coordinately regulated at the posttranscriptional level (reviewed in

Keene, 2007). Work in yeast established coordinated control of expression of functionally related mRNAs by Puf RNA-binding proteins, which control localization, translation, and stability of mRNAs (Gerber et al., 2004). Translational coregulation of subsets of genes must be highly operative during early development where transcription is very low. The miRNA network should also benefit from systems biology, since many miRNAs control the translation of multiple mRNAs.

An important area for future efforts will be translational research on translational control, whereby drugs for diseases whose origin can be explained by defects in translation factors or their modulators will be developed. Because a large number of miRNAs are implicated in cancer, it is possible that components of the miRNA machinery will also become targets for new therapeutics. Neurological diseases, such as FMR, and those with memory loss manifestations (e.g., Alzheimer's) might be targeted, especially because enhanced memory was attained in mice with defects in eIF2α phosphorylation.

The last 10 years saw tremendous progress in deciphering molecular mechanisms of translation, demonstrating translational control in development, the nervous system, and in human disease. This progress brought new talent, technologies, and strategies into the field that should ensure its vitality and continued expansion in the next decade.

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