Analysis of N-glycan glucosylation and processing using a synthetic lethal approach

Christella Munyana

Department of Biology
McGill University, Montreal
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ABSTRACT.

A large-scale procedure was used to screen for deletions affecting growth of *Saccharomyces cerevisiae* when combined to *ALG6, ALG8, ALG10, CWH41, ROT2* or *CNE1* deletions. 40 genes, grouped in 8 functional categories, were found to interact with the 6 query genes. The resulting network of 61 synthetic interactions was composed of 3 subnetworks, the N-glucosyltransferase (*ALG6, ALG8, ALG10*), the glucosidase (*CWH41, ROT2*) and *CNE1* interaction sets, respectively. Deletion in 34 interacting genes conferred calcofluor white hypersensitivity, strengthening the relationship between N-glycan glucosylation/processing and cell wall physiology. In addition, a genetic interaction was found between *ALG6* and *SEC53*, the yeast homologues of human *ALG6* and *PMM2* genes involved in congenital disorders of glycosylation. The *alg6sec53* double mutant shows a synthetic growth defect and a CPY underglycosylation. Since this synthetic interaction is conserved from yeast to mammals, this work proposes the use of SGA analysis as a tool to uncover digenic effects that may underlie complex human genetic disorders.

RÉSUMÉ

Grâce à une technique à grande échelle, le criblage des délétions qui affectent la croissance de la levure Saccharomyces cerevisiae lorsqu'elles sont combinées à une délétion de *ALG6*, *ALG8*, *ALG10*, *CWH41*, *ROT2* ou *CNE1* a été effectué. Au total, 40 gènes, regroupés en 8 catégories fonctionnelles, ont montré une interaction avec l'un des 6 gènes de départ. Un réseau de 61 interactions synthétiques a été obtenu. Il est subdivisé en 3 sous-réseaux composés des interactions avec les N-glucosyltrensferases (*ALG6, ALG8, ALG10*), les glucosidases (*CWH41*, *ROT2*), et *CNE1*. Le fait que la délétion de 34 gènes du réseau confère une hypersensibilité au calcofluor white, renforce le lien établi entre la glucosylation/maturation des chaînes de N-glycosylations et la synthèse de la paroi cellulaire. De plus, une interaction a été trouvée entre *ALG6* et *SEC53*, les homologues de levure des gènes humains *ALG6* et *PMM2*, impliqués dans des désordres de glycosylation congénitaux. Le double mutant *alg6sec53* montre une croissance réduite ainsi qu'une hypoglycosylation de CPY. Puisque cette interaction est conservée de la levure jusqu'à l'homme, la méthode utilisée ici pourrait s'avérer un outil précieux pour l'étude des effets digénique à la base de maladies humaines génétiques et complexes.

Translated by Guillaume Lesage.

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This thesis is dedicated to my parents for all they have sacrificed for me to pursue my education abroad. I know you are very proud of what I have accomplished.

PREFACE

This thesis is presented in accordance with the regulation of the Faculty of Graduate Studies and Research as well as the Department of Biology of McGill University. It includes an abstract and résumé, an introduction, material and methods section, results section and discussion. In this thesis, I present a contribution to the knowledge, namely the analysis of N-glycan glucosylation using a synthetic lethal approach.

All work presented in this thesis was conducted by myself, except for the automated SGA screens which were conducted by myself, Anne-Marie Sdicu, Shamiza Hussein and Patrice Ménard. This work is part of a collaborative effort involving the Howard Bussey and Charlie Boone laboratories (University of Toronto). The data presented here are also used to form part of a separate article (Tong *et al.* 2003).

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LIST OF ABBREVIATIONS

aa	amino acid residue(s)
Arg	arginine
ATP	adenosine trisphosphate
°C	degree Celsius
Can	canavanine
CDG	congenital disorder of glycosylation
CPY	carboxypeptidase Y
Dol-P	dolichyl phosphate
Dol-PP	dolichyl pyrophoshate
$EndoH_f$	endo-β-N-glucosiminidase H/maltose-binding protein fusion
ER	endoplasmic reticulum
ERAD	ER-associated degradation
GDP	guanosine diphoshate
Glc	glucose
GlcNAc	*N*-acetylglucosamine
GT	glucosyltransferase
His	histidine
KDa	kilodalton
Man	mannose
mRNA	messenger ribonucleic acid
NAT	nourceothricine
N-glucosylation	asparagine-linked glucosylation

N-linked	asparagine-linked
NS	non-synthetic
O-mannosylation	oxygen-linked mannosylation
ORF	open reading frame
OST	oligosaccharyltransferase
PCR	polymerase chain reaction
S. cerevisiae	*Saccharomyces cerevisiae*
SDS	sodium dodecyl sulfate
SGA	synthetic genetic array
SL	synthetic lethal
SS	synthetic sick
Tris	tris (hydroxymethyl) aminomethane
UDP	uridine diphosphate
YEPD	yeast extract peptone dextrose
YNB	yeast nitrogen base

1. INTRODUCTION

N-glycosylation is the process by which oligosaccharide chains are co-transitionally transferred to suitable protein asparagine residues in the lumen of the endoplasmic reticulum (ER). The ordered assembly and the processing of the oligosaccharide chains in the endoplasmic reticulum has been remarkably conserved from yeast to mammals. This process was first described using experiments performed with mammalian cells (Kornfeld and Kornfeld 1985, Hirschberg and Snider 1987, Verbert *et al.* 1987, Cummings *et al.* 1992, Hemming 1995). Subsequent work on the yeast *Saccharomyces cerevisiae* revealed that the basic components of the N-glycosylation pathway are conserved among eukaryotes (Kukuruzinska and Bergh 1987, Tanner and Lehle 1987, Abeijon and Hirschberg 1992, Herscovics and Orlean 1993, reviewed by Burda and Aebi 1999).

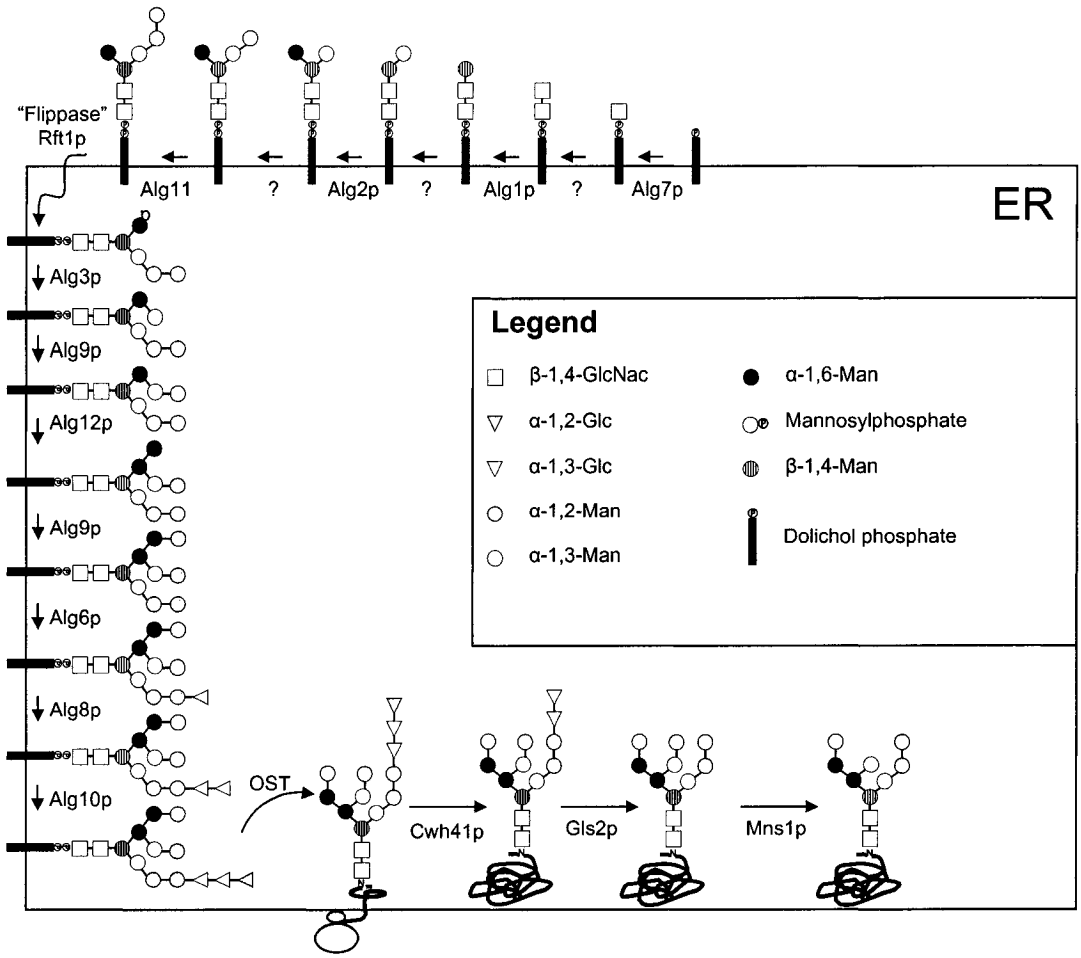
The pathway of N-linked protein glycosylation can be divided into two parts: first, the lipid-linked oligosaccharide precursor is assembled at the cytoplasmic side of the ER membrane then translocated to the luminal side of the ER membrane for further maturation. Second, the oligosaccharide moiety is transferred *en bloc* to asparagine residues of proteins and then processed by ER enzymes (Fig.1).

1.1 Assembly of the lipid-linked oligosaccharide at the ER membrane of the yeast *Saccharomyces cerevisiae*.

1.1.1. Cytoplasmic side of ER.

A series of reactions catalyzed at the cytoplasmic side of the ER lead to synthesis of a dolichol linked $GlcNAc_2Man_5$-precursor (Fig.1). Dolichyl pyrophosphate (Dol-PP) acts as the carrier in the synthesis of the core oligosaccharide. UDP-N-acetylglucosamine (UDP-GlcNAc) and GDP-mannose (GDP-Man) serve as sugar donors for the synthesis of the Dol-PP anchored $GlcNAc_2Man_5$ oligosaccharide. The first step is the addition of a GlcNAc residue from UDP-GlcNAc to Dol-PP. This is catalyzed by the UDP-N-acetyl-glucosamine-1-P transferase encoded by the essential gene *ALG7* (Rine *et al.* 1983, Winzeler *et al.* 1999). The enzyme catalyzing the addition of the second GlcNAc residue to generate Dol-PP-$GlcNAc_2$ is still uncharacterized. Then a first mannose is added onto Dol-PP-$GlcNAc_2$ by the β-1,4- mannosyltransferase encoded by the essential gene *ALG1* (Albright and Robbins 1990). The enzyme which adds the second mannose residue is unknown. Mutation in the *ALG2* gene causes an accumulation of Dol-PP-$GlcNAc_2Man_2$ suggesting that Alg2p is the mannosyltransferase adding the third α-1,6-linked mannose (Jackson *et al.* 1993).

Figure1: N-linked glycosylation in S. cerevisiae. Biosynthesis of the lipid-linked precursor oligosaccharide, its transfer to protein and processing. Adapted from Orlean (1997), Dean (1999) and Aebi and Hennet (2001).



Addition of a fourth α1,2-linked mannose residue is catalyzed by a yet unknown enzyme. Alg11p, the α-1,2-mannosyltransferase responsible for adding the fifth mannose residue has 23% identity to Alg2p (Cipollo *et al.* 2001). Even though deletion in *ALG11* shows a growth defect, *alg11Δ* mutants are viable at room temperature. The viability of strains lacking Alg11p indicates that Dol-PP-$GlcNAc_2Man_4$ and not Dol-PP-$GlcNAc_2Man_5$ represents the minimal structure for survival of yeast strains. The *alg11Δ* mutant is able to translocate Dol-PP-$GlcNAc_2Man_3$ and Dol-PP-$GlcNAc_2Man_4$ structures into the ER lumen to be further elaborated and transferred onto proteins (Cipollo *et al.* 2001).

1.1.2. Lumenal side of the ER.

Translocation of the Dol-PP-$GlcNAc_2Man_5$ precursor from the cytoplasmic side of the endoplasmic reticulum membrane to the lumen for further elongation has been proposed to be catalyzed by the flippase Rft1p (Helenius *et al.* 2002). Completion of the lipid-linked core oligosaccharide synthesis requires four mannosyltransferases and three glucosyltransferases. The mannosyltransferases Alg3p (Huffaker and Robbins 1983, Aebi *et al.* 1996), Alg9p (Burda *et al.* 1996) and Alg12p (Burda *et al.*1999) act successively to generate Dol-PP-$GlcNAc_2Man_9$.This oligosaccharide is then specifically glucosylated by three glucosyltransferases: Alg6p (Reiss *et al.* 1996) and Alg8p (Stagljar *et al.* 1994), both α-1,3-glucosyltransferase and Alg10p (Burda and Aebi 1998) an α-1,2-

glucosyltransferase. The structure of the fully assembled oligosaccharide is Dol-PP-$GlcNAc_2Man_9Glc_3$ (Fig.1).

1.2.Transfer of the oligosaccharidic chain to nascent polypeptide chains and its processing.

1.2.1. Transfer to nascent polypeptide chains.

The fully assembled oligosaccharide is subsequently transferred by the oligosaccharyl-transferase complex (OST) to selected asparagine residues of nascent polypeptide chains during their translocation across the ER membrane (Knauer and Lehle 1999). The OST complex consists of at least nine ER membrane proteins: Wbp1p, Swp1p, Ost1p, Ost2p and Stt3p which are essential for viability and Ost3p, Ost4p, Ost5p, Ost6p which are required for optimal oligosaccharide transfer *in vitro* and *in vivo* (Knauer and Lehle 1999). The specific role of the individual subunits has yet to be determined.

The recognition signal for N-glycosylation is the tripeptide sequon Asn-X-Ser/Thr, where X can be any amino acid except proline. Not all potential Asn-X-Ser/Thr sequons in a protein become N-glycosylated *in vivo*. Proximal amino acid residues and local peptide conformation are proposed to be factors that influence the substrate preference of OST (Bause and Lehle 1979, Reddy *et al.* 1988).

Even though shorter oligosaccharides including Dol-PP-$GlcNAc_2Man_2$ can serve as substrates for the OST complex, the fully assembled Dol-PP-

$GlcNAc_2Man_9Glc_3$ precursor is the preferred oligosaccharide substrate for the OST complex (Sharma *et al.* 1981).

Mutations in the late *ALG* genes do not result in obvious growth defects, but lead to protein underglycosylation due to decreased affinity of the OST towards truncated oligosaccharides. The incomplete oligosaccharides are still transferred to protein, but with reduced efficiency (Huffaker *et al.* 1983).

It is unclear how the fully assembled donor is preferentially transferred. In a recent study using proteoliposome, Karaoglu *et al.* (2001) provided evidence for two oligosaccharide binding sites in the OST complex. A model was proposed in which optimal binding is achieved through allosteric communication between two binding sites and high affinity towards Dol-PP-$GlcNAc_2Man_9Glc_3$. For example the enzyme will display a 10-fold preference for the fully assembled Dol-PP-$GlcNAc_2Man_9Glc_3$ relative to an assembly intermediate, Dol-PP-$GlcNAc_2Man_9$. This would ensure that the majority of proteins will receive a fully assembled oligosaccharide.

1.2.2. Processing of oligosaccharide by glycosidases in the lumen of ER

Processing of the N-linked oligosaccharide begins immediately following the transfer of the $GlcNAc_2Man_9Glc_3$ oligosaccharide precursor from its dolichol-bound form to nascent proteins. The terminal α1,2-linked glucose is first removed by α-glucosidase I (GI, Cwh41p/Gls1p) (Romero *et al.* 1997, Simons *et al.* 1998) and then the two α-1,3-linked glucose residues are cleaved by α-

glucosidase II (GII, Rot2p) (Trombetta *et al.* 1996). In mammalian cells, removal of the first α1,3 glucose residue by Rot2p creates a transient monoglucosylated oligosaccharide which appears to provide a recognition signal for lectin-like chaperones (calnexin and calreticulin) and improves the efficiency of protein folding (reviewed in Helenius *et al.* 1997, Ellgaard and Helenius 2001). Jakob *et al.*(1998b), proposed the existence of a related system in *S. cerevisiae*. α-glucosidases play an additional role in maintaining a normal level of β1,6-glucan of yeast cell wall, however this role is poorly understood (Abeijon and Chen 1998). The middle branch α-1,2-mannose residue is removed by α-1,2-mannosidase I (Mns1p) creating N-glycoproteins with $GlcNAc_2Man_8$ oligosaccharides (Camirand *et al.* 1991).

Trimming of the glucose residues is identical in *Saccharomyces cerevisiae* and in mammalian cells, but the number of mannose residues that are subsequently cleaved differs from one organism to another. Mannose removal is not required for outer chain synthesis and *mns1Δ* mutants lack obvious growth defects, but Knop *et al.* (1996) observed reduced degradation of misfolded carboxypeptidase Y in *mns1Δ* cells. Slow mannose removal by Mns1p has been proposed to serve as a signal in the ER associated degradation (ERAD) pathway for misfolded glycoproteins (Helenius and Aebi 2001). Jakob *et al.* (2001) and Nakatsukasa *et al.* (2001) reported the identification of a mannosidase-like protein, Mnl1p/Htm1p required for efficient degradation of misfolded N-glycoproteins. They proposed that this protein may function as a lectin recognizing and retaining malfolded proteins with $GlcNAc_2Man_8$ oligosaccharides in the ER.

Following processing of their oligosaccharide residues, glycoproteins are further modified in the Golgi.

1.3. ER quality control

In both mammalian and yeast cells, proteins entering the secretory pathway acquire their proper tertiary and in some cases quaternary structures in the ER. Proteins that fail to fold properly are not transported to the Golgi. They are first retained in the ER and eventually transported to the cytosol where they are degraded by the proteasome in a process called Endoplasmic Reticulum Associated Degradation or ERAD (Hiller *et al.* 1996, McCracken and Brodsky 1996, Werner *et al.* 1996).

In mammalian cells, glycoproteins exhibiting $GlcNAc_2Man_9Glc_1$ are substrates for an ER chaperone system called the calnexin-calreticulin cycle or ER quality control (Helenius *et al.* 1997, Zapun *et al.* 1999, Parodi 2000). Calnexin and calreticulin are related membrane and soluble ER lectins respectively, that bind transiently to newly synthesized glycoproteins (Ou *et al.* 1993, Parodi 2000). Interaction with calnexin and calreticulin exposes the folding glycoprotein to the associated co-chaperone, Erp57, a thiol oxidoreductase of the protein disulfide isomerase (PDI) family (Olivier *et al.* 1997). The opposing actions of two soluble ER enzymes, GII and UDP-Glc: glycoprotein glycosyltransferase (GT), regulate the association and dissociation of glycoproteins with complexes of ER lectins (Helenius *et al.* 1997, Olivier *et al.* 1997, Zapun *et al.* 1999). While GII removes a glucose residue from $GlcNAc_2Man_9Glc_1$ oligosaccharide, GT reglucosylates

glucose-free $GlcNAc_2Man_9$ oligosaccharides (Trombetta *et al.* 1996). Substrate recognition by GT is thought to be based on general biophysical properties of the protein shared by incompletely folded proteins. These include mobile loops and lack of compactness (Sousa *et al.* 1992, Cannon and Helenius 1999, Parodi 2000).

Overall ER quality control of glycoprotein requires three basic components: GII, GT and calnexin/calreticulin. These enzymes cooperate together to increase the folding efficiency and prevent the export of misfolded glycoproteins from the ER. The situation in *S. cerevisiae*, however, may differ from that in other organisms. First, the disruption of GII gene in *S.cerevisiae* does not lead to accumulation of misfolded glycoproteins in ER. Second, the yeast *S.cerevisiae* is the only organism known so far that appears to be devoid of GT activity. Though the *KRE5* gene product shows a limited but significant degree of similarity with GT sequences from other organisms (Fernandez *et al.* 1994, Parker *et al.* 1995, Fernandez *et al.* 1996, Jakob *et al.* 1998a,Tessier *et al.* 2000), it seems to be devoid of classical GT activity (Levinson *et al.* 2002). Third, *S. cerevisiae* Cne1p (calnexin homolog) shows only 23% similarity to mammalian counterpart, lacks any Ca^{2+}-binding motifs, has no cytoplasmic tail, and is not essential for viability though it is essential in *S. pombe* (Jannatipour and Rokeach 1995, Parlati *et al.* 1995).

1.4. Genes involved in oligosaccharide precursor glucosylation: late *ALG* genes.

- ***ALG6* (YOR002W, chromosome XV):**

ALG6 (Asparagine-Linked Glycosylation 6) encodes a 544 aa (63 KDa) ER transmembrane protein (with 12 predicted transmembrane domains) that is required for glucosylation in the N-linked glycosylation pathway (Runge *et al.* 1984, Aebi *et al.* 1996). Alg6 p adds the first α-1,3 glucose residue on the lipid-linked oligosaccharide. Alg6p preferentially glucosylates the completely mannosylated oligosaccharide even though Dol-PP-$GlcNAc_2Man_7$ is also a substrate. Mutations in this locus do not affect growth. However, the null *alg6* mutant accumulates Dol-PP-$GlcNAc_2Man_9$ (Huffacker and Robbins 1983), shows a number of cell wall phenotypes such as resistance to K1 killer toxin and hypersensitivity to calcofluor white and SDS (Pagé *et al.* 2003).

- ***ALG8* (YOR067C, chromosome XV):**

ALG8 encodes a 577 aa (67 KDa) transmembrane ER protein which catalyzes the addition of the second α-1,3 glucose residue on the lipid-linked oligosaccharide residue. Alg8p has 12 predicted transmembrane domains and 27% identity to Alg6p. This is not unexpected since both proteins encode putative glucosyltransferases that catalyze the addition of an α-1,3 glucose from Dol-P-Glc to the lipid-linked oligosaccharide. Nevertheless, the two activities are highly specific because overexpression of Alg6p does not suppress the

hypoglycosylation phenotype in an *alg8* mutant. An *alg8* mutant accumulates Dol-PP-$GlcNAc_2Man_9Glc_1$ (Runge and Robbins 1986, Staglajar *et al.* 1994), displays cell wall phenotypes such as resistance to K1 killer toxin and hypersensitivity to calcofluor white and SDS (Pagé *et al.* 2003).

- ***ALG10* (YGR227W, chromosome VII)**:

ALG10 (*DIE2*) encodes a 525aa (62 kDa) transmembrane protein (with 9 predicted transmembrane domains) which catalyzes the addition of the terminal α-1,2 glucose yielding the fully assembled core oligosaccharide (Burda and Aebi 1998). The enzymatic activity of Alg10p has been established and stringent acceptor substrate specificity was observed. Dol-PP-$GlcNAc_2Man_9Glc_1$ is not a substrate for the Alg10p either *in vivo* or *in vitro.*

The *alg10* null mutants show reduced N-linked glycosylation. This observation demonstrates the importance of the terminal α-1,2-linked glucose as a key element in oligosaccharide recognition by the OST complex. Overexpression of *ALG10* enhances expression of *ITR1*, the inositol-transport system I (Nikawa and Hosaka 1995) and *INO1* encoding inositol-1-P-synthase (Dean-Johnson and Henry 1989). The regulatory effect of Alg10p on *INO1* and *ITR1* expression has not been analyzed in detail.

The stepwise, highly ordered assembly of the lipid-linked oligosaccharide culminating in the addition of the α-1,2-linked glucose residue ensures that it is almost exclusively the fully assembled oligosaccharide which is transferred to

protein. Individual sugar residues of the protein-bound core oligosaccharide fulfill specific functions in the processing of glycoproteins in the endoplasmic reticulum (Helenius *et al.* 1997).

The importance of the ER glucosyltransferases is revealed in this work describing genetic interactions and phenotypes of mutants.

1.5. Genes encoding for ER glucosidases and calnexin.

- ***CWH41/GLS1* (YGL027C, chromosome VII):**

CWH41 gene was cloned by functional complementation and shown to encode an ER type II transmembrane protein of 833aa (96 kDa) that cleaves the terminal alpha 1,2-linked glucose residue (Romero *et al.* 1997). The *cwh41* mutant was isolated as part of a screen for calcofluor white hypersensitivity (Ram *et al.* 1994). This mutant is partially resistant to K1 killer toxin and shows a 50% reduction in cell wall β-1,6-glucan content (Jiang *et al.*1996). The *cwh41* mutant exhibits wild type growth, sporulation and mating efficiency. The *cwh41* mutation is synthetically lethal in combination with *big1*, *kre1*, *kre5* and *kre6* null mutants (Jiang *et al.* 1996, Azuma *et al.* 2002).

The yeast α-glucosidase I is inhibited by 1-deoxynojirimicin and its derivatives, by kojibiose and by Tris (Herscovics *et al.* 1982, Bause *et al.* 1986, Herscovics *et al.* 1998). Unlike its mammalian counterpart, α-glucosidase I from *S. cerevisiae* is not inhibited by removal of mannose residues from the α-1,6-branch of $GlcNAc_2Man_9Glc_3$ (Herscovics *et al.* 1982).

- ***ROT2/GLSII* (YBR229C, chromosome 2):**

The yeast α-glucosidase II gene (*ROT2*) was identified by similarity with its mammalian counterpart (Helenius *et al.* 1996). The yeast gene encodes a hydrophilic protein of 954aa (110 KDa) with a putative signal sequence, no apparent transmembrane domain or known ER retention/retrieval signal. The enzyme removes two α-1,3-linked glucose residues from $GlcNAc_2Man_9Glc_2$ oligosaccharide structure (Herscovics 1999) and may play a role in glycoprotein quality control (Jakob *et al.* 1998a). Disruption of *ROT2* does not affect growth or viability but causes a decrease in cell wall β-1,6-glucan levels similar to that observed in the *cwh41* mutant. In contrast to the *cwh41* mutant, however, levels of β-1,3-glucan are increased (< 25%) in *rot2* null mutant (Helenius *et al.* 1998, Shahinian *et al.* 1998, Pagé *et al.* 2003). The null mutation confers resistance to K1 killer toxin, hypersensitivity to calcofluor white, hygromycin B, and SDS (Pagé *et al.* 2003), increased level and altered distribution of chitin (Bickle *et al.* 1998). Lack of α-glucosidase activity in mutants or in cells treated with α-glucosidase inhibitors (castanospermine, 1-deoxynojirimycin) interferes with folding, secretion and proper localization of some, but not necessarily all, glycoproteins. The lack of glucose trimming can have important biological consequences.

- ***CNE1* (YAL058W, chromosome1):**

Saccharomyces cerevisiae CNE1 encodes a 502aa (57KDa) endoplasmic reticulum (ER) membrane protein with sequence similarity to calnexin and calreticulin and functions as a constituent of the ER quality control apparatus

(Parlati *et al.* 1995). The *cne1* null mutant has a 30% reduction of β-1,6-glucan levels into the cell wall (Shahinian *et al.* 1998), displays resistance to K1 killer toxin, and hypersensitivity to calcofluor white, hygromycin B, and SDS (Pagé *et al.* 2003). Disruption of this gene affects the quality control of misfolded glycoproteins such as the heterologously expressed hen egg white lysozyme (McCracken and Brodsky 1996, Song *et al.* 2001).

1.6 Synthetic lethality.

Two genes show a "synthetic lethal" interaction if the combination of two mutations, neither by itself lethal, causes cell death. Synthetic lethality defines a relationship where the presence of one gene allows the organism to tolerate genetic variation in another gene that would be lethal in the absence of the first gene (Hartman *et al.* 2001). With the high buffering capacity of the yeast *Saccharomyces cerevisiae*, synthetic lethality uncovers redundant and related functions through genetic interactions.

Synthetic lethal relationships may occur between genes acting at the same step of a certain biochemical pathway, at different steps of the same pathway (both intrinsic buffering), or even in completely different pathways (extrinsic buffering). Synthetic lethal interactions may be tedious to demonstrate (Hartman *et al.* 2001). The use of Synthetic Genetic Array (SGA), a collection of 4700 null viable mutants, to create double mutants is an important advancement. Work presented here focuses on the identification of synthetic interactions with N-glycan

glucosylation and processing genes using the SGA analysis (Tong *et al.* 2001) to systematically construct double mutants with the following query genes: *ALG6, ALG8, ALG10, CWH41, ROT2* and *CNE1.*

1.7 Phenotype of N-glucosylation mutants (congenital disorder of glycosylation): case of human *ALG6* and *PMM2* genes.

In humans, mutant alleles of genes involved in N-glycan biosynthesis are linked to congenital disorders of glycosylation (CDG), a group of autosomal recessive metabolic disorders (Aebi and Hennet 2001, Freeze 2001, Jaeken and Matthijs 2001). Most of the consequences of CDG are thought to result from hypoglycosylation.

CDGI disorders affect the biosynthesis of the dolichol-linked precursor oligosaccharide and its transfer to proteins. CDGIc is caused by mutations in *hALG6.* Psychomotor retardation is the main clinical symptom of CDIc disorder. Muscular hypotonia is a common symptom and nearly all patients have recurrent seizures (Grunewald *et al.* 2000). Seven mutations in *hALG6* gene are currently known to cause CDG-Ic (Imbach *et al.* 1999, Freeze *et al.* 2000, Imbach *et al.* 2000). CDGIc patients make normal amounts of lipid-linked oligosaccharide but accumulate non-glucosylated precursors. Since the incomplete oligosaccharide is a poor substrate for the OST complex, the consequence is low transfer of the oligosaccharide to proteins and hypoglycosylation. CDGIb results from a mutation in *MPI1*, a gene encoding a phosphomannomutase isomerase. Most of the other types of CDGI are rare. CDGId, CDGIe, CDGIf and CDGIg are caused

by mutations in *hALG3, DPMI, MPDUI* and *hALG12* respectively (Marquardt and Denecke 2003). The most common type, CDGIa, is caused by mutations in the *PMM2* gene, a phosphomannomutase. Some of the symptoms seen in patients with CDGIa are axial hypotonia, psychomotor retardation, internal strabismus, impaired night vision and retinitis pigmentosa (Marquardt and Denecke 2003). *PMM2* gene encodes for the phosphomannomutase which converts mannose-6-phosphate to mannose-1-phosphate (Matthijs *et al.* 1997). Mutation in this gene decreases the supply of intracellular GDP-Man and limits the amount of lipid-linked oligosaccharide precursor available for protein glycosylation (Rush *et al.* 2000). This limitation leads to unoccupied glycosylation sites and thus to protein hypoglycosylation. Recently, it has been shown that a frequent mild mutation in the *ALG6* gene may exacerbate the clinical severity of patients with CDG-Ia caused by deficiency in *PMM2* activity (Westphal *et al.* 2002).

Yeast *SEC53* gene has 60% identity to human *PMM2* and codes for a 29KDa cytoplasmic essential protein (Huffaker and Robbins 1983, Mondesert *et al.* 1997). Temperature sensitive mutants of this gene show altered protein glycosylation (Mondesert *et al.* 1997) and secretion (Ruohola and Ferro-Novick 1997).

This thesis will analyze the phenotype of the *alg6Δsec53-6* double mutant.

2. MATERIALS AND METHODS.

2.1 Strains and culture conditions.

Yeast strains used in this study are listed in Table 1. Deletant strains were from the Saccharomyces Genome Deletion Consortium (Giaver *et al.* 2002) and are available at Research Genetics (http://www.resgen.com/products/YEASTD.php3). Single mutant knockout strains were made by PCR-based gene deletion using homologous recombination (Winzeler *et al.* 1999). Each gene was replaced by a dominant selectable kanamycin (G418) resistance marker (KanMX4 cassette). Crosses, sporulation of diploids and dissection of tetrads were conducted using standard genetic methods (Sherman *et al.* 1982). Yeast were grown on YPD or on SD supplemented with appropriate amino acids. Geneticin (G418, Invitrogen, Carlsbad, CA), nourceothricin (NAT, Werner Bioagent, Jena, Germany), canavanine (Can, Sigma, Ontario, Canada) calcofluor white (Fluorescent Brightner 28, Sigma) were used at 200mg/liter, 100mg/liter, 100mg/liter and 25mg/liter respectively.

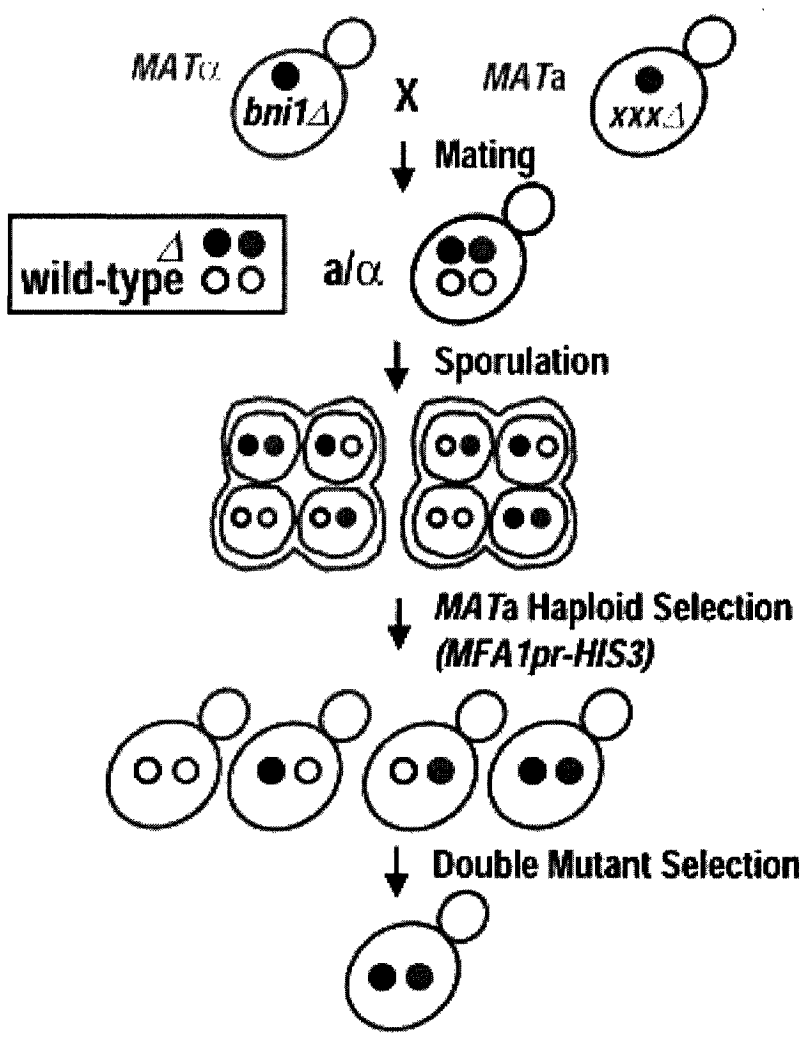
Table1: Yeast strains.

Strain	Genotype	Source
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Brachmann *et al.* 1998
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Brachmann *et al.* 1998
YD11778	BY4742 *alg6Δ* ::kanMX4	Brachmann *et al.* 1998
Haploid mutant	*MATa orf::kanMX4* as BY4741	Winzeler *et al.* 1999
SBY1	*MATα alg6*::NatR *mfα1Δ:: MFα1-prLEU2 can1Δ::MFA1-prHIS3 his3Δ leu2Δ lys2Δ met15Δ ura3Δ*	Bussey lab
SBY7	*MATα cne1*::NatR *mfα1Δ:: MFα1-prLEU2 can1Δ::MFA1-prHIS3 his3Δ leu2Δ lys2Δ met15Δ ura3Δ*	
SBY9	*MATα cwh41*::NatR *mfα1Δ:: MFα1-prLEU2 can1Δ::MFA1-prHIS3 his3Δ leu2Δ lys2Δ met15Δ ura3Δ*	
SBY26	*MATα mnl1*::NatR *mfα1Δ:: MFα1-prLEU2 can1Δ::MFA1-prHIS3 his3Δ leu2Δ lys2Δ met15Δ ura3Δ*	
SBY27	*MATα mns1*::NatR *mfα1Δ:: MFα1-prLEU2 can1Δ::MFA1-prHIS3 his3Δ leu2Δ lys2Δ met15Δ ura3Δ*	
SBY28	*MATα rot2*::NatR *mfα1Δ:: MFα1-prLEU2 can1Δ::MFA1-prHIS3 his3Δ leu2Δ lys2Δ met15Δ ura3Δ*	
SBY34	*MATα ylr057w*::NatR *mfα1Δ:: MFα1-prLEU2 can1Δ::MFA1-prHIS3 his3Δ leu2Δ lys2Δ met15Δ ura3Δ*	
SBY71	*MATα alg10*::NatR *mfα1Δ:: MFα1-prLEU2 can1Δ::MFA1-prHIS3 his3Δ leu2Δ lys2Δ met15Δ ura3Δ*	
SBY80	*MATα alg8*:NatR *mfα1Δ:: MFα1-prLEU2 can1Δ::MFA1-prHIS3 his3Δ leu2Δ lys2Δ met15Δ ura3Δ*	
HMSF331	*MATa, sec53-6*	Novick *et al.* 1984

2.2 Synthetic Genetic Array (SGA) Analysis

Double mutant combinations were obtained by means of the synthetic genetic array (SGA) analysis (Tong *et al.* 2001, see Fig.2). Briefly, the goal of this procedure is to generate haploid double mutants bearing a deletion in a query gene (*ALG6, ALG8, ALG10, CWH41, ROT2* or *CNE1*) and a deletion in one of the 4700 other non-essential single-gene deletions. In the SGA procedure, the query mutation is introduced in a *MATa* haploid starting strain containing both nourseothricin-resistance (natR) and *MFA1pr-HIS3* markers, such that the *HIS3* gene is only expressed in a *MATa* background. The *MAT*α queries were constructed using homologous recombination by replacing the KanMX4 cassette with the natR cassette at the deleted ORF locus, and by replacing the *CAN1* locus with the *MFA1pr-HIS3* marker. The single mutant query strain is crossed to each of the 4700 single mutant strains. The resultant heterozygous double mutant diploids are sporulated to obtain haploid double mutants. The haploid double mutant progeny is then selected for growth on medium with G418 and NAT. Size of haploid double mutant colonies are scored visually as slow growth (SG) or no growth (synthetic lethal, SL). If a strain is scored SG or SL in two out of three replicate screens, the interaction is kept for further confirmation.

Figure 2: SGA double selection procedure (Tong *et al.* 2001)



2.3. Confirmation procedure

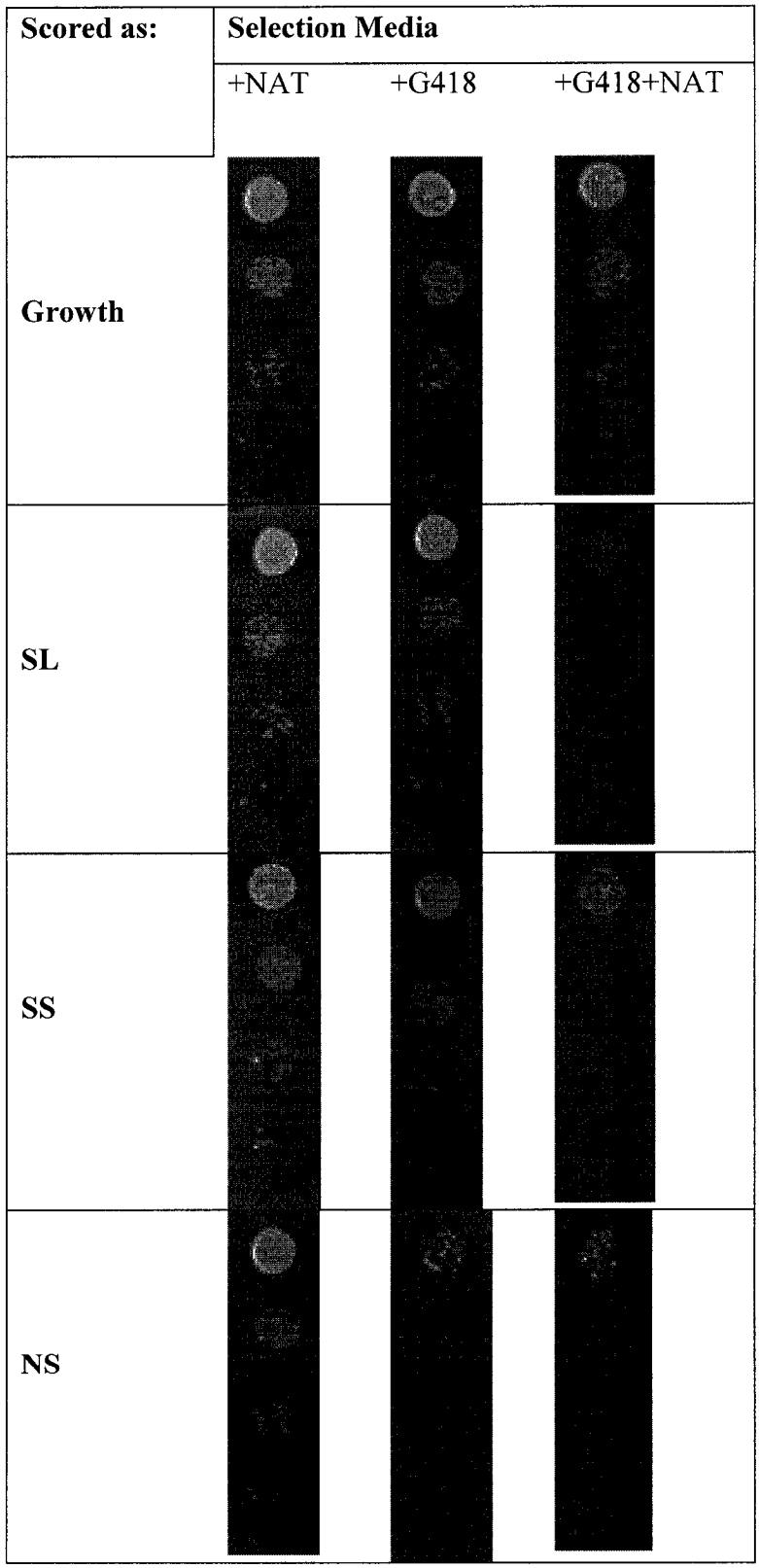
To filter out false positive interactions, all double mutants scored more than once as SG or SL in the initial SGA screens are confirmed using the "spotting" method and tetrad dissection.

2.3.1 The "spotting " method

This method allows comparison of the double mutant growth to growth of the single mutants. It requires picking cells directly from the robot-pinned sporulation array plates, resuspending them in 200 µl of liquid SD lacking histidine and arginine and supplemented with canavanine (SD-HIS-ARG+Can) in 96-well plates, and incubating for 48 hours at 30^0C. This incubation provides enough time for all strains to grow to saturation in the 200 µl well, as well as to be selected for *MATa* spores only. After 48 hours, all strains are diluted serially and 3µl of undiluted, 1:100, 1:10000 and 1:100000 dilutions is spotted on SD-HIS-ARG+G418+Can (for array mutant selection), SD-HIS-ARG+NAT+Can (for query mutant selection), and SD-HIS-ARG+G418+NAT+Can (for double mutant selection). By comparing growth on different selection plates, it is ensured that double mutant growth defects are indeed due to synthetic interactions, and not simply to the slow growth of the parental single mutant. True interactions are scored as either 'SS' (synthetic sick) or SL (Fig.3) while NS was the score for non synthetic interactions. This spotting method was used to confirm all *ALG6, ALG8,*

ALG10, CWH41, ROT2, and *CNE1* interactions marked as SG or SL in the initial SGA screens.

Figure 3: Scoring Procedure for Random Sporulation Spotting



2.3.2 Tetrad analysis.

Spores from heterozygote diploid double mutants are taken from sporulation plates. A small amount of cells is resuspended in a solution of 200µl H_2O + 7 µl zymolyase (2.5 g/liter). After a 10-minute incubation, 10µl of this solution is spotted on a dissection plate. Tetrads are picked from the plate and dissected using a Singer micromanipulator. For each strain, at least 10 tetrads are dissected, and incubated at 30^0C for 3 days. The resultant spore colonies are then photographed and velvet-replica-plated onto YEPD+G418 and YEPD+NAT to assess the genotype of the spore progeny. Double mutant haploids are identified by their ability to grow on both YEPD+G418 and YEPD+NAT. Double mutant growth is scored as SS or SL by referring back to the initial photograph.

2.4. Analysis of CPY glycosylation by immmunoblotting.

Total cell lysates were prepared from log-phase yeast cells grown in YPD at 27^0C. Cells were harvested and broken with glass beads in ice-cold lysis buffer (50mM Tris-HCl pH 7.5, Complete Protease Inhibitor Cocktail [Boehringer Mannheim, Germany]). Cell wall material and unbroken cells were removed by centrifugation at 4^0C (3,000 rpm,10 minutes). For analysis of CPY glycosylation, EndoglycosydaseH_f (New England Biolabs) treated, according to supplier's instructions, and untreated whole cells extracts were analyzed by SDS-PAGE using 8% polyacrylamide gel then transferred to Hybond-C nitrocellulose

membrane (Amersham, Oakville,ON, Canada). Immunoblotting was performed using 2μg/ml rabbit anti-CPY (Research Diagnostics, Flanders, NJ) as primary antibody and a 1: 2000 dilution of horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Amersham). Bands were detected using the chemiluminescence detection kit (Amersham).

2.5. Growth phenotype.

The "spotting" method was used to analyze the growth of yeast cells on YPD at 27^0C. For the growth curve, yeast cells were grown over night at 27^0C, diluted to an OD_{600} of 0.1 then incubated at 27^0C. Samples were taken at 3, 6, 12, 24, 48 and 54 hours, and their optical density measured.

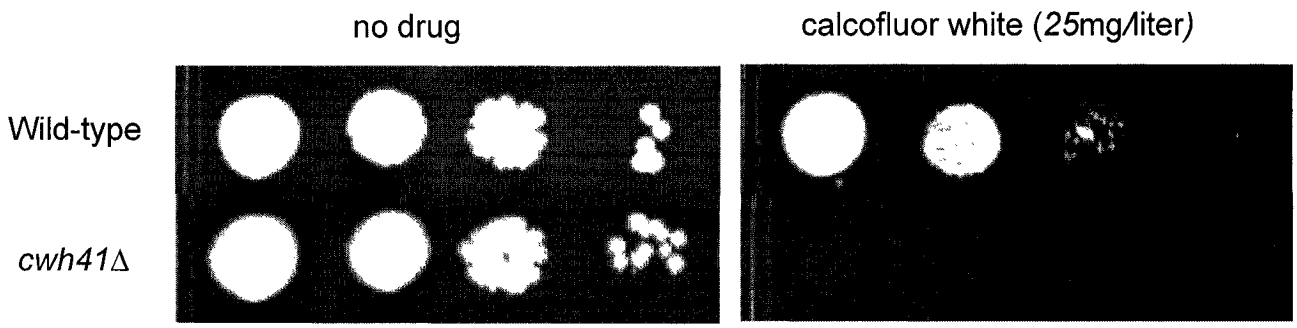
2.6. Calcofluor White Sensitivity.

Calcofluor white sensitivity was checked by using a modified version of the method described by Ram *et al.* (1994). Strains were grown over night at 30^0C in 10ml YEPD. An OD_{600} is taken the next day and each strain is diluted to an OD_{600} value of 0.5.

3 μl of the pure or 1:10, 1:100 and 1:1000 dilutions of this suspension is spotted on SD plates containing 25 mg/liter calcofluor white and control plates. As a control, the wild type BY4741 strain is spotted on each plate. Plates are

incubated at 30^{0}C for 48 hours, photographed, then checked again after 72 hours. Strains growing more slowly than the wild type are scored as sensitive.

Figure 4: Example of a calcofluor white sensitive strain



3. RESULTS.

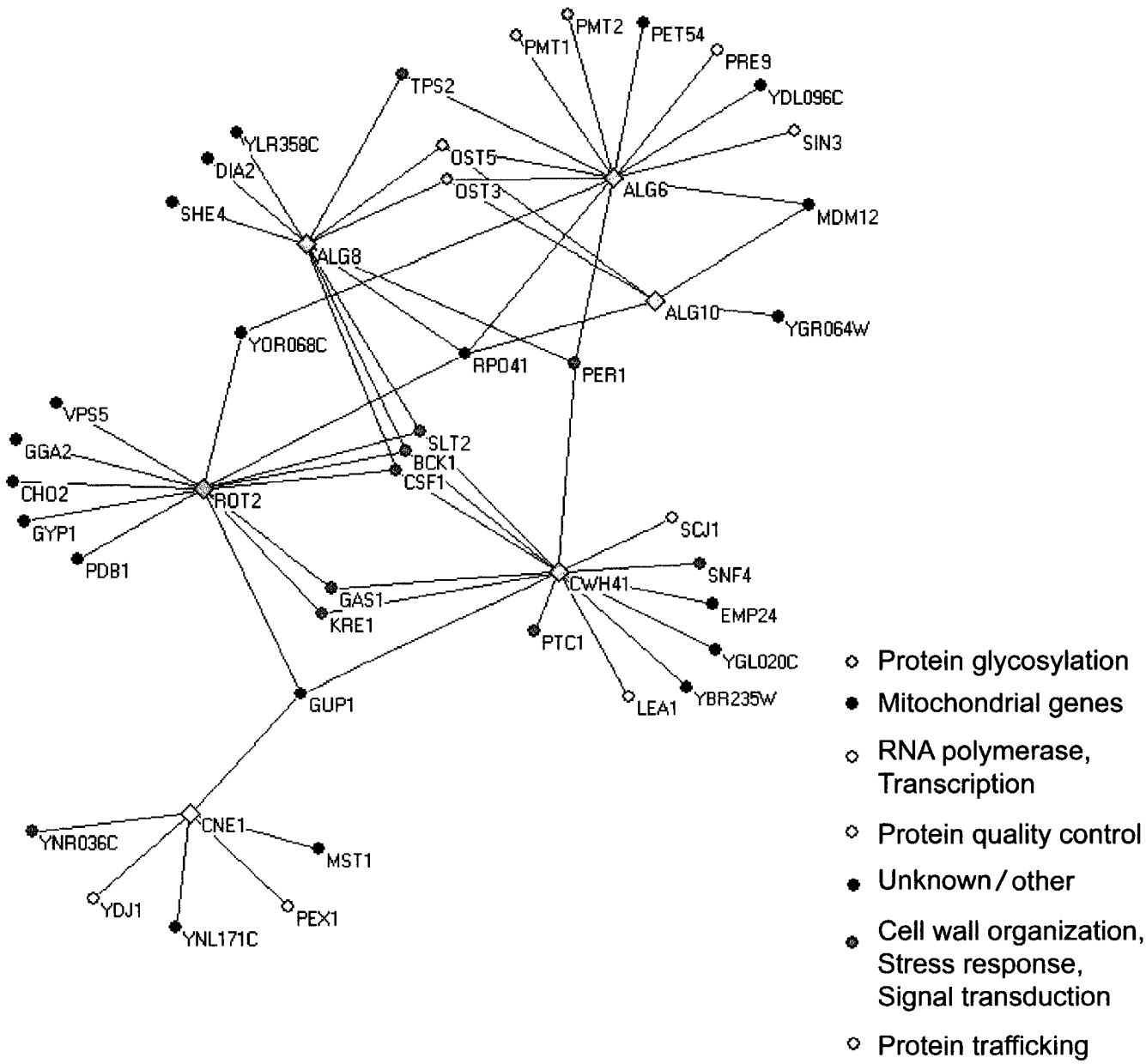
3.1 SGA analysis of N-glucosylation related genes.

With the goal of identifying factors that are functionally related to the N-glycan glucosylation and processing enzymes, I took advantage of a recently developed method to create and analyze yeast double mutants, the SGA analysis (Tong *et al.* 2001). Eight query genes involved in N-glycan glucosylation and processing and one gene of unknown function were tested: *ALG6, ALG8, ALG10, CWH41, ROT2, MNS1, MNL1, CNE1, YLR057W* (has a product with 46% similarity to Mns1p). The query mutations were deletion alleles of these non-essential genes. After crossing mutant strains carrying these query genes to an array of strains containing mutations in about 4700 genes, double mutants showing a growth defect were identified. These defined candidate genetic backgrounds in which the N-glycan glucosylation or processing enzymes are required for growth or viability.

Overall 9 SGA screens made of a total of 42,000 crosses in triplicates (each of the 9 mutant query strains was crossed to an array of 4700 mutant strains). Primary results from the 9 screens gave 1792 putative interactions. To create a high quality data set each SGA screen was done three times then all the 1792 hits were confirmed by random spore analysis and/or tetrad dissection. This confirmation gave a total of 61 interactions between 6 query genes and 41 array genes which define a network of synthetic interactions (Fig.5). Three genes (*MNS1, MNL1,* and *YLR057W*) did not show any genetic interactions. *ALG6,*

ALG8, ALG10, CWH41, ROT2 and *CNE1* showed 12, 11, 5, 14, 13 and 6 interactions respectively.

Figure 5: Global interaction network for N-glycan glucosylation and processing enzymes. Functional categories are identified by different colors.


PMT2
PMT1
PET54
PRE9
YDL096C
TPS2
SIN3
YLR358C
DIA2
OST5
OST3
ALG6
MDM12
SHE4
ALG8
ALG10
YGR064W
YOR068C
RPO41
PER1
VPS5
SLT2
GGA2
BCK1
CSF1
CHO2
ROT2
GYP1
SCJ1
PDB1
SNF4
CWH41
GAS1
EMP24
KRE1
PTC1
YGL020C
GUP1
LEA1
YBR235W
CNE1
YNR036C
MST1
YDJ1
PEX1
YNL171C
Protein glycosylation
Mitochondrial genes
RNA polymerase, Transcription
Protein quality control
Unknown / other
Cell wall organization, Stress response, Signal transduction
Protein trafficking

All the query genes and their interacting genes were grouped in 8 functional categories according to their cellular function (Table 2).

1. Protein glycosylation (9 genes):

In addition to the query genes *ALG6, ALG8, ALG10, CWH41, ROT2*, 3 other genes are assigned to this category. Two of them, *OST3* and *OST5*, are components of the oligosaccharyltransferase complex that transfers core oligosaccharide from dolichol carrier to Asn-X-Ser/Thr motifs within acceptor proteins. *OST3* is the gamma subunit while *OST5* is the zeta subunit of this complex. Mutants of *OST3* show significant underglycosylation of soluble and membrane-bound glycoproteins *in vivo* (Karaoglu *et al.* 1995). The two other genes *PMT1* and *PMT2* are mannosyltransferases. Both *PMT1* and *PMT2* initiate O-glycosylation. *PMT1* and *PMT2* form a heterodimer which accounts for the majority of protein O-glycosylation activity in yeast cells (Gentzsch *et al.* 1995). Mutants in these genes have improperly processed O-mannoproteins, and thus have cell wall defects.

2. Protein trafficking (6 genes):

Emp24p is a component of COPII-coated vesicles involved in cargo sorting of GPI-containing proteins such as Gas1p (Muniz *et al.* 2000) from ER to Golgi. Gga2p is involved in trafficking of proteins between the trans-Golgi network and the vacuole (Zhdankina *et al.* 2001). Vps5p is a member of the sorting nexin family involved in Golgi retention and recycling from the vacuolar/late endosomal compartment to the late Golgi apparatus (Nothwerh and Hindes 1997). She4p is

required for receptor mediated as well as membrane lipid endocytosis and expression of a number of mother-cell specific genes. *GYP1* encodes a GTPase activating protein for Ypt1p and Sec4p, two proteins involved in vesicular transport. Gyp1p is also involved in protein trafficking to the Golgi (Bonangelino *et al.* 2002). *GUP1*, a gene involved in active glycerol uptake, is also involved in protein trafficking to the vacuole (Bonangelino *et al.* 2002).

3. Protein quality control (5 genes):

One of the query genes, *CNE1*, falls in this category. The mitochondrial protein Ydj1p is involved in protein import into mitochondria and the ER. This chaperone is essential for the ubiquitin-dependent degradation of short-lived and abnormal proteins (Lee *et al.* 1996). Scj1p is a co-chaperone for Kar2p (a heat shock protein of the ER lumen) in normal protein folding reactions in the lumen of the rough ER (Silberstein *et al.* 1998). Pre9p is one of the 14 subunits of the core complex of the proteasome important for degradation of ubiquitinated proteins. *PEX1* encodes a peroxisomal biogenesis protein (peroxin), a member of the AAA family of ATPases associated with diverse aspects of protein folding.

Table 2: Functional categories of genes.

FUNCTIONAL CATEGORIES	GENES
1. Protein glycosylation	*ALG6, ALG8, ALG10, CWH41, OST3, OST5, PMT1, PMT2, ROT2*
2. Protein trafficking	*EMP24, GGA2, GUP1, GYP1, SHE4, VPS5*
3. Protein quality control	*CNE1, PEX1, PRE9, SCJ1, YDJ1*
4. Cell wall organization, stress response, signal transduction	*BCK1, CHO2, CSF1, GAS1, KRE1, PER1, PTC1, SLT2, SNF4, TPS2*
5. Mitochondrial genes	*MDM12, MST1, PDB1, PET54, RPO41, YGL020C, YNR036C*
6. RNA polymerase, Transcription factor	*LEA1, SIN3*
7. Unknowns	*YOR068C, YBR235W, YDL096C, YGR064W, YLR358C, YNL171C*
8. Others	*DIA2*

Table 3: Overlapping interactions between subnetworks.

Query genes	**# of shared interactions with the N-glucosyltransferase subnetwork**	**# of shared interactions with the glucosidase subnetwork**	**# shared interactions with *CNE1***
ALG6	6	3	0
ALG8	5	5	0
ALG10	4	1	0
CWH41	4	6	1
ROT2	5	6	1
CNE1	0	1	-

4. Cell wall organization, stress response and signal transduction (10 genes)

- Cell wall organization: *GAS1, BCK1, SLT2, KRE1* and *CHO2* all fall in this category. *GAS1* codes for a β-1,3 glucanosyltransferase involved in crosslinking of β-1,3 glucan chains in the cell wall (Popolo *et al.* 1997, Mouyna *et al.* 2000). Absence of Gas1p leads to increased chitin synthesis (Popolo *et al.* 1997, Ram *et al.* 1998, Valdivielo *et al.* 2000). *BCK1* and *SLT2* both encode serine/threonine protein kinases: a MAPKK kinase and a MAP kinase respectively that act in maintaining cell wall integrity (Ammerer 1994). *KRE1* is a cell wall protein needed for cell wall β-1,6 glucan assembly. Cho2p is a phosphatidylethanolamine N-methyltransferase.
- Stress response, signal transduction: *PTC1* encodes a protein serine/threonine phosphatase involved in the dephosphorylation of multiple MAP kinases including Slt2p (Huang and Symington 1995, Warmka *et al.* 2001). Ptc1p also acts as a down regulator of the HOG pathway. Csf1p is required for normal growth rate and resistance to NaCl and H_2O_2. Snf4p is involved in the derepression of glucose-repressed genes. It acts with Snf1p, a serine/threonine protein kinase. *TPS2* encodes a component of the trehalose-6-phosphatase synthase/phosphatase complex involved in response to osmotic and heat stress (Winderickx *et al.* 1996, Causton *et al.* 2001). Per1p is a suppressor of *cdc1-1* temperature-sensitive growth defect, involved in Mn^{2+} homeostasis (Paidhungat and Garrett 1998).

5. Mitochondrial genes (7genes):

MDM12 codes for a protein implicated in maintaining mitochondrial DNA (Berger and Yaffe 2000). The *PET54* gene product is a specific translational activator for mitochondrial *COX3* mRNA and a splicing factor for mitochondrial *COX1* pre-mRNA (Costanzo *et al.* 1986, Valencik *et al.* 1989). The product of *MST1* gene is a mitochondrial threonyl-tRNA synthetase. Rpo41p is an RNA polymerase while the product of YNR036C open-reading frame is a putative mitochondrial ribosomal protein of the small subunit. *PDB1* encodes the E-1 beta subunit of the pyruvate dehydrogenase complex.

6. RNA polymerase, Transcription factor (2 genes):

Lea1p is a component of the U2snRNP complex similar to the human U2A' protein. It is involved in the initial steps of pre-mRNA splicing (Caspary and Seraphin 1998). *SIN3* encodes a component of the histone deacetylase β which functions in transcriptional regulation of gene expression and silencing (Loewith *et al.* 2001).

7. Poorly characterized and genes of unknown function (6 ORFs):

Except for *YBR235W* which is known to be a member of the cation: chloride cotransporter family of membrane transporters (Andre and Scherens 1995), not much is known on the other ORFS. *VAM10* (*YOR068C*) encodes for a highly hydrophobic protein with one predicted transmembrane segment (Valens *et al.* 1997) which interact with the vacuolar iron transporter Fth1p in a high throughput

two hybrid assay (Ito *et al.*2001). Null mutation in *YOR068C* is sensitive to tert-butyl hydroxyperoxide (Begley *et al.* 2002).

8. Others (1 gene):

DIA1 encodes a protein involved in invasive and pseudohyphal growth (Palecek *et al.* 2000).

To better analyze the importance of shared interactions as compared to specific interactions in the N-glucosylation network, the N-glycan subnetwork was divided into 3 subnetwork : the N-glucosyltransferase subnetwork (*ALG6, ALG8, ALG10*), the N-glucosidase subnetwork (*CWH41, ROT2*) and CNE1 which forms a separate subnetwork (Table 3). Overall the number of overlapping interactions between a query gene and the other genes in the same subnetwork is higher than between a query gene and another subnetwork. As an example, the number of overlapping interactions between *ALG6* and the N-glucosyltransferase subnetwork is 6, while between *ALG6* and the glucosidase subnetwork it is 3 (Table 3).

3.2 Calcofluor white sensitivity testing.

A link between the query genes and cell wall has previously been found (Shahihian *et al* 1998, Pagé *et al.* 2003). To further explore the basis of this complex phenotype, the growth of mutants in the interacting genes was also assayed under conditions that affect cell wall integrity using a Calcofluor white

test. Calcofluor white is a negatively charged fluorescent dye that binds to nascent chains of chitin and, to a lesser extent, glucan through hydrogen bonding and dipole interactions and, by preventing microfibril assembly, interferes directly with the supramolecular organization of the cell wall (Elorza *et al.* 1983). A disturbed or weakened cell wall is not able to withstand a drug concentration that does not affect normal wild-type cells. Mutants in all the 6 query genes and the 39 interacting genes were tested for calcofluor white sensitivity. A wild type strain was added on each plate as a control.

Mutants in 6 query genes and 28/41 array genes showed a hypersensitive phenotype to 25 mg/liter calcofluor white concentration. Mutants in the remaining genes showed a wild type sensitivity at 25 mg/liter and 50 mg/liter. Thus these mutants did not show any altered sensitivity to calcofluor white (Fig. 6, Table 4).

Figure 6: calcofluor white test.

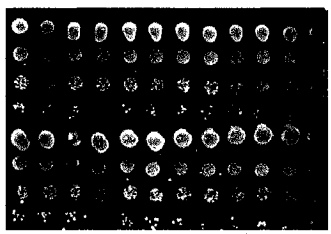
Mutant strains were grown at 30 degrees for 48h, in the absence or presence of 25mg/liter calcofluor white.

1. wt
2. *pdb1Δ*
3. *rot2Δ*
4. *pmt2Δ*
5. *ybr235wΔ*
6 *snf11Δ*
7. *cne1Δ*
8. *emp24Δ*
9. *gup1Δ*
10. *sac7Δ*
11. *ygl020Δ*
12. *cwh41Δ*
13. *spf1Δ*
14. *gga2Δ*
15. *slt2Δ*
16. *cho2Δ*
17. *pre9Δ*
18. ost5Δ
19. *csf1Δ*
20. *sic1Δ*
21. *mnn11Δ*
22. *ubi4Δ*
23. *vps24Δ*
24. *sec22Δ*
25. wt
26. *alg6Δ*
27. *yor235w*
28. *ynl171cΔ*
29. *dia2Δ*
30. *ost3Δ*
31. *kre1Δ*
32. *alg8Δ*
33. *yor068cΔ*
34. *vps5Δ*
35. *gyp1Δ*
36. *gas1Δ*
37. *ynl198cΔ*
38. *she4Δ*
39. *slg1Δ*
40. *tir4Δ*
41. *mdm2Δ*
42. *sin3Δ*
43. *bem4Δ*
44. *lea1Δ*
45. *ptc1Δ*
46. *pet54Δ*
47. *ydl096cΔ*
48. *snf4Δ*
49. wt
50. *pmt1Δ*
51. *dot1Δ*
52. *rpo41Δ*
53. *ydj1Δ*
54. *bck1Δ*
55. *van1Δ*
56. *yme1Δ*
57. *nup133Δ*
58. *tps2Δ*
59. *yor008c-aΔ*
60. *ylr358cΔ*
61. *ubp3Δ*
62. *per1Δ*
63.*ynr020cΔ*

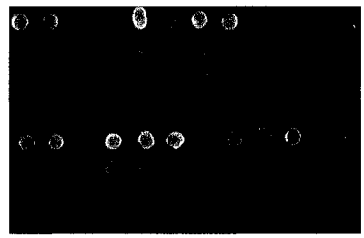
control

25 mg/l

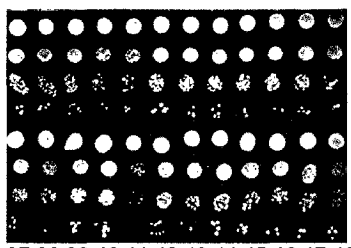
1 2 3 4 5 6 7 8 9 10 11 12



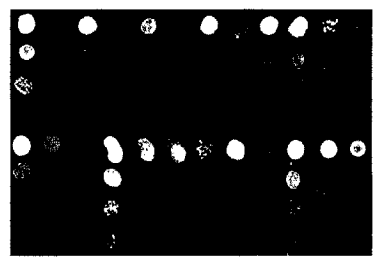
1314 15 16 17 18 19 20 21 22 23 24



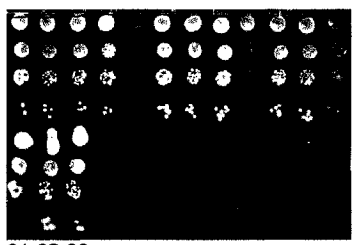
25 26 27 28 29 30 31 32 33 34 35 36



37 38 39 40 41 42 43 44 45 46 47 48



49 50 51 52 53 54 55 56 57 58 59 60



61 62 63

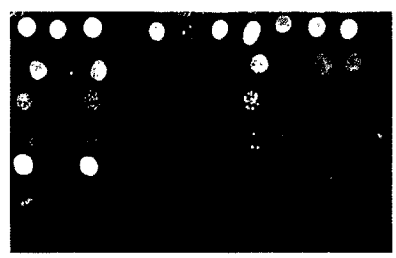


Table 4: List of genes whose deletion confers calcofluor white hypersensitivity.

ORF	GENE	interaction with query gene
YAL023C	*PMT2*	*ALG6*
YAL058W	*CNE1*	
YBR229C	*ROT2*	
YCR044C	*PER1*	*ALG8, CWH41, ALG6*
YDL006W	*PTC1*	*CWH41*
YDL095W	*PMT1*	*ALG6*
YDR074W	*TPS2*	*ALG8, ALG6*
YFL036W	*RPO41*	*ALG6, ALG8, ALG10, ROT2*
YGL020C		*CWH41*
YGL027C	*CWH41*	
YGL084C	*GUP1*	*CWH41, ROT2,CNE1*
YGL115W	*SNF4*	*CWH41*
YGL200C	*EMP24*	*CWH41*
YGL226C-A	*OST5*	*ALG6, ALG8, ALG10*
YGR227W	*ALG10*	
YHR030C	*SLT2*	*ALG8, ROT2, CWH41*
YHR108W	*GGA2*	*ROT2*
YJL095W	*BCK1*	*ALG8, CWH41, ROT2*
YLR087C	*CSF1*	*ROT2, CWH41, ALG8*
YLR358C		*ALG8*
YMR307W	*GAS1*	*CWH41, ROT2*
YNL171C		*CNE1*
YNL322C	*KRE1*	*CWH41, ROT2*
YOL004W	*SIN3*	*ALG6*
YOL009C	*MDM12*	*ALG6,ALG10*
YOR002W	*ALG6*	
YOR035C	*SHE4*	*ALG8*
YOR067C	*ALG8*	
YOR068C	*VAM10*	*ROT2*
YOR069W	*VPS5*	*ROT2*
YOR070C	*GYP1*	*ROT2*
YOR080W	*DIA2*	*ALG8*
YOR085W	*OST3*	*ALG6, ALG8, ALG10*
YPL213W	*LEA1*	*CWH41*

3.3 Synthetic interaction between *ALG6* and *SEC53.*

The results obtained by SGA analysis uncover the cellular functions required when N-glucosylation defects are present in *S. cerevisiae*. In the case of the *ALG6, ALG8* and *ALG10* genes, glucosylation is compromised but no growth defect is associated with mutants individually deleted for these genes.

Stagljar *et al.* (1994) showed that the consequences of *ALG6* mutations (complete absence of glucosylation) in yeast cells depend on the presence of other mutations (e.g. *WBP1*) in the glycosylation pathway. This effect of an *ALG6* mutant allele on mutations in other genes has been demonstrated in humans where research done by Westphal *et al.* (2002) revealed that the effect of the *ALG6* mutant allele is only evident when the glycosylation system must perform at high efficiency. They showed that a frequent mild mutation that changes a phenylalanine to a serine at position 304 in the *ALG6* gene may exacerbate the clinical severity of patients with CDG-Ia caused by *PMM2* deficiency. There is a significant greater frequency of F304S among the severely affected patients, which points to *ALG6* as a genetic modifier of the *PMM2* mutant phenotype.

To test whether this synthetic interaction may be conserved in yeast, we asked if an analogous interaction may exist between *ALG6*, the yeast homologue of *hALG6* and *SEC53 (*YFL045C),the yeast homologue of *PMM2*. Since *SEC53* is an essential gene, a thermosensitive allele, *sec53-6* was used. This mutant grows normally at a temperature between 20-25^{0}C and fails to grow above 30^{0}C. By crossing strains YD11778 and HMSF331 (Table1) an heterozygous double mutant diploid was obtained. After sporulation and tetrad analysis, 3 double

mutant *alg6Δsec53-6* strains were isolated (G418 resistant and thermosensitive). Growth of the double mutants was compared to that of the wild-type or the two parental mutants by a spotting assay. At a temperature where both parental mutants and the wild-type strain grew normally (27^0C), the double mutant *alg6Δsec53-6* showed a significant growth defect (Fig.7A). This phenotype was confirmed by growth curve analysis using 3 independent growth curve assays at 27^0C. One representative double mutant is shown on Fig.7B. Thus, *ALG6* and *SEC53* show a synthetic interaction.

Figure 7:

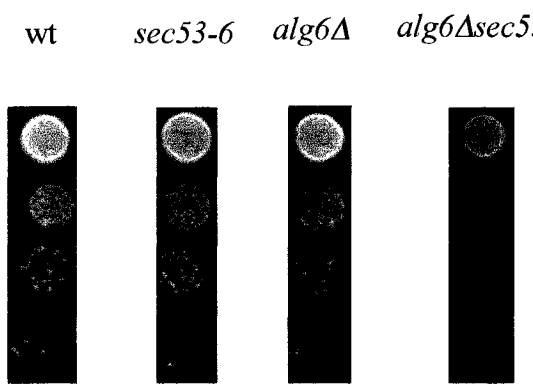
A. Spotting assay.

Cells were pre-grown at 27^{0}C to log-phase, diluted down to an OD_{600} of 0.5, serial diluted 10-fold three times, 5 μl was then spotted on YPD and incubated at 27°C for 36 hours.

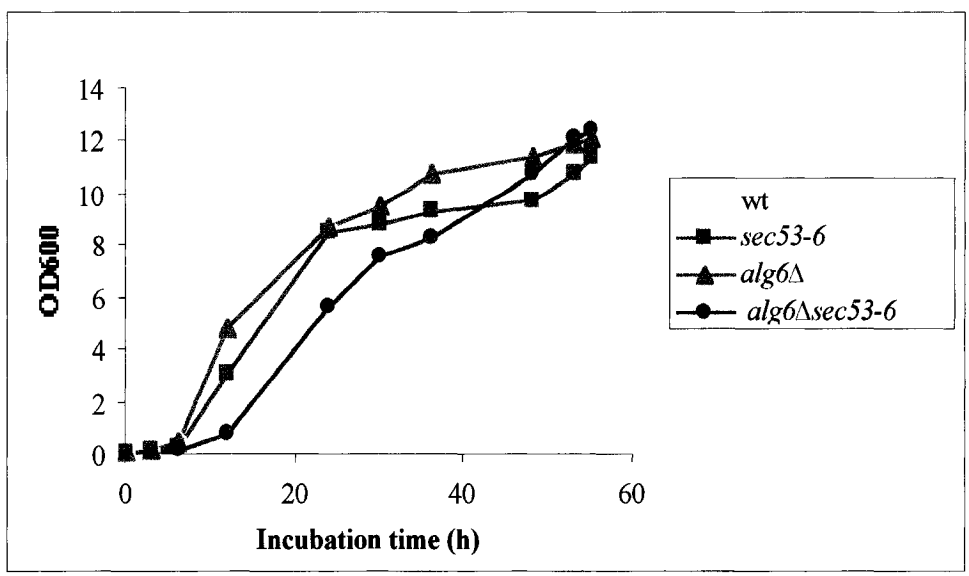
B. Growth curve.

Cells were pre-grown at 27^{0}C to log-phase, diluted to an OD_{600} of 0.1 then incubated at 27^{0}C. Aliquots were taken at indicated times and OD_{600} measured. The doubling time of each strain is shown at the bottom of the plot.

A.



B.



Doubling time (wild type)	= 2.09 h
Doubling time (*sec53-6*)	= 2.59 h
Doubling time (*alg6Δ*)	= 2.19 h
Doubling time (*alg6Δ sec53-6*)	= 4.01 h

3.4. Phenotypic analysis of the *alg6Δ sec53-6* double mutant.

The *alg6* mutant is deficient in glucosylation of lipid-linked core oligosaccharide. Deletion of *ALG6* gene results in an accumulation of lipid-linked $GlcNAc_2Man_9$ which is a suboptimal substrate for the oligosaccharyltransferase. A *sec53* mutant is defective in protein glycosylation due to reduced levels of GDP-mannose (Mondesert *et al.* 1997). The temperature sensitive *sec53-6* allele accumulates Dol-PP-$GlcNAc_2Man_{(1-8)}$ at the restrictive temperature. Underglycosylation is observed in both *alg6* and *sec53* mutants but no growth defect is associated with these mutations (at the permissive temperature for the *sec53* mutant). Combining the two mutations might lead to an enhanced underglycosylation of secreted glycoproteins explaining the slow growth phenotype of the double mutant strain.

Carboxypeptidase Y (CPY) is a 61KDa N-glycosylated vacuolar protease. This protein is widely used as N-glycosylation reporter and a defect in CPY glycosylation has been observed in *sec53-6* mutant strains at restrictive temperatures (Ferro-Novick *et al.* 1984) as well as in *alg6* mutants (Stagljar *et al.* 1994, Westphal *et al.* 2002).

To further explore the phenotype of the *alg6Δsec53-6* mutant, CPY glycosylation was analyzed by immunoblot and compared to that of the wild-type and the single mutants. The cells were also treated with $EndoH_f$, a glycosidase which cleaves the oligosaccharide chains of glycoproteins between the two GlcNAc residues leaving a monoglycosylated protein.

EndoH$_f$ treatment leaves all 4 N-glycosylation sites of CPY attached to one GlcNAc residue. As seen in Fig.8, the wild-type strain expresses only the mature form of CPY (lane1). At 27^{0}C, the intermediate temperature, *sec53-6* mutant produces mainly the mature form and a substential amount of the unglycosylated CPY (lane3). In contrast, the CPY glycosylation pattern is dramatically modified in the *alg6Δ* mutant which shows a lower expression of the mature form of CPY compared to the wild type strain. It also expresses CPY glycoforms lacking one or two oligosaccharide chains (lane 5). CPY underglycosylation in the double mutant strain *alg6Δsec53-6* is more pronounced. The mature CPY form is almost absent and the CPY glycoform lacking 2 oligosaccharide chains is mainly produced. CPY glycoform lacking 3 oligosaccharide chains as well as the monoglycosylated CPY are also observed (lane 7). The difference in CPY maturation resides only in glycosylation since EndoH$_f$ treatment of both the wild-type, the single mutants as well as the double mutant leads to the same sized band (lane 2, 4, 6, 8). This work allows me to conclude that the double mutant strain has a more severe glycosylation defect than either of the single mutants.

Figure 8: Analysis of CPY glycosylation.

Whole cells extracts from wild-type, *sec53-6*, *alg6Δ* and *alg6Δse53-6* double strains were analyzed by SDS-PAGE and Western blot analysis using an anti α-CPY antibody (lane 1, 3, 5, 7). The extracts were also treated with endoglycosidase F (lane 2, 4, 6, 8) then blotted with anti α-CPY antibody.

In agreement with Stagljar *et al.* 1994 and Wesphal *et al.* 2002 :

m : Mature form of CPY (61 KDa, has 4 oligosaccharide chains).

-1 : CPY glycoform lacking 1 oligosaccharide chain

-2 : CPY glycoform lacking 2 oligosaccharide chains.

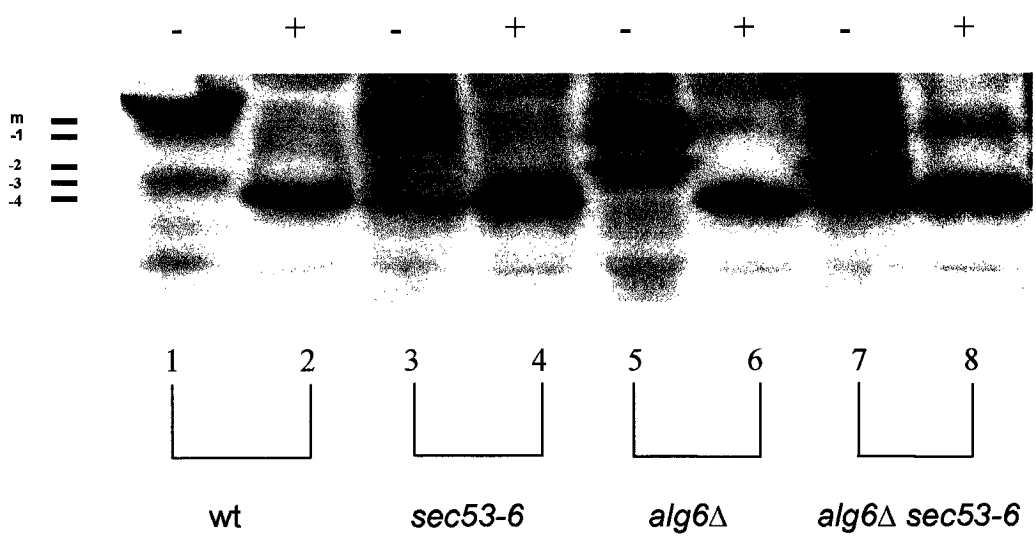
-3 : CPY glycoform lacking 3 oligosaccharide chains.

-4 : CPY glycoform lacking 4 oligosaccharide chains.

$EndoH_f$ treatment leaves a CPY protein with 4 monoglycosyl chains.

+: $EndoH_f$ treatment.

- : no $EndoH_f$ treatment.


- + - + - + - +
m
-1
-2
-3
-4
1 2 3 4 5 6 7 8
wt
sec53-6
alg6Δ
alg6Δ sec53-6

4. DISCUSSION.

4.1 Genetic interaction network analysis.

This work uses a genome wide approach which allowed me to unravel a network of interactions with genes involved in N-glycan glucosylation and processing. I identify 12 interactions with *ALG6*, 11 with *ALG8*, 5 with *ALG10*, 14 with *CWH41*, 13 with *ROT2* and 6 with *CNE1* (Fig.5). Considering that overlaps may reflect a common requirement for mutants, the global interaction network (Fig.5) can be divided in three subnetworks (Table3). In the glucosyltransferase subnework, *ALG6* shows the highest number of interactions. This indicates that many genes are required to allow optimal growth of the *alg6* mutant. In contrast, less genes are required for optimal growth of *alg10* null mutant.

Deletion of the *ALG6, ALG8* or *ALG10* creates progressively more incompletely glucosylated oligosaccharide. Even though glucosylation is not essential for yeast cell viability, it is known that a combination of defects in N-glycan glucosyltransferase and in OST activity leads to reduced growth (Stagljar *et al.* 1994, Zufferey *et al.* 1995). The present work identified genetic interactions between *ALG6, ALG8, ALG10* and *OST3* and *OST5* encoding subunits of the OST complex. The absence of glucose residues reduces the efficiency of OST activity. This may be caused by an unfavorable conformation of the oligosaccharide lacking glucose residues (Alvarado *et al.*1991) and by an affect on the apparent binding affinity for the acceptor substrate (Sharma *et al.* 1981,

Breuer and Bause 1995). *RPO41* gene encoding a mitochondrial RNA polymerase is required for optimal growth in all 3 *alg* null mutants. This could be an interesting genetic interaction to study. Some mitochondrial functions could compensate for the consequences of low glucosylation in yeast cells. This work also identified a synthetic interaction between *ALG6* and two genes encoding for mannosyltransferases (*PMT1* and *PMT2*). This shows that decreased efficiency of N-glycosylation, due to N-glucosylation defects, combined with reduced O-mannosylation is lethal to the cells. Thus, protein glycosylation (N-glycosylation and O-mannosylation) is an essential process.

Defects in N-glycan glucosylation and processing have an effect on the biosynthesis of the *S. cerevisiae* cell wall (Shahinian *et al.* 1998). Mutant yeast cells defective in this pathway display phenotypes characteristic of cell wall defects (Pagé *et al.* 2003) although the molecular basis for these effects is unclear. Results presented here show that effects of mutation in *CWH41* and *ROT2* are compensated for by genes involved in cell wall biosynthesis. This is also observed to a lower extend in the case of *ALG8.* Indeed, genes encoding cell wall components (*KRE1, GAS1*) or regulating cell wall assembly upon stress (*BCK1, GUP1, SLT2*) show an interaction with *CWH41*and *ROT2* or *ALG8.* The link between N-glycosylation and cell wall assembly has been shown by Abeijon *et al.* (1998). They showed that in the *cwh41* mutant, Kre6p, a putative Golgi glucanase, was unstable.

Other interesting interactions are identified by the SGA analysis. *CWH41* interacts with *SCJ1* encoding an ER co-chaperone. This finding shows the importance of the chaperone activity in the case of deficient N-glycan processing.

A link between N-glycan processing and membrane lipid synthesis and trafficking is uncovered by the interaction between *ROT2* and *CHO2* ,*VPS5*, *GGA2* and *GYP1*.

CNE1 forms a separate subnetwork (Table1). Genetic interaction between *CNE1* and genes implicated in protein folding (*YDJ1*, *PEX1*) or in mitochondrial physiology (*MST1*) was found. The interaction between *CNE1* and *MST1* could be explored further in order to gain insight in the requirement of mitochondrial functions in mutants with defects in ER genes.

The calcofluor white test identified that 72.5% of genes belonging to the interaction network (6 query genes and 28/41 array genes) lead to calcofluor white hypersensitivity when deleted (Table 4). This percentage is high considering that two genomewide searches for cell wall mutants using different methods by and Ram *et al.* (1994) and Lussier *et al.* (1997) identified hypersensitive mutants in 63 and 67 genes respectively. The enrichment of the N-glycan network for genes with cell wall defects reveals the importance of N-glycan glucosylation and processing for yeast cell wall biosynthesis.

In conditions used here, the major effect of *ALG* and glucosidase mutants seems to be on the cell wall. The new interactions found through the SGA analysis and the results from the calcofluor white test may be a starting point to gain better insight into this complex phenotype. A plausible explanation is that the N-glucosylation and processing gene products have a regulatory effect on the genes involved in cell wall biosynthesis. Shahinian *et al.* (1998) suggested that β-1,6 glucan, a component of the yeast cell wall, is attached to N-chains of wall

mannoproteins. Defects in N-glycan processing would cause steric hindrance (due to presence of glucose residues of N-glycans) to this attachment.

To date, however, the glucose residues addition/removal cycle has been studied mainly with respect to ER quality control (Parodi 2000, Helenius *et al.* 2001, Roth 2002) suggesting that its major role is played in this process. This work is done in an "unbiased" way since it systematically looked at all possible interactions with the query genes. Only a very small number of genes found in the interaction network are involved in ER quality control. Genes implicated in ER quality control may not be required in the conditions used here. This possibility is strengthened by the fact that *MNS1, MNL1* and *CNE1* did not show any interaction with genes important in ER quality control. The calcofluor white test identified a new phenotype for genes of unknown function found in the interaction network. This cell wall phenotype can be further explored to gain more knowledge on the possible function of these genes.

4.2 Conservation of a genetic interaction from humans to yeast.

Deficiencies in N-glycan glucosylation and processing are associated with a number of inherited human diseases called congenital disorders of glycosylation (CDGs) (Aebi and Hennet 2001). CDGIa is caused by mutation in the phosphomanomutase gene *PMM2*. Severely affected patients have a significantly greater frequency of mutation in *ALG6* suggesting that *ALG6* is a genetic modifier for *PMM2* mutation (Westphal *et al.* 2002). Work presented here

indicates a genetic interaction between the *S. cerevisiae* homologues of *hALG6* and *PMM2* (namely *ALG6 and SEC53* respectively). At a semi-permissive temperature, the growth of an *alg6sec53* double mutant was slower than that of the wild type, *alg6* or *sec53* mutants (Fig.7 A). The doubling time of the double mutant is almost twice that of the wild type or the single mutants (Fig.7 B). Thus the proposed human genetic interaction is also found in yeast, and is conserved. Here, I show that an *alg6se53-6* mutant has a reduced CPY glycosylation compared to the wild-type and single mutant strains (Fig.8). It is thus likely that, as previously shown for an *alg6wbp1*mutant (Stagljar *et al.*1994), the growth defect of the mutant *alg6sec53* resulted from severe underglycosylation of secreted proteins.

The concept of genetic modifiers illustrated in the case of the interaction between *ALG6* and *PMM2/SEC53* (Westphal *et al.* 2002) in humans can be extended to other genes interacting with *ALG6.* Patients with defects in *ALG6* present a spectrum of phenotypes of CDGIc (Imbach *et al.* 2000). We could hypothesize that the yeast genes found to interact with *ALG6* from the SGA results in yeast are candidates for conserved modifier genes in human CDGIc disease. Human homologues of some of the genes interacting with *ALG6* are implicated in human disease. For example a mutation in *POMT1*, a gene related to yeast *ALG6* interacting genes *PMT1* and *PMT2* (Fig.5) causes the Walker-Warburg syndrome, an autosomal recessive developmental disorder characterized by brain and eye abnormalities and congenital muscular dystrophy (Beltran-Valero de Bernabe *et al.* 2002).

4.3 SGA analysis in S.cerevisiae as a model for predicted human disease.

In the view of the results obtained here with respect to the *ALG6-SEC53* interaction, one can ask if the knowledge of conserved yeast synthetic interactions can be informative about the basis for inherited complex human disease. Advances in human diseases research has revealed that the traditional classification of genetic disorders into "simple mendelian" or "complex" diseases is an oversimplification (Badano and Katsanis 2002). The preferred view is that the phenotype of the so-called " single gene disorder" is not only caused by one primary gene but is also influenced by one or more independently inherited genes. An example is cystic fibrosis where a primary single gene defect in the CFTR gene is modified by several other genes, many enhancing the severity of the pulmonary phenotype (Drumm 2001). In the case of complex diseases, the phenotype results from the interaction of two or more independently inherited pairs of alleles, most likely influenced by additional modifier genes (review by Dipple and Mc Cabe 2000).

The simplest case of complex inherited diseases are "digenic" where the individual mutant genes have no phenotype but the combination of mutant alleles in two genes leads to the disease. As an example, single mutations in two genes ROM1 (encoding the retinal outer segment membrane protein1) and RDS (retinal degeneration slow) are asymptomatic, but when combined they cause retinis pigmentosa (Hariharan and Haber 2003).Congenital disorders of glycosylation (CDG) are among the reported digenic diseases (Westphal *et al.* 2002).

SGA screens allow the uncovering of synthetic or digenic interactions between one query gene pairwise with several other genes. For complex diseases, it is a great task to map a specific disease to the large possible number of contributing genes. Because elements of the yeast genetic network are likely to be conserved, SGA analysis may be a useful tool in predicting genetic interactions that are implicated in human genetic disorders (Tong *et al.* 2003 in press). For example SGA analysis could be used to study the disease related genes *PEX1, SNF4* or *YBR235W*. In the case of *PEX1,* small peroxisomal membrane-bound structures observed in the null mutant bear a resemblance to structures observed in cells of human patients with peroxisomal defects (Hettema *et al.* 2000). Mutations in the human *PEX1* gene are associated with the Zellweger spectrum disorders including Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease, which are characterized by neural, hepatic, and renal abnormalities with shortened life span (Gould and Valle 2000). Mutations in the *SNF4* human homolog (human *PRKAG2*) lead to heart disorders such as myocardial metabolic storage disease, Wolff-Parkinson-White syndrome, and hypertrophic cardiomyopathy (Arad *et al.* 2002). *YBR235W* has similarity to human *SLC12A1*, mutations in which cause Bartter's syndrome (Foury *et al.* 1997).

This work identifies *CNE1/PEX1, CWH41/SNF4* and *CWH41/YBR235W* genetic interactions. *CNE1* could be a genetic modifier for *PEX1* and *CWH41* could be a genetic modifier of *SNF4* or *YBR235W*. The use of SGA analysis could be a useful tool to identify other candidate modifier of *PEX1*, *SNF4* or *YBR235W*.

5. REFERENCES

ABEIJON C., CHEN L.Y. 1998. The role of glucosidase I (Cwh41p) in the biosynthesis of cell wall beta-1,6-glucan is indirect. Mol.Biol.Cell. 9(10):2729-38.

ABEIJON C., HIRSCHBERG C.B. 1992. Topography of glycosylation reactions in the endoplasmic reticulum. Trends Biochem. Sci. 17: 32-36.

AEBI M., GASSENHUBER J., DOMDEY H., Te HEENSEN S. 1996. Cloning and characterization of the *ALG3* gene of *Saccharomyces cerevisiae*. Glycobiology 6(4): 439-44.

AEBI M. , HENNET T. 2001. Congenital disorders of glycosylation: genetic model systems lead the way. Trends Cell. Biol. 11: 136-141

ALBRIGHT C.F., ROBBINS P.W. 1990. The sequence and transcript heterogeneity of the yeast gene *ALG1*, an essential mannosyltransferase involved in N-glycosylation. J. Biol. Chem.265: 7042-7049.

ALVARADO E., NUKADA T., OGAWA T., BALLOU C.E. 1991. Conformation of the glucotriose unit in the lipid-linked oligosaccharide precursor for protein glycosylation. Biochemistry 30(4):881-6.

AMMERER G. 1994. Sex, stress and integrity: the importance of MAP kinases in yeast. Curr. Opin.Genet. Dev. 4(1):90-5.

ANDRE B., SCHERENS B. 1995. The yeast *YBR235W* gene encodes a homologue of the mammalian electroneutral Na(+)-(K+)-C1- cotransporter family. Biochem. Biophys. Res. Commun. 217(1):150-3.

ARAD M., BENSON D.W., PEREZ-ATAYDE A.R., MCKENNA W.J., SPARKS E.A., KANTER R.J., MCGARRY K., SEIDMAN J.G., SEIDMAN C.E. 2002. Constitutively active AMP kinase mutations cause glycogen storage disease mimicking hypertrophic cardiomyopathy. J. Clin. Invest. 109(3):357-62.

AZUMA M., LEVINSON J.N., PAGE N., BUSSEY H. 2002. *Saccharomyces cerevisiae* Big1p, a putative endoplasmic reticulum membrane protein required for normal levels of cell wall beta-1,6-glucan. Yeast 19(9):783-93.

BADANO J.L., KATSANIS N. 2002. Beyond Mendel: an evolving view of human genetic disease transmission. Nat. Rev. Genet. 3(10):779-89.

BARNES G., HANSEN W.J., HOLCOMB C.L. 1984. Asparagine-linked glycosylation in *Saccharomyces cerevisiae*: genetic analysis of an early step. Mol. Cell. Biol. 4(11): 2831-8.

BAUSE E., LEHLE L. 1979. Enzymatic N-glycosylation and O-glycosylation of synthetic peptide acceptors by dolichol-linked sugar derivatives in yeast. Eur.J. Biochem. 101(2):531-40.

BAUSE E., SCHWEDEN J., BORGMANN C., LEGLER G. 1986. Characterization of calf liver glucosidase I and its inhibition by basic sugar analogs. Arch. Biochem. Bophys. 248(1):335-40.

BEGLEY T.J., ROSENBACH A.S., IDEKER T., SAMSON L.D. 2002. Damage recovery pathways in *Saccharomyces cerevisiae* revealed by genomic phenotyping and interactome mapping. Mol. Cancer Res. 1(2):103-12.

BELTRAN-VALERO DE BERNABE D., CURRIER S., STEINBRECHER A., CELLI J., VAN BEUSEKOM E., VAN DER ZWAAG B., KAYSERILI H., MERLINI L., CHITAYAT D., DOBYNS W.B., CORMAND B., LEHESJOKI A.E., CRUCES J., VOIT T., WALSH C.A., VAN BOKHOVEN H., BRUNNER H.G. 2002. Mutations in the O-mannosyltransferase gene POMT1 give rise to the severe neuronal migration disorder Walker-Warburg syndrome. Am .J. Hum. Genet. 71(5):1033-43.

BERGER K.H., YAFFE M.P. 2000. Mitochondrial DNA inheritance in *Saccharomyces cerevisiae*. Trends Microbiol. 8(11):508-13.

BERGERON J.J., BRENNER M.B., THOMAS D.Y., WILLIAMS D.B. 1994. Calnexin: a membrane-bound chaperone of the endoplasmic reticulum. Trends Biochem. Sci.19(3):124-8.

BICKLE M., DELLEY P.A., SCHMIDT A., HALL M.N. 1998. Cell wall integrity modulates RHO1 activity via the exchange factor ROM2. EMBO J. 17(8):2235-45.

BONANGELINO C.J., CHAVEZ E.M., BONIFACINO J.S. 2002. Genomic screen for vacuolar protein sorting genes in *Saccharomyces cerevisiae*. Mol. Biol. Cell. 13(7):2486-501

BRACHMANN C.B., DAVIES.A., COST G.J., CAPUTO E., LI J., HIETER P., BOEKE J.D. 1998. Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14(2):115-32.

BREUER W., BAUSE E. 1995. Oligosaccharyltransferase is a constitutive component of an oligomeric protein complex from pig liver endoplasmic reticulum. Eur. J. Biochem. 228(3):689-96.

BURDA P., AEBI M. 1998. The *ALG10* locus of *Saccharomyces cerevisiae* encodes the alpha-1, 2-glucosyltransferase of the endoplasmic reticulum: the terminal glucose of the lipid-linked oligosaccharide is required for efficient N-linked glycosylation. Glycobiology 8(5): 455-462.

BURDA P., AEBI M. 1999. The dolichol pathway of N-linked glycosylation. Biochim. Biophys. Acta 1426: 239-257.

BURDA P., JAKOB C.A., BEINHAUER J., HEGEMANN J.H., AEBI M. 1999. Ordered assembly of the asymmetrically branched lipid-linked oligosaccharide in the endoplasmic reticulum is ensured by the substrate specificity of the individual glycosyltransferases. Glycobiology 9(6): 617-25.

BURDA P., Te HEESEN S., BRACHAT A.. WATCH A., DUSTERHOFT T., AEBI M.,1996. Stepwise assembly of lipid-linked oligosaccharide in the endoplasmic reticulum of *Saccharomyces cerevisiae* identification of the *ALG9* gene encoding a putative mannosyltransferase.
Proc. Natl. Acad. Sci USA 93(14): 7160-5.

BUSSEY H., SACKS W., GALLEY D., SAVILLE D. 1982. Yeast killer plasmid mutations affecting toxin secretion and activity and toxin immunity function.
Mol. Cell. Biol. 2(4):346-54.

CAMIRAND A., HEYSEN A., GRONDIN B., HERSCOVICS A. 1991. Glycoprotein biosynthesis in *Saccharomyces cerevisiae*. Isolation and characterization of the gene encoding a specific processing α-mannosidase. J.Biol.Chem. 266: 15120-15127.

CANNON K.S., HELENIUS A. 1999. Trimming and readdition of glucose to N-linked oligosaccharides determines calnexin association of a substrate glycoprotein in living cells. J. Biol. Chem. 274(11):7537-44.

CAROTTI C., FERRARIO L., RONCERO C., VALDIVIESO M.H., DURAN A., POPOLO L. 2002. Maintenance of cell integrity in the *gas1* mutant of *Saccharomyces cerevisiae* requires the Chs3p-targeting and activation pathway and involves an unusual Chs3p localization. Yeast. 19(13):1113-24.

CASPARY F., SERAPHIN B. 1998. The yeast U2A'/U2B complex is required for pre-spliceosome formation. EMBO J. 17(21):6348-58.

CAUSTON H.C., REN B., KOH S.S., HARBISON C.T., KANIN E., JENNINGS E.G., LEE T.I., TRUE H.L., LANDER E.S., YOUNG R.A. 2001. Remodeling of yeast genome expression in response to environmental changes. Mol. Biol. Cell. 12(2):323-37.

CIPOLLO J.F., TRIMBLE R.B., CHI J.H., YAN Q., DEAN N. 2001. The yeast *ALG11* gene specifies addition of the terminal alpha 1,2-Man to the dolichol-PP $GlcNAc_2Man_5$ N-glycosylation intermediate formed on the cytosolic side of the endoplasmic reticulum. J.Biol.Chem. 276(24): 21828-40.

COSTANZO M.C., SEAVER E.C., FOX T.D. 1986. At least two nuclear gene products are specifically required for translation of a single yeast mitochondrial mRNA. EMBO J. 5(13):3637-41.

COUTO J.R., HUFFAKER T.C., ROBBINS P.W. 1984. Cloning and expression in *Escherichia coli* of a yeast mannosyltransferase from the asparagine-linked glycosylation pathway. J. Biol. Chem. 259: 378-382.

CUMMINGS R.D. 1992, in H.J. Allen, E.C. Kisalius Eds., Glycoconjugates, Composition Structure and Function, Marcel Dekker. New York, NY, 333-360.

DANG H., ELLIS S.R. 1990. Structural and functional analysis of a yeast mitochondrial ribosomal protein homologous to ribosomal protein S15 of *Eschericchia coli.* Nucleic Acids Res. 18(23): 6895-901.

DEAN N. 1999. Asparagine-linked glycosylation in the yeast Golgi. Biochim. Biophys.Acta. 1426: 309-322.

DEAN-JOHNSON M., HENRY S. A. 1989. Biosynthesis of inositol in yeast. Primary structure of myo-inositol-1-phosphate synthase and functional analysis of its structural gene, the *INO1* locus. J. Biol. Chem. 264 (2): 1274-83.

DIJKGRAAF G.J., ABE M., OHYA Y., BUSSEY H. 2002. Mutations in Fks1p affect the cell wall content of beta-1, 3- and beta-1, 6-glucan in *Saccharomyces cerevisiae.*Yeast 19(8): 671-90.

DIMMER K.S., FRITZ S., FUCHS F., MESSERSCHMITT M., WEINBACH N., NEUPERT W., WESTERMANN B. 2002. Genetic basis of mitochondrial function and morphology in *Saccharomyces cerevisiae*. Mol. Biol. Cell. 13(3):847-53.

DIPPLE K.M., MCCABE E.R. 2000. Modifier genes convert "simple" Mendelian disorders to complex traits. Mol.Genet. Metab. 71(1-2):43-50.

DRUMM M.L. 2001. Modifier genes and variation in cystic fibrosis. Respir. Res. 2(3):125-8.

ELLGAARD L., HELENIUS A. 2001. ER quality control: towards an understanding at the molecular level. Curr.Opin. Cell. Biol.13(4):431-7.

ELORZA M.V., RICO H., SENTANDREU R. 1983. Calcofluor white alters the assembly of chitin fibrils in *Saccharomyces cerevisiae* and *Candida albicans* cells. J. Gen. Microbiol. 129 (Pt 5):1577-82

FERNANDEZ F.S., JANNATIPOUR M., HELLAMAN U., ROKEACH L.A., PARODI A.J. 1996. A new stress protein: synthesis of *Schizosaccharomyces pombe* UDP-Glc:glycoprotein glucosyltransferase mRNA is induced by stress conditions but the enzyme is not essential for cell viability. EMBO J. 15: 705-713.

FERNANDEZ F.S., TROMBETTA S.E., HELLMAN O., PARODI A.J. 1994. Purification to homogeneity of UDP-glucose:glycoprotein glucosyltransferase from *Schizosaccharomyces pombe* and apparent absence of the enzyme from *Saccharomyces cerevisiae*. J.Biol.Chem. 269 (48): 30701-6.

FERRO-NOVICK S., NOVICK P., FIELD C., SCHEKMAN R. 1984. Yeast secretory mutants that block the formation of active cell surface enzymes. J.Cell. Biol. 98(1):35-43.

FREEZE H. 2001 Update and perspectives on congenital disorders of glycosylation. Glycobiology 11, 129R–143R.

FOURY F. 1997. Human genetic diseases: a cross-talk between man and yeast. Gene 195(1):1-10.

GENTZSCH M., IMMERVOLL T., TANNER W. 1995. Protein O-glycosylation in *Saccharomyces cerevisiae*: the protein O-mannosyltransferases Pmt1p and Pmt2p function as heterodimer. FEBS Lett. 377(2):128-30.

GIAEVER G., CHU A.M., NI L., CONNELLY C., RILES L., VERONNEAU S., DOW S., LUCAU-DANILA A., ANDERSON K., ANDRE B., ARKIN A.P., ASTROMOFF A., EL-BAKKOURY M., BANGHAM R., BENITO R., BRACHAT S., CAMPANARO S., CURTISS M., DAVIS K., DEUTSCHBAUER A, ENTIAN K.D., FLAHERTY P., FOURY F., GARFINKEL D.J., GERSTEIN M., GOTTE D., GULDENER U., HEGEMANN J.H., HEMPEL S., HERMAN Z., JARAMILLO D.F., KELLY D.E., KELLY S.L., KOTTER P., LABONTE D., LAMB D.C., LAN N., LIANG H., LIAO H., LIU L., LUO C., LUSSIER M., MAO R., MENARD P., OOI S.L., REVUELTA J.L., ROBERTS C.J., ROSE M., ROSS-MACDONALD P., SCHERENS B., SCHIMMACK G., SHAFER B.., SHOEMAKER D.D., SOOKHAI-MAHADEO S., STORMS R.K., STRATHERN J.N., VALLE G., VOET M., VOLCKAERT G., WANG C.Y., WARD T.R., WILHELMY J., WINZELER E.A., YANG Y., YEN G., YOUNGMAN E., YU K., BUSSEY H., BOEKE J.D., SNYDER M., PHILIPPSEN P., DAVIS R.W., JOHNSTON M. 2002. Functional profiling of the *Saccharomyces cerevisiae* genome. Nature 418:387-391.

GOULD S.J., VALLE D. 2000. Peroxisome biogenesis disorders: genetics and cell biology. Trends Genet. 16(8):340-5.

GRUNEWALD S., IMBACH T., HUIJBEN K., RUBIO-GOZALBO M.E., VERRIPS A., DE KLERK J.B., STROINK H., DE RIJK-VAN ANDEL J.F., VAN HOVE J.L., WENDEL U., MATTHIJS G., HENNET T., JAEKEN J., WEVERS R.A. 2000. Clinical and biochemical characteristics of congenital disorder of glycosylation type Ic, the first recognized endoplasmic reticulum defect in N-glycan synthesis. Ann. Neurol.47(6):776-81.

HARIHARAN I.K., HABER D.A. 2003. Yeast, flies, worms, and fish in the study of human disease. N.Engl. J .Med. 348(24):2457-63.

HARTMAN J.L. 4TH, GARVIK B., HARTWELL L. 2001. Principles for the buffering of genetic variation. Science 291(5506):1001-4.

HELENIUS A., AEBI M. 2001. Intracellular functions of N-linked glycans. Science 291: 2364-2369.

HELENIUS A., SIMONS J.F., EBERSOLD M. 1998. Cell wall 1,6-beta-glucan synthesis in *Saccharomyces cerevisiae* depends on ER glucosidases I and II, and the molecular chaperone BiP/Kar2p. EMBO J. 17(2):396-405.

HELENIUS A., TROMBETTA E.S., HEBERT A.H., SIMONS F. 1997. Calnexin, calreticulin and the folding of glycoproteins. Trends Cell. Biol. 7: 193-200.

HELENIUS J., NG D.T., MAROLDA C.L., WALTER P., VALVANO M.A., AEBI M. 2002. Translocation of lipid-linked oligosaccharides across the ER membrane requires Rft1 protein. Nature 415(6870) : 447-50.

HEMMING F. W. 1995 in J. Montreuil, J.F.G. Vliegenhas, H. Schacter Eds. Glycoproteins 299: 125-143.

HERSCOVICS A. 1999. Importance of glycosidases in mammalian glycoprotein biosynthesis. Biochim. Biophys. Acta 1473 (1):96-107.

HERSCOVICS A., ORLEAN P. 1993. Glycoprotein biosynthesis in yeast.

FASEB J. 7: 540-550.

HERSCOVICS A., SAUNIER B., KILKER R.D.Jr., TKACZ J.S., QUARONI A. 1982. Inhibition of N-linked complex oligosaccharide formation by 1-deoxynojirimycin, an inhibitor of processing glycosidases.

J.Biol.Chem. 257(23): 14155-61.

HERSCOVICS A., TREMBLAY L.O., CAMPELL DYKE N. 1998. Molecular cloning, chromosomal mapping and tissue-specific expression of a novel human alpha 1,2-mannosidase gene involved in N-glycan maturation.

Glycobiology 8(6): 585-95.

HETTEMA E.H., GIRZALSKY W., VAN DEN BERG M., ERDMANN R., DISTEL B. 2000. *Saccharomyces cerevisiae* Pex3p and Pex19p are required for proper localization and stability of peroxisomal membrane proteins.

EMBO J. 19(2):223-33.

HILLER M.M., FINGER A., SCHWEIGER M., WOLF D. 1996. ER degradation of a misfolded luminal protein by the cytosolic ubiquitin-proteasome pathway.

Science 273(5282): 1725-8.

HIRSCHBERG C.B., SNIDER M. D. 1987. Topography of glycosylation in the rough endoplasmic reticulum and Golgy apparatus.

Annu. Rev. Biochem. 56: 63-87.

HUANG K.N., SYMINGTON L.S. 1995. Suppressors of a *Saccharomyces cerevisiae pkc1* mutation identify alleles of the phosphatase gene PTC1 and of a novel gene encoding a putative basic leucine zipper protein.

Genetics 141(4):1275-85.

HUFFAKER T.C., ROBBINS P.W. 1983. Yeast mutants deficient in protein glycosylation. Proc. Natl. Acad. Sci U S A 80(24): 7466-70.

IMBACH T., BURDA P., KUHNERT P., WEVERS R.A., AEBI M., BERGER E.G. AND HENNET T. 1999 A mutation in the human ortholog of the *Saccharomyces cerevisiae ALG6* gene causes carbohydrate-deficient glycoprotein syndrome type-Ic. Proc. Natl. Acad. Sci. USA,96: 6982–6987.

IMBACHT., GRUNEWALD S., SCHENK B., BURDA P., SCHOLLEN E., WEVERS R.A., JAEKEN J., DE KLERK J.B., BERGER E.G., MATTHIJS G., AEBI M. AND HENNET T. 2000 Multi-allelic origin of congenital disorder of glycosylation (CDG)-Ic. Hum. Genet. 106: 538–545.

ITO T., CHIBA T., OZAWA R., YOSHIDA M., HATTORI M., SAKAKI Y. 2001. A comprehensive two-hybrid analysis to explore the yeast protein interactome. Proc Natl Acad.. Sci. USA. 98(8):4569-74.

JACKSON B.J., KUKURUZINSKA M.A., ROBBINS P.W. 1993. Biosynthesis of asparagine-linked oligosaccharides in *Saccharomyces cerevisiae*: the *alg2* mutation. Glycobiol.3: 357-369.

JAEKEN J. AND MATTHIJS G. 2001 Congenital disorders of glycosylation. Annu. Rev. Genomics Hum. Genet. 2, 129–15

JAKOB C.A., BODMER D., SPIRIG U., BATTIG P., MARCIL A., DIGNARD D., BERGERON J. J., THOMAS D.Y., AEBI M. 2001. Htm1p, a mannosidase-like protein, is involved in glycoprotein degradation in yeast. EMBO Rep. 5: 423-430.

JAKOB C.A., BURDA P., ROTH J., AEBI M. 1998a. Degradation of misfolded endoplasmic reticulum glycoproteins in *Saccharomyces cerevisiae* is determined by a specific oligosaccharide structure. J.Cell.Biol. 142(5):1223-33.

JAKOB C.A., BURDA P., TE HEESEN S., AEBI M., ROTH J.1998b. Genetic tailoring of N-linked oligosaccharides: the role of glucose residues in glycoprotein processing of *Saccharomyces cerevisiae* in vivo. Glycobiology 8(2):155-64

JANNATIPOUR M., ROKEACH L.A. 1995. The *Schizosaccharomyces pombe* homologue of the chaperone calnexin is essential for viability.

J.Biol.Chem. 270(9): 4845-53.

JIANG B., SHERATON J., RAM A.F., DIJKGRAAF G.J., KLIS F.M., BUSSEY H. 1996. *CWH41* encodes a novel endoplasmic reticulum membrane N-glycoprotein involved in beta-1, 6- glucan assembly.

J. Bacteriol. 176 (4): 1162-71.

KARAOGLU D., KELLEHER D.J., GILMORE R. 1995. Functional characterization of Ost3p. Loss of the 34-kD subunit of the *Saccharomyces cerevisiae* oligosaccharyltransferase results in biased underglycosylation of acceptor substrates. J.Cell. Biol. 130(3):567-77.

KARAOGLU D., KELLEHER D.J., GILMNORE R. 2001. Allosteric regulation provides molecular mechanism for preferential utilization of the fully assembled dolichol-linked oligosaccharide by the yeast oligosaccharyltransferase.

Biochem. 40: 12193-12206.

KNAUER R., LEHLE L. 1999. The oligosaccharyltransferase complex from yeast. Biochim. Biophys. Acta 1426(2): 259-73.

KNOP M., HAUSER N., WOLF D. 1996. N-glycosylation affects endoplasmic reticulum degradation of a mutated derivative of carboxypeptidase Y scy in yeast.

Yeast 12: 1229-1238.

KORNFELD R., KORNFELD S. 1985. Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. 54: 631-64.

KUKURUZINSKA M.A., BERGH M.L.E. 1987. Protein glycosylation in yeast. Annu. Rev. Biochem. 56: 914-944.

KUKURUZINSKA M.A., ROBBINS P. W. 1987. Protein glycosylation in yeast: transcript heterogeneity of the *ALG7* gene.
Proc. Natl. Acad. Sci USA 84(8): 2145-9.

LAGORCE A., LE BERRE-ANTON V., AGUILAR-USCANGA B., MARTIN-YKEN H., DAGKESSAMANSKAIA A., FRANCOIS J. 2002. Involvement of GFA1, which encodes glutamine-fructose-6-phosphate amidotransferase, in the activation of the chitin synthesis pathway in response to cell-wall defects in *Saccharomyces cerevisiae*. Eur. J. Biochem. 269(6):1697-707.

LEE D.H., SHERMAN M.Y., GOLDBERG A.L. 1996. Involvement of the molecular chaperone Ydj1 in the ubiquitin-dependent degradation of short-lived and abnormal proteins in *Saccharomyces cerevisiae*.
Mol.Cell.Biol. 16(9):4773-81.

LEVINSON J.N., SHAHINIAN S., SDICU A.M., TESSIER D.C., BUSSEY H. 2002. Functional, comparative and cell biological analysis of *Saccharomyces cerevisiae* Kre5p. Yeast 19(14):1243-59.

LOEWITH R., SMITH J.S., MEIJER M., WILLIAMS T.J., BACHMAN N., BOEKE J.D., YOUNG D. 2001. Pho23 is associated with the Rpd3 histone deacetylase and is required for its normal function in regulation of gene expression and silencing in *Saccharomyces cerevisiae*. J. Biol. Chem. 276(26):24068-74.

LUSSIER M., GENTZSCH M., SDICU A.M, BUSSEY H., TANNER W. 1995. Protein O-glycosylation in yeast. The *PMT2* gene specifies a second protein O-mannosyltransferase that functions in addition to the *PMT1*-encoded activity. J. Biol. Chem.;270(6):2770-5.

LUSSIER, M., WHITE A. M., SHERATON J., DI PAOLO T., AND TREADWELL J., SOUTHARD S.B. HORENSTEIN C.I. CHEN-WEINER J., RAM A.F., KAPTEYN J.C., ROEMER T.W., VO D.H., BONDOC D.C., HALL J., ZHONG W.W., SDICU A.M., DAVIES J., KLIS F.M., ROBBINS P.W., BUSSEY H. 1997 Large scale identification of genes involved in cell surface biosynthesis and architecture in *Saccharomyces cerevisiae.* Genetics 147:435-450.

MARQUARDT T., DENECKE J. 2003. Congenital disorders of glycosylation: review of their molecular bases, clinical presentations and specific therapies. Eur. J. Pediatr. 162(6):359-79.

MATTHIJS G., SCHOLLEN E., PARDON E., VEIGA-DA-CUNHA M., JAEKEN J., CASSIMAN J.J., VAN SCHAFTINGEN E. 1997. Mutations in *PMM2*, a phosphomannomutase gene on chromosome 16p13, in carbohydrate-deficient glycoprotein type I syndrome (Jaeken syndrome). Nat .Genet.16(1):88-92.

McCRACKEN A.A., BRODSKY J.L. 1996. Assembly of ER-associated protein degradation in vitro: dependence on cytosol, calnexin and ATP. J.Cell.Biol. 132(3): 291-8.

MERIIN A.B., ZHANG X., HE X., NEWNAM G.P., CHERNOFF Y.O., SHERMAN M.Y. 2002. Huntington toxicity in yeast model depends on polyglutamine aggregation mediated by a prion-like protein Rnq1. J.Cell. Biol. 157(6):997-1004.

MONDESERT G., CLARKE D.J., REED S.I. 1997. Identification of genes controlling growth polarity in the budding yeast *Saccharomyces cerevisiae*: a possible role of N-glycosylation and involvement of the exocyst complex. Genetics.147 (2):421-34.

MOUYNA I., MONOD M., FONTAINE T., HENRISSAT B. LECHENNE B., LATGE J.P. 2000. Identification of the catalytic residues of the first family of beta(1-3)glucanosyltransferases identified in fungi. Biochem. J. 347 Pt 3:741-7.

MUNIZ M., NUOFFER C., HAURI H..P, RIEZMAN H. 2000. The Emp24 complex recruits a specific cargo molecule into endoplasmic reticulum-derived vesicles. J. Cell. Biol. 148(5):925-30.

MURGUI A., ELORZA M.V., SENTANDREU R. 1985. Effect of papulacandin B and calcofluor white on the incorporation of mannoproteins in the wall of *Candida albicans* blastospores. Biochim. Biophys. Acta. 841(2):215-22.

NAKATSUKASA K., NISHIKAWA S., HOSOKAWA N., NAGATA K. 2001. Mnl1p, an α-mannosidase-like protein in yeast *Saccharomyces cerevisiae*, is required for endoplasmic reticulum-associated degradation of glycoproteins. J.Biol.Chem.276:8635-8638.

NIKAWA J., HOSAKA K. 1995. Isolation and characterization of genes that promote the expression of inositol transporter gene ITR 1 in *Saccharomyces cerevisiae.* Mol. Microbiol. 16 (2): 301-8.

NOTHWEHR S.F., HINDES A.E. 1997. The yeast VPS5/GRD2 gene encodes a sorting nexin-1-like protein required for localizing membrane proteins to the late Golgi. J. Cell. Sci. 110 (Pt 9):1063-72.

OLIVER J.D., VAN DER WAL F.J., BULLEID J.J., STIGH S. 1997. Interaction of the thiol-dependent reductase Erp57 with nascent glycoproteins. Science 275: 86-88.

ORLEAN P. IN PRINGLE J. R., BROACH J. R. AND JONES

ORLEAN P. IN PRINGLE J. R., BROACH J. R. AND W. E. (EDS.). 1997. Molecular and cellular biology of the yeast *Saccharomyces*. Cell cycle and cell biology, Vol. 3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

OU W.J., CAMERON P.H., THOMAS D.Y., BERGERON J.J. 1993. Association of folding intermediates of glycoproteins with calnexin during protein degradation. Nature 364 (6440): 771-6

PAGE N., GERARD-VINCENT M., MENARD P., BEAULIEU M., AZUMA M., DJIKGRAAF G.J., LI H., MARTCOUX J., NGUYEN T., DOWSE T., SDICU A.M., BUSSEY H. 2003. A *Saccharomyces cerevisiae* Genome-Wide-Mutant Screen for altered sensitivity to K1 killer toxin. Genetics 163 (3): 875-94.

PAIDHUNGAT M., GARRETT S. 1998. Cdc1 and the vacuole coordinately regulate Mn2+ homeostasis in the yeast *Saccharomyces cerevisiae*. Genetics. 148(4):1787-98.

PALECEK S.P., PARIKH A.S., KRON S.J. 2000. Genetic analysis reveals that *FLO11* upregulation and cell polarization independently regulate invasive growth in *Saccharomyces cerevisiae*. Genetics 156(3):1005-23.

PARKER C.G., FESSLER L.I., NELSON R., FESSLER J.H. 1995. Drosophilia UDP-glucose: glycoprotein glucosyltransferase: sequence and characterization of an enzyme that distinguishes between denatured and native proteins.
EMBO J. 14: 1294-1303.

PARLATI F., DOMINGUEZ M., BERGERON J.J., THOMAS D.Y. 1995. *Saccharomyces cerevisiae* CNE1 encodes an endoplasmic reticulum (ER) membrane protein with sequence similarity to calnexin and calreticulin and functions as a constituent of the ER quality control apparatus.
J.Biol.Chem. 270(1): 244-53.

PARODI AJ. 1998. The quality control of glycoprotein folding in the endoplasmic reticulum, a trip from trypanosomes to mammals.
Braz. J. Med.Biol.Res. 31(5):601-14.

PARODI A.J. 2000. Protein glucosylation and its role in protein folding.
Annu. Rev. Biochem. 69: 69-93.

PARODI A.J. 2000. Role of N-oligosaccharide endoplasmic reticulum processing reactions in glycoprotein folding and degradation. Biochem J. 15, 34Pt 1:1-13.

POPOLO L., GILARDELLI D., BONFANTE P., VAI M. 1997. Increase in chitin as an essential response to defects in assembly of cell wall polymers in the *ggp1*delta mutant of *Saccharomyces cerevisiae*. J. Bacteriol. 179(2):463-9.

RAM A.F., KAPTEYN J.C., MONTIJN R.C., CARO L.H., DOUWES J.E, BAGINSKY W., MAZUR .P, VAN DEN ENDE H., KLIS F.M. 1998. Loss of the plasma membrane-bound protein Gas1p in Saccharomyces cerevisiae results in the release of beta1,3-glucan into the medium and induces a compensation mechanism to ensure cell wall integrity. J. Bacteriol. 180(6):1418-24.

RAM A.F., WOLTERS A., Ten HOOPEN R., KLIS F.M. 1994. A new approach for isolating cell wall mutants in *Saccharomyces cerevisiae* by screening for hypersensitivity to calcofluor white. Yeast 10 (8):1019-30.

REDDY A.V., JOHNSON R.S., BEIMANN K., WILLIAMS R.S., ZIEGLER F.D., TRIMBLE R.B., MALEY F. 1988. Characterization of the glycosylation sites in yeast external invertase. N-linked oligosaccharide content of the individual sequons. J. Biol. Chem. 263: 6978-6985.

REISS G., Te HEESEN S., ZIMMERMAN J., ROBBINS P.W., AEBI M. 1996. Isolation of the *ALG6* locus of *Saccharomyces cerevisiae* required for glucosylation in the N-linked glycosylation pathway. Glycobiology. 6(5):493-8.

RINE J., HANSEN W., HARDENMANT E., DAVIS R.W. 1983. Targeted selection of recombinant clones through gene dosage effects. Proc. Natl. Acad. Sci. USA. 80(22):6750-4.

ROMERO P.A., DIJKGRAAF G.J.P., SHAHINIAN S., HERSCOVICS A., BUSSEY H. 1997. The yeast *CWH41* gene encodes glucosidase I. Glycobiology. 7(7):997-1004.

ROTH J. 2002. Protein N-glycosylation along the secretory pathway: relationship to organelle topography and function, protein quality control, and cell interactions. Chem. Rev. (2):285-303.

RUNGE K.W., HUFFAKER T.C., ROBBINS P.W. 1984. The yeast mutations in glucosylation steps of the asparagine glycosylation pathway. J.Biol.Chem. 259(1):412-7.

RUNGE K.W., ROBBINS P.W. 1986. A new yeast mutation in glycosylation steps of the asparagine-linked glycosylation pathway. Formation of a novel asparagine-linked oligosaccharide containing two glucose residues. J.Biol. Chem. 261 (33):15582-90.

RUOHOLA H., FERRO-NOVICK S. 1987. Sec53, a protein required for an early step in secretory protein processing and transport in yeast, interacts with the cytoplasmic surface of the endoplasmic reticulum.

Proc. Natl. Acad. Sci. USA. (23):8468-72.

RUSH J.S., PANNEERSELVAM K., WAECHTER C.J., FREEZE H.H. 2000. Mannose supplementation corrects GDP-mannose deficiency in cultured fibroblasts from some patients with Congenital Disorders of Glycosylation (CDG). Glycobiology.10(8):829-35.

SHAHINIAN S., DIJKGRAAF G.J., SDICU A.M., THOMAS D.Y., JAKOB C.A, AEBI M., BUSSEY H. 1998. Involvement of protein N-glycosyl chain glucosylation and processing in the biosynthesis of cell wall beta-1,6-glucan of *Saccharomyces cerevisiae*. Genetics 149(2):843-56.

SHARMA C.B., LEHLE L., TANNER W. 1981. N-glycosylation of yeast proteins characterization of the solubilized oligosaccharyltransferase.

Eur. J. Biochem. 116: 101-108.

SHERMAN F. 1991. Getting started with yeast. Methods Enzymol. 194:3-21.

SHERMAN F., FINK G. HICKS JB. 1982. Methods in yeast genetics.

Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SILBERSTEIN S., SCHLENSTEDT G., SILVER P.A., GILMORE R. 1998. A role for the DnaJ homologue Scj1p in protein folding in the yeast endoplasmic reticulum. J Cell Biol. 143(4):921-33.

SIMONS J.F., EBERSOLD M., HELENIUS A. 1998. Cell wall 1, 6 beta-glucan synthesis in *Sacharomyces cerevisiae* depends on ER glucosidases I and II, and the molecular chaperone Bip/Kar2p. Embo J. 17: 396-405.

SONG Y., SATA J., SAITO A., USUI M., AZAKAMI H., KATO A.2001. Effects of calnexin deletion in Saccharomyces cerevisiae on the secretion of glycosylated lysozymes. J. Biochem. (Tokyo) 130(6):757-64.

SOUSA M.C., FERRERO GARCIA M.A., PARODI A.J. 1992. Recognition of the oligosaccharide and protein moieties of glycoproteins by the UDP-Glc:glycoprotein glucosyltransferase. Biochem. 31: 97-105.

SPIRO R.G. 2000. Glucose residues as key determinants in the biosynthesis and quality control of glycoproteins with N-linked oligosaccharide.
J. Biol .Chem. 275(46):35657-60.

STAGLJAR I., Te HEESEN S., AEIBI M. 1994. New phenotype of mutations deficient in glucosylation of lipid-linked oligosaccharide cloning of the *ALG8* locus. Proc. Natl. Acad. Sci U S A 91(3): 5977-81.

STEINMETZ L.M., SCHARFE C., DEUTSCHBAUER A.M., MOKRANJAC D., HERMAN Z.S., JONES T., CHU A.M., GIAEVER G., PROKISCH H., OEFNER P.J., DAVIS R.W. 2002. Systematic screen for human disease genes in yeast.
Nat Genet (4):400-4.

TANNER W., LEHLE L. 1987. Protein glycosylation in yeast.
Biochim. Biophys. Acta 906: 81-99.

TEOH K.L., MACKAY I.R., ROWLEY M.J., FUSSEY S.P. 1994. Enzyme inhibitory autoantibodies to pyruvate dehydrogenase complex in primary biliary cirrhosis differ for mammalian, yeast and bacterial enzymes: implications for molecular mimicry. Hepatology 19(4):1029-33.

TESSIER D.C., DIGNARD D., ZAPUN A., PARODI A., BERGERON J.J., THOMAS D.Y., RADOMINSKA-PANDAY A. 2000. Cloning and characterization of mammalian UDP-glucose glycoprotein: glucosyltransferase and the development of a specific substrate for this enzyme. Glycobiol. 10: 403-412.

TONG A.H., EVANGELISTA M., PARSONS A.B., XU H., BADER G.D., PAGE N., ROBINSON M., RAGHIBIZADEH S., HOGUE C.W., BUSSEY H., ANDREWS B., TYERS M., BOONE C. 2001. Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science 294(5550):2364-8

TONG A., LESAGE G., BADER G., DING H., XU H., XIN X., YOUNG J. *et al.* 2003. Genetic interaction networks: large-scale mapping of synthetic genetic interactions in yeast (in press).

TRIMBLE R.B., MALEY F., TARENTINO A.L. 1980. Characterization of large oligosaccharide-lipids synthesis in vitro by microsomes from *Saccharomyces cerevisiae*. J. Biol. Chem. 255: 10232-10237.

TROMBETTA E.S., SIMONS J.F., HELENIUS A. 1996. Endoplasmic reticulum glucosidaseII is composed of a catalytic subunit conserved from yeast to mammals, and a tightly bound non-catalytic HDEL-containing subunit.
J. Biol. Chem. 271(44):27509-16.

VALDIVIESO M.H., FERRARIO L., VAI M., DURAN A., POPOLO L. 2000. Chitin synthesis in a *gas1* mutant of *Saccharomyces cerevisiae.* J. Bacteriol. 182(17):4752-7.

VALENS M., BOHN C., DAIGNAN-FORNIER B., DANG V.D., BOLOTIN-FUKUHARA M. 1997. The sequence of a 54.7 kb fragment of yeast chromosome XV reveals the presence of two tRNAs and 24 new open reading frames. Yeast. 13(4):379-90.

VALENCIK M.L., KLOECKENER-GRUISSEM B., POYTON R.O., MCEWEN J.E. 1989. Disruption of the yeast nuclear PET54 gene blocks excision of mitochondrial intron al5 beta from pre-mRNA for cytochrome c oxidase subunit I. EMBO J. 8(12):3899-904.

VERBERT A., CACAN R., CACCHELI R. 1987. Membrane transport of sugar donors to the glycosylation sites. Biochimie 69: 91-99.

WARMKA J., HANNEMAN J., LEE J., AMIN D., OTA I. 2001. Ptc1, a type 2C Ser/Thr phosphatase, inactivates the HOG pathway by dephosphorylating the mitogen-activated protein kinase Hog1. Mol. Cell. Biol. 21(1):51-60.

WERNER E.D., BRODSKY J.L., McCRACKEN A. 1996. Proteasome-dependent endoplasmic reticulum-associated protein degradation an unconventional route to a familiar fate. Proc. Natl. Acad. Sci USA 93(24): 13797-801.

WESTPHAL V., KJAERGAAD S., SCHOLLEN E., MARTENS K., GRUNEWALD S., SCHWARTZ M., MATTHIJS G., FREEZE H.H. 2002. A frequent mild mutation *in ALG6* may exacerbate the clinical severity of patients with congenital disorder of glycosylation Ia (CDG-Ia) caused by phosphomanomutase deficiency. Hum. Mol. Genet.11(5): 599-604.

WESTPHAL V., SCHOTTSTADT C., MARQUARDT T., FREEZE H.H. (2000) Analysis of multiple mutations in the *hALG6* gene in a patient with congenital disorder of glycosylation Ic. Mol. Genet. Metab. 70: 219–223.

WINDERICKX J., DE WINDE J.H., CRAUWELS M., HINO A., HOHMANN S., VAN DIJCK P., THEVELEIN J.M. 1996. Regulation of genes encoding subunits of the trehalose synthase complex in *Saccharomyces cerevisiae*: novel variations of STRE-mediated transcription control? Mol. Gen. Genet. 252(4):470-82.

WINZELER E. A., SHOEMAKER D. D., ASTROMOFF A., LIANG H., ANDERSON K. , ANDRE B., BANGHAM R., BENITO R., BOEKE J.D., BUSSEY H., CHU A.M., CONNELLY C., DAVIS K., DIETRICH F., DOW S.W., EL BAKKOURY M., FOURY F., FRIEND S.H., GENTALEN E., GIAEVER G., HEGEMANN J.H., JONES T., LAUB M., LIAO H., DAVIS R.W., *et al.* 1999 Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. Science 285:901-906.

ZAPUN A., JAKOB C.A., THOMAS D.Y., BERGERON J.J. 1999. Protein folding in a specialized compartment: the endoplasmic reticulum. Structure Fold. Des. 7 (8):173-82.

ZHDANKINA O., STRAND N.L., REDMOND J.M., BOMAN A.L. 2001. Yeast GGA proteins interact with GTP-bound Arf and facilitate transport through the Golgi. Yeast.18(1):1-18.

ZUFFEREY R., KNAUER R., BURDA P., STAGLJAR I., TE HEESEN S., LEHLE L., AEBI M. 1995. STT3, a highly conserved protein required for yeast oligosaccharyl transferase activity in vivo. EMBO J. 14(20):4949-60.