

*i*n mammalian cells, translation of secretory proteins is initiated on cytoplasmic ribosomes, which are subsequently targeted to the endoplasmic reticulum (ER) membrane by signal recognition particle (SRP) and the ER membrane protein, SRP receptor (reviewed in Ref. 1). Nascent secretory proteins are then co-translationally inserted into the ER and subjected to a battery of covalent modifications, including signal peptide cleavage and core glycosylation on asparagine residues (reviewed in Refs 2, 3). These events are summarized in Fig. 1. Whereas much is known about the mechanism of targeting secretory proteins to the ER, little is known about how proteins are translocated across the ER membrane. The number and arrangement of the components in the putative 'translocation pore' are unknown. Crosslinking experiments suggest that the signal sequence of preprolactin interacts with at least one protein of the ER membrane (signal sequence receptor, SSR) during its passage across the lipid bilayer⁴.

Somewhat more is known about the enzymatic properties of the ER membrane proteins that participate in the cleavage of signal peptides and in the biosynthesis and transfer of core oligosaccharides, since independent assays have been devised to monitor their activities. However, little is known about the organization of these proteins within the ER membrane. Since protein translocation, core glycosylation and signal sequence cleavage are kinetically coupled, the molecules that catalyse these events may be intimately associated within the ER membrane bilayer.

To understand further the process of protein secretion in eukaryotic cells, many cell biologists have turned their attention to the yeast *Saccharomyces cerevisiae*. Genetic screens and selections have allowed the identification of a large collection of genes required for both post-translational modification and inter-compartmental transport of secretory proteins (reviewed in Ref. 5). In this review, we focus on genes required for the translocation, signal peptide processing and asparagine-linked core glycosylation of secretory proteins in yeast. We describe the progress that has been made by applying genetics, molecular biology and biochemistry to the study of these three aspects of protein secretion.

PROTEIN TRANSLOCATION INTO THE YEAST ENDOPLASMIC RETICULUM

Recently, several groups have reconstituted protein translocation *in vitro* using components extracted from

GENETIC DISSECTION of the EARLY STAGES of PROTEIN SECRETION in YEAST

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*The earliest events in the export of secretory proteins from eukaryotic cells are their insertion into and transport across the membrane of the endoplasmic reticulum, followed by signal peptide cleavage and transfer of core oligosaccharides to specific asparagine residues. Much has been learned through reconstitution of these processes in vitro using cell-free extracts prepared from mammalian and yeast cells. Now, a combination of genetic, molecular and biochemical approaches are being employed to study the early stages of protein secretion in the yeast *Saccharomyces cerevisiae*.*

*S. cerevisiae*⁶⁻⁸. A translation extract supplemented with mRNA encoding the precursor of the secreted mating pheromone α -factor (prepro- α -factor) directs the synthesis of an intact precursor that can insert

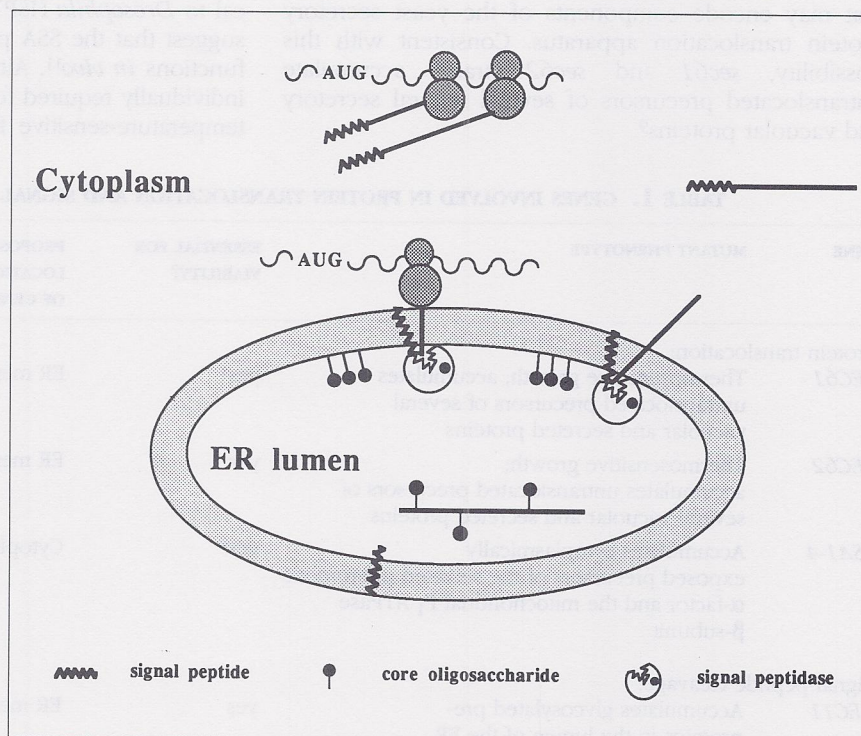


FIG 1

SECRETORY PROTEIN TRANSLOCATION AND POST-TRANSLATIONAL MODIFICATION. PRECURSORS OF SECRETORY PROTEINS SYNTHESIZED IN THE CYTOPLASM ARE IMPORTED EITHER CO- OR POST-TRANSLATIONALLY INTO THE LUMEN OF THE ENDOPLASMIC RETICULUM (ER). AS THE TRANSLOCATING POLYPEPTIDE EMERGES ON THE LUMINAL SIDE OF THE ER MEMBRANE, THE SIGNAL SEQUENCE IS REMOVED BY SIGNAL PEPTIDASE, AND CORE OLIGOSACCHARIDES ARE TRANSFERRED TO ASPARAGINE RESIDUES BY OLIGOSACCHARYLTRANSFERASE. THE MODIFIED POLYPEPTIDE THEN FOLDS INTO ITS NATIVE CONFORMATION BEFORE IT IS TRANSPORTED TO THE GOLGI BODY.

co- or post-translationally into yeast microsomes, become core glycosylated, and have its signal peptide cleaved. Because prepro- α -factor can be translocated post-translationally, it has been possible to show that protein translocation into the yeast ER requires nucleotide hydrolysis^{7,8}. The reconstitution of protein translocation with yeast extracts presents an opportunity to combine biochemical analyses with genetic approaches aimed at identifying genes whose products participate in the reconstituted reaction. Several genes that are required for import of secretory proteins into the ER have recently been identified (Table 1).

Isolation of translocation-defective mutants

To identify genes that potentially encode components of the ER translocation machinery, a direct selection for temperature-sensitive translocation-defective mutants of *S. cerevisiae* was developed⁹. The rationale for this selection is shown in Fig. 2. *HIS4* protein (His4p), which converts histidinol to histidine, normally resides in the cytoplasm. A modified His4p derivative bearing an amino-terminal signal peptide is targeted to the ER lumen. Wild-type cells expressing the ER-localized His4p chimera fail to grow on minimal medium supplemented with histidinol, presumably because the substrate cannot gain access to the luminal His4p (Fig. 2A). By selecting for mutants that can grow on histidinol, recessive temperature-sensitive lethal mutations that impede translocation of the hybrid protein into the ER were obtained (Fig. 2B). These mutations define two genes, *SEC61* and *SEC62*, that may encode components of the yeast secretory protein translocation apparatus. Consistent with this possibility, *sec61* and *sec62* strains accumulate untranslocated precursors of several natural secretory and vacuolar proteins⁹.

Evaluation of *sec61* and *sec62* cytosols and microsomes in the yeast *in vitro* protein translocation assay should reveal whether these mutations affect soluble or membrane-associated components of the translocation machinery. Preliminary data suggest that the *sec62* mutation compromises the translocation competence of yeast microsomal membranes (R. Deshaies, unpublished). DNA sequence analysis of *SEC61* and *SEC62* indicates that both genes encode polypeptides with potential membrane-spanning domains (C. Stirling and R. Deshaies, unpublished).

The role of 70 kDa stress proteins in protein translocation into the yeast ER

α -Factor precursor synthesized in a wheat-germ lysate is poorly assembled into yeast ER vesicles, but uptake is stimulated approximately fivefold by a soluble extract from yeast. Purification of this cytosolic translocation-promoting activity yielded two related 70 kDa polypeptides¹⁰. These proteins cross-react with antibodies raised against the *Drosophila* heat shock protein HSP70 and bind ATP, suggesting that they are members of the yeast HSP70 family. Comparison (by two-dimensional gel electrophoresis) of the purified 70 kDa proteins with extracts of yeast cells lacking individual members of the HSP70 family established that the translocation-promoting polypeptides correspond to the constitutively expressed *SSA1* and *SSA2* gene products¹⁰.

SSA1 and *SSA2* are two members of a group of four yeast genes (*SSA1* – *SSA4*) that are 60–70% identical to *Drosophila* HSP70. Gene disruption experiments suggest that the SSA proteins perform interchangeable functions *in vivo*¹¹. Although none of the SSA genes is individually required for cell growth, *ssa1 ssa2* cells are temperature-sensitive for growth, and *ssa1 ssa2 ssa4*

TABLE 1. GENES INVOLVED IN PROTEIN TRANSLOCATION AND SIGNAL PEPTIDE CLEAVAGE

GENE	MUTANT PHENOTYPE	ESSENTIAL FOR VIABILITY?	PROPOSED LOCATION OF GENE PRODUCT	REFS
Protein translocation:				
<i>SEC61</i>	Thermosensitive growth; accumulates untranslocated precursors of several vacuolar and secreted proteins	yes ^a	ER membrane ^b	9
<i>SEC62</i>	Thermosensitive growth; accumulates untranslocated precursors of several vacuolar and secreted proteins	yes ^c	ER membrane ^d	9
<i>SSA1-4</i>	Accumulates cytoplasmically exposed precursors of the secreted pheromone α -factor and the mitochondrial F ₁ ATPase β -subunit	yes ^e	Cytoplasm	10–12
Signal peptide cleavage:				
<i>SEC11</i>	Accumulates glycosylated pre-proteins in the lumen of the ER	yes	ER membrane	16

^aC. Stirling and R. Schekman, unpublished data.

^bThe DNA sequence of *SEC61* predicts a protein with multiple transmembrane domains (C. Stirling and R. Schekman, unpublished data).

^cR. Deshaies and R. Schekman (unpublished).

^dThe DNA sequence of *SEC62* predicts a protein with two transmembrane domains (R. Deshaies and R. Schekman, unpublished data).

^e*ssa1*, *ssa2*, *ssa4* strains are inviable.

spores are inviable. SSA1 protein (Ssa1p) expressed from a galactose-inducible promoter rescues the lethal phenotype of *ssa1 ssa2 ssa4* cells on galactose-based medium; when glucose is added to repress transcription from this promoter, the levels of Ssa1p decline, and the cells eventually stop growing¹¹. Depletion of Ssa1p *in vivo* results in pleiotropic defects in transmembrane protein transport; precursors of the secretory protein α -factor, the vacuolar protein carboxypeptidase Y, and the mitochondrial F₁ATPase β -subunit accumulate in an unprocessed, untranslocated form¹². These results suggest that SSA proteins participate in the import of precursor proteins into both the ER lumen and the mitochondrial matrix.

How do the SSA proteins promote translocation of precursor proteins across organellar membranes? A clue to the mechanism of action of SSA proteins comes from work showing that their effect can be mimicked by treating the α -factor precursor with urea before adding yeast membranes¹⁰. Although the effect of urea on the structure of the precursor is unclear, this result suggests that α -factor precursor must be unfolded before it is translocated into the ER. Perhaps the SSA proteins use energy derived from ATP hydrolysis to inhibit folding of nascent chains into a translocation-incompetent form, or to unfold precursor molecules before membrane insertion. Besides the SSA proteins, one or more soluble proteins are required for maximal import of wheat-germ-synthesized prepro- α -factor into yeast microsomes^{10,12}. Although the function of these proteins is unknown, they may either target secretory proteins to the ER membrane, or facilitate the interaction or dissociation of SSA proteins and secretory precursors.

SIGNAL PEPTIDE PROCESSING IN YEAST

Arrival of newly synthesized proteins in the lumen of the ER or the matrix of mitochondria is accompanied by removal of the amino-terminal targeting peptides. This task is performed by endopeptidases with high cleavage specificities.

Genetic evaluation of signal peptidase function

Removal of a protein's signal peptide can be blocked by either amino acid substitutions at the cleavage site or by short deletions that span the cleavage site. Secretory proteins with mutated cleavage sites accumulate as core-glycosylated precursors in the ER lumen¹³⁻¹⁵. Although prevention of signal peptide processing does not impair translocation into the ER, further transport from the ER lumen to subsequent compartments is dramatically delayed. This transport retardation may be due to an association between the mutant protein and the ER membrane via the unprocessed signal peptide¹⁴.

Since transport of mutant proteins that retain their signal peptide is delayed, but not blocked, precursors eventually reach their final destinations¹⁵. These observations suggest that mutations in yeast signal peptidase should result in a dramatically reduced rate of secretion of proteins that have cleavable signal peptides. The temperature-sensitive lethal *sec11* mutant fulfills this prediction. At the restrictive temperature of 37°C, *sec11* cells externalize secretory glycoproteins at a

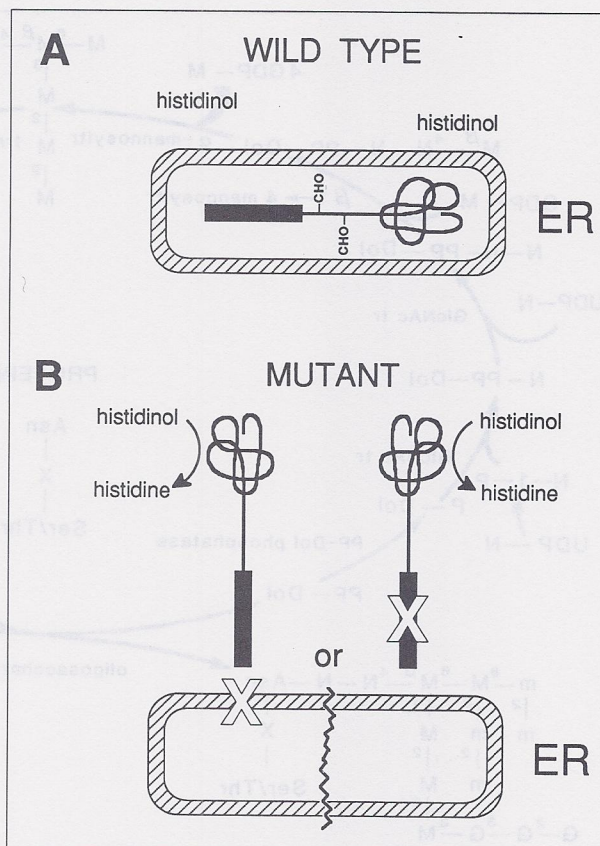


FIG 2

PREDICTED TOPOLOGY OF A SIGNAL PEPTIDE-CONTAINING HIS4 HYBRID PROTEIN IN WILD-TYPE AND TRANSLOCATION-DEFECTIVE MUTANT YEAST CELLS. CHO, ASPARAGINE-LINKED CARBOHYDRATE; BLACK RECTANGLE, THE SIGNAL SEQUENCE; FOLDED BLACK TRACE, THE CATALYTIC DOMAIN OF THE HIS4 FUSION PROTEIN. X, A MUTATION THAT EITHER INACTIVATES A CELLULAR COMPONENT (CYTOSOLIC OR MEMBRANE-BOUND) REQUIRED FOR PROTEIN TRANSLOCATION, OR ABROGATES THE FUNCTION OF THE SIGNAL SEQUENCE.

severely reduced rate. Pulse-labelling experiments indicate that newly synthesized secretory proteins accumulate as core-glycosylated, signal sequence-containing precursors in the ER lumen of *sec11* cells¹⁶. Many yeast plasma membrane proteins lack cleavable amino-terminal signal sequences; it is not yet known whether efficient transport of these membrane proteins to the cell surface requires *SEC11* function.

Gene disruption experiments indicate that the *SEC11* locus is essential for yeast cell growth¹⁶. The DNA sequence of the *SEC11* gene predicts an open reading frame encoding an 18.8 kDa protein containing one potential asparagine-linked glycosylation site and an amino-terminal hydrophobic sequence reminiscent of a signal peptide¹⁶. The predicted *SEC11* polypeptide (Sec11p) shares features with signal peptidase subunits isolated from either hen oviduct or dog pancreas microsomes. The molecular mass of Sec11p is similar to the molecular masses of the glycosylated subunits of the vertebrate enzymes: hen oviduct signal peptidase activity copurifies with a 19 kDa unglycosylated protein and a 22-24 kDa glycoprotein; canine signal peptidase preparations contain six polypeptides, including two glycoproteins with molecular masses of 23 kDa and 22 kDa. Enzymatic removal of the carbo-

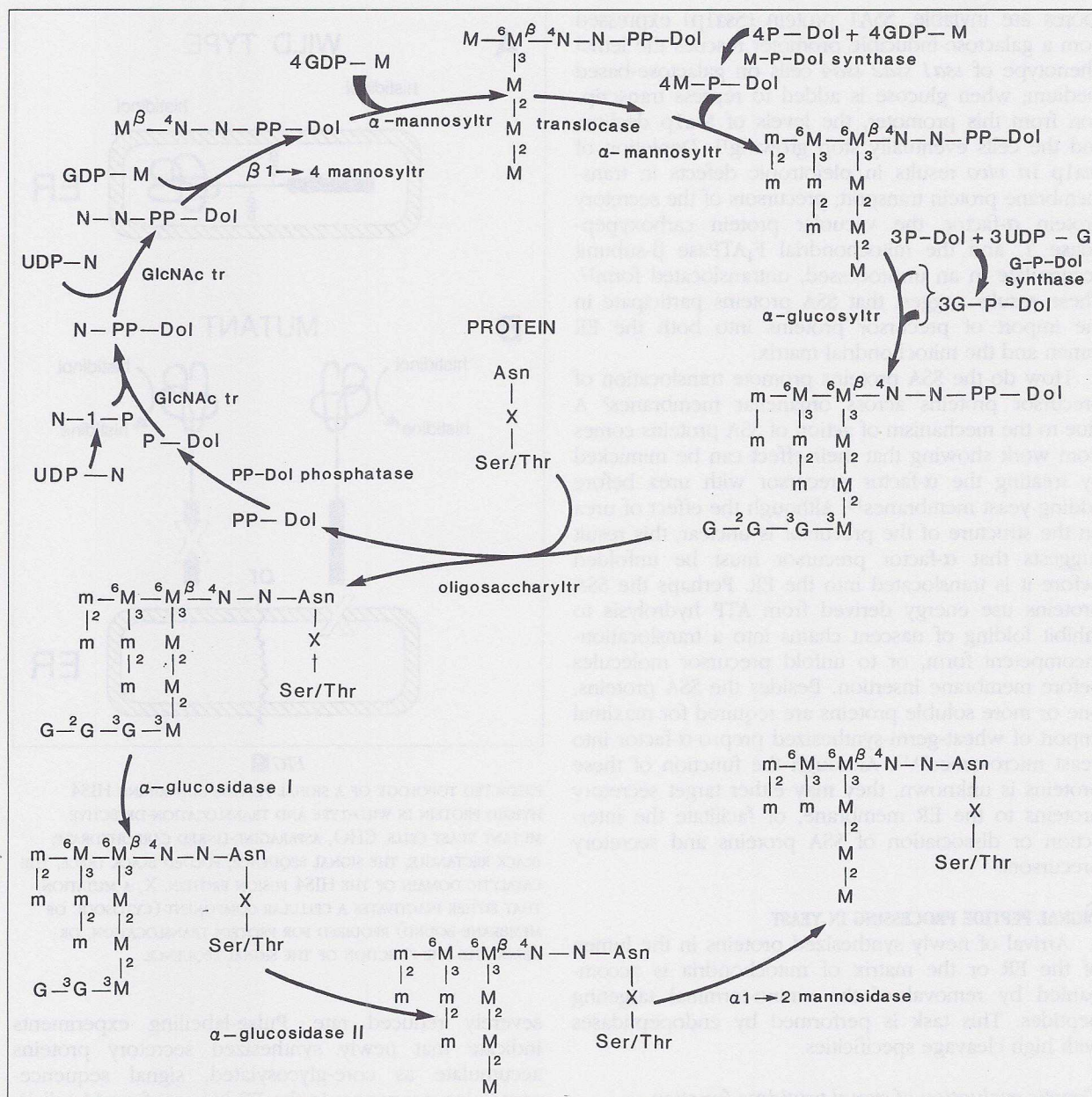
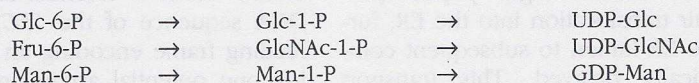


FIG 3

BIOSYNTHESIS OF THE DOLICHOL-LINKED OLIGOSACCHARIDE, TRANSFER ONTO A PROTEIN AND PROCESSING OF THE OLIGOSACCHARYL UNIT. N, N-ACETYLGLUCOSAMINE; M or m, MANNOSE; G, GLUCOSE; TR, TRANSFERASE. LOWER CASE 'm' INDICATES A MANNOSE ORIGINATING FROM MANNOSE-P-DOL. THE TYPES OF $\alpha 1 \rightarrow \text{X}$ LINKAGE ARE SIMPLY INDICATED BY 'X'. THE ONLY HEXOSE IN β -LINKAGE IS THE FIRST MANNOSE ADDED, AS SHOWN. THE MONOSACCHARIDE DONORS ARE SUGAR-NUCLEOTIDES THAT ARE PRODUCED BY THE FOLLOWING REACTIONS. NOTE THAT THE HEXOSES-6-PHOSPHATE ARE INTERCONVERTED BY ISOMERASES:



hydrates on either the avian or canine glycoprotein subunits reveals a common polypeptide backbone with a molecular mass of 19 kDa^{17,18}. A cDNA encoding the glycoprotein subunit of canine signal peptidase has been cloned and sequenced¹⁹. Although the predicted amino acid sequence is not homologous to that of Sec11p, the two proteins have a strikingly similar arrangement of hydrophilic and hydrophobic domains.

Interestingly, both canine and avian signal peptidases appear to be multisubunit complexes, in contrast

to the two prokaryotic single subunit enzymes, leader peptidase and lipoprotein signal peptidase (reviewed in Ref. 20). Despite these structural differences between prokaryotic leader peptidase and canine signal peptidase, the substrate specificities are remarkably similar, since either enzyme can cleave prokaryotic or eukaryotic substrates at the correct peptide bond *in vitro*. This conservation of processing function has led to the suggestion that some of the proteins associated with the vertebrate peptidases may perform other

functions, resulting in either a high cleavage specificity, an increase in the efficiency of cleavage, a tight association with the substrate in a cleavage-competent conformation, or an association with components involved in protein translocation and core glycosylation. Unfortunately, it is not yet known whether yeast signal peptidase is a single or multisubunit enzyme.

Genetic evaluation of mitochondrial processing protease function

Genetic approaches have also been successfully used to study the matrix-localized mitochondrial processing protease responsible for cleaving transit peptides from imported mitochondrial precursor proteins. Biochemical and genetic evidence indicates that this protease is composed of two distinct subunits. Conditionally lethal mutations in the yeast genes encoding both subunits (*mas1* and *mas2*) have been isolated, and the wild-type *MAS* genes have been cloned and sequenced (see Ref. 21 and references therein).

Although import of precursors into yeast mitochondria *in vitro* does not require processing protease activity²², *mas1* and *mas2* cells accumulate untranslocated mitochondrial preproteins at the nonpermissive temperature (see Ref. 21). Potentially, *MAS1* and *MAS2* proteins are components of the mitochondrial import apparatus, or the sustained failure to process translocated precursors indirectly interferes with further import. Since import into the ER and mitochondria and export from bacteria can occur in the absence of signal peptide cleavage^{13,14,16,20,22}, it is unlikely that there is an obligatory coupling between the transmembrane movement and amino-terminal processing of precursor proteins.

CORE GLYCOSYLATION OF SECRETORY PROTEINS

The yeast core oligosaccharide is assembled stepwise on phosphorylated dolichol (P-Dol) and is then transferred to the polypeptide chain. Biosynthesis of core oligosaccharides in yeast and mammalian cells proceeds by similar pathways; the differences between

TABLE 2. MUTATIONS AFFECTING DOLICHOL-LINKED OLIGOSACCHARIDE ASSEMBLY AND PROCESSING

MUTANT	MEANS OF IDENTIFICATION	LETHALITY AT ELEVATED TEMPERATURE	ACCUMULATED DOL-LINKED OLIGOSACCHARIDE	ACTIVITY AFFECTED BY MUTATION	REFERENCES
<i>sec53</i>	Failure to secrete active glycoproteins	+	Man ₁₋₈ -GlcNAc ₂	Phosphomannomutase	24, 28
<i>alg4</i>	Survive exposure to ³ H-mannose ^a	+	Man ₁₋₈ -GlcNAc ₂	Phosphomannomutase	24, 27
<i>ALG7</i>	Tunicamycin resistance	-	ND ^b	Overexpression of first GlcNAc transferase	25
<i>alg1</i>	Survive exposure to ³ H-mannose ^a	+	GlcNAc ₂	β1→4 Mannosyl transferase	26
<i>alg2</i>	Survive exposure to ³ H-mannose ^a	+	Man ₁₋₂ -GlcNAc ₂	α-Mannosyl transfer?	27
<i>sec59</i>	Failure to secrete active glycoproteins	+	Man ₅₋₆ -GlcNAc ₂	α-Mannosyl transfer	28, 29
<i>alg3</i>	Survive exposure to ³ H-mannose ^a	-	Man ₅ -GlcNAc ₂	α-Mannosyl transfer from Man-P-Dol	27
<i>alg5</i>	Survive exposure to ³ H-mannose ^a	-	Man ₉ -GlcNAc ₂	Synthesis of Glc-P-Dol	27 ^c
<i>dpg1</i>	Suppressor of <i>gls1</i>	-	Man ₉ -GlcNAc ₂	Synthesis of Glc-P-Dol	30
<i>alg6</i>	Survive exposure to ³ H-mannose ^a	-	Man ₉ -GlcNAc ₂	First α-glucosyl transferase	27 ^c
<i>alg8</i>	Survive exposure to ³ H-mannose ^a	-	Glc ₁ -Man ₉ -GlcNAc ₂	Second α-glucosyl transferase	- ^c
<i>gls1</i>	Aberrant migration of core-glycosylated invertase ^d	-	ND	α-Glucosidase I	34

^aThese cells survived prolonged exposure to ³H-mannose because they could not efficiently incorporate the radiolabeled sugar into glycoproteins. Cells that efficiently incorporated the labeled sugar were killed by self-irradiation.

^bNot determined.

^cK. Runge, PhD thesis.

^dThe *gls1* allele was fortuitously discovered in a secretion-defective *sec19* mutant. Invertase accumulated in a *sec19 gls1* strain has an unusually low mobility on polyacrylamide gels resulting from the presence of untrimmed glucose residues on the core oligosaccharides.

mammalian and yeast glycoprotein synthesis reside in the modifications that the sugar moieties undergo during later processing steps.

Core oligosaccharide assembly in yeast

Oligosaccharide synthesis involves the stepwise transfer of 14 sugars (three glucose, nine mannose and two *N*-acetylglucosamine residues) to P-Dol, to generate Glc₃-Man₉-GlcNAc₂-PP-Dol (Fig. 3); 11 yeast genes that are required for the proper assembly and trimming of core oligosaccharides have been identified (Table 2). Genes involved in core oligosaccharide assembly in mammalian cells have also been identified, and this work has been reviewed elsewhere²³.

Synthesis of the Dol-linked oligosaccharide requires only three sugar nucleotide donors: UDP-GlcNAc, GDP-Man and UDP-Glc. The biosynthetic pathways of these three precursors are shown schematically in the legend to Fig. 3. The *sec53* and *alg4* mutations block the production of GDP-Man; these mutations are allelic, and they reside in the gene encoding phosphomannomutase, an enzyme that catalyses the interconversion of Man-6-phosphate and Man-1-phosphate²⁴.

The first step in the assembly of the Dol-linked oligosaccharide involves the transfer of GlcNAc-1-P from UDP-GlcNAc to P-Dol, a reaction that is inhibited by the antibiotic tunicamycin. This inhibitor has been used to select dominant tunicamycin-resistant mutations in the transferase gene, *ALG7* (Ref. 25). The sequence of the *ALG7* gene predicts a 51 kDa protein (J. Rine, pers. commun.). Assembly of the core oligosaccharide proceeds by transfer of a second GlcNAc from UDP-GlcNAc to GlcNAc-PP-Dol.

GDP-Man is the direct donor for the first five mannose residues. The first mannose addition is mediated by a β 1 \rightarrow 4 mannosyltransferase, and a mutation in the gene encoding this transferase (*ALG1*) causes accumulation of GlcNAc₂-PP-Dol²⁶. The predicted *ALG1* gene product is a 50 kDa protein with one potential membrane-spanning domain at the amino terminus and no apparent site for signal peptide cleavage (P. W. Robbins, pers. commun.).

The next four mannose residues are added in various α -linkages. Two conditionally lethal mutations, *alg2* (Ref. 27) and *sec59* (Ref. 28), might affect some of these steps; both mutants transfer abnormal oligosaccharide species to secretory proteins, possibly because one or a few mannose residues are added by nonmutated mannosyltransferases acting beyond the mutant block. For instance, the Man₅-GlcNAc₂ species transferred to protein in *sec59* cells is mostly sensitive to digestion by endoglycosidase H (Ref. 28), and thus must differ in structure from the authentic Man₅-GlcNAc₂ biosynthetic intermediate (see Fig. 3), which is resistant to digestion. The DNA sequence of *SEC59* predicts a very hydrophobic 59 kDa protein; the *SEC59* gene product has no apparent amino-terminal signal sequence, but has numerous potential membrane-spanning domains separated by clusters of charged amino acids²⁹. *In vitro* translocation/core glycosylation assays suggest that the *sec59* mutation affects the transfer of at least one mannose to the Dol-linked oligosaccharide²⁹.

Based on data from animal cells, it is believed that the Man₅-GlcNAc₂-PP-Dol species is translocated from the cytoplasmic to the luminal side of the ER membrane before further elongation. After this transfer, four more mannose residues are added from Man-P-Dol onto the growing core oligosaccharide (Fig. 3). Although *alg3* cells can synthesize Man-P-Dol, they accumulate an endoglycosidase-H-resistant Man₅-GlcNAc₂-PP-Dol which appears to be identical in structure to the normal biosynthetic intermediate and is transferred to protein²⁷. Thus, the *alg3* mutation appears to interfere directly with the first of these four mannose additions, and possibly with the others as well. The final steps in the assembly of the lipid-linked oligosaccharide are glucosylations via Glc-P-Dol; four genes involved in this process have been identified (Refs 27, 30; K. Runge, PhD Thesis, MIT, 1985) (Table 2).

Oligosaccharide transfer to protein and trimming

The transfer of the Dol-linked Glc₃-Man₉-GlcNAc₂ to secretory polypeptides is catalysed by oligosaccharyltransferase. The yeast enzyme has been solubilized, but not purified³¹; its molecular mass under denaturing conditions is 80 kDa, whereas that of the corresponding mammalian enzyme is 60 kDa³². There is good evidence that the oligosaccharyltransferase active site is oriented towards the ER lumen³². As in mammalian cells, the acceptor sequence on yeast polypeptide chains is Asn-X-Ser/Thr, where X may be any amino acid but Pro³³. After its transfer to protein, the core oligosaccharide is modified by trimming enzymes located in the ER; the three Glc residues are sequentially removed by α -glucosidases I (potentially encoded by the *GLS1* gene³⁴) and II, while one mannose is subsequently removed by an α 1 \rightarrow 2 mannosidase to yield a core oligosaccharide with the structure Man₈-GlcNAc₂ (Refs 2, 3). Unfortunately, mutations that block either core oligosaccharide transfer to protein or subsequent α 1 \rightarrow 2 mannosidase trimming have not yet been isolated.

Besides signal peptide processing and core glycosylation, proteins translocated into the lumen of the ER experience other post-translational covalent modifications, including O-linked glycosylation and disulfide bond formation. A complete understanding of the early events in protein secretion will require the identification and characterization of genes whose products participate in these reactions.

CONCLUSIONS

Genetic analyses of secretory protein translocation, signal peptide processing and core glycosylation in yeast have contributed valuable information to our understanding of the transport and processing of eukaryotic secretory proteins. First, characterization of strains deficient in the expression of members of the yeast HSP70 gene family has revealed that these stress proteins participate in the translocation of precursor polypeptides across the membrane of at least two different organelles.

Second, an analysis of the *sec11* mutation indicates that although proteolytic removal of a signal sequence is not absolutely required for either translocation into

the ER or secretion, mutational inactivation of signal peptidase function severely retards intercompartmental secretory protein transport, resulting in cell death.

Third, examination of various glycosylation mutants has revealed that both protein transport and cell viability require the addition of core oligosaccharides to protein. Although incompletely assembled core oligosaccharides can be transferred to proteins, the core oligosaccharide precursor must attain a minimum size to allow protein secretion and cell growth to proceed. These observations imply that it is not the exact structure of the core oligosaccharide that is important for protein secretion and cell growth, but rather a bulk chemical property inherent in the monosaccharide units. Perhaps core sugars increase the polarity of secretory proteins, thereby reducing aggregation or deleterious associations with membrane. On the other hand, the presence of asparagine-linked sugars may influence protein folding patterns by retaining flanking amino acids on the solvent-accessible surface of the folding polypeptide.

S. cerevisiae has proven to be a convenient organism for identifying genes that influence secretory protein translocation and post-translational modification. To date, at least 15 gene products have been implicated in these processes. Detailed characterization of these polypeptides should reveal insights into their structure and topography with respect to the ER membrane. This knowledge, in turn, will lead to a more refined understanding of the functional organization of the ER.

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