Review Article

Protein targeting in yeast

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Introduction

The eukaryotic cell has a wonderfully organized structure with many functionally specialized organelles, each bounded by one or more membranes that separate the organelle interior from other cellular compartments. Since the vast majority of polypeptides are synthesized in the cytosol, the cell is presented with a major twofold problem of protein targeting. Newly synthesized proteins must be specifically localized to the correct destination and not elsewhere within the cell, so there is a requirement for an efficient, specific addressing system. Furthermore, organelle proteins have to be translocated into or across biological membranes by processes which are as yet poorly understood. A considerable body of work over the last thirty years or so has been aimed at understanding the cellular and molecular events involved in protein targeting and my aim in this review is to focus on how baker's yeast, *Saccharomyces cerevisiae*, has proved to be an extremely useful experimental organism in furthering our knowledge of various targeting pathways.

Several different targeting pathways coexist within eukaryotic cells (Fig. 1). The biogenesis of many organelles, such as vacuoles, Golgi bodies and the plasma membrane, depends upon delivery of proteins via branches of the secretory pathway and in these cases the initial targeting steps occur at the endoplasmic reticulum. Transport of proteins into other organelles, including mitochondria and the nucleus, occurs directly from the cytoplasm and thus demands a range of different primary targeting sequences. There may be some functional analogies between the mechanisms used to direct proteins to different cellular destinations, both in the recognition of targeting information and in the

physical movement of proteins across biological membranes. For example chaperone proteins such as the Hsp70 family have been shown to be important in maintaining proteins in an 'unfolded' state that renders them competent for translocation (Deshaies *et al.*, 1988). Nevertheless the types of signal that are used for targeting to different organelles appear to vary substantially and the precise mechanism of transmembrane translocation remains poorly understood in all cases.

Until relatively recently it appeared that protein localization was directed solely by sequences within each polypeptide but in the past three or four years it has become clear that several eukaryotic proteins become

Fig. 1. Protein targeting pathways in yeast. Proteins synthesized in the cytosol may be targeted to various organelles dependent on the specific targeting signals that they contain. Pathways lead directly from the cytosol to the nucleus (N), peroxisomes (P), mitochondria (M) and the endoplasmic reticulum (ER) as indicated by the thick arrows. The secretory pathway then directs proteins (thin arrows) from the ER via the Golgi apparatus (G) and secretory vesicles to the plasma membrane (PM). Sorting signals direct proteins to the vacuole (V) or specify retention in the ER (and presumably in other compartments) by a recovery mechanism that involves recycling of membrane components (dashed arrow). The endocytotic pathway is not indicated.

Abbreviations: CPY, carboxypeptidase Y; ER, endoplasmic reticulum; NSF, *N*-ethylmaleimide-sensitive fusion protein; SRP, signal recognition particle.

covalently modified by the addition of lipid or glycolipid groups and that this is essential for their correct localization to membranes (McIlhinney, 1990; Glomset *et al.*, 1990; Ferguson & Williams, 1988).

The secretory pathway

All organisms have a secretory system that releases enzymes and other proteins into the extracellular medium. Secretion is a fundamental biological activity with a long evolutionary history and the basic patterns of secretion appear to be well conserved in all eukaryotes, from micro-organisms to plants and animals. Much of the early work on secretion centred on higher animals, both for reasons of medical interest and for the fact that some specialized tissues are essentially devoted to secretion of hormones or digestive enzymes and thus provide a rich source of active experimental material. Micro-organisms secrete several proteins including hydrolytic enzymes essential for nutrient uptake, protein toxins and polypeptides involved in cell-cell communication such as mating factors. Although *S. cerevisiae* secretes relatively little protein and is therefore not particularly well suited to biochemical or ultrastructural studies, it has the important advantage of a well-developed system of genetic analysis and can be subjected to genetic manipulation. These advantages have led to yeast becoming a very important system for a molecular investigation of the secretory pathway and have also enabled the exploitation of yeast for secretion of heterologous proteins (Bigelis & Das, 1988; Boyd, 1989).

The secretory pathway in eukaryotes entails an initial translocation of proteins across the membrane of the endoplasmic reticulum (ER) followed by movement from the ER to the Golgi apparatus and on through secretory vesicles to the cell surface. Randy Schekman and his colleagues have isolated many mutants of *S. cerevisiae* that are specifically defective in protein secretion (Schekman, 1985). These *sec* mutations are lethal, affecting membrane biogenesis and cell surface growth as well as secretion, so mutants have been isolated with a conditional lethal, generally temperature-sensitive, phenotype. Different *sec* mutants grown at the non-permissive temperature (37 °C) accumulate different types of intracellular organelle (e.g. ER, secretory vesicles) and proteins that are normally secreted remain intracellular, associated with the accumulated organelles. These polypeptides, such as invertase and acid phosphatase, normally undergo covalent modification during their progress through the secretory pathway: the N-terminal signal sequence is removed in the ER by signal peptidase and N-linked glycosylation begins here

also. Further modification of glycosyl chains occurs progressively as the proteins move through the Golgi apparatus and thus the covalent structure of a protein accumulated in a *sec* mutant indicates the extent of its progress along the secretory pathway.

Mutations in many genes block the final steps in secretion where vesicles fuse with the plasma membrane; some block exit from the Golgi apparatus and others prevent exit from the ER. These steps in the secretory pathway, subsequent to the initial transmembrane transport events at the ER, involve extensive budding of vesicles from donor membranes, directed movement through the cytosol and fusion with acceptor membranes. Although the biochemical details of these events remain poorly understood, several proteins involved in membrane trafficking have recently been identified, particularly those involved in transport from the ER to the Golgi apparatus (Hicke & Schekman, 1990). The *SEC23* gene encodes a polypeptide (Sec23p) of 85 kDa that is loosely associated with the cytosolic face of an intracellular membrane and has been shown to be essential for transport of proteins from ER to Golgi in an *in vitro* assay. This assay follows the fate of a radiolabelled secretory protein, the mating pheromone precursor, prepro- α -factor, that has been synthesized *in vitro* and translocated into yeast ER membranes where it is modified by core-glycosylation. Protein that is subsequently transported to the Golgi apparatus undergoes outer-chain glycosylation and can be identified by antibodies specific for outer-chain carbohydrate. When these reactions are performed with membranes from a *sec23* mutant, ER to Golgi transport is temperature-sensitive (Baker *et al.*, 1988) but this defect can be overcome by addition of soluble fractions containing wild-type Sec23p (Hicke & Schekman, 1989). This assay has allowed purification of functional Sec23p as a complex of about 400 kDa containing at least one other polypeptide. Kaiser & Schekman (1990) have recently shown strong genetic interactions amongst *SEC12*, *SEC13*, *SEC16* and *SEC23*, suggesting that the polypeptides encoded by these genes may also interact, perhaps as components of a complex. The *sec17* and *sec18* mutations also interact strongly and mutations in either of these genes or *sec22* cause accumulation of 50 nm vesicles, in addition to ER, at the non-permissive temperature whereas no vesicles accumulate in *sec12*, *sec13*, *sec16*, *sec21* or *sec23* mutants. These and other results implicate the 50 nm vesicles in the transport of proteins from ER to Golgi and it appears that some mutations block the membrane budding reactions that result in vesicle formation whereas others block the fusion of transport vesicles with the *cis* Golgi membrane (Fig. 2). The products of several other genes (*SEC19*, *SEC20*, *BET1*, *BET2* and *YPT1*) are also required for ER

Fig. 2. Vesicular transport between organelles. Vesicle budding and fusion reactions are essential in the transfer of proteins from ER to Golgi apparatus and elsewhere in the secretory pathway. The products of several SEC genes have been shown to be required for these processes in yeast. Genetic interaction (synthetic lethality) between pairs of SEC genes is indicated by the connecting lines and their involvement in vesicle formation and fusion reactions is indicated.

to Golgi transport (Newman & Ferro-Novick, 1987; Segev *et al.*, 1988) but it is not yet clear whether they are required for vesicle formation, movement or fusion.

Similar budding and fusion events are probably involved in the transport of proteins between Golgi cisternae and at later stages of the secretory pathway. An *in vitro* assay for transport between the cisternae of the mammalian Golgi apparatus has allowed identification of several proteins that promote vesicle fusion (Malhotra *et al.*, 1988). One of these, NSF (*N*-ethylmaleimide-sensitive fusion protein), is a cytoplasmic protein that has also been shown to be required for ER to Golgi transport (Beckers *et al.*, 1989). NSF and Sec18p appear to be functionally homologous and molecular analysis of the respective genes reveals 48% sequence identity (Wilson *et al.*, 1989). It therefore seems likely that Sec18p functions not only in ER to Golgi transport but also in fusion events later in the secretory pathway. When *sec18* mutants are grown at the non-permissive temperature they accumulate ER and 50 nm vesicles, presumably because these organelles are at the first site of action of Sec18p and secretory proteins are unable to progress beyond the block in ER to Golgi transport.

Hierarchical sorting signals

Proteins that have been translocated across the ER membrane have many possible destinations. Some polypeptides are transported all the way to the cell surface whereas others are retained within organelles *en route*. Furthermore, the secretory pathway is not linear but branched, with several proteins being diverted to the lysosome-like vacuole in yeast. Thus beyond the initial targeting signals that direct proteins into the secretory pathway there must exist a range of sorting signals that determine the final destinations of individual proteins.

All of these proteins enter the secretory pathway by virtue of a primary targeting signal, the N-terminal signal sequence, but many also contain secondary sorting signals. In the absence of such sorting signals, proteins are transported to the cell surface (the default pathway) and there must therefore be efficient systems for specific targeting to the various organelles of the secretory pathway. Two examples of these are described below.

Retention of proteins in the ER

Soluble proteins of the ER in animal cells share a common C-terminal tetrapeptide sequence, usually Lys-Asp-Glu-Leu (KDEL) that is essential for retention in the ER (Munro & Pelham, 1987). Variations on the KDEL theme are found in different organisms but the principles for function are no doubt very similar in all cases. Among the yeasts, *Saccharomyces cerevisiae* uses the C-terminal sequence HDEL, *Schizosaccharomyces pombe* uses ADEL and *Kluyveromyces lactis* recognizes both HDEL and DDEL (Pelham, 1990). Removal of these C-terminal motifs results in secretion of the protein whereas attachment of the appropriate tetrapeptide to a protein that is normally secreted results in its retention in the ER. This retention system appears to be quite specific for soluble ER proteins, though Sec20p is predicted to be a transmembrane protein and it also terminates with HDEL (Pelham, 1990). The mechanism whereby other ER membrane proteins that lack the HDEL motif are retained in the ER is not understood.

Although soluble ER proteins are referred to as 'resident', there is considerable evidence that they are able to leave the ER but are rapidly retrieved from a later compartment on the secretory pathway and returned to the ER lumen. Most strikingly, fusion proteins tagged with HDEL undergo glycosylation in *S. cerevisiae* that is diagnostic of transport to the Golgi apparatus (Dean & Pelham, 1990). It thus appears that the critical step in ER retention takes place in the Golgi apparatus where HDEL-bearing proteins are recognized by a receptor that is then recycled with the protein ligand to the ER. Hugh Pelham and colleagues have identified a putative HDEL receptor (Erd2p) in *S. cerevisiae* by selecting mutants that were defective in retention of an HDEL-bearing fusion protein. The *ERD2* gene encodes a membrane protein with several of the properties expected of the receptor (Semenza *et al.*, 1990). The best evidence that Erd2p is indeed the receptor comes from the following experiment which takes advantage of the finding that *K. lactis* uses both HDEL and DDEL as ER retention signals whereas *S. cerevisiae* uses HDEL and fails to recognize DDEL. When the *S. cerevisiae* *ERD2* gene was replaced by the equivalent gene from *K. lactis*,

both HDEL and DDEL were recognized efficiently, demonstrating that the specificity of the ER retention signal is determined by Erd2p (Lewis *et al.*, 1990). The Erd2 protein has been found largely in a post-ER, Golgi-like compartment, a distribution that is consistent with the retrieval model for ER retention.

It is not yet known how resident proteins of the various cisternae of the Golgi apparatus and of other organelles of the secretory pathway are retained in the correct compartment but the ER retention system may serve as a useful working model for the general principles involved. It is clear that, whatever mechanisms are used, there must be several distinct signals that determine the fates of individual proteins.

Targeting to the vacuole

The yeast vacuole is functionally comparable with the lysosome of animal cells, having an acid interior and containing several hydrolytic enzymes (Klionsky *et al.*, 1990). Proteins destined for the vacuole enter the secretory pathway and are transported through the Golgi apparatus along with secreted proteins (Rothman *et al.*, 1989a). A branch point in the pathway separates the two streams of traffic and the specificity of this segregation is mediated by vacuolar targeting signals that are presumably recognized by one or more receptors. Lysosomal protein targeting in mammals is mediated by the attachment in the Golgi apparatus of mannose 6-phosphate residues to glycosyl chains but, although phosphorylated mannose residues are found in yeast vacuolar proteins, carbohydrate is not required for vacuolar targeting: localization of carboxypeptidase Y (CPY) to the vacuole is unaffected by tunicamycin, an inhibitor of N-linked glycosylation.

CPY is synthesized with a typical signal sequence (residues 1–20) but also has a propeptide (residues 21–111) that prevents the attainment of proteolytic activity before the protein reaches the vacuole. Once there, preproCPY is activated by the *PEP4* gene product, proteinase A. Site-directed mutagenesis of the preproCPY coding sequence has localized vacuolar targeting information to the N-terminal segment of the propeptide and the tetrapeptide Gln-Arg-Pro-Leu (QRPL) at positions 4–7 of proCPY (i.e. 24–27 of preproCPY) appears particularly important (Valls *et al.*, 1987, 1991; Johnson *et al.*, 1987). Vacuolar targeting information has also been localized to the propeptide of proteinase A (Klionsky *et al.*, 1988) but the sequence of this segment shows no significant similarity to that of the CPY propeptide. The two proteins are either sorted by distinct mechanisms or the vacuolar targeting signal consists of a structural feature that has not yet been identified.

When preproCPY is over-expressed in yeast much of the enzyme appears at the cell surface. This indicates that the sorting of proteins to the vacuole is saturable, presumably at the level of a receptor. In an attempt to identify receptors and other components of the vacuolar targeting machinery, a genetic selection was developed for mutants that mislocalize CPY to the cell surface. This and another selection procedure have led to the identification of 40 *vps* (vacuolar protein sorting) complementation groups, and several other previously identified mutations also block vacuolar targeting (Klionsky *et al.*, 1990). It is perhaps unlikely that the products of all of these genes are directly involved in vacuolar protein sorting but many proteins are presumably required as receptors or for vesicle budding, transport and fusion reactions. Although none of these components has yet been identified biochemically, one important feature of vacuolar protein targeting has become clear using this genetic approach. The requirement for acidification of the vacuole interior was initially suggested by the effects of NH_4^+ ions or inhibitors of the vacuolar H^+ -ATPase, which both dissipate the transmembrane pH gradient and cause mislocalization of vacuolar proteins. It has since been shown that some of the *vps* mutants are defective in vacuolar acidification but it is not clear whether the lack of targeting results in a loss of acidification or, conversely, the absence of acidification causes loss of targeting (Banta *et al.*, 1988; Rothman *et al.*, 1989b). Recent cloning of the rat microtubule-associated, force-producing GTPase, dynamin, shows a 45% sequence identity with Vps1p (Obar *et al.*, 1990), suggesting a possible role for cytoskeletal elements in protein sorting, perhaps by catalysing the movement of particular groups of vesicles between donor and acceptor membranes.

Early steps in ER targeting

The protein targeting events described above take place after the initial translocation into or across the membrane of the ER. The mechanism of transmembrane movement of proteins is of immense interest in this and other biological systems and has been studied both biochemically and genetically. Targeting of proteins to the ER of animal cells involves a large, cytoplasmic ribonucleoprotein, signal recognition particle (SRP), that binds nascent polypeptides with signal sequences as they emerge from the ribosome (reviewed by Rapoport, 1990). It is suggested that this interaction results in a slowing of translation, maintaining the protein in a translocation-competent state until it reaches the ER where binding to the SRP receptor (docking protein) initiates translocation. The maintenance of proteins in

an incompletely folded state is an apparently universal requirement for efficient translocation across biological membranes. The role of molecular chaperones in this process has been described in many systems (Deshaies *et al.*, 1988; Rothman, 1989). Most chaperones function in a manner that is not specific for transported proteins nor for any particular type of targeting signal. SRP behaves in many respects as a chaperone but with a clear specificity for proteins that bear a signal sequence. Mammalian SRP contains 7S L-RNA and six different polypeptides but no equivalent complex has yet been identified biochemically in yeast.

The original selection procedures for *sec* mutations failed to identify any that affected steps involved in initial targeting to the ER and translocation. Deshaies & Schekman (1987) devised a more specific selection for such mutants and this has allowed the identification of a few genes encoding ER membrane proteins. The products of the *SEC61*, *SEC62* and *SEC63* genes have recently been shown biochemically to assemble with other proteins as part of a multisubunit complex within the ER membrane (Deshaies *et al.*, 1991). Unfortunately there is no evidence as yet for a correlation between any of the biochemically identified components of the mammalian targeting pathway and the genetically defined components from yeast. Similarities are however suggested by the identification in yeasts (*S. cerevisiae* and *Sch. pombe*) of a homologue of the 54 kDa subunit of SRP (Hann *et al.*, 1989) and by preliminary evidence that the *SEC65* gene encodes a 73 kDa protein, a putative domain of which is a homologue of the 19 kDa SRP subunit (C.J. Stirling, personal communication).

A soluble ER protein (BiP, a member of the Hsp70 heat-shock family) has been shown to be essential for transport into the ER (Vogel *et al.*, 1990) and this protein may be analogous in function to the mitochondrial Hsp70 (see below) in binding to proteins as they emerge on the luminal face of the ER membrane. The importance of heat-shock proteins in protein translocating and folding reactions appears to be universal (Ellis & Hemmingsen, 1989).

Mitochondrial protein targeting

Saccharomyces cerevisiae has long been used for the investigation of mitochondrial biogenesis largely because of its ability to grow independently of oxidative phosphorylation, i.e. by fermentation. It has therefore been possible to isolate mutants with specific mitochondrial defects. Nevertheless much of our understanding of mitochondrial protein targeting is derived from biochemical analysis using yeast as a convenient source of material (reviewed by Pfanner & Neupert, 1990). As

with other protein targeting systems, little variation is found between species in the way that proteins are transported into mitochondria.

Unlike other organelles in yeast, the mitochondrion has its own protein synthesis system but this accounts for only a small fraction of all the polypeptides that mitochondria contain. Thus most mitochondrial proteins are transported from their site of synthesis in the cytosol. Most cytoplasmically synthesized mitochondrial proteins contain an N-terminal presequence that is required for targeting and is removed proteolytically within the organelle. These presequences are characterized by a lack of acidic amino acid residues and are rich in basic residues that are apparently arranged such that the presequence has been predicted to form an amphiphilic alpha helix (von Heijne, 1986). Mitochondrial targeting sequences must clearly be recognized as distinct from secretory signal sequences by components of the cytosol and the target membrane.

Recent work has resulted in the identification of several components of the mitochondrial protein transport pathway in both *S. cerevisiae* and *Neurospora crassa* (Baker & Schatz, 1991; Pfanner *et al.*, 1991). By raising antibodies against individual polypeptides from the mitochondrial outer membrane and testing the effects of these on the ability of cytoplasmically synthesized proteins to bind to the mitochondrial surface and to be transported across the mitochondrial membranes, Walter Neupert and his colleagues have shown that a 19 kDa polypeptide (MOM19) acts as a receptor for many imported mitochondrial proteins (Söllner *et al.*, 1989). A second receptor of 72 kDa (MOM72) is involved in the recognition and transport of other proteins. Very similar receptors are found in yeast mitochondria.

Jeff Schatz and colleagues have examined the transport of an artificial fusion protein containing a cleavable presequence at the N-terminus, the complete dihydrofolate reductase sequence and chemically attached bovine pancreatic trypsin inhibitor, a small, tightly folded protein with internal disulphide bonds. This hybrid protein begins to be transported across the mitochondrial membranes and undergoes N-terminal cleavage in the matrix. The C-terminal portion of this fusion protein remains folded under the experimental conditions used and cannot be translocated across the mitochondrial membranes. It thus becomes stuck at some point on the transport pathway, associated with translocation contact sites where the two membranes are in close proximity, and it was thought likely that the interaction between the protein undergoing translocation and one or more components of the transport pathway might be sufficiently stable to allow them to be chemically cross-linked. This procedure led to the identification of a

42 kDa polypeptide (ISP42) that is an integral component of the outer membrane but is not enriched within translocation contact sites (Vestweber *et al.*, 1989). Antibodies raised against ISP42 inhibit mitochondrial protein transport, indicating that this polypeptide has a genuine function in the transport pathway.

The gene encoding ISP42 has recently been cloned and sequenced, as has the homologous gene from *N. crassa*, encoding MOM38, a protein that has also been implicated in transport. Kiebler *et al.* (1990) isolated a complex of 400–600 kDa from *N. crassa* mitochondrial membranes that contained MOM38 in association with MOM19, MOM72 and another outer-membrane polypeptide, MOM22. A possible mechanism for transport of proteins across the mitochondrial membranes has been suggested by Neupert *et al.* (1990) whereby precursor polypeptides synthesized in the cytosol are recognized by receptors on the mitochondrial surface. Translocation is initiated by insertion of the N-terminal targeting peptide into the outer membrane and in response to the transmembrane electrical potential difference ($\Delta\psi$) generated by the respiratory chain the N-terminus crosses the inner membrane, resulting in unfolding of the membrane-spanning domain. By binding Hsp70 proteins or other chaperones in the cytoplasm, at least some precursor proteins are maintained in a translocation-competent state, i.e. they are prevented from misfolding. As polypeptides appear in the mitochondrial matrix they become associated, during translocation, with mitochondrial Hsp70 (Kang *et al.*, 1990; Scherer *et al.* 1990) and it is suggested that this interaction provides the driving force for transport subsequent to the translocation initiation event mediated by the targeting sequence (Fig. 3). Proteins are then passed from Hsp70 to Hsp60 in the mitochondrial matrix, which assists in correct folding. ATP hydrolysis is associated with release of polypeptides from each of these heat-shock proteins. It is suggested that this model may have relevance for the transport of proteins across other eukaryotic membranes, and heat-shock proteins are certainly involved in other transport pathways.

As with the secretory pathway, mitochondrial protein transport uses a hierarchical system of targeting signals that direct proteins to one of the two membranes or to the matrix or intermembrane space. Almost all mitochondrial proteins have an N-terminal sequence that is capable of directing 'passenger' proteins such as the normally cytosolic dihydrofolate reductase to the mitochondrial matrix (Hurt & van Loon, 1986). This targeting sequence thus specifies interaction with the MOM19 or MOM72 receptor and MOM38-dependent transport. Subsequent sorting information then determines localization to non-matrix compartments. Outer membrane localization is specified by a stop-transfer

Fig. 3. Translocation of proteins across the mitochondrial membranes. After binding to a receptor (hatched) in the outer membrane (OM), protein transport is initiated by $\Delta\psi$ -dependent translocation of the N-terminus through contact sites between the outer and inner membrane (IM). Proteins are prevented from adopting a stably-folded structure in the cytosol by interaction with Hsp70 proteins. Mitochondrial Hsp70 is required for translocation and it is suggested (Neupert *et al.*, 1990) that the binding of Hsp70 in the mitochondrial matrix provides the driving force for translocation of the bulk of the polypeptide chain.

sequence immediately adjacent to the matrix targeting sequence and targeting to the inner membrane generally depends on hydrophobic membrane insertion sequences. Soluble components of the yeast mitochondrial intermembrane space, such as flavocytochrome b_2 , cytochrome c peroxidase and an inner-membrane protein, cytochrome c_1 , that has its bulk exposed to the intermembrane space, undergo a rather more complex targeting pathway involving proteolytic removal of the matrix targeting signal within the mitochondrial matrix. Each of these three polypeptides contains secondary sorting information between the sites of this and a second cleavage. These sorting signals operate either as stop transfer sequences, allowing the extreme N-terminus but not the bulk of the protein to cross the inner membrane (Reid *et al.*, 1982) or, according to an alternative model, by directing export of the processed intermediate form out from the matrix to the intermembrane space where a second proteolytic cleavage releases the mature polypeptide. This latter model has been suggested to have evolved from the protein export system of the prokaryotic ancestors of mitochondria (Hartl & Neupert, 1990).

Targeting of proteins to nuclei, peroxisomes and other organelles

In this review I have focussed on the best-understood pathways of protein localization but I should stress that the eukaryotic cell must cope with other targeting systems which operate simultaneously. Proteins are

targeted to the nucleus by virtue of a nuclear targeting signal that consists of a short stretch of peptide sequence which is generally rich in basic amino acid residues (Silver, 1991). The nuclear targeting signal is present in the mature protein and this reflects a fundamental biological difference between targeting of proteins to the nucleus and other targeting pathways. In the other cases of transmembrane transport described here, newly-synthesized proteins are irreversibly removed from the cytosol. In many organisms the nuclear envelope breaks down into vesicles during mitosis with the result that soluble components of the nucleus are distributed around the cell but these are rapidly imported when the nuclear envelope is re-formed. Although the nuclear envelope in *S. cerevisiae* does not break down during mitosis, it has been shown that transport of the *SWI5* transcription factor into the nucleus is regulated in a cell-cycle-dependent manner (Nasmyth *et al.*, 1990), a considerable time after its synthesis. Proteins enter the nucleus through the nuclear pore complex and the role of pore components in recognition and transport is the subject of intense study (Silver, 1991). Again the yeast genetic system has allowed the isolation of mutants defective in targeting (Sadler *et al.*, 1989). The *npl1* mutation affects targeting to the nucleus and, less predictably, to the ER. In fact it has been shown that *npl1* is allelic with *sec63* (see above), a gene encoding a membrane protein that may not be directly involved in nuclear protein targeting but might, for example, be required for correct assembly of the nuclear pore complex. In this context it should be noted that the outer membrane of the nuclear envelope is contiguous with the ER membrane. Yeast mutants deficient in nuclear protein localization have been isolated with defects in at least five further genes but these have yet to be characterized at the molecular level and the function of their products is unknown.

Peroxisomes are vesicular organelles of the microbody family (along with glyoxysomes, glycosomes and other organelles) that contain several oxidative enzymes. Peroxisomes are found universally in eukaryotes but are particularly prolific in methylotrophic yeasts such as *Hansenula polymorpha*. When grown with methanol as sole carbon source these yeasts undergo a massive induction of methanol oxidase which forms crystalline arrays in enlarged peroxisomes. Proteins are transported into peroxisomes directly from the cytosol. Many of these proteins in higher eukaryotes have been found to contain a C-terminal tripeptide targeting signal, Ser-Lys-Leu or a closely related sequence (Gould *et al.*, 1989). Firefly luciferase, an insect peroxisomal protein, is efficiently targeted to peroxisomes when expressed in plants and in the yeasts *H. polymorpha* and *S. cerevisiae* (Gould *et al.*, 1990), indicating that targeting to peroxisomes has been well conserved throughout eukaryotic evolution.

Direct secretion of proteins from the yeast cytosol

Several proteins have been shown to undergo post-translational covalent attachment of lipids and these modifications can affect both localization and function (McIlhinney, 1990; Glomset *et al.*, 1990; Ferguson & Williams, 1988). For example, many cell surface proteins are attached to the plasma membrane by a glycosylphosphatidylinositol (GPI) tail that is attached in the ER, so GPI-modified proteins must also have secretory signal sequences. Some intracellular proteins, such as ras, are attached to membranes by other lipid modifications, notably prenylation, palmitoylation and myristoylation. In one case it has been shown that lipid modification is essential for secretion of a protein into the extracellular medium, the mating pheromone **a**-factor from *S. cerevisiae*.

Unlike the **a**-factor, which is secreted by the conventional secretory pathway described above, **a**-factor secretion is unaffected by *sec* mutations. After its synthesis in the cytosol **a**-factor undergoes attachment of a farnesyl group to a cysteine residue located at the C-terminus. Mutations that block prenyl biosynthesis (e.g. *hmg1*, *hmg2*) also block **a**-factor secretion, demonstrating that farnesylation is essential for transport. Mutations in another gene, *ste6*, also block export of **a**-factor and molecular analysis of the *STE6* gene demonstrates a striking resemblance to a family of transport ATPases (McGrath & Varshavsky, 1989) that includes the *E. coli* *HlyB* gene product that is required for the protein toxin haemolysin A. It appears that **a**-factor is secreted directly from the cytosol by a pathway that may be more widespread than generally assumed (Muesch *et al.*, 1990).

Conclusion

Of the few thousand polypeptides synthesized in the yeast cytosol, most are removed by a range of protein targeting pathways to various intracellular destinations or are secreted out of the cell. These targeting events demand specificity and proteins contain within their structures targeting signals that determine where they are to be localized. We are beginning to understand the biochemistry of some of the protein transport reactions and several components involved in catalysing these reactions have been identified. Nevertheless we have, as yet, little knowledge of the molecular details of the mechanisms of recognition of targeting sequences and of transmembrane translocation of proteins. Some targeting sequences consist of well-defined peptide sequences (e.g. HDEL) that might be recognized by typical

protein-protein interactions, analogous in some respects to the specificity of antibody-antigen interactions. On the other hand, secretory signal sequences and mitochondrial targeting sequences show remarkable variability and we can only assume at this stage that some element of three-dimensional structure is recognized in these cases.

S. cerevisiae has been and continues to be a particularly useful organism for the investigation of eukaryotic protein targeting pathways, particularly because of the possibilities for genetic analysis and genetic manipulation. It should be stressed, however, that protein transport pathways have a long evolutionary history and are of obvious importance to cell function, and it is therefore not surprising that they are well conserved amongst widely divergent groups of eukaryotes. For example, yeast mitochondrial proteins can be transported into animal mitochondria and vice versa, human secretory proteins can be expressed in and secreted by yeast and so on. The information gleaned from studies of the targeting pathways in yeast thus presents us with a useful model for the analogous pathways in 'higher' eukaryotes. Furthermore, our understanding of the molecular biology of protein targeting in yeast has enabled these pathways to be manipulated and exploited, most obviously by directing the secretion of heterologous proteins.

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