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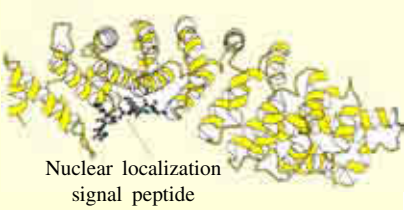

Protein targeting signal recognition
The structure of the nuclear localization signal-binding protein α-karyopherin (or α-importin) with a nuclear localization signal peptide bound to its major recognition site.

CHAPTER 29

Protein Targeting and Degradation

GENERAL CONSIDERATIONS

Protein turnover – that is synthesis and degradation – occurs constantly in eukaryotic cells but it is a highly selective process with different rates of turnover for various proteins. Turnover of proteins can control the level of certain enzymes, furnish amino acids in times of need and degrade faulty or damaged proteins that are generated during synthesis or arise from deleterious activities in the cell. Nascent proteins contain signals that determine their ultimate destination. A newly synthesized protein in the prokaryotic *Escherichia coli* cell, for example, can stay in the cytosol or it can be sent to the plasma membrane, the outer membrane, the space between them, or the extracellular medium.

The eukaryotic cell is made up of many structures, compartments and organelles, each with specific functions requiring different types of proteins and enzymes. The synthesis of most of these proteins begins on free ribosomes in the cytosol. Therefore, eukaryotic cells must direct proteins to internal sites such as lysosomes, mitochondria, chloroplasts, nucleus etc. How then is sorting accomplished? In eukaryotes, a key choice is made soon after the synthesis of a protein begins. A ribosome remains free in the cytosol unless it is directed to the

endoplasmic reticulum (ER) by a signal sequence in the protein being synthesized. Nascent polypeptide chains formed by ribosomes are translocated across the ER membrane. In the lumen of the ER, many of them are glycosylated and modified in other ways. These are then transported to Golgi complex where they are further modified. Finally, they are sorted for delivery to lysosomes, secretory vesicles, and the plasma membrane. Transported proteins are carried by vesicles that bulge out from donor compartments and fuse with target compartments. *The signals used to target eukaryotic proteins for transfer across the ER membrane are ancient*, for bacteria also use similar sequences or signals for sending proteins to their plasma membrane and to secrete them.

The transported proteins must reach their assigned cellular locations. This is of utmost importance because mistakes in transport can severely affect cellular metabolism and the cumulative effect may prove fatal to an organism. For instance, *I-cell disease*, a rare human disease, is characterized by export from the cell of at least 8 enzymes which should be transported to lysosomes.

FREE AND MEMBRANE-BOUND RIBOSOMES

In eukaryotic cells, one may distinguish free ribosomes in the cytosol and ribosomes bound to the membranes of the endoplasmic reticulum (ER). The **cytosolic ribosomes** are responsible for the a synthesis of the proteins which will be released in the cytosol, whereas the **membrane-bound ribosomes** (Fig 29–1) synthesize 3 major classes of proteins: secretory proteins (which are secreted outside the cell), lysosomal proteins and proteins spanning the plasma membrane. *ER amounts to about half of the total membrane of a cell*. The region of ER that binds ribosomes is called the **rough ER** because of its beaded appearance in comparison to the **smooth ER,** which is devoid of ribosomes. Virtually all integral membrane proteins of the cell, barring those located in the mitochondrial and chloroplast membranes, are formed by ribosomes bound to the ER. George Palade's (1975–Nobel Laureate) pioneering studies on the mechanism of secretion of zymogens by the pancreatic acinar cells opened a new field of enquiry – protein targeting – and delineated the pathway taken by the secretory proteins.

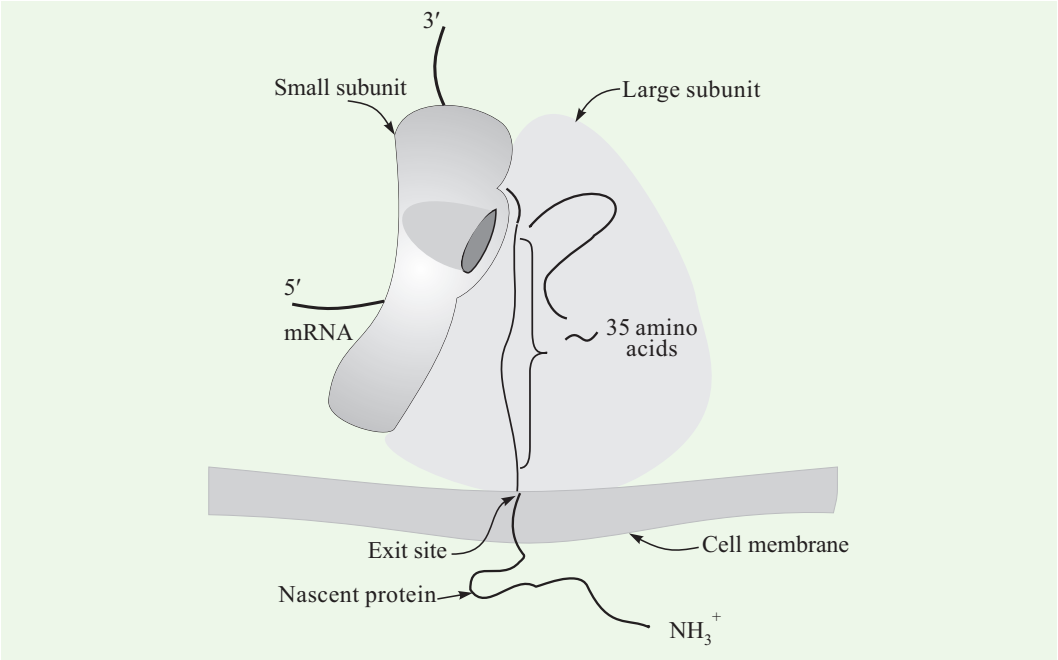

Fig. 29–1. A membrane-bound ribosome

(*After James A. Lake, 1981*)

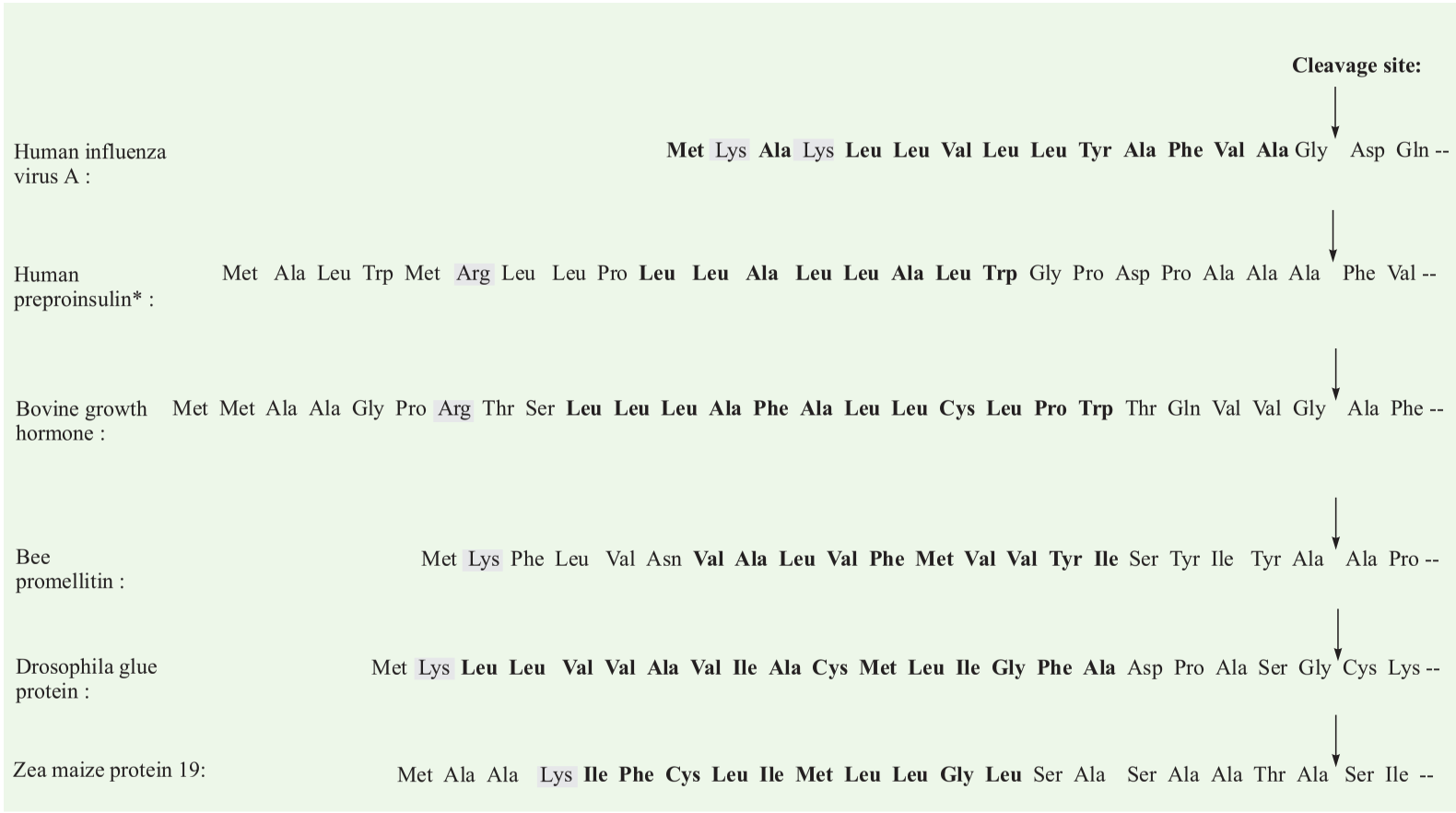

Fig. 29–2. Amino terminal signal sequences of some eukaryotic proteins (secretory and plasma membrane), directing translocation into the endoplasmic reticulum

The hydrophic core (in boldface) is preceded by one or more basic residues (shaded). Note the presence of polar and short-side-chain residues immediately preceding the cleavage sites (indicated by arrows).

* Insulin is synthesized as preproinsulin and the prefix pre refers to the 24-residue signal sequence, preceding the cleavage site. Note that leaving Arg, Gly and Asp, the remaining 21 residues have hydrophobic side chains (a higher percentage than is found in most signal sequences).

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Studies conducted on the protein-synthesizing activities of ribosomes in cell-free systems confirmed that *the membrane-bound ribosomes and free cytosolic ribosomes are intrinsically identical*. In fact, free ribosomes from the cytosol were isolated and then added to rough ER membranes that had been stripped of their ribosomes. This reconstituted system actively synthesized secretory proteins when supplied with the proper mRNAs and other soluble factors. Similarly, ribosomes isolated from the rough ER were fully active in synthesizing proteins that are normally freed into the cystosol. Whether a particular ribosome is free or attached to the rough ER depends only on the kind of protein it is synthesizing.

SIGNAL HYPOTHESIS

The pathways by which proteins are sorted and transported to their proper cellular locations are referred to as *protein targeting pathways*. A characteristic feature of these targeting pathways (with the exception of cytosolic and nuclear proteins) is the presence of a short amino acid sequence at the amino terminus of a newly synthesized polypeptide called the **signal sequence** or **signal peptide**. In many cases, the targeting capacity of particular signal sequences has been confirmed by fusing the signal sequence from one protein, say protein A, to a different protein B, and showing that the signal directs protein B to the location where protein A is normally found. The signal sequence, whose function was first postulated by David Sabatini and Günter Blobel (1970), directs a protein to its proper location in the cell and is removed by a *signal peptidase* during transport or when the protein reaches its final destination. Obviously, the signal sequence is absent in the protein once secreted.

At present, the signal sequences of more than 100 secretory proteins from a wide variety of eukaryotic species have been determined; some of which have been presented in Fig. 29–2. A well-defined consensus sequence such as the TATA box guiding the initiation of transcription, is not evident. However, signal sequences do exhibit certain common characteristics;

1. They range in length from 13 to 36 amino acid residues.
2. The amino terminal part of the signal contains at least one or more positively charged amino acid residues, preceding the hydrophobic sequence.
3. A sequence of highly hydrophobic amino acids (10 to 15 residues long) forms the centre of the signal sequence. Ala, Val Leu, Ile and Phe residues are common in this region.
4. There is present a region of more polar short sequence (of about 5 residues) at the carboxyl terminus, upstream the cleavage site. The amino acid residues having short side chains (*esp*, Ala) predominate in this region at positions closest to the cleavage site.

However, in certain secretory and plasma membrane proteins, the signal sequence is not situated at the amino terminus. These proteins contain an **internal signal sequence** that serves the same role. For example, in the case of ovalbumin, the sequence is located between residues 22 and 41 and is critical for the transfer of nascent albumin across the ER membrane.

In 1975, George Palade, at the Rockfeller Institute in New York, demonstrated that proteins with these signal sequences are synthesized on ribosomes attached to the ER membrane. The overall pathway, summarized in Fig. 29–3, proceeds in following 8 steps:

1. First of all, the ribosomal subunits assemble in an initiation complex at the initiation codon and begin protein synthesis.
2. Later, a proper signal sequence appears early in the synthetic process because it is at the amino terminus of the nascent polypeptide.
3. Then, this signal sequence and the ribosome itself are rapidly bound by a large rod-shaped complex called **signal recognition particle (SRP).** This binding event halts elongation and the signal sequence has completely emerged from the ribosome. The SRP receptor is

a heterodimer of α (M*r* 69,000) and β (M*r* 30,000) subunits and consists of a 305-nucleotide RNA (called 7 SL-RNA) and 6 different proteins, with a combined molecular weight of 3,25,000. One protein subunits binds directly to the signal sequence, inhibiting elongation by sterically blocking entry of aminoacyl-tRNAs and inhibiting peptidyl transferase.

4. The ribosome-SRP complex with the incomplete polypeptide is bound by two receptors (ribosome receptor and SRP receptor) present on the cytosolic face of the ER. For transport of a polypeptide into the ER lumen, the signal sequence attaches to the **SRP receptor**. The hydrophobicity of the signal sequence is postulated to be the molecular key for the polypeptide's interaction with the ER membrane, which is also a hydrophobic structure. The second recognition site, **ribosome receptor**, serves to anchor the organelle (ribosome) to the ER membrane. The interaction between the signal sequence and the ER membrane is believed to open a channel in the membrane through which the polypeptide is transported into the ER lumen. Thus, the molecular instructions for transport into the ER (in the form of a hydrophobic sequence) are furnished by the polypeptide.
5. The SRP dissociates and is recycled.
6. Protein synthesis then resumes, along with translocation of the polypeptide chain into the lumen of the ER. The nascent polypeptide is delivered to a **peptide translocation complex** in the ER. The translocation complex feeds the growing polypeptide chain into the lumen of the ER in a reaction driven by the energy of ATP.
7. The signal sequence is cleaved by a membrane enzyme, **signal peptidase** which is located on the lumenal side of the ER.

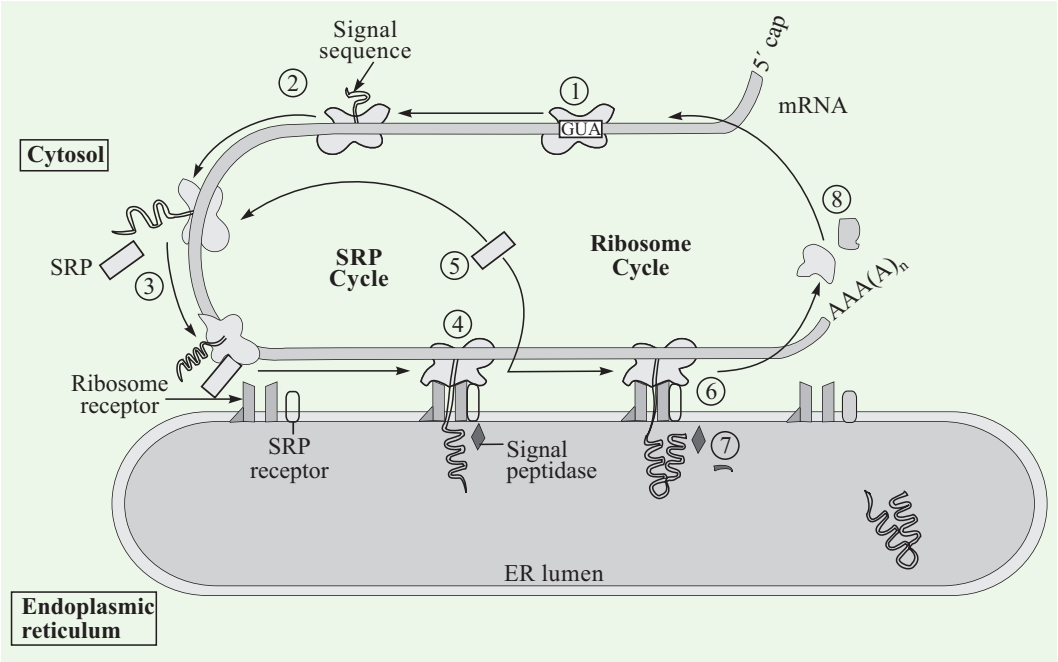


Fig. 29–3. The SRP cycle and nascent polypeptide translocation and cleavage

The signal sequence of a nascent polypeptide chain is recognized by SRP. The complex consisting of SRP, the nascent peptide chain, and the ribosome binds to the SRP receptor in the ER membrane. The ribosome is then transferred to the translocation machinery, which actively threads the polypeptide chain across the ER membrane. SRP released from its receptor is free to bind another emerging signal sequence. The steps conform to the description in the text.

(*Adapted from Lehninger AL, Nelson DL and Cox MM, 1993*)

8. Once the complete protein has been synthesized, the ribosome dissociates from the ER and is recycled.

The proteins to be secreted and the lysosomal proteins completely pass through the membrane of the ER. On the contrary, other proteins must form part of a membrane. Such proteins, in the lumen of the ER, are modified in several ways. Besides the removal of signal sequences, polypeptide chains fold and disulfide bonds form. Many proteins are also glycosylated.

As a result of about 20 years of strenuous work, Günter Blobel formulated in 1980 general principles for the sorting and targeting of proteins to particular cell compartments. Each protein carries in its structure the information needed to specify its proper location in the cell. Specific amino acid sequences (**topogenic signals**) determine whether a protein will pass through a membrane into a particular organelle, become integrated into the membrane, or be exported out of the cell. In essence, the **signal hypothesis** may be summarized below :

Proteins which are to be exported out of the cell are synthesized by ribosomes, associated with the ER. The genetic information from DNA is transferred *via* RNA. This information determines how the amino acids build up the proteins. First, a signal peptide is formed as a part of the protein. With the help of binding proteins, the signal peptide directs the ribosome to a channel in the ER. The growing protein chain penetrates the channel, the signal peptide is cleaved and the completed protein is released into the lumen of ER. The protein is subsequently transported out of the cell.

Infact, the signal hypothesis explains how new polypeptides scheduled for intracellular transport are routed into the ER lumen. The signal hypothesis was originally proposed for the transport of secretory proteins. But it is also applicable to storage proteins. An important feature of this hypothesis is that the membrane transport of the protein depends on the simultaneous protein synthesis by the membrane-bound ribosomes, thus causing the polypeptide to migrate through the tunnel in the endoplasmic reticular membrane. Thus, it may be emphasized that the signal hypothesis is both correct and universal, since the various processes associated with it operate in the same way in yeast, plant and animal cells.

GÜNTER BLOBEL

Blobel, a cell and molecular biologist at the Rockfeller Institute in New York fetched him **1999's Nobel Prize for physiology or Medicine** for his work proving that the signal sequences in the form of a chain of amino acids present either as a short 'tail' at one end of the protein, guide the proteins to their correct destination. In fact, at the end of the 1960s, Blobel joined the famed Cell Biology Laboratory of George Palade (himself, Nobel Laureate, 1975) at the Rockfeller Institute. Here he formulated the first version of the **signal hypothesis.** He postulated that a short sequence of amino acids attached to the end of a newly-synthesized protein could serve as an address label to enable them to pass through the membrane of the cellular organelle in which they are synthesized. He also opined that the protein traverses the membrane of the ER through a channel. In collaboration with other research groups, Blobel also showed that similar intrinsic signals target the transport of proteins also to other intracellular organelles.

Blobel's discovery has had an immense impact on modern cell biological research. It has helped explain the molecular mechanisms behind several genetic diseases. If a sorting signal in a protein is changed, the protein could end up in a wrong location in the cell. One example is the hereditary disease called *primary hyperoxaluria*, which causes kidney stones at the early age. In some forms of *familial hypercholesterolemia*, a very high level of cholesterol in the blood is due to deficient transport signals. Other hereditary diseases, *e.g.*, *cystic fibrosis* are caused by the fact that proteins do not reach their proper destination.

Blobel's discovery will also have a profound effect in the field of drug manufacture. With efforts being on to map the entire human genome, it will be easy to deduce the structure and topogenic signals of the proteins. This knowledge will increase our understanding of processes leading to disease and can be used to develop new therapeutic strategies.

GLYCOSYLATION OF PROTEINS AT THE LEVEL OF ER

While the individual polypeptides are in the ER lumen, the biochemical processes start for their cellular distribution. Whereas hydrophobicity provides the first molecular instructions for intracellular transport, it is **glycosylation** (*i.e.*, addition of carbohydrates) that establishes the molecular patterns acquired by polypeptides to continue their intracellular routing (Armstrong, 1989). The addition of oligosaccharide units, which convert polypeptides into glycoproteins, commences in the ER lumen and continues when they are transported from the ER to the Golgi apparatus. The particular oligosaccharide unit, attached to a glycoprotein, furnishes the molecular instructions for its cellular destination. Acquisition of oligosaccharide units by polypeptides may be compared to the assignment of Pin Codes to mailing addresses with each type of oligosaccharide, representing a distinct Pin Code.

Glycosylation begins soon after a nascent polypeptide enters the ER lumen. Carbohydrates bind to either the amide group of an asparagine (Asn) or the hydroxyl group of a serine (Ser) or threonine (Thr). Oligosaccharides attached to asparaginyl residues are referred to as **N-linked** and those to seryl or threonyl residues as **O-linked.** The following discussion will make it amply clear that, in the case of N-linked glycosylation, the molecular instructions dictating which oligosaccharide unit a protein will attain reside in the sequence and composition of the protein. For example, which asparaginyl residues will be glycosylated and which of the diverse oligosaccharides it will bear.

A. Core Glycosylation

Carbohydrate processing in the ER is called **core glycosylation** to distinguish it from *terminal glycosylation* (described in the subsequent Section), which takes place in the Golgi complex. In the ER lumen, an N-linked oligosaccharide is not added to a polypeptide by a series of one-

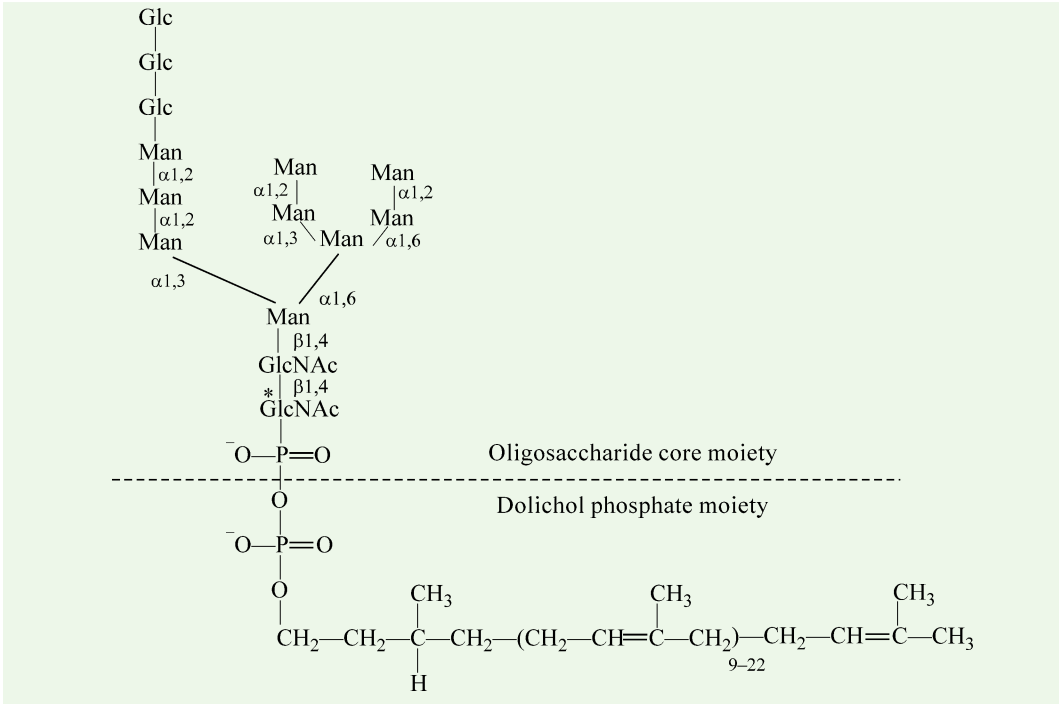


Fig. 29–4. Activated oligosaccharide core

Dolichol phosphate is a highly hydrophobic lipid carrier, whose terminal phosphate group is the site of attachment of the activated oligosaccharide. Note that the first carbohydrate, *N*-acetylglucosamine, GlcNAc (indicated by an asterisk) is added to the dolichol phosphate moiety as a phosphorylated derivative.

carbohydrate addition, but instead as an intact unit, called the *common oligosaccharide* core (Fig. 29–4), consisting of 14 residues (2 N-acetylglucosamine + 9 mannose + 3 glucose residues.) However, this oligosaccharide core is constructed by the successive addition of single monosaccharide units to dolichol phosphate (Fig 29–4). Dolichol is an unusually long-chain lipid, containing from 9 to 22 isoprene units. Phosphorylation of dolichol at the nonolefinic end produces dolichol phosphate. Dolichol phosphate is used to carry activated sugars in the membrane-associated synthesis of glycoproteins and some polysaccharides.

When the oligosaccharide core is completely synthesized on dolichol phosphate moiety, the whole structure is now called the *activated* oligosaccharide core (Fig. 29–4). The synthesis of activated oligosaccharide core and its transfer to the protein in the ER is depicted in Fig. 29–5. Once this oligosaccharide core is completely synthesized, it is enzymatically transferred en bloc from dolichol phosphate to a specific asparagine residue of the growing polypeptide chain. The enzyme *transferase* is located on the lumenal face of the ER and thus does not catalyze glycosylation of cytosolic proteins. An asparagine residue can accept the oligosaccharide only if it is a part of

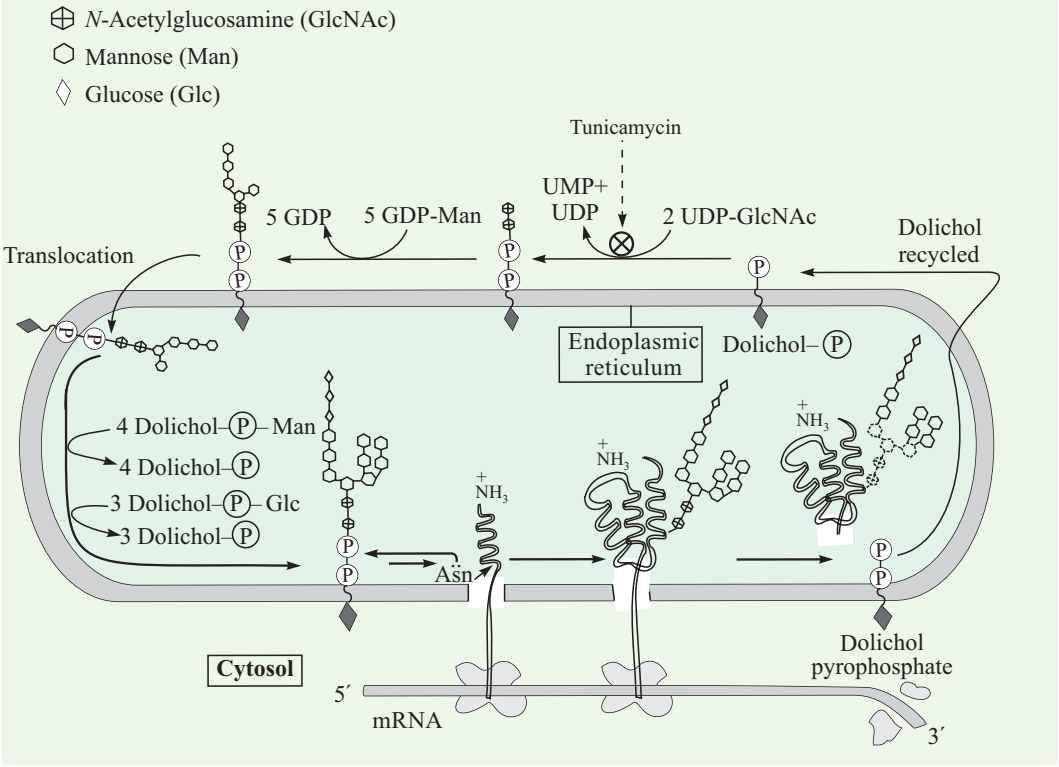


Fig. 29–5. Synthesis of the oligosaccharide core of glycoproteins and its transfer to the protein in the endoplasmic reticulum

The oligosaccharide core is built up in a series of steps as shown. The first few steps occur on the cytosolic face of the ER. Completion occurs within the lumen of the ER after a translocation step (upper left) in which the incomplete oligosaccharide is moved across the membrane. The synthetic precursors that provide additional mannose and glucose residues to the growing oligosaccharide in the ER lumen are themselves dolichol phosphate derivatives. Dolichol-phosphate-mannose and dolichol-phosphate-glucose are synthesized from dolichol phosphate and GDP-mannose or UDP-glucose, respectively. After it is transferred to the protein, the oligosaccharide core is further modified in the ER and the Golgi complex in pathways that differ for different proteins. The released dolichol pyrophosphate is recycled. The 5-sugar residues (shown in a dotted enclosure on lower right side) are retained in the final structure of all N-linked oligosaccharides.

(*Adapted from Lehninger AL, Nelson DL and Cox MM, 1993*)

an Asn-X-Ser or Asn-X-Thr sequence, and if it is sterically accessible to the transferase. Dolichol pyrophosphate, released in the transfer of the oligosaccharide to the protein, is recycled to dolichol phosphate by the action of a *phosphatase*. After the transfer, the oligosaccharide core is trimmed (*i.e.*, carbohydrates removed) in the ER but all linked oligosaccharides retain a pentasaccharide core derived from the original 14-residue oligosaccharide. Trimming continues when the polypeptides are transferred to the Golgi apparatus. For some polypeptides, trimming produces the required oligosaccharide units; for others, trimming and subsequent addition of new carbohydrates are needed for these polypeptides to acquire their characteristic glycosylated patterns. It is in the Golgi apparatus that most of the final trimming and additions take place (called terminal glycosylation).

Several antibiotics interfere with one or more steps in the core glycosylation process. The best-characterized is **tunicamycin**, which blocks the first step (*i.e.*, addition of *N*-acetylglucosamine to dolichol phosphate). Tunicamycin (Fig. 29–6) is a hydrophobic analogue of UDP-*N*-acetylglucosamine which blocks the fixation of N-acetylglucosamine on dolichol phosphate, and therefore prevents the glycosylation of proteins. Tunicamycin, thus, mimics UDP-*N*-acetylglucosamine. Another antibiotic, **bacitracin** blocks the hydrolysis of dolichol pyrophosphate to dolichol phosphate by a phosphatase.

B. Terminal Glycosylation

Proteins are transported from the ER to the Golgi complex in transport vesicles (Fig. 29–7). Golgi complex (Fig. 29–7) is an asymmetric stack of flattened membranous sacs called **cisternae**. A typical mammalian cell has 3 or 4 cisternae, whereas many plant cells usually have about twenty. The

The Golgi complex or Golgi apparatus is named for **Camillo Golgi** (1906-Nobel Laureate) who first detected the vesicles in 1898 by staining brain cells of a barn owl with silver salts.

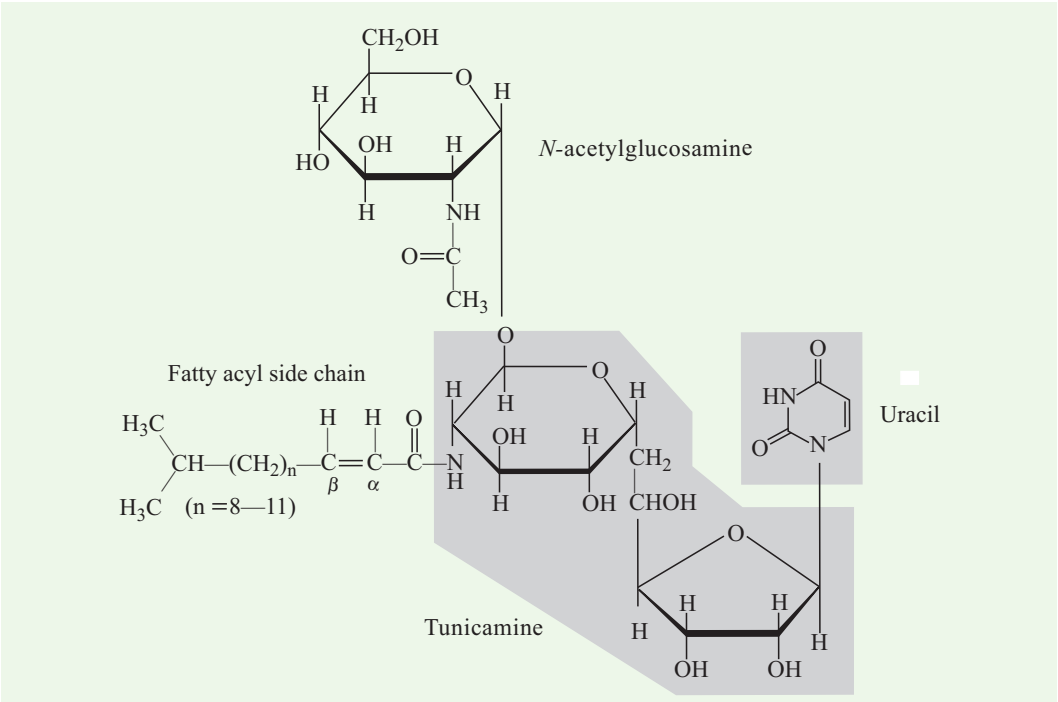


Fig. 29–6. Structure of tunicamycin

Tunicamycin is actually a family of antibiotics produced by (and isolated as a mixture from) *Streptomyces lysosuperficens*. They all contain uracil-*N*-acetylglucosmine, an 11-carbon aminodialdose called tunicamine and a fatty acyl side chain. The structure of the fatty acyl side chain varies in the different compounds within the family. In addition to the variation in length of the fatty acyl side chain, some homologues lack the isopropyl group at the end and/or α, β-unsaturation.

Golgi (*pronounced* as GOAL-gee) is differentiated into (1) a *cis* compartment, the receiving end, which is closed to the ER; (2) *medial* compartments; and (3) a *trans* compartment, which exports proteins to various destinations. These compartments contain different enzymes and carry out distinctive functions. Different vesicles transfer proteins from one Golgi compartment to another and then to lysosomes, secretory vesicles, and the plasma membrane. The transport of proteins between the ER and Golgi, and between the Golgi and subsequent destinations is mediated by small (~ 50 to 100 nm in diameter) membrane-bound compartments called *transport vesicles* (or *transfer vesicles*). The Golgi complex performs two main roles. First, carbohydrate units of glycoproteins are altered and elaborated in the Golgi. O-linked sugar units are trimmed there, and N-linked ones are modified in many different ways. Second, the Golgi is the major sorting and packaging center of the cell. It sends proteins to lysosomes, secretory granules, or the plasma membrane according to signals encoded by their 3-dimensional structures (Fig. 29–7).

The carbohydrate moieties of glycoproteins are modified in each of the compartments of the Golgi complex (Fig. 29–8). In the *cis* compartment, 3 mannoses are removed from the oligosaccharide chains of proteins destined for secretion or for insertion in the plasma membrane. The carbohydrate moieties of glycoproteins targeted to the lysosomal lumen are modified differently (described later). In the medial compartments, 2 or more mannoses are removed, and 2 *N*-acetylglucosamines and a fucose are added. Finally, in the *trans* compartment, another N-acetylglucosamine is added, followed by galactose and sialic acid, to form a complex oligosaccharide unit.

Although the biochemical mechanisms involved in "sorting and packaging" is not fully understood, however, with respect to *N*-linked oligosaccharides, a unified concept about the types of units attached is developing. As a rule, *N*-linked oligosaccharides have the same *inner core* which is the branched pentasaccharide

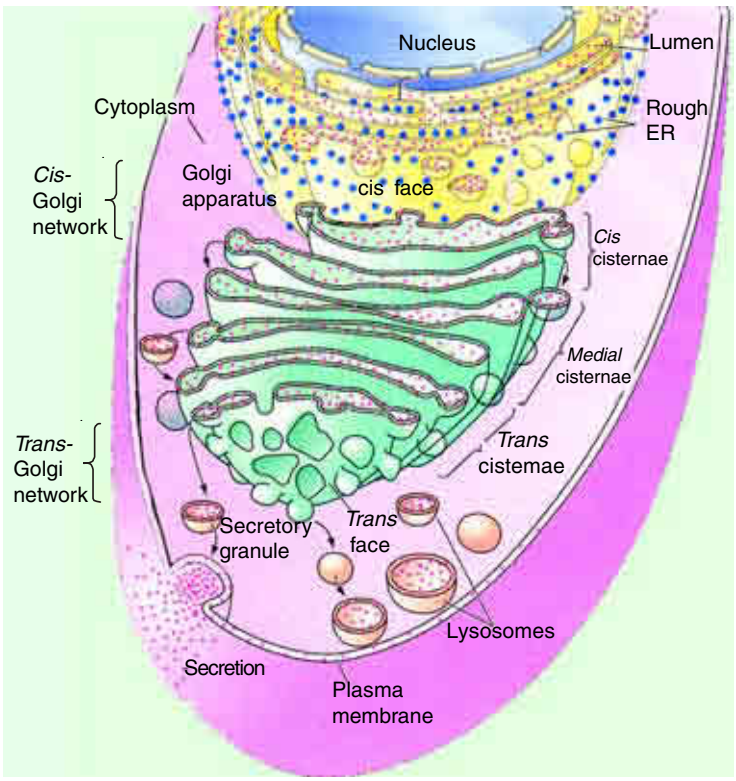


Fig. 29–7. Different pathways adopted by proteins destined for lysosomes, the plasma membrane or secretion

Most proteins destined for secretion of insertion into a membrane are synthesized by ribosomes (*blue dots*) attached to the rough endoplasmic reticulum (rough ER; *top*). As they are synthesized, the proteins (*red dots*) are either injected into the lumen of the endoplasmic reticulum or inserted into its membrane. After initial processing, the proteins are encapsulated in vesicles formed from endoplasmic reticulum membrane, which subsequently fuse with the *cis* Golgi network. The proteins are progressively processed according to their cellular destinations, in the *cis*, *medial,* and *trans* cisternae of the Golgi, between which they are transported by other membranous vesicles, Finally, in the *trans* Golgi network (*bottom*), the completed glycoproteins are sorted for delivery to the final destinations, for example, lysosomes, the plasma membrane, or secretory granules, to which they are transported by yet other vesicles.

containing 3 mannose and 2 acetylglucosamine (Fig. 29–9). Apparently, trimming of the common oligosaccharide core can proceed to the level of the inner core.The *N*-linked oligosaccharides generally fall into one of the following two categories;

(*a*) **Simple mannose-rich units:** These possess the inner core either with short or long mannose oligosaccharides attached (chicken albumin) or with one or few carbohydrates attached (human immunoglobulin M, IgM).

(*b*) **Complex N-acetyllactosamine units:** These are oligosaccharides with *N*-acetylgalactosamine (disaccharide unit of galactose and *N*-acetylglucosamine) linked to the mannosyl residues of the inner core, since they generally have additional sialate (NAN) residues bonded to their galactosyl residues. The two common examples are the oligosaccharide units of human transferrin and immunoglobulin G, IgG, which, unlike the simple units, have been found only in animals.

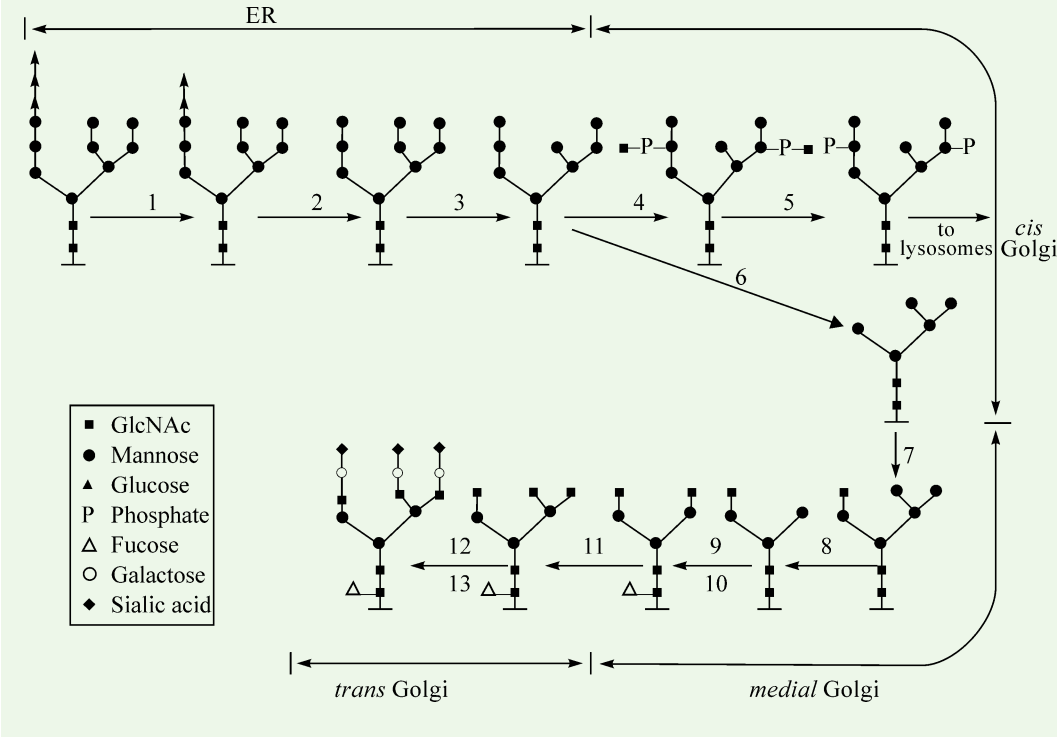


Fig. 29–8. Processing of asparagine-linked (or N-linked) oligosaccharides in the ER and the 3 compartments of the Golgi complex

Steps 4 and 5 apply only to proteins destined for delivery to lysosomes. The enzymes catalyzing all the 13 steps are:

1. glucosidase I
2. glucosidase II
3. ER -1, 2-mannosidase
4. N-acetylglucosaminyl-phosphotransferase
5. phosphodiester glycosidase
6. Golgi mannosidase I
7. GlcNAc transferase I
8. mannosidase II
9. GlcNAc transferase II
10. fucosyl transferase
11. GlcNAc transferase IV
12. galactosyltransferase
13. sialyltransferase

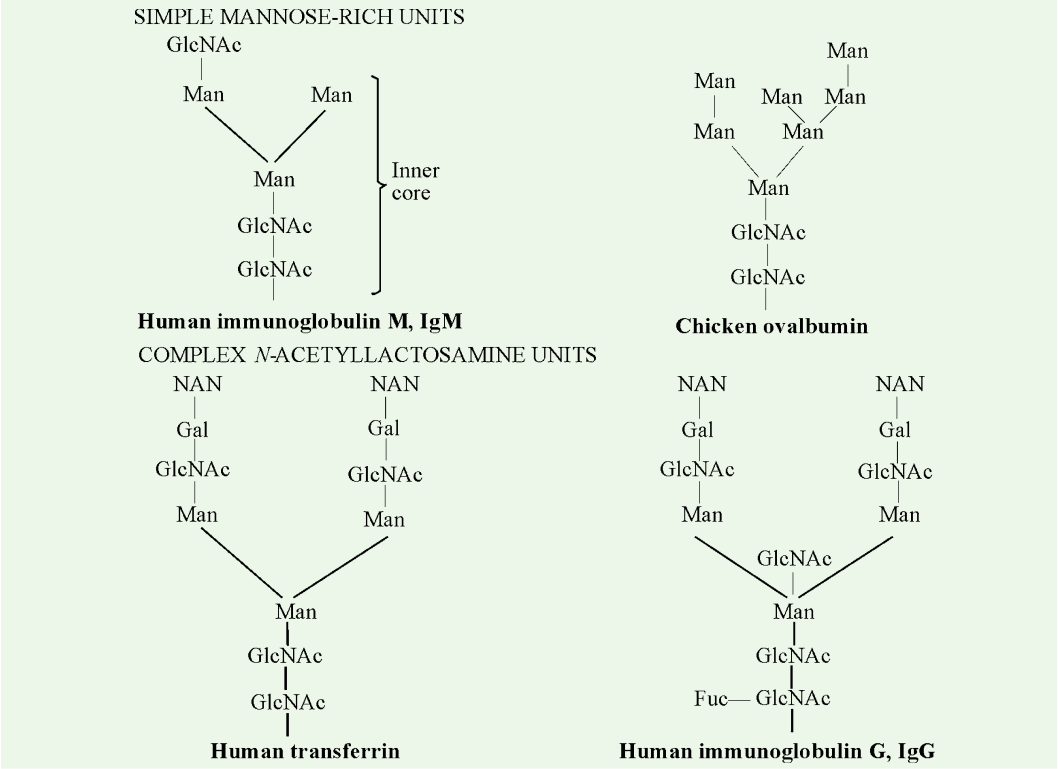


Fig. 29–9. Four oligosaccharide units of glycoproteins

Note that the two examples of complex units have 3 carbohydrates (galactose, sialate, fucose), not found in the common core.

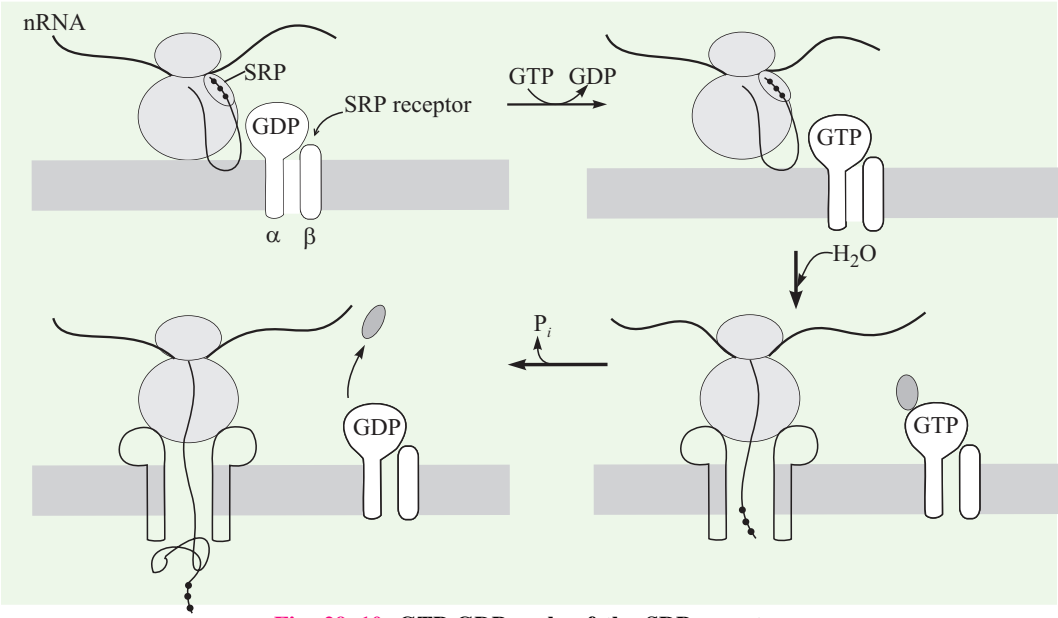


Fig. 29–10. GTP-GDP cycle of the SRP receptor

The cycle drives the delivery of the signal sequence to the translocation machinery of the ER membrane. (*Adapted from Rapoport TA, 1992*)

GTP-GDP Cycle and the Signal Sequence

The signal sequence on the nascent polypeptide is protected rather sequestered by SRP until it is delivered to the translocation machinery on the ER membrane. The exact timing of the release of the polypeptide by SRP is achieved by a GTP-GDP cycle in the SRP receptor, which is an integral membrane protein consisting of two submits, α (68 kd) and β (30 kd). The binding of SRP-signal peptide to the receptor triggers the replacement of GDP (bound to the α subunit) by GTP (Fig. 29–10). The GTP form of the receptor firmly binds SRP, which loses its grip on the signal peptide. The released signal peptide quickly binds to the *translocon*, a multisubunit assembly of integral and peripheral membrane proteins, which act as translocation machinery. The α subunit of the receptor then hydrolyzes its bound GTP to GDP, which releases SRP. The delay in GTP hydrolysis gives the signal peptide enough time to find its new partner so that the signal peptide is not recaptured by SRP. *Ribosomes bearing signal sequences are targeted to the ER membrane because of the unidirectionality of the GTP-GDP cycle.*

Although elongation of a polypeptide and its translocation across the ER membrane are two separate processes, yet they do occur simultaneously. This is because the synthesized proteins become folded and cannot be efficiently translocated as they do not fit in the protein conducting channel. *Unfolded polypeptide chains are the optimal substrates for translocation across the ER membrane*. Also, binding of SRP to ribosomes arrests elongation so that the premature folding of the nascent chain is prevented. Moreover, the ribosomes keep the nascent polypeptide chain fully stretched out in the narrow tunnel of the large subunit.

The translocation process for integral membrane proteins is more complex than for secretory and lysosomal proteins which are threaded through in entirety. The integral membrane proteins have either one or many membrane-spanning helices (Fig. 29–11). Moreover, the amino and carboxy termini can be on either side of the membrane in such proteins. The translocation machinery acts restlessly unless stopped by a specific instruction, which in this case is a **stop transfer sequence** (also called **a membrane anchor sequence**) present on the nascent polypeptide chain. A second signal sequence is also required to start another round of translocation of a chain that spans the membrane more than once. Furthermore, the translocation machinery must be able to thread the nascent chains in the reverse direction also. All this is yet unexplored and needs investigation.

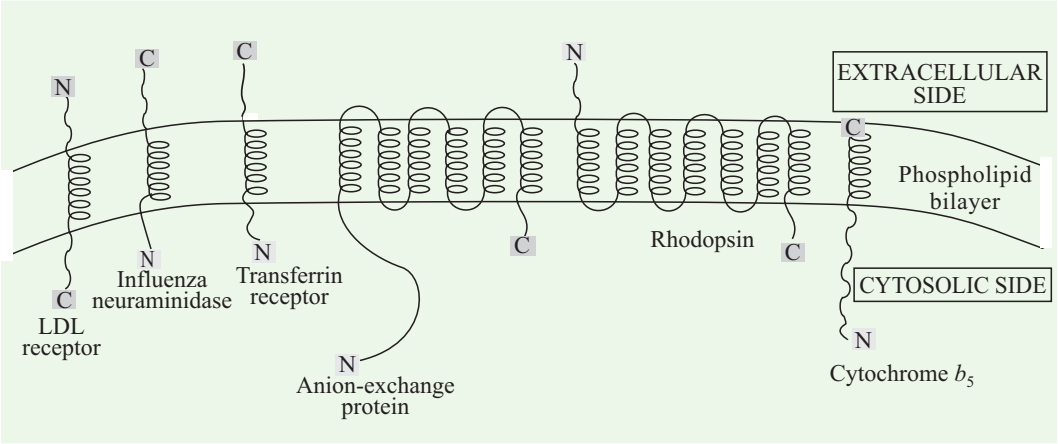


Fig. 29–11. Different topological arrangements of integral membrane proteins

(*Adapted from Wickner WT and Lodish HF, 1985*)

Chaperones and the Nascent Protein Folding

The newly-synthesized polypeptide chains in the ER do not immediately fold. Rather, they bind to some specific proteins called **polypeptide chain binding proteins** or **chaperone proteins** that keep the nascent polypeptides unfolded for minutes. In the absence of chaperons, the nascent proteins would become hopelessly entangled. The chaperons assist folding of the polypeptide chain by preventing nonspecific aggregation of weak-bonding side chains. The chaperon proteins were originally identified as members of "heat-shock" protein (hsp) family because they are induced in many cells when heat stress is applied and apparently help stabilize other proteins.

> Also spelt as *chaperon*. The word **chaperone** [Fr < OFr, head, covering, hood (hence protection, protector) < chape : sea chape] means a person, especially an older or married woman who accompanies young unmarried ladies in public, or is present at their parties, dances etc to supervise their behaviour. This refers to the function which these protein molecules perform with regard to the folding of the polypeptide chain.

When does a chaperone releases its bound nascent chain? Dissociation of chaperons from polypeptides is often coupled to ATP hydrolysis. In fact, *chaperone proteins are slow ATPases*. The ADP-chaperone complex (Fig. 29–12) has high affinity for unfolded peptides but not for the folded (or native) proteins. The binding of unfolded peptide to the chaperone induces the release of ADP and the entry of ATP into the chaperone's active site. The ATP-chaperone complex releases the peptide portion. The subsequent hydrolysis of bound ATP enables the chaperone to again bind an unfolded peptide. Thus, the interval between release and binding of the unfolded peptide chain is determined by the hydrolytic rate. In essence, chaperones buy time before proper folding proceeds.

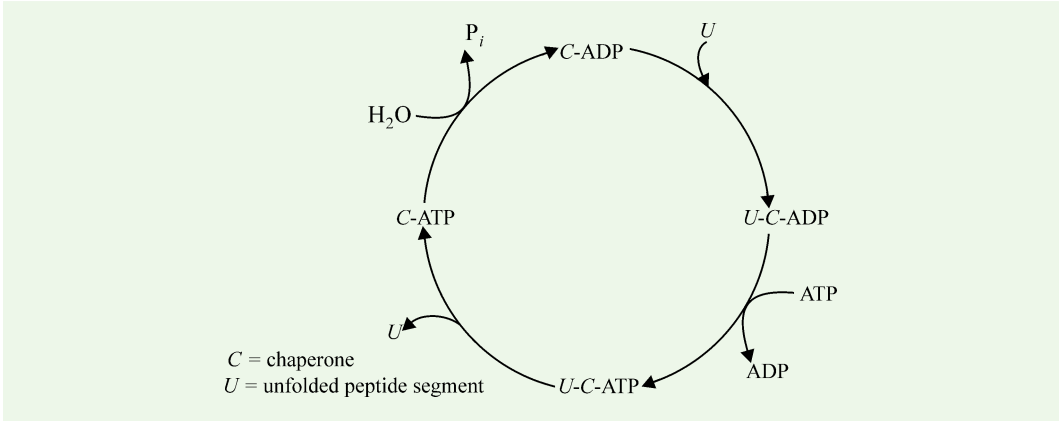


Fig. 29–12. Mechanism of chaperone action

Note that the chaperone action is driven by ATP.

The major chaperone in the lumen of the ER is BiP (*binding protein*), a 78-kd member of hsp family. Besides heat, other stresses such as free-radical damage, also induce all the prokaryotic and eukaryotic cells to synthesize new proteins that belong to this family. If should, however, be noted that many members of this family are *not* induced – rather they are normally present in virtually all cellular compartments (Table 29–1).

The Hsp 60 class of heat-shock protein from the photosynthetic bacterium *Rhodobacter sphaeroides* (Fig 29-13) consists of 14 identical ~ 60-kD subunits arranged to form two apposed rings of 7 subunits, each surrounding a central cavity.

Table 29–1. Heat shock proteins

Hsp60 (chaperon-60) family	
	GroEL (in bacterial cytosol)
	Hsp60 (in mitochondrial matrix)
	Rubisco binding protein (in chloroplasts)
Hsp70 (stress-70) proteins	
	Hsp70 (in mammalian cytosol)
	BiP (in ER of eukaryotes)
	Grp75 (in mitochondria)
	DnaK (in bacterial cytosol)
Hsp90 (stress-90) proteins	
	Hsp83 (in eukaryotic cytosol)
	Grp94 (in mammalian ER)
	Htgp (in bacterial cytosol)

(*Adapted from Getting M-J and Sambrook J, 1992*)

The hsp70 class of heat-shock proteins, which includes BiP, is highly conserved in evolution– the amino acid sequences of the *E.coli* and human proteins are 50% identical. Hsp70 proteins (Fig. 29–14) consist of an ATPase domain and a peptide-binding domain. The ATPase moiety has 2 lobes with a cleft in between.

ENVELOPE CARRIER HYPOTHESIS

The envelope carrier hypothesis is linked with the transport of proteins to mitochondria and chloroplasts. Initially, this hypothesis was put forth to explain the transport of smaller subunit of ribulose bisphosphate carboxylase-oxygenase (RUBISCO) enzyme from cytoplasm to chloroplasts. The hypothesis proposes that there are certain proteins in the chloroplastic envelope which can detect and bind with all chloroplast-bound proteins. The smaller subunit of RUBISCO can also bind with these protein in the envelope (Fig. 29–15). This subunit is synthesized as a slightly bigger precursor protein (MW = 20kd) than the actual size (MW = 16kd). The bigger precursor is transported through the chloroplastic envelope by the receptor protein, which also removes the extra segment from the protein through proteolytic action. The portion so removed is especially rich in acidic amino acids, Asp and Glu. Removal of this segment, which contains about 50 amino acid residues, induces a configurational change in the protein, leading to its transport across the chloroplastic envelope and into the stroma.

Fig. 29–13. An electron micrograph–derived 3D image of the Hsp60 chaperonin from the photosynthetic bacterium *Rhodobacter sphaeroides*

The image of Hsp60, which is viewed with its 7-fold axis tipped towards the viewer, reveals that each subunit consists of two major domains, one in contact with the opposing heptameric ring, and the other at the end of the cylindrical protein molecule. The spherical density occypying the protein's central cavity is thought to represent a bound polypeptide. The cavity presumably provides a protected microenvironment in which a polypeptide can progressively fold itself.

[*Courtesy*: *Helen Saibil and Steve Wood, Birbeck College, London*]

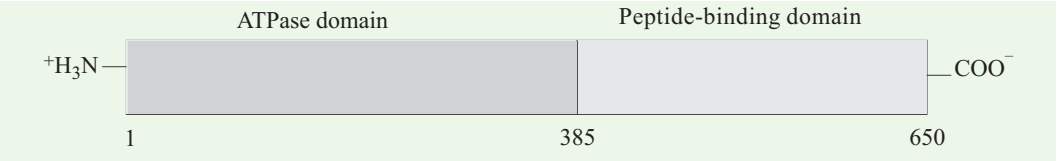


Fig. 29–14. Structure of Hsp 70 proteins

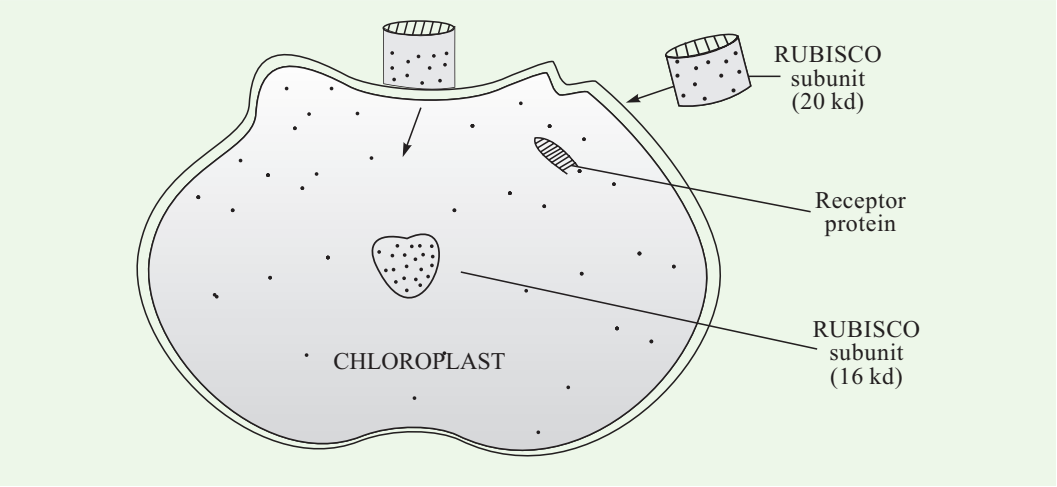


Fig. 29–15. Envelope carrier hypothesis of protein transport

PROTEINS WITH A CARBOXYL-TERMINAL KDEL SEQUENCE (=RECYCLING OF RESIDENT PROTEINS OF THE ER)

The ER is rich in chaperones and other proteins that help folding of the nascent peptides. What prevents the ER from losing these essential resident proteins ? In principle, either the resident or the secreted set of proteins could contain a distinguishing tag. However, the experimental evidences indicate that no special tag is needed for needed. A cytosolic protein given a signal sequence will emerge in the ER and then be efficiently secreted. *The resident proteins of the ER lumen carry a retention signal*. More than 50 resident ER proteins from vertebrates, plants, arthropods and nematodes have been shown to a carboxyl-terminal Lys-Asp-Glu-Leu (KDEL) sequence or a closely-related tetrapeptide sequence. In yeast, an HDEL (His-Asp-Glu-Leu) sequence does the same work. Annexation of KDEL sequence to the C-terminus of a secretory protein keeps it (the protein) in the ER of the higher eukaryotes. Conversely, removal of KDEL sequence from the C terminus of a resident ER protein changes its fate: the protein is secreted rather than retained in the ER. Thus, *KDEL sequences retain the resident proteins in the ER*.

In fact, a KDEL sequence does not block the departure of a proteins from the ER. Rather, it serves as a restoring or *retrieval tag*. On reaching the Golgi, KDEL proteins bind to membrane receptors that recognize their C terminal tail (Fig. 29–16). These protein-receptor complexes then incorporate into vesicles that return them to the ER. This recycling scheme operates only when the receptor has high affinity for KDEL in the Golgi but low affinity in the ER. The affinity of the KDEL for its receptor increases about 10 times when the pH is lowered from 7 to 5. As the lumen of the Golgi complex is more acidic than that of the ER, KDEL proteins are membrane-bound in the Golgi but free in solution is the ER. Tight binding in the Golgi ensures efficient retrieval, whereas weak binding in the ER gives resident proteins the freedom to promote the folding and modification of nascent proteins.

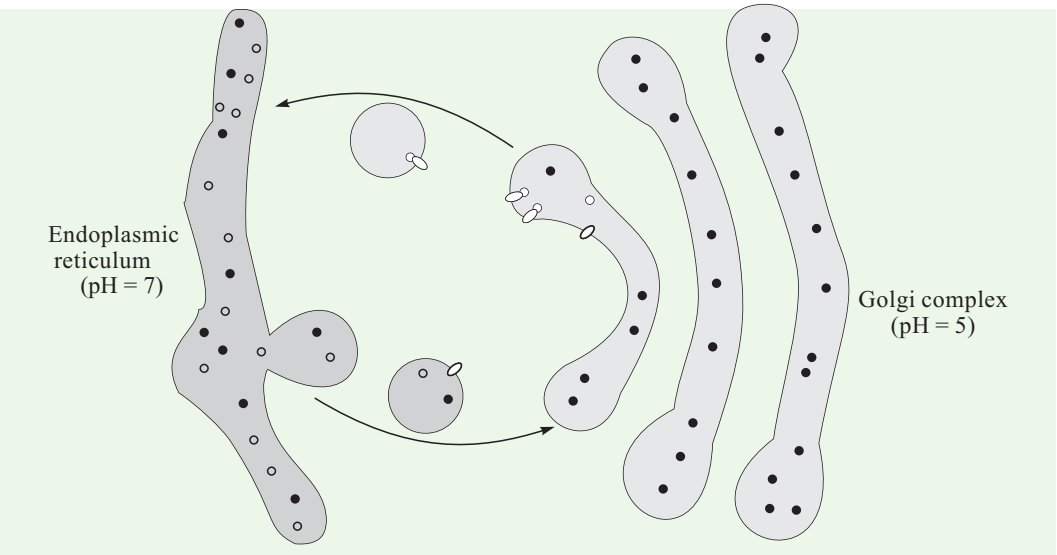


Fig. 29–16. Recycling of resident proteins of the endoplasmic reticulum

Both resident proteins (•) and secretory proteins (o) are carried from the ER to the Golgi complex by transport vesicles. The KDEL sequences of resident proteins enable them to be restored by a membrane-bound protein that acts as a recycling receptor.

A. Protein Transport to Lysosomes

Proteins from the Golgi to lysosomes are sent by a different king of marker. In fact, a glycoprotein destined for delivery to lysosomes acquires a phosphoryl marker in the *cis* Golgi. This (*i.e.*, phosphorylation) is a two-step process (Fig. 29–17). In the first step, a *phosphorylase* adds a phospho-N-acetylglucosamine unit to the 6-OH group of a mannose; in the second step, a *phosphodiesterase* removes the added sugar to produce a mannose-6-phosphate residue in the core oligosaccharide. Phosphotransferase is a highly discriminating enzyme. It does not act on a mannose residue in an unattached oligosaccharide or on a short peptide containing such a unit. Rather, the phosphotransferase recognizes a 3-'D' structure (sometimes called a '**signal patch**') that is present only in glycoproteins destined to lysosomes.

How is the lysosomal targeting accomplished by mannose-6-phosphate?. The Golgi membrane contains a receptor that specifically recognizes the mannose-6-phosphate residue and binds proteins marked by it. The vesicles containing this protein-receptor complex bud from the *trans* side of the Golgi. These vesicles then fuse with pre-lysosomal vesicles which are more acidic than the Golgi. A decrease in pH triggers dissociation of the marked glycoprotein from its receptor, which now returns to the Golgi. Pre-lysosomes mature into lysosomes by fusing with lysosomes and receiving their enzymes. The membrane-bound mannose-6-phosphate receptor returns to the Golgi by a different set of vesicles. This receptor is recycled so as to be used again and again. Lysosomal targeting is blocked by agents that make sorting vesicles less acidic. Chloroquine and ammonium chloride, for example, raise the pH of sorting vesicles and thus lead to the export of lysosomal enzymes from the cell, rather than to lysosomes. In the absence of receptors, newly-formed glycoproteins (containing mannose-6-phosphate) continue to be exported from the cell.

However, it does not seem that for transport of other proteins, namely those which must be either secreted or integrated in the plasma membrane, particular monosaccharides serve as markers. And it is rather believed that elements of 3-'D' structure play a role in directing these glycoproteins to their correct destination. These targeting pathways are not hindered by tunicamycin, indicating that the signals are not carbohydrates.

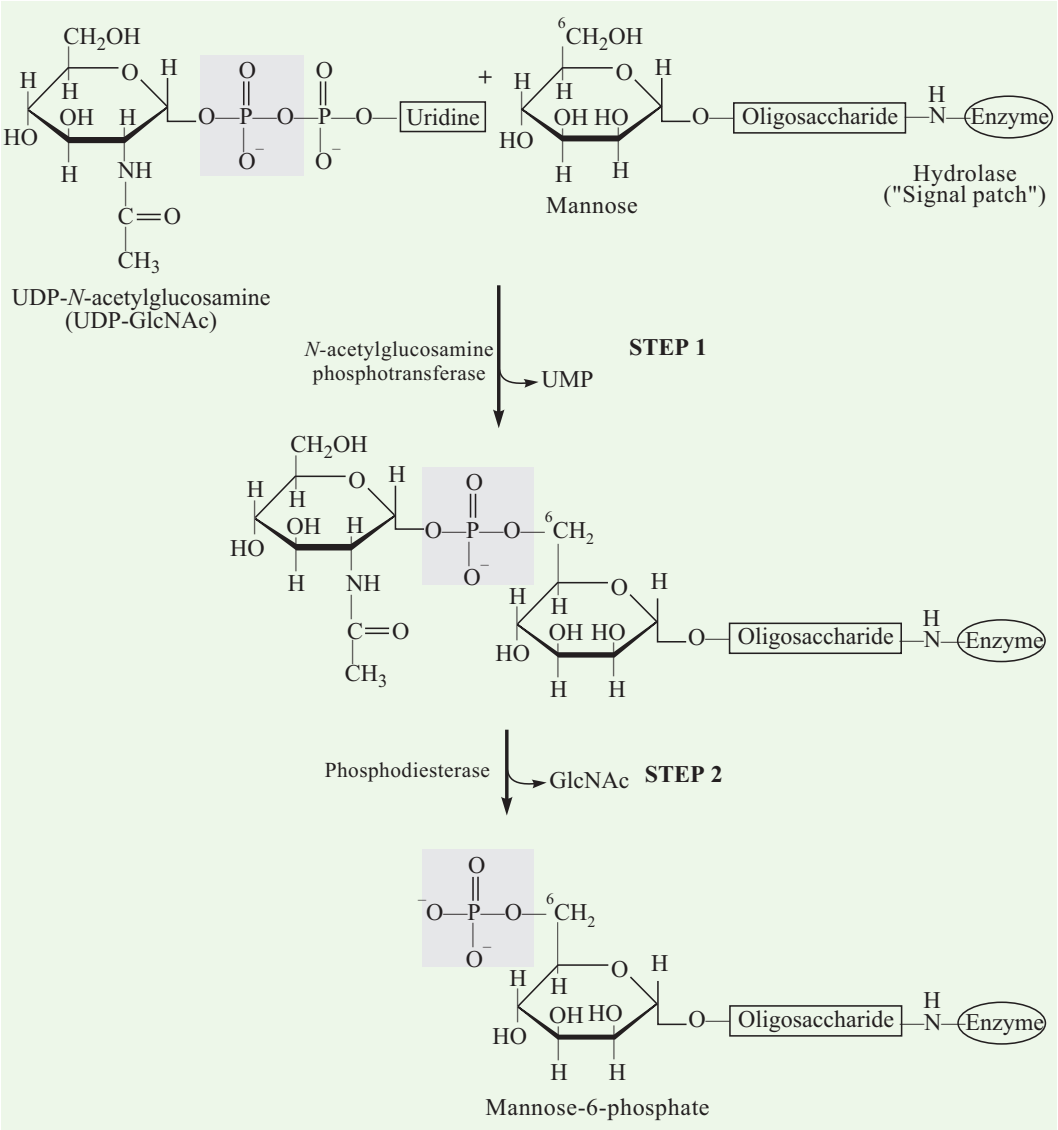


Fig. 29–17. Phosphorylation of mannose residues on lysosome-targeted enzymes such as hydrolases
Note that phosphorylation occurs in 2 steps. A phosphotransferase and a phosphodiesterase in the *cis* Golgi catalyze the addition of a phosphoryl tag. Mannose-6-phosphate, in fact, acts as a marker that normally directs many hydrolytic enzymes from the Golgi to lysosomes.

Mistakes in transport of lysosomal enzymes can lead to a rare inborn error in humans, in which at least 8 hydrolytic enzymes (hydrolases) are exported out of the cell, instead of going to the lysosomes. This disease is called as **I-cell disease** (or **mucolipidosis II**). Since the function of lysosomes is to degrade cellular debris (*e.g.,* glycolipids), lack of hydrolytic enzymes in them results in cells accumulating *inclusion bodies, i.e.,* becoming *I-cells* (I for inclusion). An *I-cell is, thus, defective because it exports lysosomal enzymes*. In contrast, high levels of enzymes are present in the urine and blood. The inclusion bodies are bloated lysosomes filled with cellular debris that cannot be degraded. I-cell disease is characterized by severe psychomotor retardation and skeletal deformities in patients, often leading to their death in childhood. This lysosomal storage disease is inherited as an autosomal recessive trait.

B. Protein Transport to Mitochondria

The mitochondrial genome encodes all the RNA but only a very small number of mitochondrial proteins (about 12) with the result that the great majority of mitochondrial proteins is encoded by the nuclear genome, synthesized in the cytosol by free ribosomes and then imported into the mitochondria. Indeed about 10% of the proteins in a eukaryotic cell are imported into mitochondria. How do these majority proteins reach their mitochondrial destinations? The problem becomes more complex as mitochondrial proteins reside in 4 locations; the outer membrane, the inner membrane, the intermembrane space, and the matrix. Gottfried Schatz (1993) discovered that the import of a protein into the mitochondrion requires the presence of a particular sequence at its amino-terminal end, called the **presequence** or **matrix targeting sequence**. This sequence must be recognized by receptors situated on the external face of the outer membrane of the mitochondrion and leads to the import of the protein bearing it into the matrix. The presequences (Fig. 29–18) are typically 15 to 35 residues long. They are rich in positively-charged residues, and in serines and threonines. No consensus sequence has been found. In fact, matrix targeting sequences, like prokaryotic and eukaryotic signal sequences, are highly degenerate – about 20% of randomly-generated sequences allow proteins to enter mitochondria.

$^{+}H_3N$—Met—**Leu**—Arg—Thr—Ser—Ser—**Leu**—**Phe**—Thr—Arg—Arg—**Val**—Gln—Pro—Ser
—**Leu**—**Phe**—Arg—Asn—**Ile**—**Leu**—Arg—**Leu**—Gln—Ser—Thr

Fig. 29–18. A mitochondrial matrix targeting sequence

The 9 hydrophobic residues are shown in boldface. There are 5 basic residues (Arg) also.

The precursor proteins are imported into the mitochondrial matrix through mediation by proteins in the cystosol, outer and inner mitochondrial membranes, and the matrix. The fully folded proteins cannot enter mitochondria. Hence, chaperones play a key role in maintaining the precursor proteins in unfolded or partly folded state. A cytosolic *hsp70 protein* hands the precursor to an *import receptor* in the outer mitochondrial membrane (Fig. 29–19). The receptor-bound precursor then moves to a site where the outer and inner mitochondrial membranes adhere to one another. The precursor protein threads through a channel formed by several subunits from each membrane. Translocation is driven by the membrane potential across the inner mitochondrial membrane and by the ATP-powered untate of the nascent by mhsp70, a 70 kd heat-shock protein in the mitochondrial matrix. Finally, the amino-terminal signaling sequence is cleaved in the matrix by a metalloprotease to produce the mature protein.

The import of proteins into mitochondria (and plasts) is a post-translational process which may be performed *in vitro* by incubating organelles in presence of the protein precursor, and therefore differs from the transport of proteins through the membrane of the ER which takes place concomitantly with the translocation, *i.e.,* while the protein chain is being synthesized.

C. Protein Transport to Chloroplasts

Most proteins of chloroplasts, like those of mitochondria, are coded by the nuclear genome, synthesized by the cytosolic ribosomes and imported in the plast. But there are more possible localizations in the plast (6) than in the mitochondria : the outer membrane, the inner membrane, the intermembrane space, the stroma, the thylakoid membrane and the lumen of the thylakoid. The existence of the thylakoid membranes, separate from the inner membrane, gives rise to two more destinations than are present in mitochondria. As in the case of mitochondria, the import of proteins requires a **presequence** (also called **transit sequence** or **transit peptide**) at the amino-terminal end. Chloroplast presequences resemble mitochondrial presequences in being positively-charged and rich in hydroxylated residues (*i.e.*, serine and threonine). This chloroplast presequence

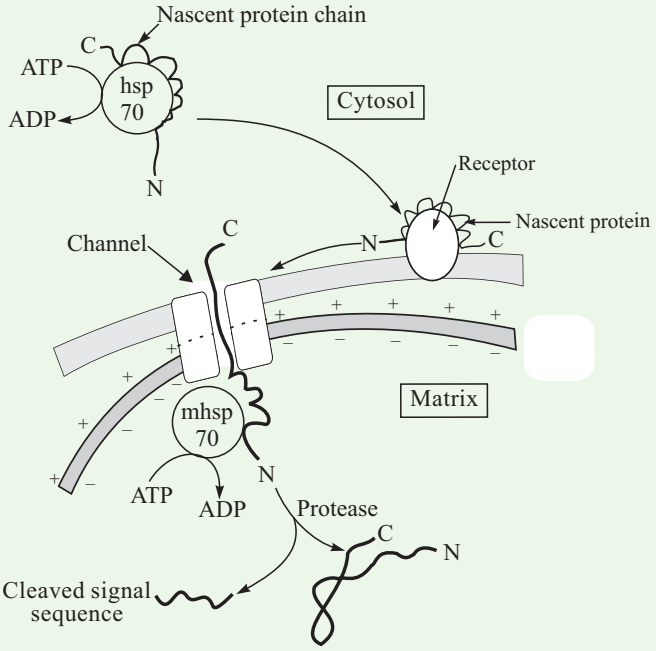


Fig. 29–19. Proposed mechanism for the delivery of a protein from the cytosol to the mitochondrial matrix

The nascent chains is delivered by a chaperone to a receptor in the outer mitochondrial membrane. The nascent protein threads through a channel that traverses both membranes at a contact site. A trans-membrane potential across the inner mitochondrial membrane is essential for transport. The matrix-trageting sequence is removed by protease.

(*After Gottfried Schatz, 1993*)

is cleaved during the transport process through stroma and thylakoids. Transport across the outer and inner chloroplast membranes is powered by ATP hydrolysis, whereas transport across the thylakoid is driven by the pH gradient. The presequences of proteins destined for the thylakoid lumen appear to contain two signals (Fig. 29–20). The amino-terminal signal leads to the import of the precursor protein into the chloroplast stroma. This part of the presequence (*first signal*) is cleaved in the stroma, or en route to it, exposing a *second signal* that directs the translocation of the modified precursor across the thylakoid membrane. A protein targeted to the stroma lacks this second signal, which contains a hydrophobic core reminiscent of bacterial and ER signal sequences.

D. Protein Transport to Peroxisomes

Peroxisomes are small membrane-bound compartments, present in most eukaryotic cells. These organelles contain *oxidases* that produce H_2O_2 and *catalase* which decomposes H_2O_2 to water and oxygen. Peroxisomes perform a variety of functions. They bring about detoxication. They also catalyze the first two steps in the synthesis of plasmalogens. In humans, β-oxidation of fatty acids longer than C_{18} occurs primarily in the peroxisomes, rather than in mitochondria. In plants, similar organelles (also called **glyoxysomes**) play a key role in the recycling of phosphoglycolate, which is generated by the oxygenase action of RUBISCO. Peroxisomes and glyoxysomes are together known as **microbodies**.

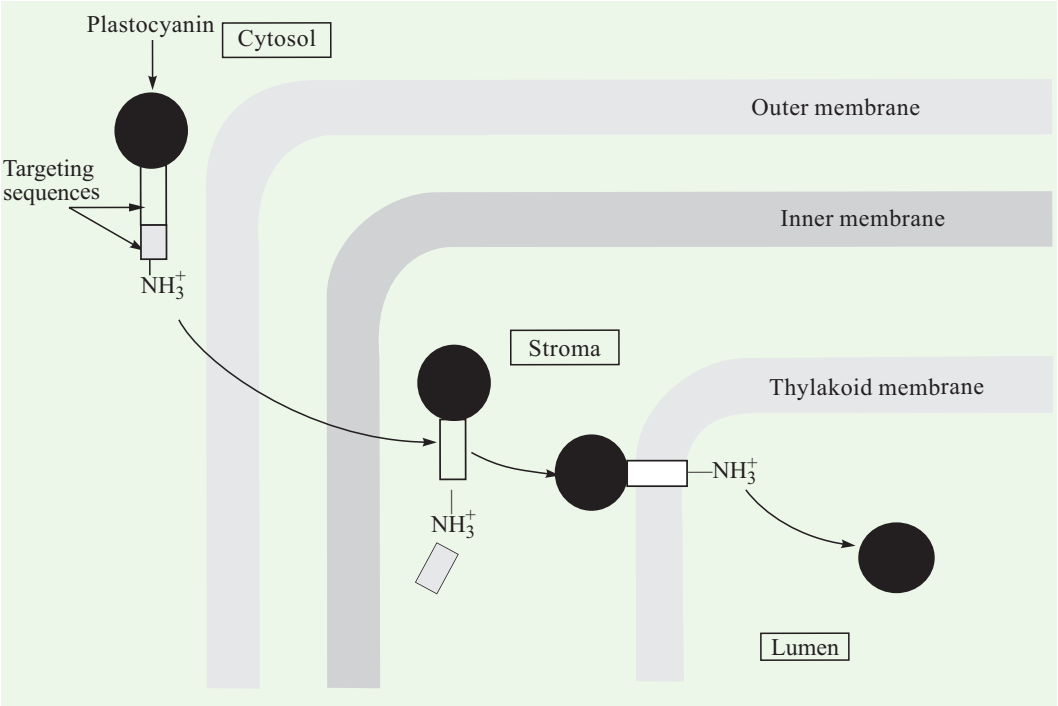


Fig. 29–20. Targeting of the plastocyanin to the thylakoid lumen of chloroplasts by the sequential action of two amino-terminal sequences

The first sequence enables plastocyanin to enter the stroma and the second (exposed by proteolysis) enables it to cross the thylakoid membrane.

(*After Smeeckens, Bauerle, Hagemann, Keegstra and Weisbeck, 1986*)

Both peroxisomes and glyoxysomes are devoid of DNA. The soluble proteins in their matrix are imported from the cytosol. The targeting signal for many peroxisomal matrix proteins is simply a carboxyl-terminal Ser-Lys-Phe or SKF (the one-letter symbol for the 3 amino acids) **tripeptide sequence**. Mutation of the SKF sequence of a cytosolic precursor protein blocks its import into peroxisomes. The SKF signal, in contrast with mitochondrial and chloroplast import signals, is *not* cleaved. Two other differences are its brevity and carboxyl-terminal location.

E. Protein Transport to Nucleus

All nuclear proteins (*esp*, histones, DNA polymerases, RNA polymerases and all proteins participating in the replication of DNA and transcription) are synthesized in the cystosol by free ribosomes and must pass through the nuclear envelope of eukaryotes comprising an outer membrane and an inner membrane. This transport, for the small proteins (*e.g*, histones), seems to take place through nuclear pores of 70 Å diameter; but for larger proteins (> 90 kd), a short peptide sequence (or signal sequence) appears to be necessary. For example, the T antigen of SV 40 virus is a protein of molecular weight 92,000 daltons (or 92 kd) that regulates the replication and transcription of viral DNA. And studies have shown that the transport of this large protein, depends on the presence of a **nuclear localization sequence**, containing five consecutive positively-charged residues (shown in red):

-Pro-Lys-Lys-Lys-Arg-Lys-Val-
128

A change of even a single amino acid residue can render this sequence inactive. As an instance, T antigen containing Thr or Asn in place of Lys at residue 128 stays in the cystol and

not transported to the nucleus. The nuclear localization sequences can also accelerate the entry of small proteins. The transport of large proteins into nuclei is powered by ATP hydrolysis. Interestingly, none of the nuclear localization signals are cleaved on entry into the nucleus. It was also possible to bring about the transport of proteins to the nucleus by grafting (at the DNA level) this heptapeptide sequence (shown above) on pyruvate kinase or on other cytosolic proteins.

It is moteworthy that fully-folded proteins can be imported into nuclei but not into mitochondria or chloroplasts, which must maintain a tight permeability barrier to sustain a proton-motive force. No bilayer membrane is crossed on entering the nucleus– hence, unfolding is not essential. The nucleus can afford to be more relaxed about its border since the pH and ionic composition of the nucleoplasm is essentially the same as that of the cytosol.

BACTERIAL SIGNAL SEQUENCES AND PROTEIN TARGETING

Protein targeting is not confined to eukaryotes. Bacteria also target proteins to destinations encoded in their sequences. A Gram-negative microorganism such as *E.coli* can translocate nascent proteins to the inner and outer membranes, the periplasmic space between the membranes or (rarely) the extracellular medium (secretion) as depicted in Fig. 29–21. As in eukaryotes, translocation

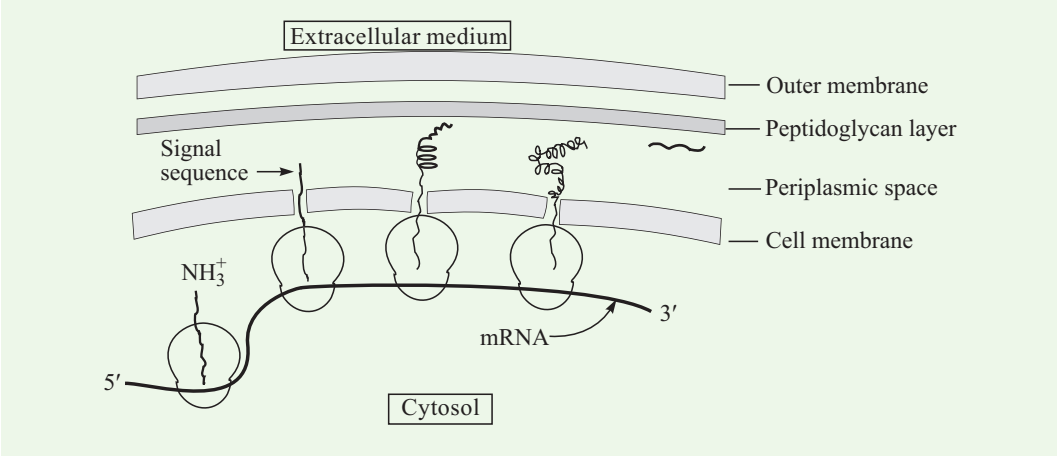


Fig. 29–21. Schematic of the synthesis of noncytosolic proteins by ribosomes bound to the plasma membrane in prokaryotes

A signal sequence (shown by bold line) on the nascent chain directs the ribosomes to the plasma membrane and enables the protein to be translocated. The translocation machinery is not depicted in this schematic diagram.

is not mechanistically coupled to chain elongation. This targeting uses signal sequences (also called **leader sequences**) at the amino terminus of the proteins, much like those found on eukaryotic proteins targeted to the ER (Fig. 29–22). These signal sequences are usually 16 to 26 residues long. Though diverse, the prokaryotic signal sequences have a positively-charged amino-terminal region, and a helix-breaking segment. Like eukaryotes, the signal sequence is usually cleaved by a signal peptidase at the helix-breaking site. Another similarity is that polypeptide chain elongation and translocation usually take place at about the same time but are not mechanistically coupled.

Translocation of a polypeptide chain through the cell membrane of *E. coli* is catalyzed by a soluble chaperone and a membrane-bound, multisubunit translocase (Fig. 29–23). The bacterium contains a major chaperone, *SecB protein* which keeps nascent chains in unfolded or partially folded state to enable them to traverse the membrane. SecB presents the nascent chain to Sec A,

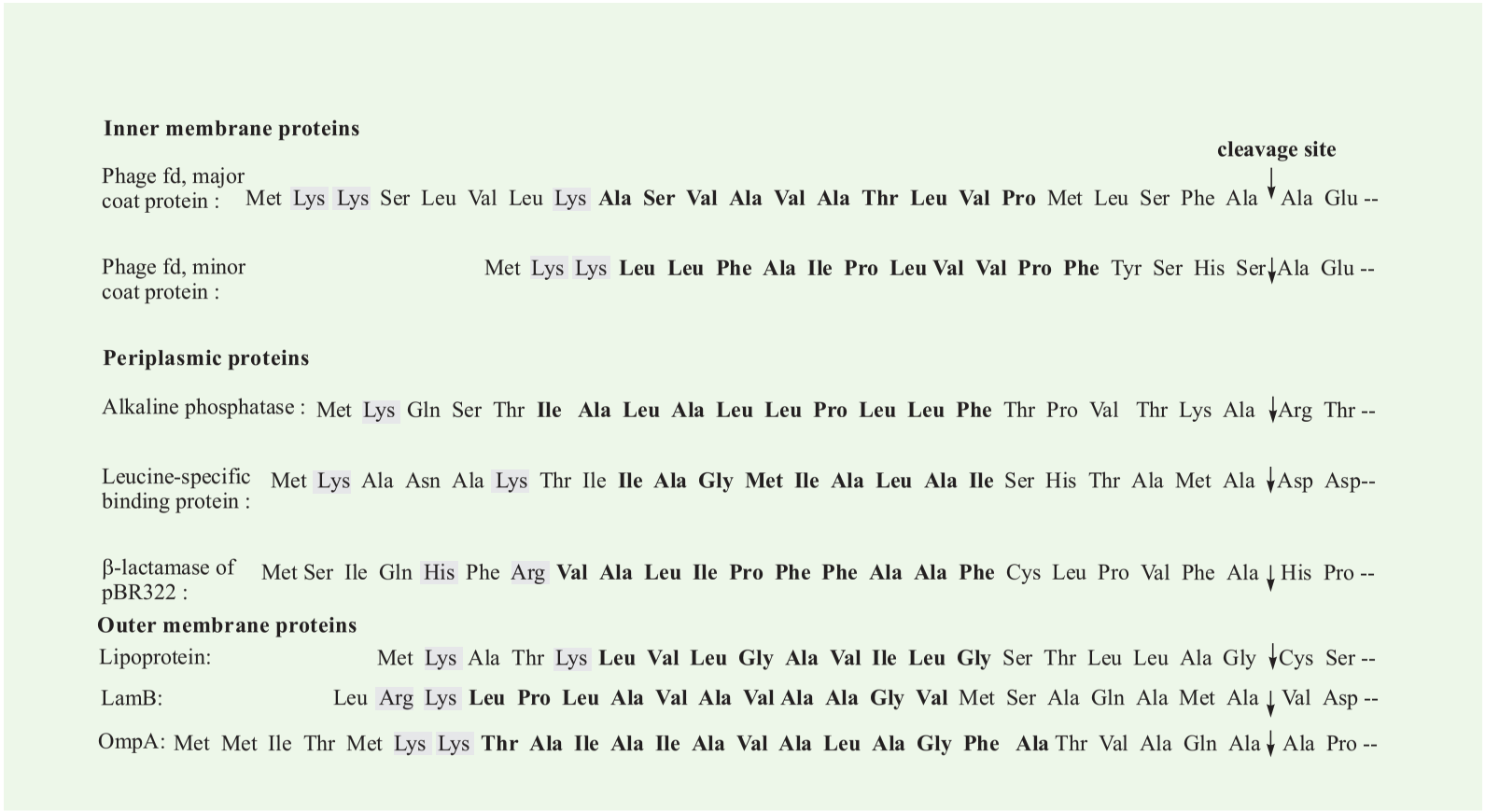


Fig. 29–22. Amino-terminal signal sequences of some bacterial proteins used for targeting to different locations

Basic amino acids (shaded) near the amino terminus and hydrophobic core amino acids (in boldface) are highlighted. The cleavage sites denoting the ends of the signal sequences are marked by arrows.

Contents

a peripheral membrane component of the translocase. SecA works in unison with SecY and SecE, the membrane-embedded portion of the translocase. Two forms of free energy (ATP and proton-motive force) drive protein translocation in *E. coli*. SecA is an ATPase– the ATP state has high affinity for the protein to be translocated, whereas the ADP state has low affinity. Portions of the nascent chain are successively handed from from SecA to SecY-SecE channel as a result of many cycles of ATP hydrolysis. The proton-motive force across the cell membrane then drives the threading of the nascent chain through the membrane.

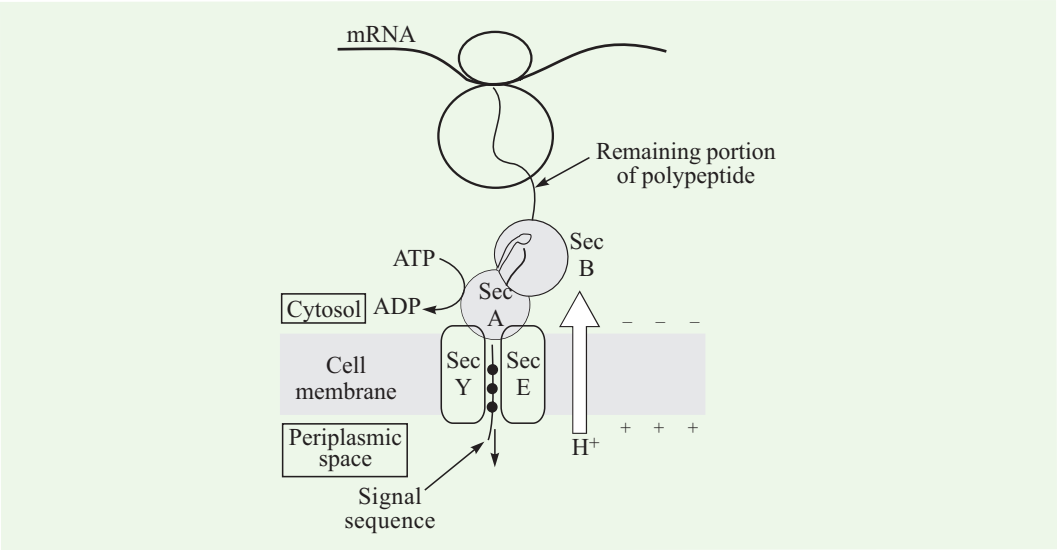


Fig. 29–23. Schematic diagram showing the interplay of Sec proteins in protein translocation across the cell membrane

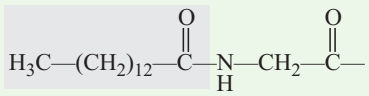
Proton-motive force powers the unidirectional translocation of the polypeptide from the cytosolic to the periplasmic side of the membrane

Some proteins that are translocated through one or more membranes to reach their final destinations are maintained in a distinct "*translocation-competent*" *conformation* until this process is complete. The functional conformation is assumed after translocation, and proteins purified in this final form are now longer capable of translocation. Available evidences indicate that the translocation conformation is stabilized by a specialized set of proteins in all bacterial cells. These bind to the protein to be translocated while it is being synthesized, preventing it from folding into its final 3-'D' structure. In *E. coli*, a protein called trigger factor (Mr 63,000) appears to facilitate the translocation of at least one outer membrane protein through the inner membrane.

EUKARYOTIC PROTEIN TRANSPORT ACROSS MEMBRANES

The eukaryotic organisms employ different strategies to send proteins to the *cytosolic face* of either the plasma membrane or a compartment membrane. A fatty acyl or prenyl group is attached covalently to a soluble cytosolic protein. This membrane-anchoring may be accomplished in following 4 ways (Lubert Stryer, 1995):

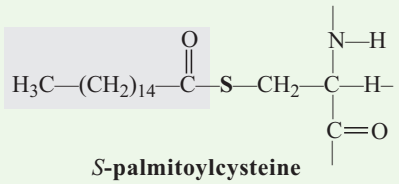
1. Myristoylation at the N terminus. The amino terminus of many proteins is acylated with a myristoyl (C_{14}) or a similar fatty acyl group; the acyl group being donated by myristoyl-CoA. This reaction is catalyzed by *N-myristoyl transferase*. The N-terminal residue (residue 1) must be glycine, residue 5 is usually serine or threonine, and residues 6 and 7 are typically basic. Myristoylation enables a modified protein to interact with a membrane receptor or the lipid bilayer itself.



N- **myristoylglycine**

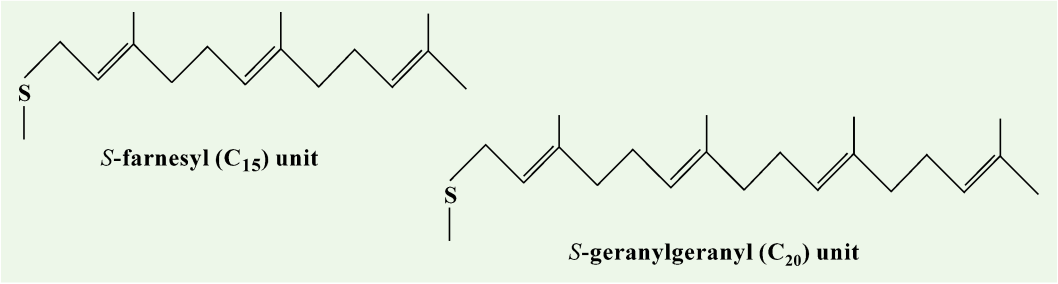
2. Palmitoylation of cysteine residues. The thiol (– SH) group of some cysteine residues in proteins can be acylated by palmitoyl-CoA to form an C_{16} *S*-palmitoyl derivative. Rhodopsin, for example, contains two adjacent *S*-palmitoyl groups that act as membrane anchors."

3. Farnesylation at the C terminus. Many of the proteins that participate in signal transduction and protein targeting contain either a farnesyl (C_{15}) or a geranylgeranyl (C_{20}) unit at their C terminus. These prenyl groups are attached to C-terminal cysteine residues by thioester linkages. Farnesylation occurs at **CaaX sequences** in which cysteine (C) is followed by two



S-**palmitoylcysteine**

aliphatic residues (*a*) and a C-terminal residue (X) (Fig. 29–24). After attachment of the C_{15} unit to this cysteine, the aaX residues are proteolytically removed and the new terminal carboxylate group is methylated. Thus, a highly hydrophobic C terminus is fashioned by a series of modifications. The ras protein does not insert in the plasma membrane unless it is farnesylated. In fact, unfarnesylated ras is unable to transduce growth signals.



S-**farnesyl (C_{15}) unit**

S-**geranylgeranyl (C_{20}) unit**

4. Geranylgeranylation at the C terminus. When the C-terminal sequence is CC, CXC, or CCXX, a geranylgeranyl (C_{20}) unit, rather than a farnesyl unit, becomes attached to one or both cysteines. The rab family of small GTP-binding proteins, which participate in membrane targeting, are geranylgeranylated. The attachment of this highly hydrophobic prenyl unit is necessary for membrane binding.

PROTEIN IMPORT BY RECEPTOR-MEDIATED ENDOCYTOSIS

Specific proteins are imported into a cell by their binding to receptors in the plasma membrane and their inclusion into vesicles. Such a process is called **receptor-mediated endocytosis** and has a great number of biological applications:

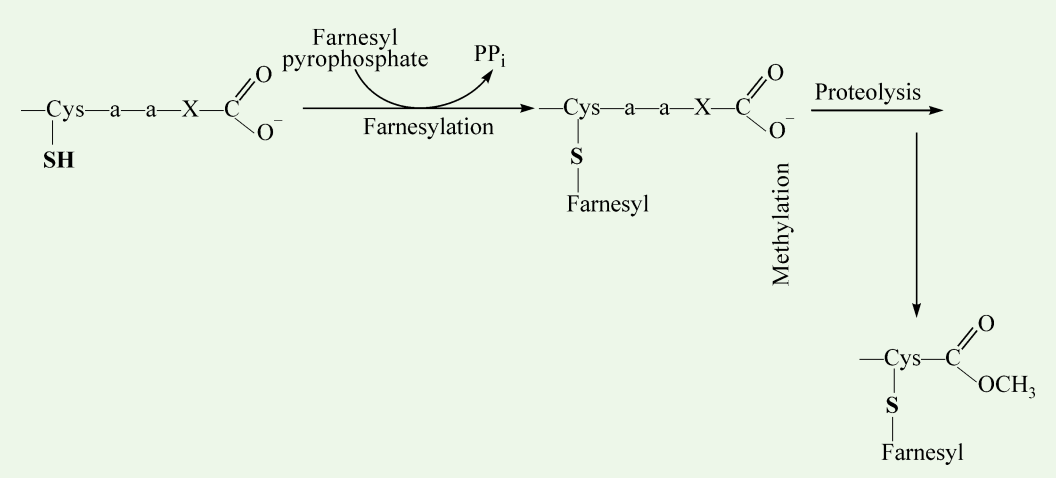


Fig. 29–24. Farnesylation of a cytosolic protein at the C terminus

Farnesylation is followed by trimming of 3 C-terminal residues and methylation of the terminal carboxylate.

1. It is a means of delivering essential metabolites to cells. For instance, the low-density lipoprotein (LDL) carrying cholesterol is taken up by the LDL receptor in the plasma membrane and internalized.
2. Endocytosis regulates responses to many protein hormones and growth factors. Epidermal growth factor and nerve growth factor are taken into the cell and degraded together with their receptors.
3. Proteins destined for degradation are taken up and delivered to lysosomes for digestion. Phagocytes, for example, have receptors that enable them to take up antigen-antibody complexes.
4. Receptor-mediated endocytosis is employed by many viruses and toxins to gain entry into cells, as exemplified by the ingenious mode of entry and departure of Semliki Forest Virus (SFV), a membrane-enveloped virus.
5. Disorders of receptor-mediated uptake can lead to diseases, such as some forms of familial hypercholesterolemia.

Cell-surface Receptors and Clathrin

The **cell-surface receptors**, which mediate endocytosis, are transmembrane glycoproteins. They have a large extracellular domain and a small cytosolic region and contain either one (*e.g.*, asialoglycoproteins) or two (*e.g.*, transferrin) transmembrane helices (Fig. 29-25). Many of the receptors are located in specialized regions of the plasma membrane called *coated pits*. The cytosolic side of these pits has a thick coat of *clathrin*, a protein designed to form lattices around membranous vesicles. Many receptors (such as those for LDL, transferrin, asialogycoproteins, insulin) congregate in coated pits; others (such as the receptor for epidermal growth factor) cluster there after binding their cognate protein.

Clathrin (Fig. 29-26) is a trimeric protein, consisting of 3 heavy chains (H ; M*r* = 180,000) and 3 light chains (L; M*r* = 35,000). The $(HL)_3$ clathrin unit (8 S; M*r* = 650,000) is organized as a three-legged structure, called a *triskelion*. The carboxy termini of the 3 heavy chains (each about 500 Å long) come together at a vertex. A bend in the heavy chain divides it into a proximal arm, closest to the vertex, and a distal arm. Each of 3 the light chains is aligned with the proximal arm of a heavy chain. Many clathrin assemble into closed shells having a polyhedral structure. The polyhedra are made of both pentagons and hexagons. A single edge of a pentagon or hexagon is made of parts of four triskelions, 2 proximal arms and 2 distal arms. The flexibility of a triskelion is important in enabling it to fit into a pentagon or hexagon.

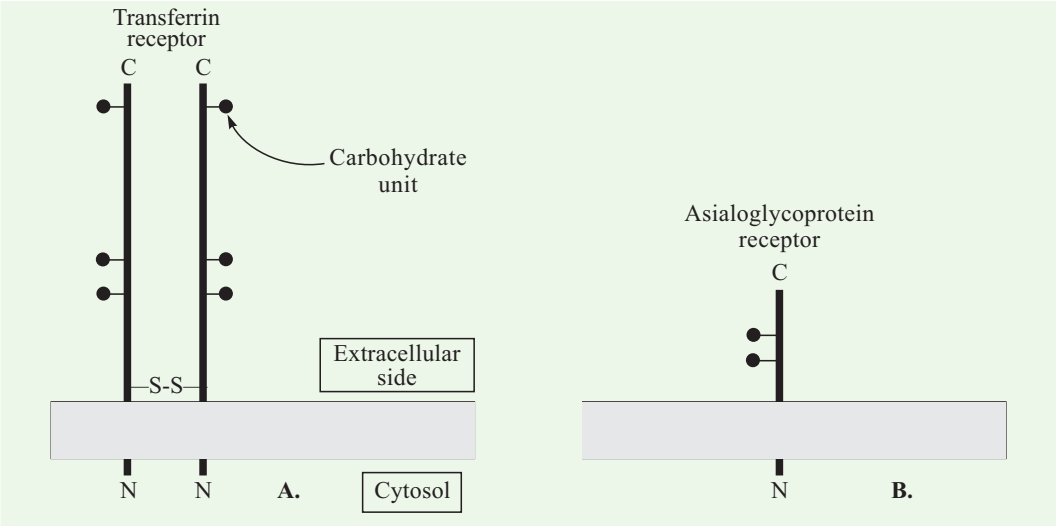


Fig. 29–25. Schematic of two cell-surface receptors that are internalized at coated pits

A. Transferrin receptor B. The asialoglycoprotein receptor

The short N-terminal tails of these receptors are critical for internalization.

Receptor-mediated Endocytosis

Receptor-mediated endocytosis (Fig. 29–27) begins with the binding of certain proteins (such as LDL, transferrin, peptide hormones etc) to receptors on the outer face of the plasma membrane. The receptors are concentrated in invaginations of the membrane called coated pits, which are coated on their cytosolic face with a lattice made up of the protein clathrin. The clathrin lattice grows up until a complete membrane-bound endocytic vesicle, with a diameter of about 80 nm, buds off the plasma membrane and moves into the cytosol. The endocytic vesicle then rapidly loses its clathrin shell by uncoating enzymes and fuses with an endosome. The endosomes, in turn, fuse with one another to form bigger vesicles, ranging between 200 and 600 nm. The membrane ATPases present in the endosomes lower the pH, so that the receptors dissociate from their target proteins. Proteins and receptors then follow separate paths, their fates varying according to the system (Table 29–2). The protein transferrin transports iron from sites of absorption and storage to sites of utilization. Two Fe^{3+} ions are bound to the protein which contains two similar domains. The protein devoid of iron is called *apotransferrin*. Transferrin, but not apotransferrin, binds to a dimeric receptor (Fig. 29–25). The low pH within the endosome causes dissociation of Fe^{3+} from transferrin. The acidity lowers the affinity of transferrin for Fe^{3+} more than a millionfold. However, apotransferrin remains bound to the receptor.

Sorting then takes place : part of the vesicle bearing apotransferrin bound to the receptor pinches off and proceeds towards plasma membrane, whereas the remaining Fe^{3+} is stored in ferritin in the cytosol. When the pinched off vesicle fuses with the plasma membrane, apotransferrin is released from the receptor because of the sudden increase in pH. Apotransferrin has little affinity for the receptor at pH 7.4. Thus, *pH changes are used twice to drive the transferrin transport cycle*: first to release iron from transferrin in the endosome, and then to discharge apotransferrin into the extracellular fluid. *The cycle takes about 16 minutes* : 4 minutes for the binding of transferrin, 5 minutes for transport to endosomes, and 7 minutes for the return of the iron carrier and the receptor to the cell surface. Toxins (diphtheria toxin, cholera toxin) as well as viruses (influenza virus) enter cells by receptor-mediated endocytosis.

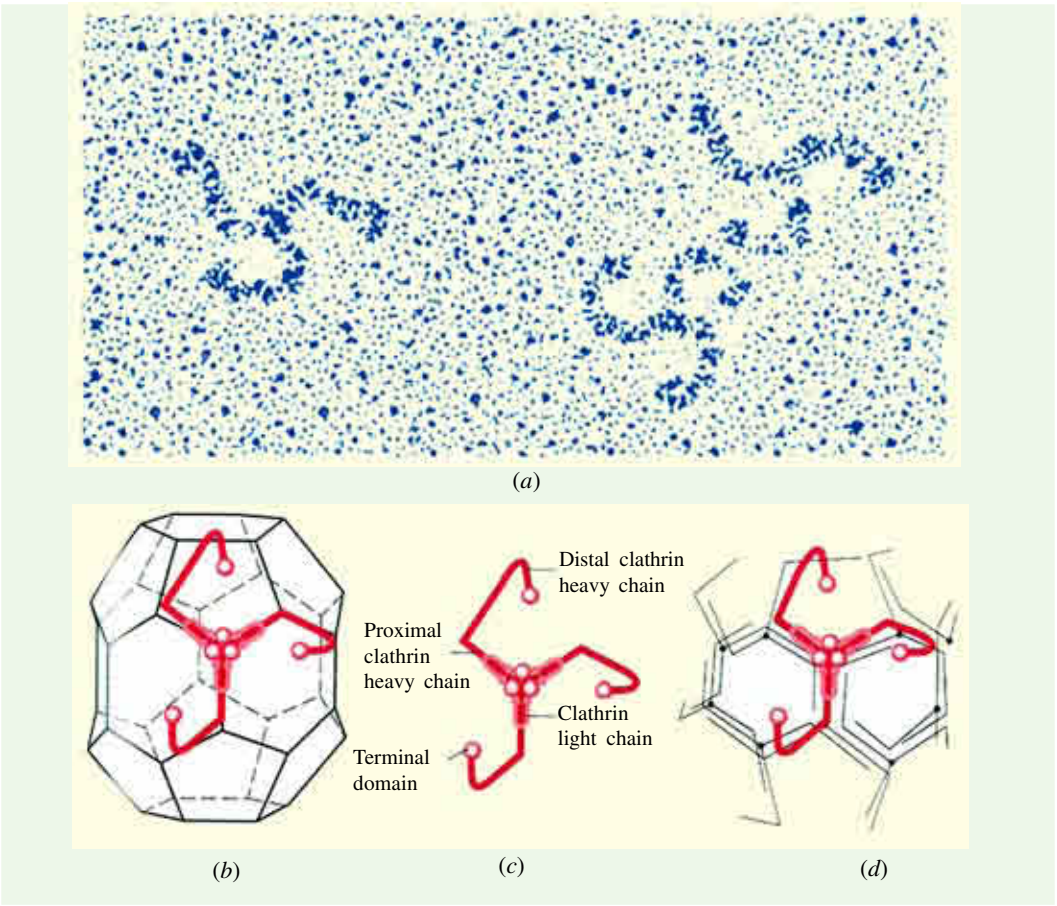


Fig. 29–26. Structure and assembly of a coated vesicle or clathrin

(*a*) **Electron micrograph of a metal-shadowed preparation of clathrin triskelions.**

(*b*) **A typical coated vesicle containing a membrane vesicle about 40 nm in diameter surrounded by a fibrous network of 12 pentagons and 8 hexagons.** The fibrous coat is constructed of 36 clathrin triskelions. One clathrin triskelion is centered on each of the 36 vertices of the coat. Coated vesicles having other sizes and shapes are believed to be constructed similarly: each vesicle contains 12 pentagons but a variable number of hexagons.

(*c*) **Detail of a clathrin triskelion.** Each of three clathrin heavy chains is bent into proximal arm and a distal arm. A clathrin light chain in attached to each heavy chain, most likely near the center

(*d*) **An intermediate in the assembly of a coated vesicle, containing 10 of the final 36 triskelions, illustrates the packing of the clathrin triskelions.** Each of the 54 edges of a coated vesicle is constructed of two proximal and two distal arms intertwined. The 36 triskelions contain 36 × 3 = 108 proximal and 108 distal arms, and the coated vesicle has precisely 54 edges.

[*Courtesy : (a) Ernst Ungewickell and Daniel Branton, 1981, and (b), (c) and (d) Nathke, IS, Heuser J, Lupas A, Stock J., Turck CW and Brodsky EM 1992 and Darnell J, Lodish H, and Baltimore D, 1986*]

PROTEIN DEGRADATION

Proteins are constantly being degraded in all cells so as to prevent the buildup of the abnormal or unwanted proteins and to facilitate the recycling of amino acids. *Degradation is a selective process*. The lifetime of any particular protein is regulated by proteolytic systems meant for this

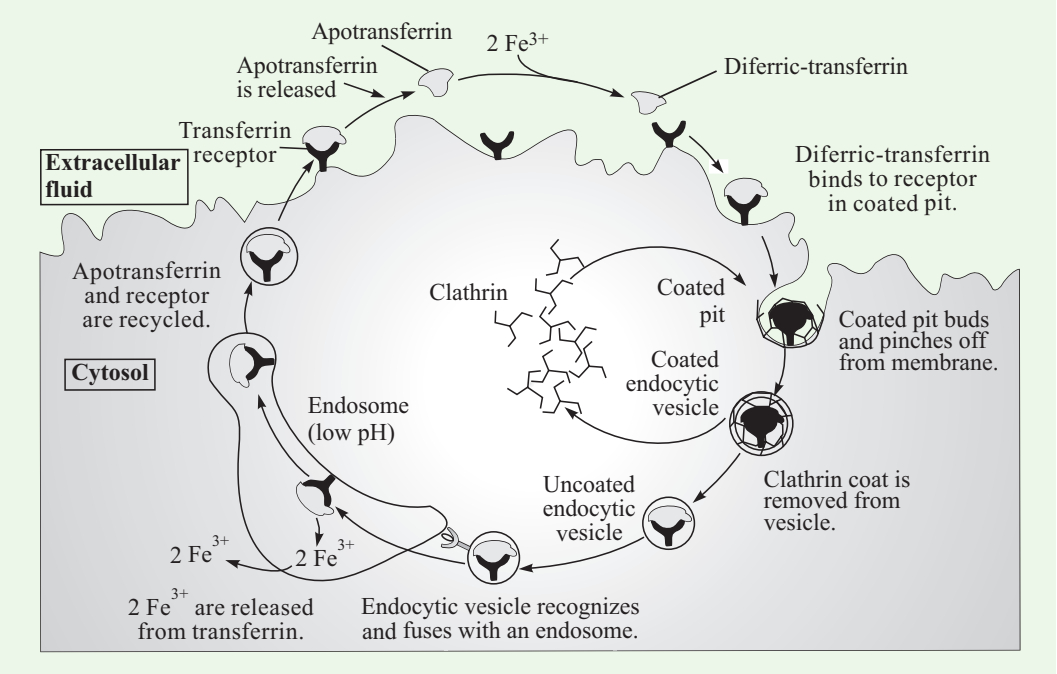


Fig. 29–27. Endocytic pathway for transferrin

Iron is released in acidic endosomes. Apotransferrin and the receptor are recycled. The transferrin cycle, thus, transports iron into the cells.

purpose as opposed to proteolytic events that might occur during post-translational processing. The proteins differ markedly in their half-lives from half a minute to many hours or even days in eukaryotes. Most proteins are turned over rapidly in relation to the lifetime of a cell, although a few stable proteins (hemoglobin, for example) can last for the life span of a cell, *i.e.*, about 110 days for an erythrocyte. The rapidly-degraded proteins include :

Table 29–2. Four modes of receptor-mediated endocytosis*

Mode	*Fate of receptor*	*Fate of protein*	*Examples*
1	Recycled	Recycled	Transferrin, Histocompatibility proteins
2	Recycled	Degraded	Low-density lipoprotein, Transcobalamin II
3	Degraded	Degraded	Epidermal growth factor, Immune complexes
4	Transported	Transported	Maternal immunoglobulin G, Immunoglobulin A

* As is evident from the table, the protein transferrin and its receptor both are eventually recycled, whereas LDL is degraded after the associated cholesterol has been delivered to its destination, but its receptor is recycled. Epidermal growth factor and its receptor are both degraded, whereas immunoglobulin A and its receptor are both transported. Hence, it may be concluded that *sorting decisions are made in endosomes*.

a. the defective proteins because of incorrect amino acid(s) insertion during synthesis, or because of a damage occurring during normal cell functioning , and

b. also many enzymes that act at key regulatory points in metabolic pathways.

Defective proteins and proteins with short half-lives are generally degraded in both bacteria and eukaryotes by *ATP-dependent cytosolic systems*. In vertebrates, however, a second system also operates in lysosomes which serves to recycle membrane proteins, extracellular proteins and proteins with long half-lives.

A. Protein Degradation in Prokaryotes

In *Escherichia coli*, many proteins are degraded by an ATP-*dependent protease* called La. The ATPase is activated only in the presence of defective proteins or those destined for rapid turnover. Two ATP moles are hydrolyzed for each peptide bond cleaved. The precise molecular function of ATP hydrolysis during peptide-bond cleavage is unclear.

B. Protein Degradation in Eukaryotes

i. Ubiquitin Proteolytic System

In eukaryotes, the ATP-dependent pathway is quite different. A key component in this system is a small protein (76 amino acids, 8.5 kd) called **ubiquitin**, so named because of its presence throughout the eukaryotic kingdoms. *Ubiquitin is one of the most highly conserved protein in evolution*, so much so that yeast and human ubiquitins differ at only 3 of 76 residues.

In vitro studies on reticulocytes (red blood cells) have established that the C-terminal glycine of ubiquitin becomes covalently linked to the -amino group of lysine residues of proteins destined for destruction. The energy for the formation of these **isopeptide bonds** (*iso* because ε-rather than α-amino groups are partners) come from ATP. This *ATP-dependent pathway* involves 3 steps, each catalyzed by a separate enzyme (Fig. 29–28). The **first step** involves activation of ubiquitin and then its linkage to the *ubiquitin activating enzyme* (E-SH). This step is reminiscent of fatty acid oxidation and amino acid activation. Activation requires ATP and formation of AMP ~ ubiquitin (an enzyme-bound intermediate) which, because of its mixed anhydride bond, is a high-energy molecule. Then, ubiquitin bonds to a specific sulfhydryl group of E_1–SH, establishing a high-energy thioester bond.

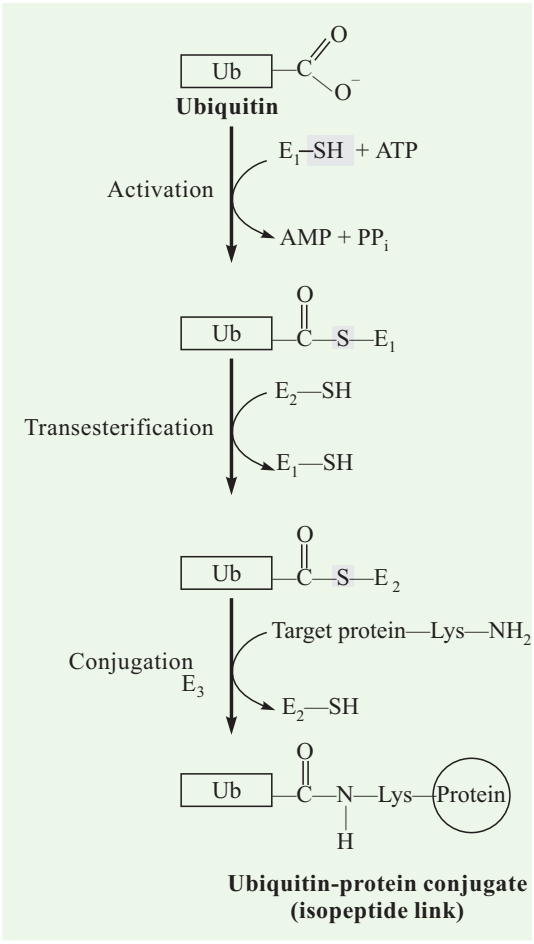
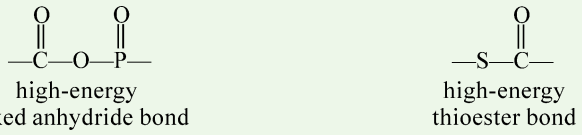


Fig. 29–28. Activation and attachment of ubiquitin to a protein targeted for degradation in eukaryotes

Two different enzyme-ubiquitin intermediates are involved. Note that the free –COOH of ubiquitin's carboxyl-terminal glycine residue is ultimately linked through an amide (isopeptide bond) to an ε-amino group of a lysine residue of the target protein.



In the **second step**, activated ubiquitin is then transferred by transesterification to a ubiquitin carrier (E_2–SH), *i.e.*, to a sulfhydryl of E_2. In the **third and last step**, E_2–SH donates the ubiquitin moiety to an acceptor protein ; the reaction being catalyzed by a conjugation enzyme (E_3). A protein tagged for destruction usually acquires several molecules of ubiquitin. The ε-amino group of a lysine residue of one ubiquitin molecule can become linked to the terminal carboxylate of another. After ubiquitin attachment, a protein is degraded, or, interestingly, deubiquitinated. Details concerning the proteolysis of ubiquitin-protein conjugates, which requires ATP, remain unclear. For example, it is not known definitely whether ubiquitin is degraded, released or reutilized. It is noteworthy that, *in the proteolytic system, ATP is required for both the formation and degradation of the ubiquitin-protein conjugate*.

The signals that trigger ubiquitination are also not all understood, but one simple one has been found. The amino terminal residue determines to a large extent the half-life of a cytosolic protein (Table 29–3). A yeast protein with Met residue at its N terminus has a half-life of more than 20 hours, whereas one with Arg residue at this position has a half-life of about 2 minutes. A highly destabilizing N-terminal residue (Arg, Leu) favours rapid ubiquitinylation, whereas a stabilizing residue (Met, Pro) does not. Proteins with an N-terminal Asp or Glu residue react with arginyl-tRNA to acquire highly destabilizing Arg as their new N terminus. Similarly, Asn and Gln are destabilizing because they are deamidated to Asp and Glu, respectively. The E_3 enzyme in the conjugation reaction is the reader of the N-terminal residues. These N-terminal residues (or signals) have been conserved during billions of years of evolution : the signals are the same in bacterial proteins degradation systems and in the human ubiquitination pathway.

Finally, the ubiquitinylated protein is digested by a 26 *S protease complex*. This ATP-driven multisubunit enzyme spares ubiquitin, which is then recycled. ATP hydrolysis is repeated many times to enable the protease to unfold the ubiquitinylated protein and gradually digest it. It is, thus, evident that *protein degradation is controlled and conducted by sophisticated molecular devices*. Also, the degradation of proteins is as important to a cell's survival in a changing environment as is the protein synthetic process.

Table 29–3. Dependence of the half-lives of cytosolic yeast proteins on the nature of their amino-terminal residue

Amino-terminal residue	*Half-life* ($t_{1/2}$)*
Highly stabilizing residues	
Met, Gly, Ala, Ser, Thr, Val, Pro, Gly	>20 hours
Intrinsically destabilizing residues	
Arg, His, Leu, Ile, Lys, Phe, Trp, Tyr	2 to 30 minutes
Destabilizing following chemical modification	
Asn, Asp, Gln, Glu	3 to 30 minutes

* Half-lives were measured in yeast for a single protein that was modified so that in each experiment it had a different N-terminal amino acid residue. Half-lives may vary for different proteins and in different organisms.

(*Adapted from Tobias JW, Schrader TE, Rocap G and Varshavsky A, 1991*)

ii Polyubiquitin System

DNA sequencing of eukaryotic genes coding for ubiquitin unexpectedly revealed that the protein is synthesized initially as a polyubiquitin precursor molecule, which is then processed to

produce individual ubiquitin molecules. For example, polyubiquitin of yeast (Fig. 29–29) contains 6 exact repeats of the 76-amino acid sequence of ubiquitin which are arranged, without spacing, in a *head-to-tail sequence*. The repeats are joined directly by the C-terminal glycine of one ubiquitin to the N-terminal methionine of the next. The only variation is the C-terminal residue of the precursor molecule which is asparagine (Asn). *Polyubiquitin organization is a common feature of eukaryotic coding sequences for ubiquitin*; only the number of repeats differ, e.g., human sequence has 9 repeats. The single residue difference at the C-terminus is also found in other eukaryotic precursor molecules, e.g., human and chicken polyubiquitin have valine and tyrosine, respectively. It has been suggested that the nonubiquitin residue at the C-terminus prevents polyubiquitin from participating in the protein-conjugating reactions before processing. However, the *Xenopus laevis* (a South African clawed toad) precursor has only ubiquitin sequences, *i.e.*, no variant C-terminus residue.

Xenopus laevis is, in fact, a species of frog, not a toad, which is frequently used in studies of early vertebrate development.

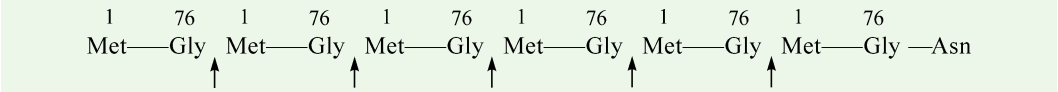


Fig 29–29. Polyubiquitin precursor protein of yeast

The 3-dimensional x-ray structures of ubiquitin and tetraubiquitin are presented in Figs. 29-30 and 29-31, respectively.

Fig. 29–30. The ribbon model of the 3–'D'structure of ubiquitin

The *white* ribbon represents the polypeptide backbone and the *red* and *blue* curves, respectively, indicate the directions of the carbonyl and amide groups.

[*Courtesy of Charles Bugg*]

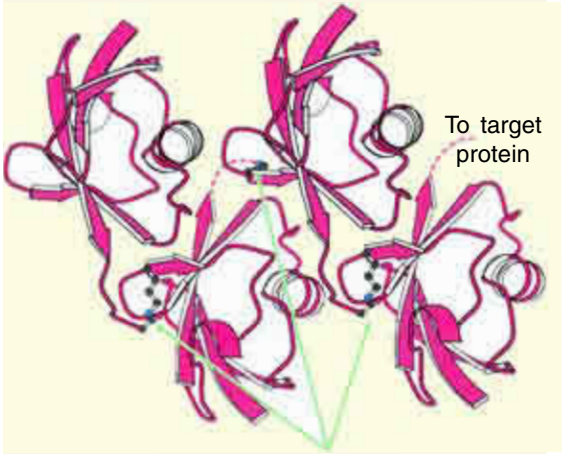


Fig. 29–31.Ribbon model of the 3–'D' structure of tetraubiquitin

Four ubiquitin molecules are linked by isopeptide bonds. This unit is the primary signal for degradation when linked to a target protein.

REFERENCES

1. **Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD :** Molecular Biology of the Cell. *Garland 1994*
2. **Bachmair A, Finlay D, Varshavsky A :** In vivo half-life of a protein is a function of its amino-terminal residue. *Science.* **234: 179-186, 1986.**

3. **Bennett MK, Scheller RH :** A molecular description of synaptic vesicle membrane trafficking. *Ann Rev. Biochem.* **63:** *63-100, 1994.*
4. **Bradshaw RA :** Protein translocation and turnover in eukaryotic cells. *Microbiol. Rev.* **55:** *543-560, 1989.*
5. **Blobel G, Walter P, Chang CN et al :** Translocation of proteins across membrane: the signal hypothesis and beyond. *Symp. Soc. Exp. Biol.* **33:** *9-36, 1979.*
6. **Blobel G, Dobberstein B :** Transfer of proteins across membrane. *J. Cell Biol.* **67:** *835-851, 1985.*
7. **Choe S, Benneth MJ, Fujii G, Curmi PM, Kantardjieff KA, Collier RJ, Eisenberg D:** The crystal structure of diphtheria toxin. *Nature.* **357:** *216-222, 1992.*
8. **Ciechanover A:** The ubiquitin-proteasome proteolytic pathway. *Cell.* **79:** *13-21, 1994.*
9. **Clarke S:** Protein isoprenylation and methylation at carboxyl-terminal cysteine residues. *Ann. Rev. Biochem.* **61:** *355-386, 1992.*
10. **Dahms NM, Lobel P, Kornfeld S:** Mannose 6-phosphate receptors and lysosomal enzyme targeting *J. Biol, Chem.* **264:** *12115-12118, 1989.*
11. **Dautry-Varsat A, Lodish HF:** How receptors bring proteins and particles into cells. *Sci. Amer.* **250 (5)** : *51-58, 1984.*
12. **Dingwall C:** Transport across the nuclear envelope: Enigmas and explanations. BioEssays. **12:** *213-218,1991.*
13. **Ferro-Novick S, Jahn R:** Vesicle fusion from yeast to man. *Nature.* **370:** *191-193, 1994.*
14. **Forbes DJ:** Structure and function of the nuclear pore complex *Ann. Rev. Cell Biol.* **8:** *495-527, 1992.*
15. **Gal S, Raikhel NV:** Protein sorting in the endomembrane system of plant cells. *Curr. Opin. Cell Biol.* **5:** *636-640, 1993.*
16. **Gething MJ, Sambrook J:** Protein folding in the cell. *Nature.* **355:** *33-45, 1992.*
17. **Gilmore R, Kellaris KV:** Translocation of proteins across and intergration of membrane proteins into the rough endoplasmic reticulum. *Ann. N.Y. Acad. Sci.* **674:** *27-37,1992.*
18. **Glick BS, Beasley EM, Schatz G:** Protein sorting in mitochondria. *Trends Biochem. Sci.* **17:** *453-459,1992.*
19. **Glick BS, Schatz G:** Import of proteins into mitochondria. *Ann. Rev. Genet.* **25:** *21-44, 1991.*
20. **Goldberg AL, Rock KL:** Proteolysis, proteasomes and antigen presentation. *Nature.* **357 :** *375-379, 1992.*
21. **Goldstein JL, Brown MS, Anderson RGW, Russell DW, Schneider WJ:** Receptor-mediated endocytosis: Concepts emerging from the LDL receptor system. *Ann. Rev. Cell Biol.* **1**:*1-39, 1985.*
22. **Hammond C, Breakman I, Helenius A**: Role of N-linked oligosacchride recognition, glucose trimming, and calnexin in glycoprotein folding and quality control. *Proc. Nat. Acad. Sci.* **91:** *913-917, 1994*
23. **Hart GW**: Glycosylation. *Curr. Opin Cell Biol.* **4:** *1017-1023, 1992*
24. **Hartmann E, Rapoport TA, Lodish HF:** Predicting the orientation of eukaryotic membrane-spanning proteins. *Proc. Nat. Acad. Sci.* **86:** *5786-5790, 1989.*
25. **Hartmann E, Sommer T, Prehn S, Gorlich D, Jentsch S, Rapoport TA:** Evolutionary conservation of components of the protein translocation complex. *Nature.* **367:** *654-657,1994.*
26. **Hendrick JP, Hartl F-U:** Molecular chaperon functions of heat-shock proteins. *Ann. Rev. Biochem.* **62:** *349-384,1993.*

27. **Hershko A, Ciechanover A:** The ubiquitin system for protein degradation. *Ann. Rev. Biochem.* **61:** *761-807,1992.*

28. **Hill WE, Dahlberg A, Garrett RA, Motre PB, Schlessinger D, Warner JR:** The Ribosome: Structure, Function and Evolution *The American Society for Microbiology, Washington, DC. 1990.*

29. **Hinshaw JE, Carragher BO, Milligan RA:** Architecture and design of the nuclear pore complex. *Cell.* **69:** *1133-1141,1992.*

30. **Hirschberg CB, Snider MD:** Topography of glycosylation in the rough endoplasmic reticulum and Golgi apparatus. *Ann. Rev. Biochem.* **56:** *63, 1987*

31. **Hurt EC, van Loon APGM:** How proteins find mitochondria and intramitochondrial compartments. *Trends Biochem. Sci.* **11:** *204-207, 1986.*

32. **Jentoff N:** Why are proteins O-glycosylated ? *Trends Biochem. Sci.* **15:** *291-294, 1990*

33. **Knight JS, Madueno F, Gray JC:** Import and sorting of protein by chloroplasts. *Biochem. Soc. Trans.* **21:** *31-36, 1993*

34. **Machamer C:** Targeting and retention of Golgi membrane proteins . *Curr. Opin. Cell Biol.* **5:** *606-612, 1993.*

35. **McKay DB:** Structure and mechanism of 70-kDa heat-shock related proteins. *Adv. Prot. Chem.* **44:** *67-98, 1993.*

36. **Mellman I, Simons K:** The Golgi complex: In vitro veritas? *Cell.* **68:** *829-840, 1992.*

37. **Miller JD, Wilhelm H, Gierasch L, Gilmore R, Walter P:** GTP binding and hydrolysis by the signal recognition particle during initiation of protein translocation. *Nature.* **366:** *351-354, 1993.*

38. **Neupert W, Hill R (editors):** New Comprehensive Biochemistry: Membrane Biogenesis and Protein Targeting *Vol. 22 Elsevier, Amsterdam. 1992.*

39. **Nunnhari J, Walter P:** Protein targeting to and translocation across the membrane of the endoplasmic reticulum. Curr. Opin. *Cell Biol* **4:** *573-580, 1992.*

40. **Nuoffer C. Balch WE:** GTPases: Multifunctional molecular switches regulating vesicular traffic. *Ann. Rev. Biochem.* **63:** *949-990, 1994.*

41. **Palade G:** Intracellular aspects of the process of protein system. *Science.* **189:** *347-358, 1975.*

42. **Palmer DJ, Helms JB, Beckers CJ, Orci L, Rothman JE:** Binding of coatomer to Golgi Membranes requires ADP-ribosylation factor. *J. Biol. Chem.* **268:** *12083-12089, 1993.*

43. **Pastan I, Willingham MC (editors):** Endocytosis. *Plenum. 1985.*

44. **Pearse BM, Robinson MS:** Clathrin, adaptors, and sorting, *Ann. Rev. Cell Biol.* **6:** *151-171,1990.*

45. **Pelham HR:** The secretion of proteins by cells. *Proc. Roy. Soc. Lond. Sec. B* **250:** *1-10, 1992.*

46. **Peters J-M: Proteosomes:** Protein degradation machines of the cell. *Trends Biochem. Sci.* **19:** *377-382, 1994.*

47. **Pfanner N, Rasson J, vander Klei IJ, Neupert W:** A dynamic model of the mitochondrial protein import machinery. *Cell.* **68:** *999-1002, 1992.*

48. **Pfeffer SR:** GTP-binding proteins in intracellular transport. *Trends Biochem. Sci.* **2:** *41-46, 1992.*

49. **Pfeffer SR, Rothman JE:** Biosynthetic protein transport and sorting by the endoplasmic reticulum and Golgi. *Ann, Rev. Biochem.* **56:** *829-852, 1987.*

50. **Pryer NK, Wuestehube LJ, Schekman R:** Vesicle-mediated protein sorting. *Ann. Rev. Biochem.* **61:** *471-516, 1992.*

51. **Pugsley AP:** The complete general secretory pathway in gram-negative bacteria. *Microbiol. Rev.* **57:** *50-108, 1993.*

52. **Randall LL, Hardy SJS:** Export of protein in bacteria. *Mirorbiol. Rev.* **48:** *290-298, 1984.*

53. **Rapoport TA:** Transport of proteins across the endoplasmic reticulum membrane *Science.* **258:** *931-936, 1992.*

54. **Rothman JE:** The compartmental organization of the Golgi apparatus. *Sci. Amer.* **253(3):** *74-89, 1985.*

55. **Rothman JE (editor):** Methods in Enzymology. vol. 219: Reconstitution of Intracellular Transport. *Academic Press, Inc., New York. 1992.*

56. **Rothman JE:** Mechanisms of intracellular protein transport. *Nature.* **372:** *59-67, 1994.*

57. **Schafer WR, Rine J:** Protein prenylation : Genes, enzymes, targets, and functions. *Ann. Rev. Genet.* **26:** *209-237, 1992.*

58. **Schatz G:** The protein import machinery of mitochondria. *Protein Sci.* **2:** *141-146, 1993.*

59. **Schmidt GW, Mishkind ML:** The transport of proteins into chloroplasts. *Ann. Rev. Biochem.* **55:** *879-912, 1986.*

60. **Silver PA:** How proteins enter the nucleus. *Cell* **64:** *489-497, 1991.*

61. **Simon SM, Blobel G:** A protein-conducting channel in the endoplasmic reticulum. *Cell.* **65:** *371-380, 1991.*

62. **Simon SM, Blobel G:** Signal peptides open protein-conducting channels in *E coli. Cell.* **69:** *677-684, 1992.*

63. **Simons K, Garoff H, Helenius A:** How an animal virus gets into and out of its host cell. *Sci. Amer.* **246(2):** *58-66, 1982.*

64. **Smeeckens S, Weisbeek P, Robinson C:** Protein transport into and within chloroplasts. *Trends Biochem. Sci.* **15:** *73-76, 1990.*

65. **Spirin AS:** Ribosomal translocation: Factors and models. *Nucleic Acid Res. Mol. Biol.* **32:** *75, 1985.*

66. **van HeijneG:** Signal sequences: the limits of variation. *J. Mol. Biol.* **184:** *99-105, 1985.*

67. **van HeijneG:** Protein targeting signals. *Curr. Opin Cell Biol.* **2:** *604-608,1990.*

68. **van Meer G:** Transport and sorting of membrane lipids. *Curr. Opin. Cell Biol.* **5:** *661-673, 1993.*

69. **Varshavsky A:** The N-end rule. *Cell.* **69:** *725-735, 1992.*

70. **Ward WHJ:** Diphtheria toxin: A novel cytocidal enzyme. *Trends Biochem. Sci.* **12:** *28-31, 1987.*

71. **Welch W, Georgopoulos C:** Heat-shock proteins or chaperones. *Ann. Rev. Cell Biol.* **9:** *601-634, 1993.*

72. **Wickner W, Driessen AJ, Hartl FU:** The enzymology of protein translocation across the Escherichia coli plasma membrane. Ann. Rev. Biochem. **60:** *101-124, 1991.*

73. **Wickner WT, Lodish HF:** Multiple mechanisms of protein insertion into and across membranes. *Science.* **230:** *400-407, 1985.*

74. **Wolin SL:** From the elephant to *E.coli:* SRP-dependent protein targeting. *Cell* **77:** *787-790, 1994.*

PROBLEMS

1. I-cell (inclusion bodies) disease results from a defect in the enzyme that transfers *N*-acetylglucosamine phosphate to proteins containing high mannose-type oligosaccharides. What enzymes would be deficient in I-cell patients ? How does the disease name correspond to the problem in these patients ?
2. What is the significance of the following structures ? A positively-charged amphiphilic α-helix, a cluster of lysine and arginine residues, and a carboxy-terminal Ser-Lys-Leu (SKL) sequence ?