Rapid Translation of a Single Message by Polysomes Large clusters of 10 to 100 ribosomes that are very active in protein synthesis can be isolated from both eukaryotic and bacterial cells. Electron micrographs show a fiber between adjacent ribosomes in the cluster, which is called a **polysome (Fig. 27-34a)**. The connecting strand is a single molecule of mRNA that is being translated simultaneously by many closely spaced ribosomes, allowing the highly efficient use of the mRNA.

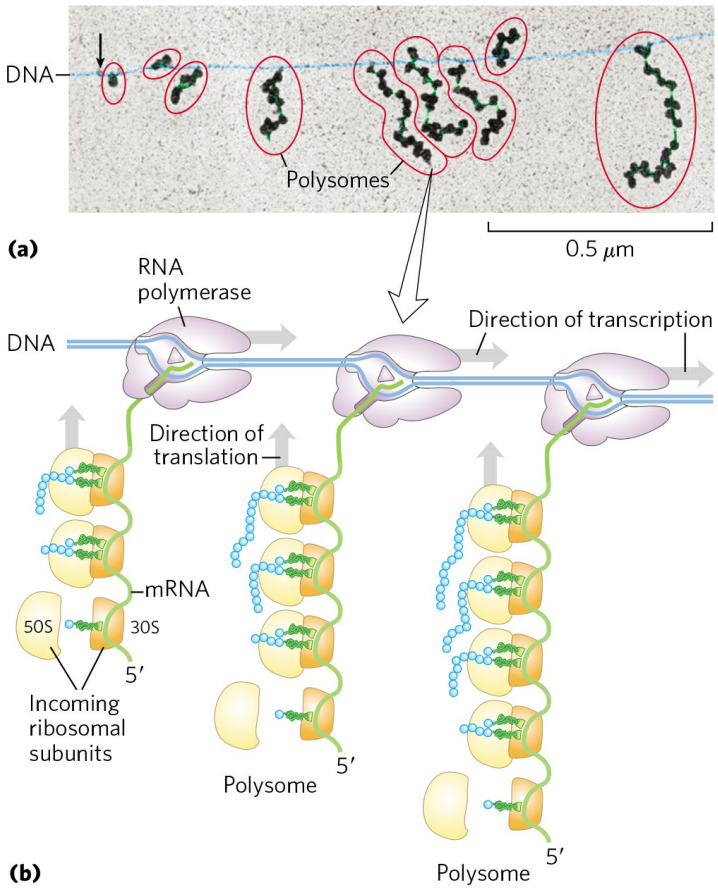


FIGURE 27-34 Proof that polypeptides grow by addition of amino acid residues to the carboxyl end: the Dintzis experiment. Reticulocytes (immature erythrocytes) actively synthesizing hemoglobin were incubated with radioactive leucine (selected because it occurs frequently in both the α- and β-globin chains). Samples of completed α chains were isolated from the incubating reticulocytes at various times, and the distribution of radioactivity determined. The dark red zones show the portions of completed α-globin chains containing radioactive Leu residues. At 4 min, only a few residues at the carboxyl end of α-globin were labeled, because the only *complete* globin chains with incorporated label after 4 min were those that had nearly completed synthesis at the time the label was added. With longer incubation times, successively longer segments of the polypeptide contained labeled residues, always in a block at the carboxyl end of the chain. The unlabeled end of the polypeptide (the amino terminus) was thus defined as the initiating end, which means that polypeptides grow by successive addition of amino acids to the carboxyl end.

In bacteria, transcription and translation are tightly coupled. Messenger RNAs are synthesized and translated in the same 5′→3′ direction. Ribosomes begin translating the 5′ end of the mRNA before transcription is complete

(Fig. 27-34b). The situation is quite different in eukaryotic cells, where newly transcribed mRNAs must leave the nucleus before they can be translated.

Bacterial mRNAs generally exist for just a few minutes (p. 1062) before they are degraded by nucleases. To maintain high rates of protein synthesis, the mRNA for a given protein or set of proteins must be made continuously and translated with maximum efficiency. The short lifetime of mRNAs in bacteria allows a rapid cessation of synthesis when the protein is no longer needed.

Stage 5: Newly Synthesized Polypeptide Chains Undergo Folding and Processing

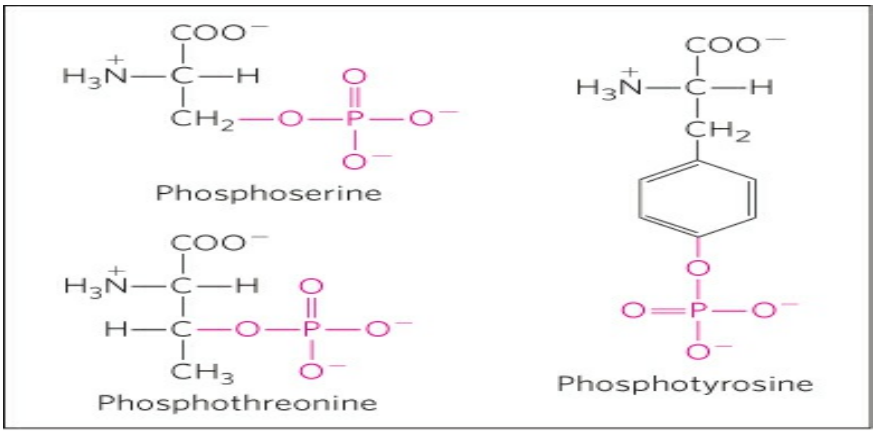
In the final stage of protein synthesis, the nascent polypeptide chain is folded and processed into its biologically active form. During or after its synthesis, the polypeptide progressively assumes its native conformation, with the formation of appropriate hydrogen bonds and van der Waals and ionic interactions, and through the hydrophobic effect. Protein chaperones play an important role in correct folding in all cells. Some newly madeproteins, bacterial, archaeal, and eukaryotic, do not attain their final biologically active conformation until they have been altered by one or more processing reactions called **posttranslational modifications**.

Amino-Terminal and Carboxyl-Terminal Modifications

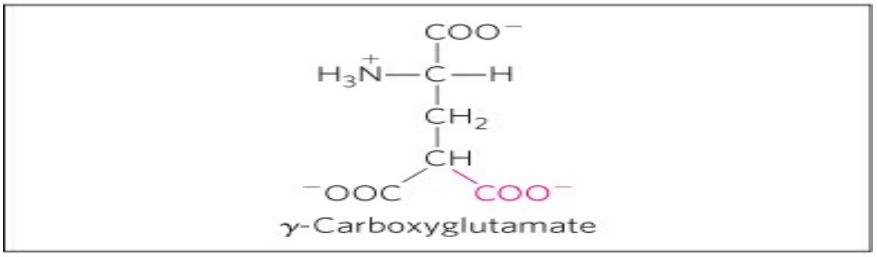
The first residue inserted in all polypeptides is *N*-formylmethionine (inbacteria) or methionine (in eukaryotes). However, the formyl group, theamino-terminal Met residue, and often additional amino-terminal (and, insome cases, carboxyl-terminal) residues may be removed enzymatically information of the final functional protein. In as many as 50% of eukaryoticproteins, the amino group of the amino-terminal residue is *N*-acetylated aftertranslation. Carboxyl-terminal residues are also sometimes modified.

Loss of Signal Sequences As we shall see in Section 27.3, the 15 to 30 residues at the amino-terminal end of some proteins play a role in directing the protein to its ultimate destination in the cell. Such **signal sequences** are eventually removed by specific peptidases.

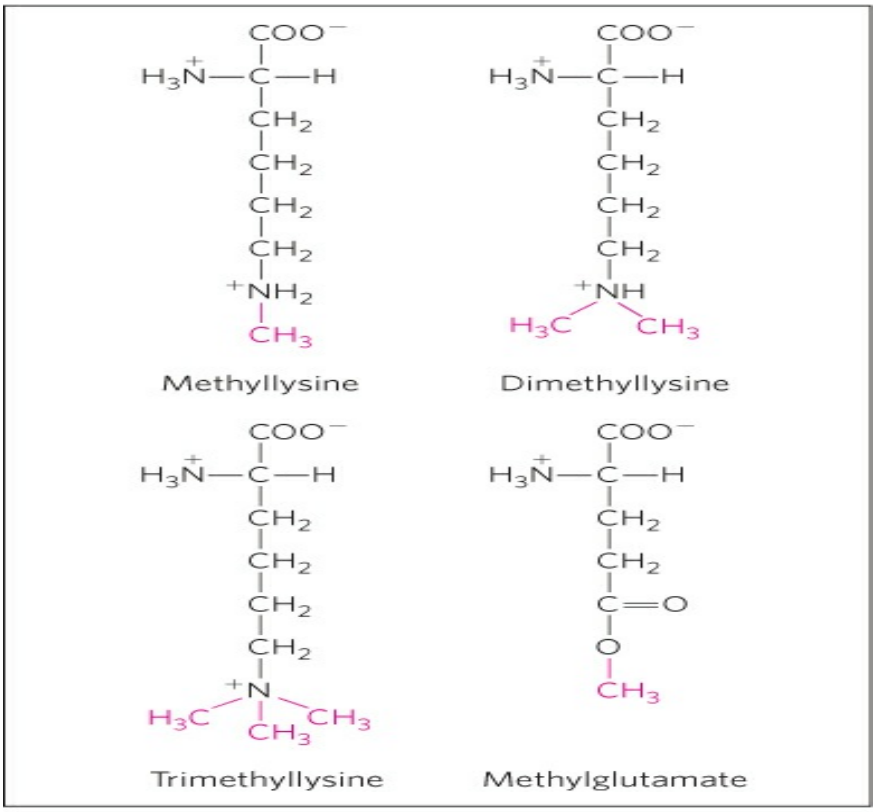
Modification of Individual Amino Acid Residues The hydroxyl groups of certain Ser, Thr, and Tyr residues of some proteins are enzymatically phosphorylated by ATP **(Fig. 27-35a)**; the phosphate groups add negative charges to these polypeptides. The functional significance of this modification varies from one protein to the next. For example, the milk protein casein has many phosphoserine groups that bind Ca^{2+}. Calcium, phosphate, and amino acids are all valuable to suckling young, so casein efficiently provides three essential nutrients. And as we have seen in numerous instances, phosphorylation-dephosphorylation cycles regulate the activity of many enzymes and regulatory proteins.


Phosphoserine
Phosphothreonine
Phosphotyrosine

(a)


γ-Carboxyglutamate

(b)


Methyllysine
Dimethyllysine
Trimethyllysine
Methylglutamate

(c)

FIGURE 27-35 Some modified amino acid residues. (a) Phosphorylated amino acids. **(b)** A carboxylated amino acid. **(c)** Some methylated amino acids.

Extra carboxyl groups may be added to Glu residues of some proteins. For example, the blood-clotting protein prothrombin contains γ-carboxyglutamate residues (Fig. 27-35b) in its amino-terminal region; the γ-carboxyl groups are introduced by an enzyme that requires vitamin K. These carboxyl groups bind Ca^{2+}, which is required to initiate the clotting mechanism.

Monomethyl- and dimethyllysine residues (Fig. 27-35c) occur in some muscle proteins and in cytochrome *c*. The calmodulin of most species contains one trimethyllysine residue at a specific position. In other proteins, the carboxyl groups of some Glu residues undergo methylation, removing their negative charge.

Attachment of Carbohydrate Side Chains The carbohydrate side chains of glycoproteins are attached covalently during or after synthesis of the polypeptide. In some glycoproteins, the carbohydrate side chain is attached enzymatically to Asn residues (*N*-linked oligosaccharides), in others to Ser or Thr residues (*O*-linked oligosaccharides) (see Fig. 7-30). Many proteins that function extracellularly, as well as the lubricating proteoglycans that coat mucous membranes, contain oligosaccharide side chains (see Fig. 7-28).

Addition of Isoprenyl Groups Some eukaryotic proteins are modified by the addition of groups derived from isoprene (isoprenyl groups). A thioether bond is formed between the isoprenyl group and a Cys residue of the protein (see Fig. 11-13). The isoprenyl groups are derived from pyrophosphorylated intermediates of the cholesterol biosynthetic pathway (see Fig. 21-35), such as farnesyl pyrophosphate **(Fig. 27-36)**. Proteins modified in this way include the Ras proteins (small G proteins), which are products of the *ras* oncogenes and proto-oncogenes, and the trimeric G proteins (both discussed in Chapter 12), as well as lamins, proteins found in the nuclear matrix. The isoprenyl group helps to anchor the protein in a membrane. The transforming (carcinogenic) activity of the *ras* oncogene is lost when isoprenylation of the Ras protein is blocked, a finding that has

stimulated interest in identifying inhibitors of this posttranslational modification pathway for use in cancer chemotherapy.

Addition of Prosthetic Groups Many proteins require for their activity covalently bound prosthetic groups. Two examples are the biotin molecule of acetyl-CoA carboxylase and the heme group of hemoglobin or cytochrome *c*.

Proteolytic Processing Many proteins are initially synthesized as large, inactive precursor polypeptides that are proteolytically trimmed to form their smaller, active forms. Examples include proinsulin, some viral proteins, and proteases such as chymotrypsinogen and trypsinogen (see Fig. 6-39).

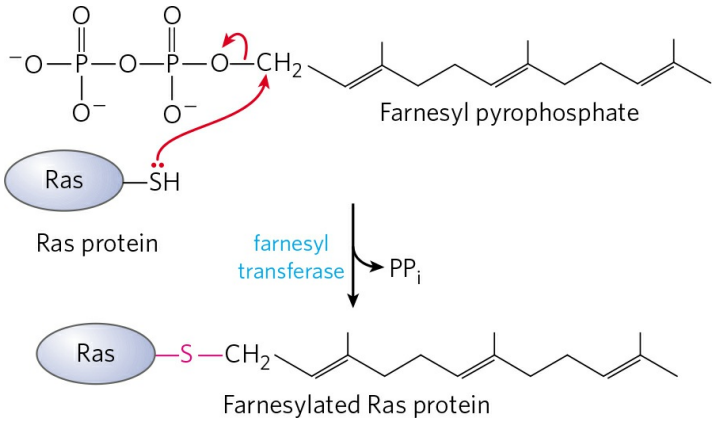


FIGURE 27-36 Farnesylation of a Cys residue. The thioether linkage is shown in red. The Ras protein is the product of the ras *oncogene*.

Formation of Disulfide Cross-Links After folding into their native conformations, some proteins form intrachain or interchain disulfide bridges between Cys residues. In eukaryotes, disulfide bonds are common in proteins to be exported from cells. The cross-links formed in this way help to protect the native conformation of the protein molecule from denaturation in the extracellular environment, which can differ greatly from intracellular conditions and is generally oxidizing.

Ribosome Profiling Provides a Snapshot of Cellular Translation

Modern DNA sequencing methods can be applied in a variety of creative ways to allow researchers to study information pathways. One application, called **ribosome profiling**, identifies the mRNA sequences that are being translated at a particular moment in a cell **(Fig. 27-37)**. Researchers harvest the cells or tissue and rapidly isolate the ribosomes, with their bound mRNAs. Ribonucleases are used to remove any RNA that is not bound by the ribosomes, and the RNA bound and protected by the ribosomes is then isolated. This RNA is converted into DNA by reverse transcriptase (see Chapter 26), then subjected to deep sequencing (p. 310). Not only do the resulting sequences reveal the parts of genes that are being translated at a particular moment, but the relative number of reads for each segment indicates the relative proportions in which the segments are being translated. As an example, all of the eight different subunits of the bacterial F_0F_1-ATPase (see Fig. 19-25) are encoded in a single operon and translated from a single, polycistronic mRNA. We might assume, then, that all the subunit proteins, translated from this mRNA, would be synthesized at similar levels. But the subunits are not present in equal numbers in the final complex; the bacterial F_0F_1-ATPase has 10 c subunits, 3 each of the α and β subunits, and 1 or 2 of the other subunits. Ribosome profiling shows that the eight genes within the mRNA are translated in the ratio 1:1:1:1:2:3:3:10. Translation of the individual genes is thus adjusted to produce the amounts of each subunit that correspond precisely to the subunit ratios in the final complex. Similar results have been obtained for many different macromolecular protein complexes in a variety of organisms.

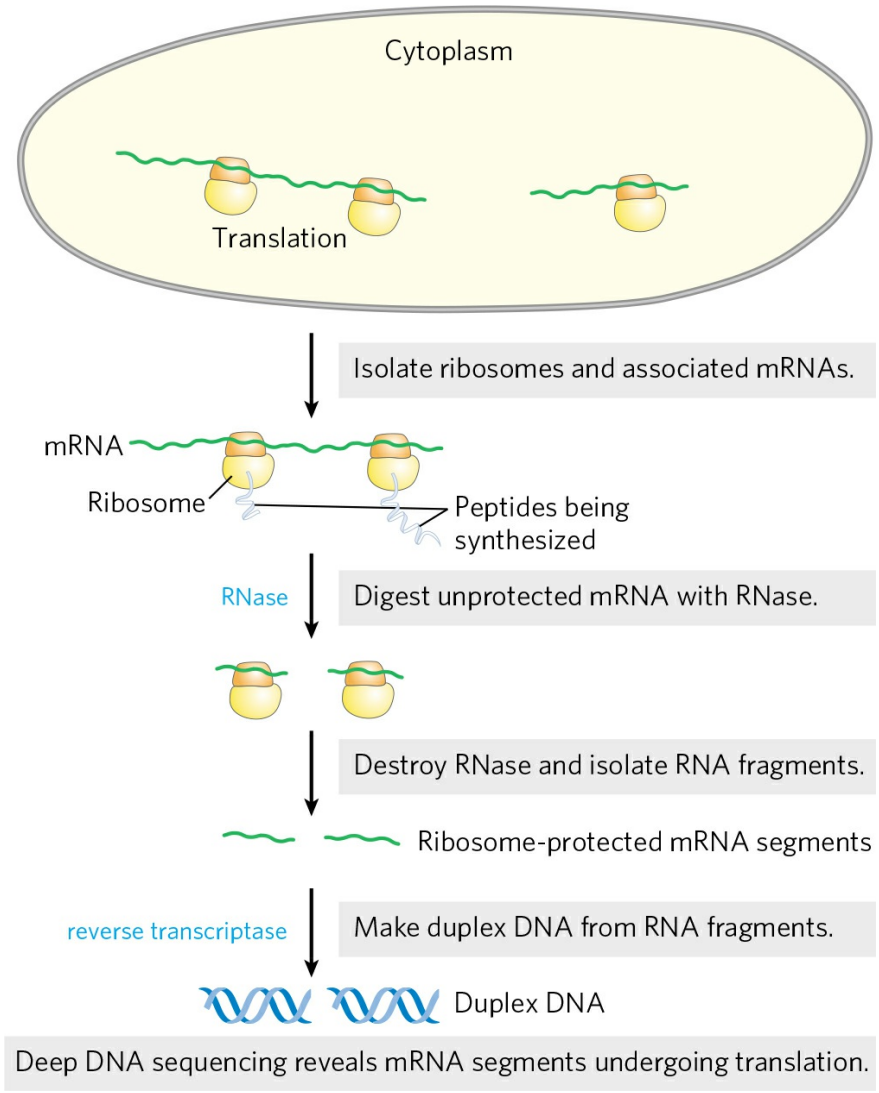


FIGURE 27-37 Ribosome profiling. This technique makes use of modern DNA sequencing methods to determine the mRNAs in a cell that are being translated at a given time. After ribosomes and associated RNA are isolated, ribonucleases are used to remove all RNA that is not bound to and thus protected by the ribosomes. The protected RNA segments are then separated from the ribosomes and converted to DNA by reverse transcriptase, and the DNA is subjected to deep sequencing (see Chapter 8).

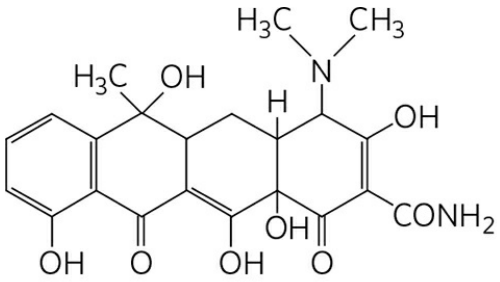
Protein Synthesis Is Inhibited by Many Antibiotics and Toxins

Protein synthesis is a central function in cellular physiology and is the primary target of many naturally occurring antibiotics and toxins. Except as noted otherwise, these antibiotics inhibit protein synthesis in bacteria. The

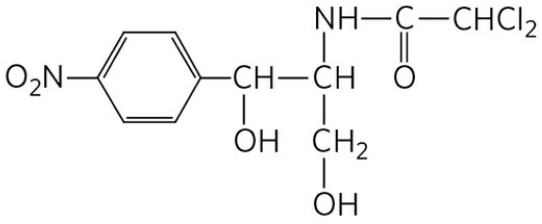
differences between bacterial and eukaryotic protein synthesis, though in some cases subtle, are such that most of the compounds discussed below are relatively harmless to eukaryotic cells. Natural selection has favored the evolution of compounds that exploit minor differences in order to affect bacterial systems selectively, so that these biochemical weapons are synthesized by some microorganisms and are extremely toxic to others. Because nearly every step in protein synthesis can be specifically inhibited by one antibiotic or another, antibiotics have become valuable tools in the study of protein biosynthesis.

Puromycin, made by the mold *Streptomyces alboniger*, is one of the best-understood inhibitory antibiotics. Its structure is very similar to the 3′ end of an aminoacyl-tRNA, enabling it to bind to the ribosomal A site and participate in peptide bond formation, producing peptidylpuromycin **(Fig. 27-38)**. However, because puromycin resembles only the 3′ end of the tRNA, it does not engage in translocation and dissociates from the ribosome shortly after it is linked to the carboxyl terminus of the peptide. This prematurely terminates polypeptide synthesis.

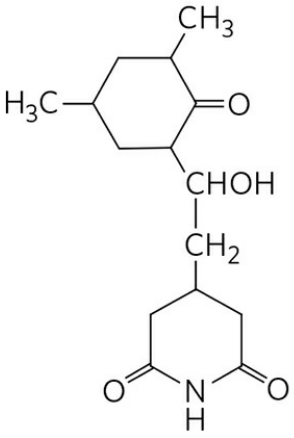
Tetracyclines inhibit protein synthesis in bacteria by blocking the A site on the ribosome, preventing the binding of aminoacyl-tRNAs. **Chloramphenicol** inhibits protein synthesis by bacterial (and mitochondrial and chloroplast) ribosomes by blocking peptidyl transfer; it does not affect cytosolic protein synthesis in eukaryotes. Conversely, **cycloheximide** blocks the peptidyl transferase of 80S eukaryotic ribosomes but not that of 70S bacterial (and mitochondrial and chloroplast) ribosomes. **Streptomycin**, a basic trisaccharide, causes misreading of the genetic code (in bacteria) at relatively low concentrations and inhibits initiation at higher concentrations.



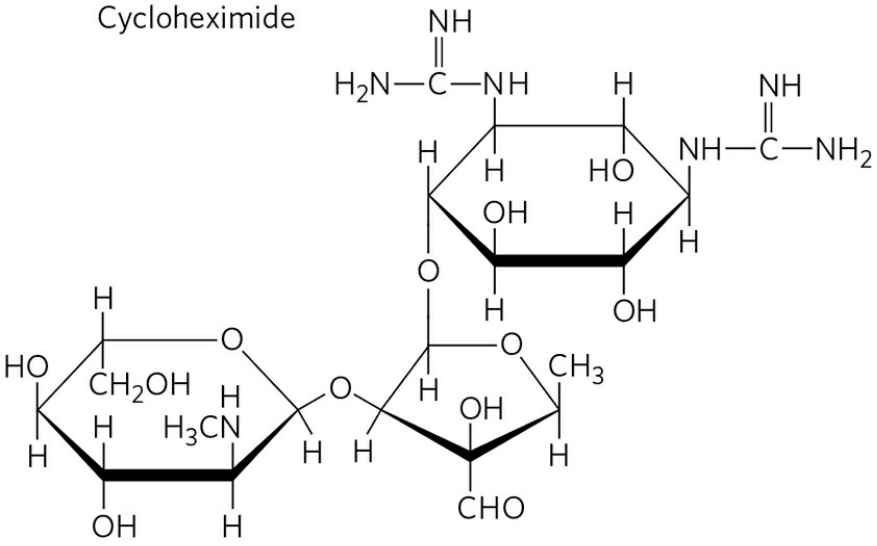
Tetracycline



Chloramphenicol



Cycloheximide



Streptomycin

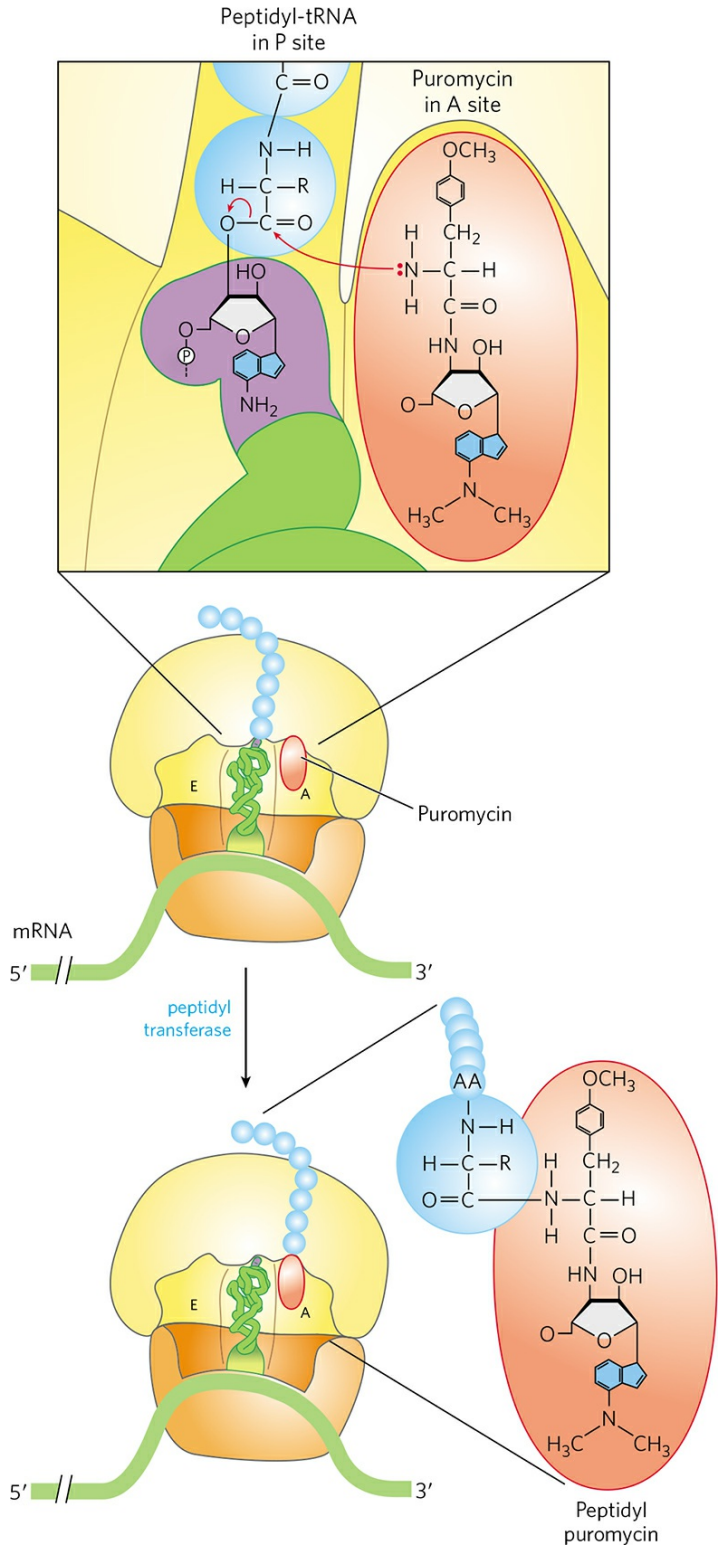


FIGURE 27-38 Disruption of peptide bond formation by puromycin. The antibiotic puromycin resembles the aminoacyl end of a charged tRNA, and it can bind to the ribosomal A site and participate in peptide bond formation. The

product of this reaction, peptidyl puromycin, is not translocated to the P site. Instead, it dissociates from the ribosome, causing premature chain termination.

Several other inhibitors of protein synthesis are notable because of their toxicity to humans and other mammals. **Diphtheria toxin** (M_r 58,330) catalyzes the ADP-ribosylation of a diphthamide (a modified histidine) residue of eukaryotic elongation factor eEF2, thereby inactivating it. **Ricin** (M_r 29,895), an extremely toxic protein of the castor bean, inactivates the 60S subunit of eukaryotic ribosomes by depurinating a specific adenosine residue in 28S rRNA. Ricin was used in the infamous 1978 murder of BBC journalist and Bulgarian dissident Georgi Markov, presumably by the Bulgarian secret police. Using a syringe hidden at the end of an umbrella, a member of the secret police injected Markov in the leg with a ricin-infused pellet. He died four days later.

SUMMARY 27.2 Protein Synthesis

■ Protein synthesis occurs on the ribosomes, which consist of protein and rRNA. Bacteria have 70S ribosomes, with a large (50S) and a small (30S) subunit. Eukaryotic ribosomes are significantly larger (80S) and contain more proteins.

■ Transfer RNAs have 73 to 93 nucleotide residues, some of which have modified bases. Each tRNA has an amino acid arm with the terminal sequence CCA(3′) to which an amino acid is esterified, an anticodon arm, a TψC arm, and a D arm; some tRNAs have a fifth arm. The anticodon is responsible for the specificity of interaction between the aminoacyl-tRNA and the complementary mRNA codon.

■ The growth of polypeptides on ribosomes begins with the amino-terminal amino acid and proceeds by successive additions of new residues to the carboxyl-terminal end.

■ Protein synthesis occurs in five stages.

1. Amino acids are activated by specific aminoacyl-tRNA synthetases in the cytosol. These enzymes catalyze the formation of aminoacyl-tRNAs, with simultaneous cleavage of ATP to AMP and PP_i. The fidelity of protein synthesis depends on the accuracy of this reaction, and some of these enzymes carry out proofreading steps at separate active sites.

2. In bacteria, the initiating aminoacyl-tRNA in all proteins is *N*-formylmethionyl-$tRNA^{fMet}$. Initiation of protein synthesis involves formation of a complex between the 30S ribosomal subunit, mRNA, GTP, fMet-$tRNA^{fMet}$, three initiation factors, and the 50S subunit; GTP is hydrolyzed to GDP and P_i.

3. In the elongation steps, GTP and elongation factors are required for binding the incoming aminoacyl-tRNA to the A site on the ribosome. In the first peptidyl transfer reaction, the fMet residue is transferred to the amino group of the incoming aminoacyl-tRNA. Movement of the ribosome along the mRNA then translocates the dipeptidyl-tRNA from the A site to the P site, a process requiring hydrolysis of GTP. Deacylated tRNAs dissociate from the ribosomal E site.

4. After many such elongation cycles, synthesis of the polypeptide is terminated with the aid of release factors. At least four high-energy phosphate equivalents (from ATP and GTP) are required to generate each peptide bond, an energy investment required to guarantee fidelity of translation.

5. Polypeptides fold into their active, three-dimensional forms. Many proteins are further processed by posttranslational modification reactions.

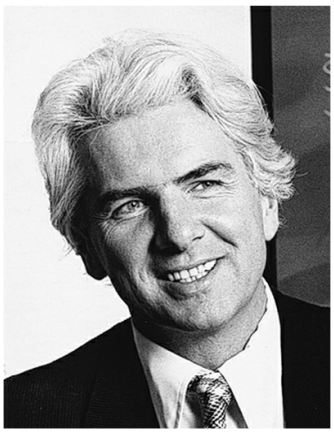
- Ribosome profiling allows investigators to determine which gene sequences are being translated at any particular moment.
- Many well-studied antibiotics and toxins inhibit some aspect of protein synthesis.

27.3 Protein Targeting and Degradation

The eukaryotic cell is made up of many structures, compartments, and organelles, each with specific functions that require distinct sets of proteins and enzymes. These proteins (with the exception of those produced in mitochondria and plastids) are synthesized on ribosomes in the cytosol, so how are they directed to their final cellular destinations?

We are now beginning to understand this complex and fascinating process. Proteins destined for secretion, integration in the plasma membrane, or inclusion in lysosomes generally share the first few steps of a pathway that begins in the endoplasmic reticulum. Proteins destined for mitochondria, chloroplasts, or the nucleus use three separate mechanisms. And proteins destined for the cytosol simply remain where they are synthesized.

The most important element in many of these targeting pathways is a short sequence of amino acids called a **signal sequence**, whose function was first postulated by Günter Blobel and colleagues in 1970. The signal sequence directs a protein to its appropriate location in the cell and, for many proteins, is removed during transport or after the protein has reached its final destination. In proteins slated for transport into mitochondria, chloroplasts, or the ER, the signal sequence is at the amino terminus of a newly synthesized polypeptide. In many cases, the targeting capacity of particular signal sequences has been confirmed by fusing the signal sequence from one protein to a second protein and showing that the signal directs the second protein to the location where the first protein is normally found. The selective degradation of proteins no longer needed by the cell also relies largely on a set of molecular signals embedded in each protein's structure.



Günter Blobel
[Source: Courtesy of Günter Blobel, The Rockefeller University.]

In this concluding section we examine protein targeting and degradation, emphasizing the underlying signals and molecular regulation that are so crucial to cellular metabolism. Except where noted, the focus is now on eukaryotic cells.

Posttranslational Modification of Many Eukaryotic Proteins Begins in the Endoplasmic Reticulum

Perhaps the best-characterized targeting system begins in the ER. Most lysosomal, membrane, or secreted proteins have an amino-terminal signal sequence **(Fig. 27-39)** that marks them for translocation into the lumen of the ER; hundreds of such signal sequences have been determined. The carboxyl terminus of the signal sequence is defined by a cleavage site, where protease action removes the sequence after the protein is imported into the ER. Signal sequences vary in length from 13 to 36 amino acid residues, but all have the following features: (1) about 10 to 15 hydrophobic amino acid residues; (2) one or more positively charged residues, usually near the amino terminus, preceding the hydrophobic sequence; and (3) a short sequence at the carboxyl terminus (near the cleavage site) that is relatively polar, typically having amino acid residues with short side chains (especially Ala) at the positions closest to the cleavage site.

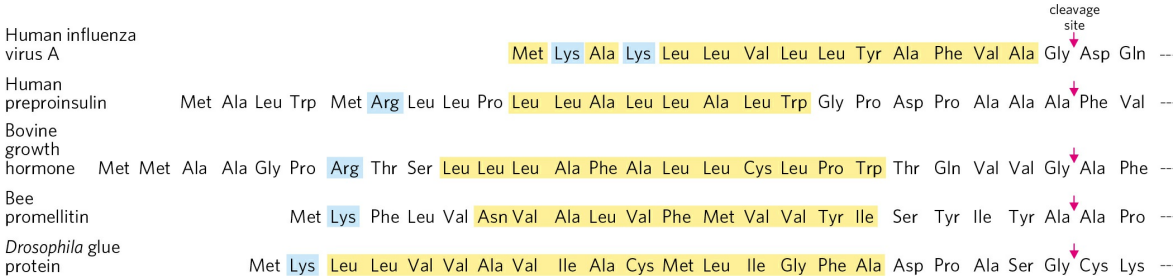


FIGURE 27-39 Amino-terminal signal sequences of some eukaryotic proteins that direct their translocation into the ER. The hydrophobic core (yellow) is preceded by one or more basic residues (blue). Polar and short-side-chain residues immediately precede (to the left, as shown here) the cleavage sites (indicated by red arrows).

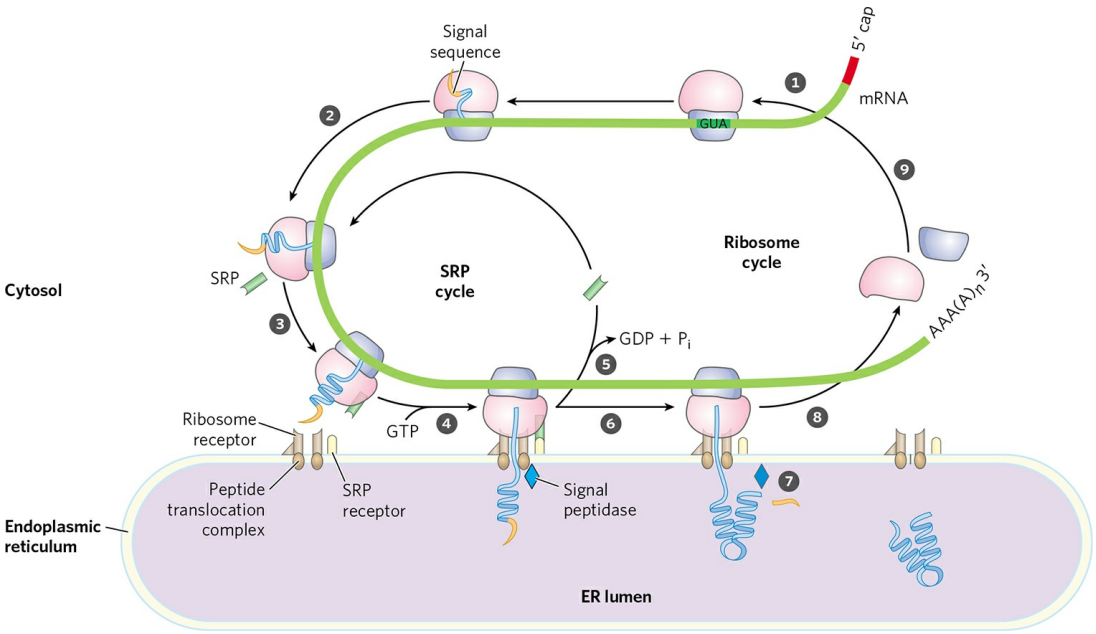
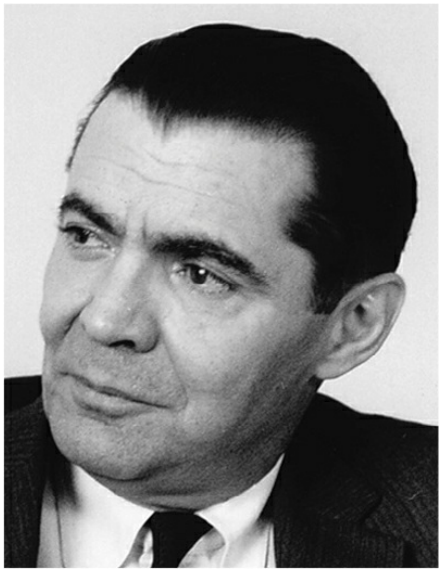


FIGURE 27-40 Directing eukaryotic proteins with the appropriate signals to the endoplasmic reticulum. This process involves the SRP cycle and the translocation and cleavage of the nascent polypeptide. The steps are described in the text. SRP is a rod-shaped complex containing a 300 nucleotide RNA (7SL-RNA) and six different proteins (combined M_r 325,000). One protein subunit of SRP binds directly to the signal sequence, obstructing elongation by sterically blocking the entry of aminoacyl-tRNAs and inhibiting peptidyl transferase. Another protein subunit binds and hydrolyzes GTP. The SRP receptor is a heterodimer of α (M_r 69,000) and β (M_r 30,000) subunits, both of which bind and hydrolyze multiple GTP molecules during this process.



George Palade, 1912–2008

[Source: AP Photo.]

As originally demonstrated by George Palade, proteins with these signal sequences are synthesized on ribosomes attached to the ER. The signal sequence itself helps to direct the ribosome to the ER, as illustrated in Figure 27-40. The targeting pathway begins in step 1, with initiation of protein synthesis on free ribosomes. The signal sequence appears early in the synthetic process (step 2), because it is at the amino terminus, which, as we have seen, is synthesized first. As it emerges from the ribosome (step 3), the signal sequence—and the ribosome itself—is bound by the large **signal recognition particle (SRP)**; SRP then binds GTP and halts elongation of the polypeptide when it is about 70 amino acids long and the signal sequence has completely emerged from the ribosome. In step 4, the GTP-bound SRP directs the ribosome (still bound to the mRNA) and the incomplete polypeptide to GTP-bound SRP receptors in the cytosolic face of the ER; the nascent polypeptide is delivered to a **peptide translocation complex** in the ER, which interacts directly with the ribosome. In step 5, SRP dissociates from the ribosome, accompanied by hydrolysis of GTP in both SRP and the SRP receptor. Elongation of the polypeptide now resumes (step 6), with the ATP-driven translocation complex feeding the growing polypeptide into the ER lumen until the complete protein has been synthesized. In step 7, the signal sequence is removed by a signal peptidase within the ER lumen. The ribosome dissociates (step 8) and is recycled (step 9).

Glycosylation Plays a Key Role in Protein Targeting

In the ER lumen, newly synthesized proteins are further modified in several ways. Following the removal of signal sequences, polypeptides are folded, disulfide bonds formed, and many proteins glycosylated to form glycoproteins. In many glycoproteins, the linkage to their oligosaccharides is through Asn residues. These *N*-linked oligosaccharides are diverse (Chapter 7), but the pathways by which they form have a common first step. A 14 residue core oligosaccharide is built up stepwise, then transferred from a dolichol phosphate donor molecule to certain Asn residues in the protein **(Fig. 27-41)**. The transferase is on the lumenal face of the ER and thus cannot catalyze glycosylation of cytosolic proteins. After transfer, the core oligosaccharide is trimmed and elaborated in different ways on different proteins, but all *N*-linked oligosaccharides retain a pentasaccharide core

derived from the original 14 residue oligosaccharide. Several antibiotics act by interfering with one or more steps in this process and have aided in elucidating the steps of protein glycosylation. The best characterized is **tunicamycin**, which mimics the structure of UDP-*N*-acetylglucosamine and blocks the first step of the process (Fig. 27-41, step 1). A few proteins are *O*-glycosylated in the ER, but most *O*-glycosylation occurs in the Golgi complex or in the cytosol (for proteins that do not enter the ER).

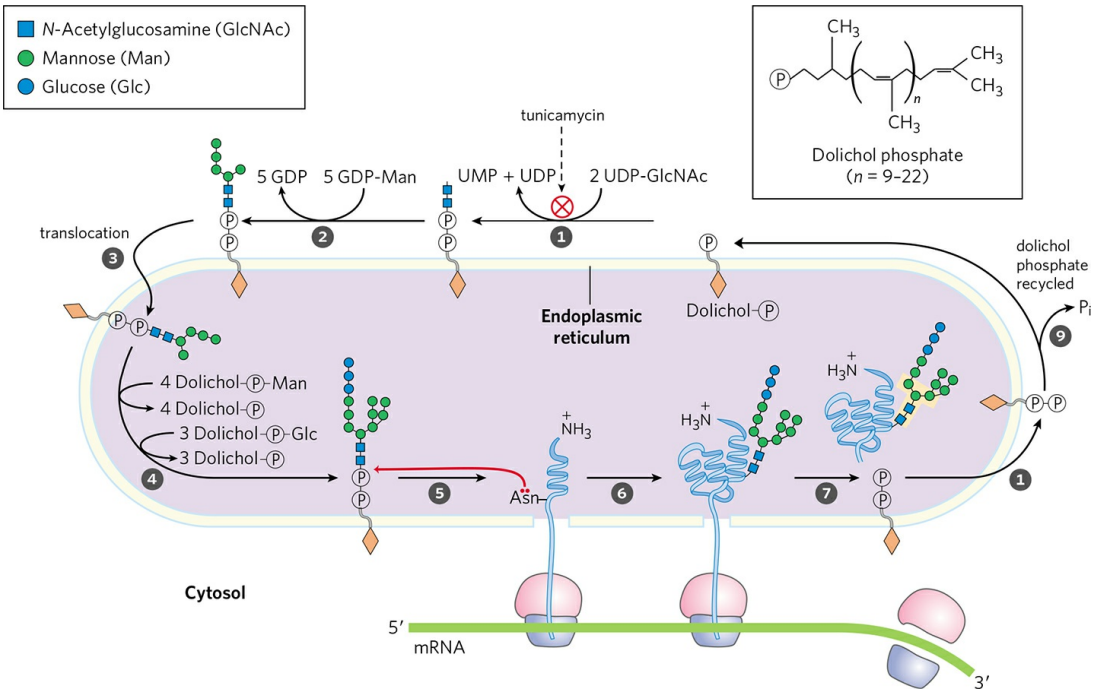
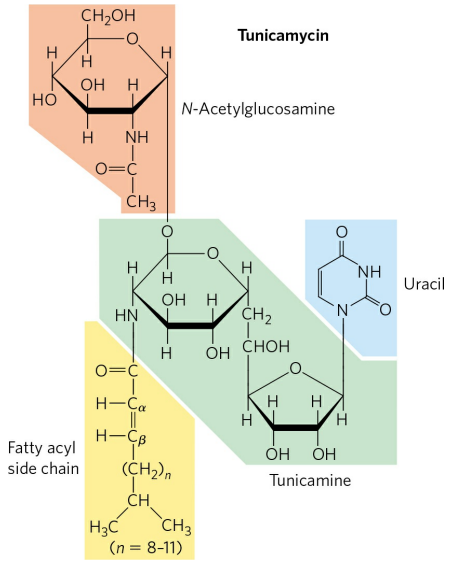


FIGURE 27-41 Synthesis of the core oligosaccharide of glycoproteins. The core oligosaccharide is built up by the successive addition of monosaccharide units. 1, 2 The first steps occur on the cytosolic face of the ER. 3 Translocation moves the incomplete oligosaccharide across the membrane (mechanism not shown), and 4 completion of the core oligosaccharide occurs within the lumen of the ER. The precursors that contribute additional mannose and glucose residues to the growing oligosaccharide in the lumen are dolichol phosphate derivatives. In the first step in construction of the *N*-linked oligosaccharide moiety of a glycoprotein, 5, 6 the core oligosaccharide is transferred from dolichol phosphate to an Asn residue of the protein within the ER lumen. The core oligosaccharide is then further modified in the ER and the Golgi complex in pathways that differ for different proteins. The five sugar residues shown surrounded by a beige screen, after step 7, are retained in the final structure of all *N*-linked oligosaccharides. 8 The released dolichol pyrophosphate is again translocated so that the pyrophosphate is on the

cytosolic face of the ER, then ❾ a phosphate is hydrolytically removed to regenerate dolichol phosphate.



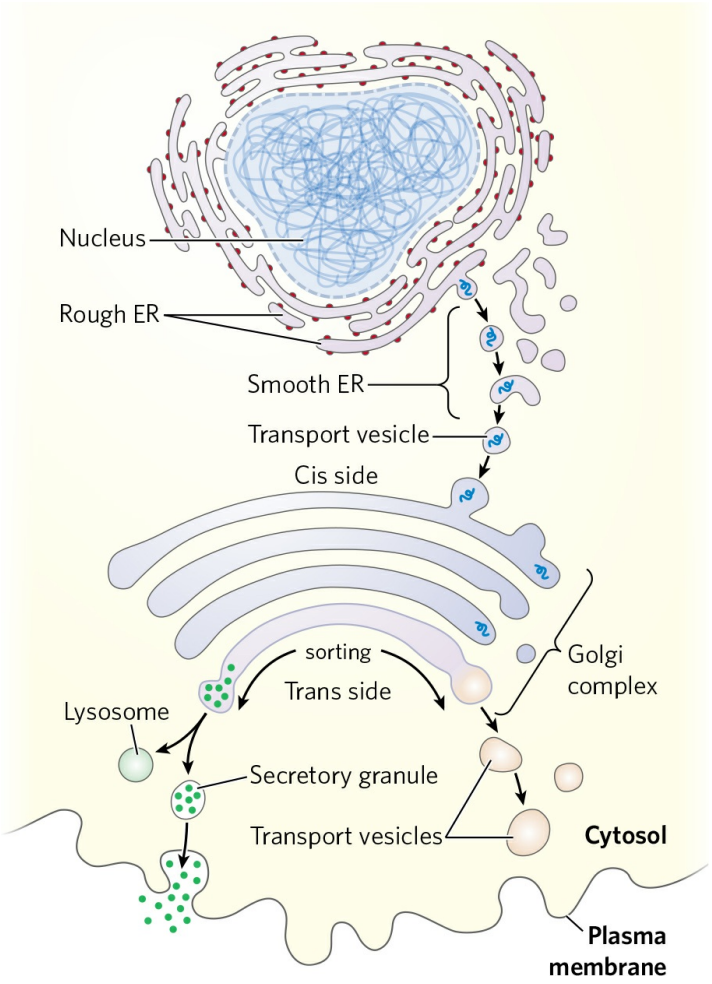


FIGURE 27-42 Pathway taken by proteins destined for lysosomes, the plasma membrane, or secretion. Proteins are moved from the ER to the cis side of the Golgi complex in transport vesicles. Sorting occurs primarily in the trans side of the Golgi complex.

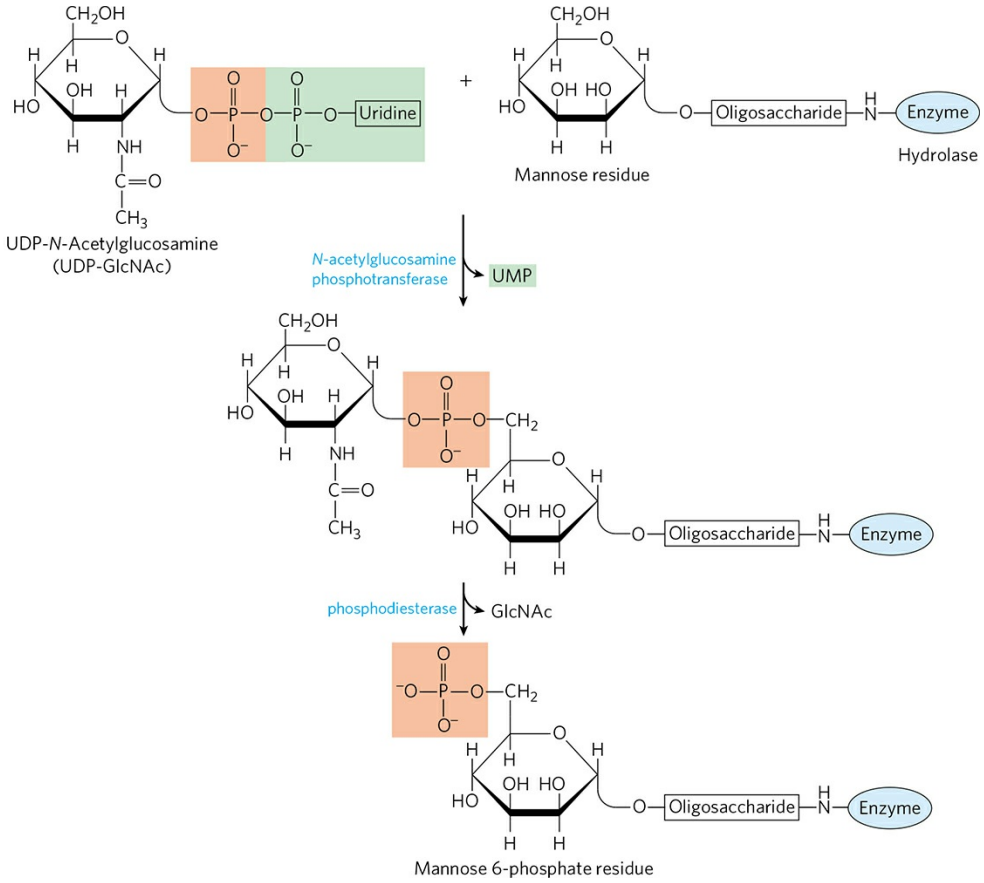


FIGURE 27-43 Phosphorylation of mannose residues on lysosome-targeted enzymes. *N*-Acetylglucosamine phosphotransferase recognizes some as yet unidentified structural feature of hydrolases destined for lysosomes.

Suitably modified proteins can now be moved to a variety of intracellular destinations. Proteins travel from the ER to the Golgi complex in transport vesicles (Fig. 27-42). In the Golgi complex, oligosaccharides are *O*-linked to some proteins, and *N*-linked oligosaccharides are further modified. By mechanisms not yet fully understood, the Golgi complex also sorts proteins and sends them to their final destinations. The processes that segregate proteins targeted for secretion from those targeted for the plasma membrane or lysosomes must distinguish among these proteins on the basis of structural features other than signal sequences, which were removed in the ER lumen.

This sorting process is best understood in the case of hydrolases destined for transport to lysosomes. On arrival of a hydrolase (a glycoprotein) in the Golgi complex, an as yet undetermined feature (sometimes called a signal patch) of the three-dimensional structure of the hydrolase is recognized by a phosphotransferase, which phosphorylates terminal mannose residues in the oligosaccharides **(Fig. 27-43)**. The presence of one or more mannose 6-phosphate residues in its *N*-linked oligosaccharide is the structural signal that targets a protein to lysosomes. A receptor protein in the membrane of the Golgi complex recognizes the mannose 6-phosphate signal and binds the hydrolase so marked. Vesicles containing these receptor-hydrolase complexes bud from the trans side of the Golgi complex and make their way to sorting vesicles. Here, the receptor-hydrolase complex dissociates in a process facilitated by the lower pH in the vesicle and by phosphatase-catalyzed removal of phosphate groups from the mannose 6-phosphate residues. The receptor is then recycled to the Golgi complex, and vesicles containing the hydrolases bud from the sorting vesicles and move to the lysosomes. In cells treated with tunicamycin (Fig. 27-41, step 1), hydrolases that should be targeted to lysosomes are instead secreted, confirming that the *N*-linked oligosaccharide plays a key role in targeting these enzymes to lysosomes.

The pathways that target proteins to mitochondria and chloroplasts also rely on amino-terminal signal sequences. Although mitochondria and chloroplasts contain DNA, most of their proteins are encoded by nuclear DNA and must be targeted to the appropriate organelle. Unlike other targeting pathways, however, the mitochondrial and chloroplast pathways begin only *after* a precursor protein has been completely synthesized and released from the ribosome. Precursor proteins destined for mitochondria or chloroplasts are bound by cytosolic chaperone proteins and delivered to receptors on the exterior surface of the target organelle. Specialized translocation mechanisms then transport the protein to its final destination in the organelle, after which the signal sequence is removed.

Signal Sequences for Nuclear Transport Are Not Cleaved

Molecular communication between the nucleus and the cytosol requires the movement of macromolecules through nuclear pores. RNA molecules

synthesized in the nucleus are exported to the cytosol. Ribosomal proteins synthesized on cytosolic ribosomes are imported into the nucleus and assembled into 60S and 40S ribosomal subunits in the nucleolus; completed subunits are then exported back to the cytosol. A variety of nuclear proteins (RNA and DNA polymerases, histones, topoisomerases, proteins that regulate gene expression, and so forth) are synthesized in the cytosol and imported into the nucleus. This traffic is modulated by a complex system of molecular signals and transport proteins that is gradually being elucidated.

In most multicellular eukaryotes, the nuclear envelope breaks down at each cell division, and once division is completed and the nuclear envelope reestablished, the dispersed nuclear proteins must be reimported. To allow this repeated nuclear importation, the signal sequence that targets a protein to the nucleus—the **nuclear localization sequence (NLS)**—is not removed after the protein arrives at its destination. An NLS, unlike other signal sequences, may be located almost anywhere along the primary sequence of the protein. NLSs can vary considerably in structure, but many consist of four to eight amino acid residues and include several consecutive basic (Arg or Lys) residues.

Nuclear importation is mediated by several proteins that cycle between the cytosol and the nucleus **(Fig. 27-44)**, including importin α and β and a small GTPase known as Ran (*Ra*s-related *n*uclear protein). A heterodimer of importin α and β functions as a soluble receptor for proteins targeted to the nucleus, with the α subunit binding NLS-bearing proteins in the cytosol. The complex of the NLS-bearing protein and the importin docks at a nuclear pore and is translocated through the pore by an energy-dependent mechanism. In the nucleus, the importin β is bound by Ran GTPase, releasing importin β from the imported protein. Importin β is bound by Ran and by CAS (*c*ellular *a*poptosis *s*usceptibility protein) and separated from the NLS-bearing protein. Importin α and β, in their complexes with Ran and CAS, are then exported from the nucleus. Ran hydrolyzes GTP in the cytosol to release the importins, which are then free to begin another importation cycle. Ran itself is also cycled back into the nucleus by the binding of Ran-GDP to nuclear transport factor 2 (NTF2). Inside the nucleus, the GDP bound to Ran is replaced with GTP through the action of Ran guanosine nucleotide–exchange factor (RanGEF; see Box 12-1).

During mitosis, when the nuclear envelope transiently breaks down, the Ran GTPase and the importins play additional roles. The Ran GTPase–

importin β complex helps to position the spindle microtubules on the cell perimeter to facilitate chromosome segregation as the cell divides, and this complex also regulates microtubule interaction with other cellular structures.

Bacteria Also Use Signal Sequences for Protein Targeting

Bacteria can target proteins to their inner or outer membranes, to the periplasmic space between these membranes, or to the extracellular medium. They use signal sequences at the amino terminus of the proteins **(Fig. 27-45)**, much like those on eukaryotic proteins targeted to the ER, mitochondria, and chloroplasts.

Most proteins exported from *E. coli* make use of the pathway shown in **Figure 27-46**. Following translation, a protein to be exported may fold only slowly, the amino-terminal signal sequence impeding the folding. The soluble chaperone protein SecB binds to the protein's signal sequence or other features of its incompletely folded structure. The bound protein is then delivered to SecA, a protein associated with the inner surface of the plasma membrane. SecA acts as both a receptor and a translocating ATPase. Released from SecB and bound to SecA, the protein is delivered to a translocation complex in the membrane, made up of SecY, E, and G, and is translocated stepwise through the membrane at the SecYEG complex in lengths of about 20 amino acid residues. Each step requires the hydrolysis of ATP, catalyzed by SecA.

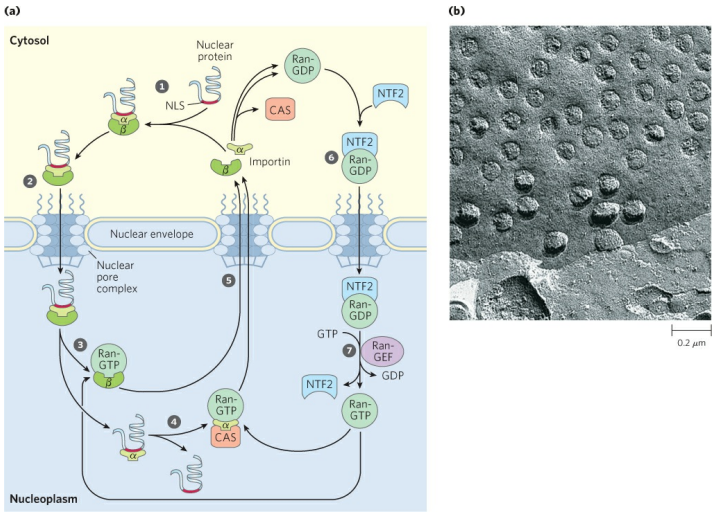


FIGURE 27-44 Targeting of nuclear proteins. (a) ❶ A protein with an appropriate nuclear localization signal (NLS) is bound by a complex of importins α and β. ❷ The resulting complex binds to a nuclear pore and translocates. ❸ Inside the nucleus, dissociation of importin β is promoted by the binding of Ran-GTP. ❹ Importin α binds to Ran-GTP and CAS (cellular apoptosis susceptibility protein), releasing the nuclear protein. ❺ Importins α and β and CAS are transported out of the nucleus and recycled. They are released in the cytosol when Ran hydrolyzes its bound GTP. ❻ Ran-GDP is bound by NTF_2, and transported back into the nucleus. [7] RanGEF promotes the exchange of GDP for GTP in the nucleus, and Ran-GTP is ready to process another NLS-bearing protein-importin complex. **(b)** Scanning electron micrograph of the surface of the nuclear envelope, showing numerous nuclear pores. The nuclear pore complex is one of the largest molecular aggregates in the cell (M_r, ~5×10^7). It is made up of multiple copies of more than 30 different proteins. [Sources: (a) Information from C. Strambio-De-Castillia et al., *Nature Rev. Mol. Cell Biol.* 11:490, 2010, Fig. 1. (b) Don W. Fawcett/Science Source.]