OUTLINE

A typical mammalian cell contains up to 10,000 different kinds of proteins; a yeast cell, about 5000. The vast majority of these proteins are synthesized by cytosolic ribosomes, and although many newly synthesized proteins remain within the cytosol (see Chapter 5), as many as half of the different kinds of proteins produced in a typical cell are delivered to one or another of the various membrane-bounded compartments of the cell. For example, many receptor proteins and transport proteins must be delivered to the plasma membrane, digestive enzymes and polypeptide signaling molecules must be directed to the cell surface for secretion from the cell, and enzymes such as RNA and DNA polymerases must be targeted to the nucleus. These and all the other proteins produced by a cell must reach their correct locations for the cell to function properly.

The delivery of newly synthesized proteins to their proper cellular destinations, usually referred to as *protein targeting* or *protein sorting*, encompasses two very different kinds of processes: **signal-based targeting** to a variety of organelles and **vesicle-based trafficking** in the secretory pathway. The first kind of process involves the targeting of a newly synthesized protein

from the cytoplasm to an intracellular organelle. Targeting can occur during translation or soon after synthesis of the protein is complete. For membrane proteins, targeting leads to insertion of the protein into the lipid bilayer of the membrane, whereas for water-soluble proteins, targeting leads to translocation of the entire protein across the membrane into the aqueous interior of the organelle. Proteins are sorted to the endoplasmic reticulum (ER), mitochondria, chloroplasts, peroxisomes, and nucleus by this general process (Figure 13-1).

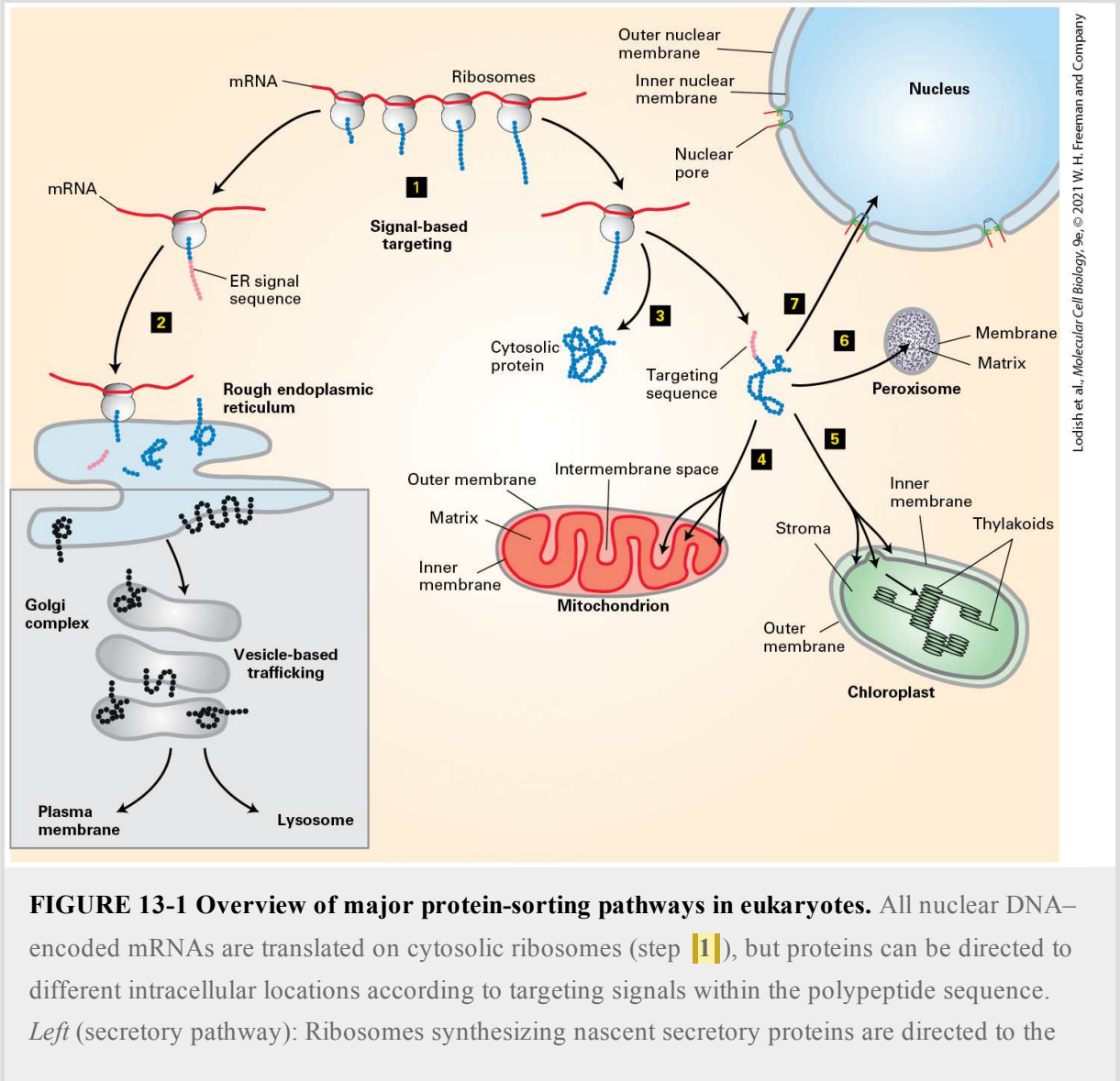

FIGURE 13-1 Overview of major protein-sorting pathways in eukaryotes. All nuclear DNA–encoded mRNAs are translated on cytosolic ribosomes (step 1), but proteins can be directed to different intracellular locations according to targeting signals within the polypeptide sequence. *Left* (secretory pathway): Ribosomes synthesizing nascent secretory proteins are directed to the

rough endoplasmic reticulum (ER) by an ER signal sequence (pink; step 2). After translation is completed on the ER, these proteins can move by vesicle-based transport processes to the Golgi complex and on to the plasma membrane or to lysosomes. The vesicle-based processes underlying the secretory pathway (shaded box) are discussed in Chapter 14. *Right* (signal-based targeting): Synthesis of proteins that contain no targeting sequence are released into the cytosol and remain there (step 3). Proteins with an organelle-specific targeting sequence (pink) are first released into the cytosol but are then imported into mitochondria, chloroplasts, peroxisomes, or the nucleus (steps 4–7). Mitochondrial and chloroplast proteins typically pass through the outer and inner membranes to enter the matrix or stromal space, respectively. Other proteins are sorted to other subcompartments of these organelles by additional sorting steps. Nuclear proteins enter and exit through visible pores in the nuclear envelope.

Description
The steps are as follows: Step 1. Signal-based targeting. Four ribosomes bound m R N A produces peptide chains. An arrow toward left lead to step 2 while toward right lead to steps 3 through 7. Ste 2. A ribosome bound m R N A produces a peptide chain with E R signal sequence in start. This reaches rough endoplasmic reticulum where the ribosome bound m R N A attaches to its surface. The signal sequence along with peptide chain are separated in the rough endoplasmic reticulum. Next, they reach Golgi complex and are transported to plasma membrane and lysosome by vesicle-based trafficking. Ste 3. A ribosome bound m R N A produces a cytosolic protein and a peptide chain with targeting sequence. Steps 4, 5, 6, and 7 show that this peptide chain with targeting sequence can be transported to mitochondrion, chloroplast, peroxisome, and nucleus, respectively.

The second general sorting process, known as the **secretory pathway**, involves transport of proteins from the ER to their final destination within membrane-enclosed vesicles. For many proteins, including those that make up the extracellular matrix, the final destination is the outside of the cell (hence the name); integral membrane proteins are also transported to the Golgi complex, lysosomes, and plasma membrane by this process. The secretory pathway begins in the ER; thus all proteins slated to enter the secretory pathway are initially targeted to this organelle.

Targeting to the ER usually involves *nascent* proteins still in the process of being synthesized on a ribosome. Newly made proteins are thus extruded from the ribosome directly into the ER membrane. Once translocated across the ER membrane, proteins are assembled into their native conformation by protein-folding catalysts present in the lumen of the ER. Indeed, the ER is the location where about one-third of the proteins in a typical cell fold into their native conformations, and most of the resident ER proteins either directly or indirectly contribute to the folding process. As part of the folding process, proteins also undergo specific post-translational modifications in the ER. These processes are monitored carefully, and only after their folding and assembly is complete are proteins permitted to be transported out of the ER to other destinations along the secretory pathway. Proteins whose final destination is the lysosome, plasma membrane, or cell exterior are transported along the secretory pathway by the action of small vesicles that bud from the membrane of one organelle and then fuse with the membrane of another (see Figure 13-1 [shaded box] and Figure 14-1). We discuss vesicle-based protein trafficking in Chapter 14 because mechanistically it differs significantly from non-vesicle-based protein targeting to intracellular organelles.

In this chapter, we examine how proteins are targeted to five intracellular organelles: the ER, mitochondria, chloroplasts, peroxisomes, and nucleus. Two features of this protein-targeting process were initially quite baffling: How could a given protein be directed to only one specific membrane, and how could relatively large hydrophilic protein molecules be translocated across a hydrophobic membrane without disrupting the function of the bilayer as a barrier to ions and small molecules? Using a combination of biochemical purification methods and genetic screens for identifying mutants unable to execute particular translocation steps, cell biologists have identified many of

the cellular components required for translocation across each of the different intracellular membranes. In addition, many of the major translocation processes in the cell have been reconstituted by incorporating their purified protein components into artificial lipid bilayers, using in vitro systems that can be freely manipulated experimentally.

These studies have shown that, despite some variations, the same basic mechanisms govern protein sorting to all the various intracellular organelles. As shown in Table 13-1, the mechanism of targeting to five organelles considered in this chapter can be described by four basic elements. (1) The information to target a protein to a particular organelle destination is encoded within the amino acid sequence of the protein itself, usually within a sequence of about 20 amino acids, known generically as a **targeting sequence**; these sequences are also called **signal sequences** or **signal peptides**. Such targeting sequences are usually located at the N-terminus of a protein and are thus the first part of the protein to be synthesized. More rarely, targeting sequences are located at either the C-terminus or within the interior of a protein sequence. (2) Each organelle carries a set of receptor proteins that bind (directly or indirectly) only to specific kinds of targeting sequences, thus ensuring the specificity of targeting. (3) Once a protein containing a targeting sequence has interacted with the corresponding receptor, the polypeptide chain is transferred to some kind of *translocation channel* that allows the protein to pass into or through the membrane bilayer. (4) Finally, the unidirectional transfer of a protein into an organelle, without its sliding back out into the cytoplasm, is usually achieved by coupling translocation to an energetically favorable process such as hydrolysis of GTP or ATP. In some cases, proteins are sorted further to reach a subcompartment within the target organelle; such sorting depends on yet other signal sequences and other receptor proteins.

TABLE 13-1 • Targeting Sequences Direct Proteins from the Cytosol to Organelles

Target Organelle	Targeting Sequence	Receptor	Translocation Channel	Energy Source
Endoplasmic reticulum (lumen)	At N-terminus, 6–12 hydrophobic amino acids, often preceded by one or more basic amino acids (Arg, Lys)	SRP (ribonucleoprotein complex associated with ribosome) and SRP receptor in the ER membrane; SRP and SRP receptor are GTPases	Sec61 complex; proteins translocate as unfolded chain, as channel remains sealed to small molecules	Translation elongation powered by GTP hydrolysis
Mitochondrion[i] (matrix)	At N-terminus an amphipathic helix, 20–50 residues in length, with Arg and Lys residues on one side and hydrophobic residues on the other	Tom20/22 import receptor in the outer mitochondrial membrane	Channels composed of Tom40 in the outer mitochondrial membrane and Tim23/17 in the inner membrane	ATP hydrolysis by Hsp70 in the matrix
Chloroplast[i] (stroma)	At N-terminus, but no common motifs; generally rich in Ser,	Toc159 and Toc34, GTPases in the outer membrane	Channels composed Toc75 in the outer membrane and Tic20 in	ATP hydrolysis by Hsp70 in the stroma

	Thr, and small hydrophobic residues and poor in Glu and Asp		the inner membrane	
Peroxisome (matrix)	PTS1 signal (Ser-Lys-Leu) at extreme C-terminus; PTS2 signal at N-terminus	Pex5, which cycles between the cytoplasm and peroxisomal membrane	A complex of Pex5 and the peroxisomal membrane protein Pex14; cargo proteins can be transported in a folded state	ATP hydrolysis coupled to ubiquitination and deubiquitination of Pex5
Nucleus (nucleoplasm)	NLS sequences can function at any location within a protein sequence; a common motif includes a short segment rich in Lys and Arg residues	Nuclear transport receptors, which cycle between the cytoplasm and the nuclear interior	Central channel of the nuclear pore complex which is filled with a fluidlike phase of FG-repeat proteins; proteins and RNA can traverse in folded state when bound to a nuclear transport receptor	GTP hydrolysis coupled to cycling of Ran GTPase into and out of the nucleus

[i]Targeting to subcompartments of the mitochondria (such as the inner membrane) and the chloroplast (such as the thylakoid) require additional targeting sequences, receptors, and translocation channels as discussed in this chapter.

In the first part of the chapter, we cover targeting of proteins to the ER, including the post-translational modifications that proteins undergo as they enter the secretory pathway. Targeting of proteins to the ER is the best understood example of protein targeting and will serve as a model of the process in general. We then describe targeting of proteins to mitochondria, chloroplasts, and peroxisomes. Finally, we cover the transport of proteins into and out of the nucleus through nuclear pores.

An important consequence of knowing the nature of the different kinds of targeting sequences is that from just the amino acid sequence encoded by a gene it is possible to reliably deduce the final cellular location of the gene product. Indeed, the cellular location of most proteins encoded by the human genome has been accurately predicted using the information on the nature of different kinds of targeting sequences that we will discuss in this chapter.