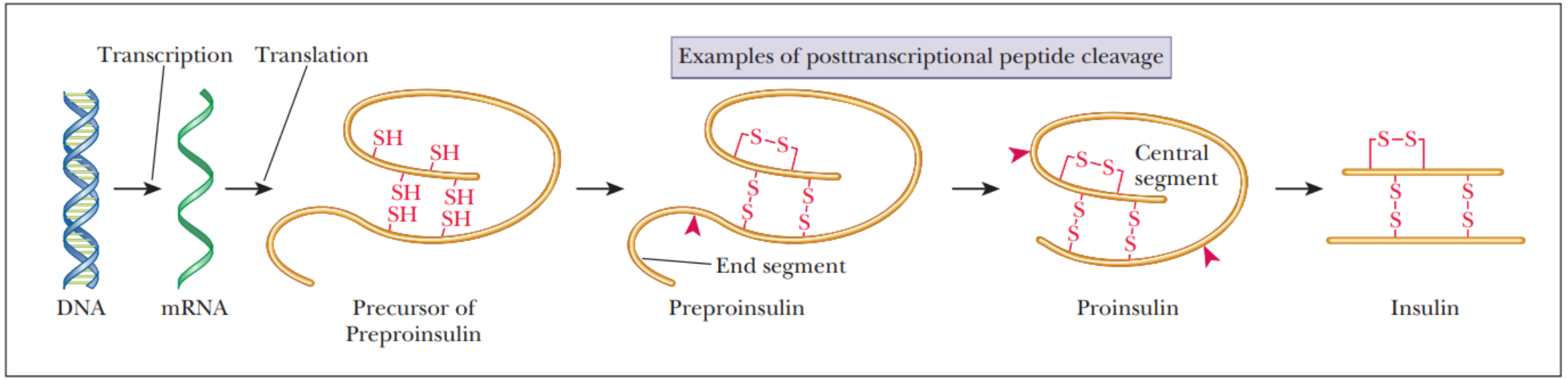
Post translational modification of Proteins, and Protein Sorting Pathways

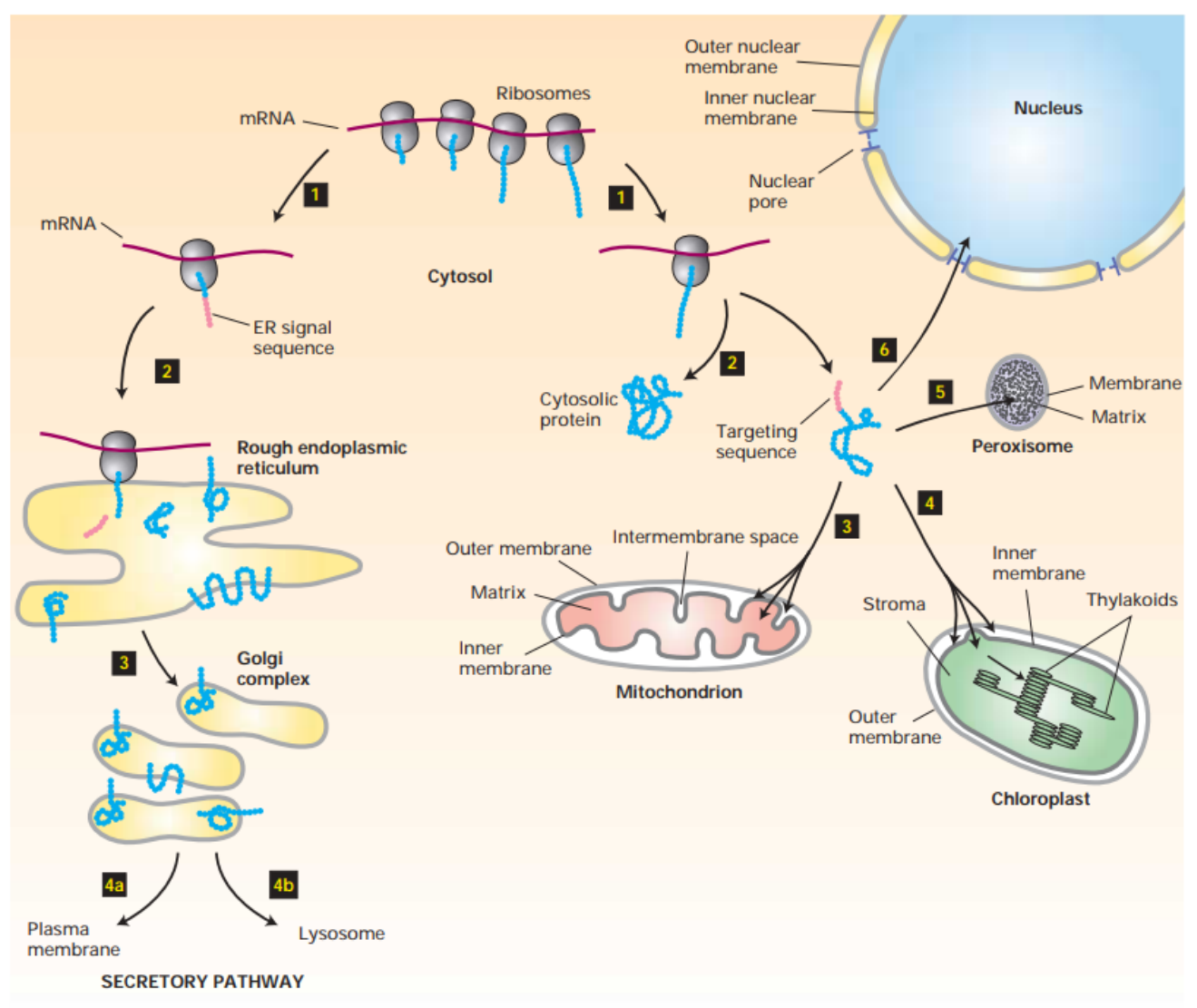
Post translational modification of proteins

- Newly synthesized polypeptides are frequently processed before they reach the form in which they have biological activity. We have already mentioned that, in prokaryotes, N-formylmethionine is cleaved off. Specific bonds in precursors can be hydrolyzed, as in the cleavage of pre-proinsulin to proinsulin and of proinsulin to insulin (Figure).
- Proteins destined for export to specific parts of the cell or from the cell have leader sequences at their N-terminal ends. These leader sequences, which direct the proteins to their proper destination, are recognized and removed by specific proteases associated with the endoplasmic reticulum.
- The finished protein then enters the Golgi apparatus, which directs it to its final destination. In addition to the processing of proteins by breaking bonds, other substances can be linked to the newly formed polypeptide. Various cofactors, such as heme groups, are added, and disulfide bonds are formed (Figure).
- Some amino acid residues are also covalently modified, as in the conversion of proline to hydroxyproline. Other covalent modifications can take place, an example being the addition of carbohydrates or lipids to yield an active final form of the protein in question. Proteins can also be methylated, phosphorylated, and ubiquitinylated.



Some examples of posttranslational modification of proteins. After a precursor of preproinsulin is formed by the transcription–translation process, it is transformed into preproinsulin by formation of three disulfide bonds. Specific cleavage that removes an end segment converts preproinsulin to proinsulin. Finally, two further specific cleavages remove a central segment, with insulin as the end result.

Overview of major protein-sorting pathways in eukaryotic cells



Overview of major protein-sorting (1) pathways in eukaryotic cells. All nuclear-encoded mRNAs are translated on cytosolic ribosomes. Left (secretory pathway): Ribosomes synthesizing nascent proteins in the secretory pathway are directed to the rough endoplasmic reticulum (ER) by an ER signal sequence (pink; steps 1, 2). After translation is completed on the ER, these proteins can move via transport vesicles to the Golgi complex (step 3). Further sorting delivers proteins either to the plasma membrane or to lysosomes (steps 4a, 4b). Right (nonsecretory pathways): Synthesis of proteins lacking an ER signal sequence is completed on free ribosomes (Step 1). Those proteins that contain no targeting sequence are released into the cytosol and remain there (step 2). Proteins with an organelle-specific targeting sequence (pink) first are released into the cytosol (step 2) but then are imported into mitochondria, chloroplasts, peroxisomes, or the nucleus (steps3–6). 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.

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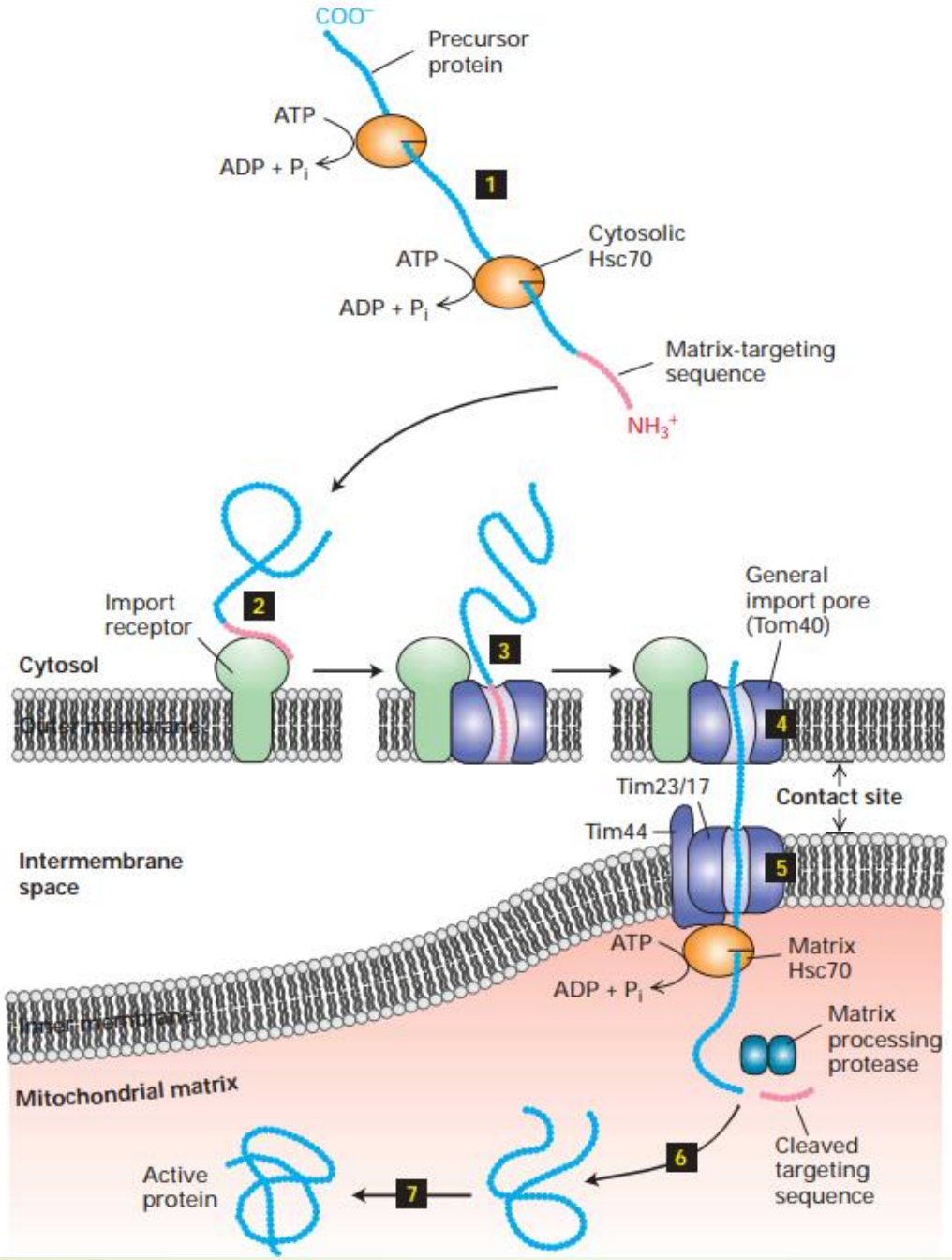
Sorting of Proteins to Mitochondria and Chloroplasts

- ■ Most mitochondrial and chloroplast proteins are encoded by nuclear genes, synthesized on cytosolic ribosomes, and imported post-translationally into the organelles.
- ■ All the information required to target a precursor protein from the cytosol to the mitochondrial matrix or chloroplast stroma is contained within its N-terminal uptake-targeting sequence. After protein import, the uptake-targeting sequence is removed by proteases within the matrix or stroma.
- ■ Cytosolic chaperones maintain the precursors of mitochondrial and chloroplast proteins in an unfolded state. Only unfolded proteins can be imported into the organelles. Translocation occurs at sites where the outer and inner membranes of the organelles are close together.
- ■ Proteins destined to the mitochondrial matrix bind to receptors on the outer mitochondrial membrane, and then are transferred to the general import pore (Tom40) in the outer membrane. Translocation occurs concurrently through the outer and inner membranes, driven by the proton-motive force across the inner membrane and ATP hydrolysis by the Hsc70 ATPase in the matrix (see Figure).
- ■ Proteins sorted to mitochondrial destinations other than the matrix usually contain two or more targeting sequences, one of which may be an N-terminal matrix targeting sequence (see Figure).
- ■ Some mitochondrial proteins destined for the intermembrane space or inner membrane are first imported into the matrix and then redirected; others never enter the matrix but go directly to their final location.
- ■ Protein import into the chloroplast stroma occurs through inner-membrane and outer-membrane translocation channels that are analogous in function to mitochondrial channels but composed of proteins unrelated in sequence to the corresponding mitochondrial proteins.
- ■ Proteins destined for the thylakoid have secondary targeting sequences. After entry of these proteins into the stroma, cleavage of the stromal-targeting sequences reveals the thylakoid-targeting sequences.
- ■ The three known pathways for moving proteins from the chloroplast stroma to the thylakoid closely resemble translocation across the bacterial inner membrane (see Figure). One of these systems can translocate folded proteins.



◀ **FIGURE 16-26 Protein import into the mitochondrial matrix.** Precursor proteins synthesized on cytosolic ribosomes are maintained in an unfolded or partially folded state by bound chaperones, such as Hsc70 (step 1). After a precursor protein binds to an import receptor near a site of contact with the inner membrane (step 2), it is transferred into the general import pore (step 3). The translocating protein then moves through this channel and an adjacent channel in the inner membrane (steps 4, 5). Note that translocation occurs at rare "contact sites" at which the inner and outer membranes appear to touch. Binding of the translocating protein by the matrix chaperone Hsc70 and subsequent ATP hydrolysis by Hsc70 helps drive import into the matrix. Once the uptake-targeting sequence is removed by a matrix protease and Hsc70 is released from the newly imported protein (step 6), it folds into the mature, active conformation within the matrix (step 7). Folding of some proteins depends on matrix chaperonins. See the text for discussion. [See G. Schatz, 1996, *J. Biol. Chem.* **271**:31763, and N. Pfanner et al., 1997, *Ann. Rev. Cell Devel. Biol.* **13**:25.]

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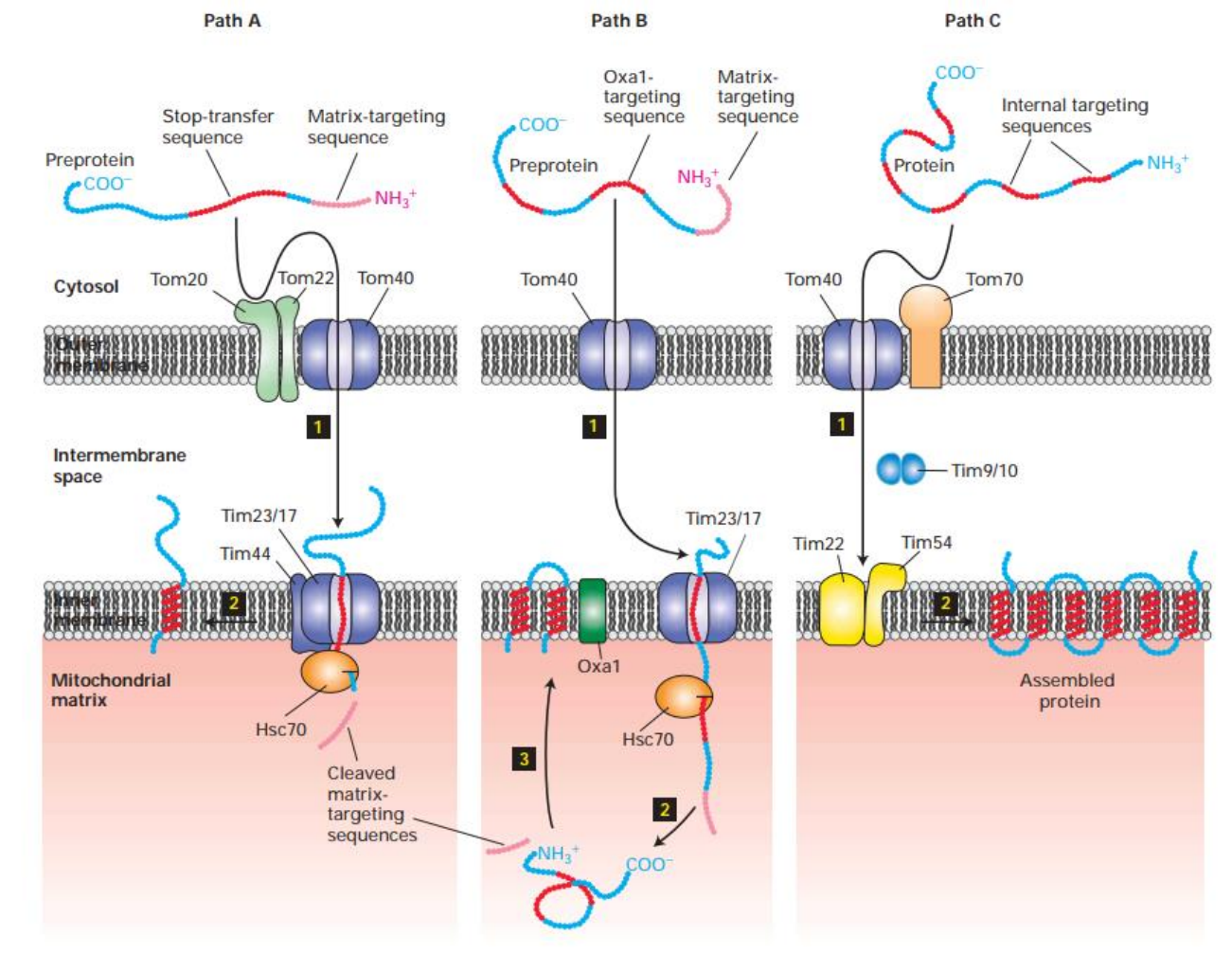

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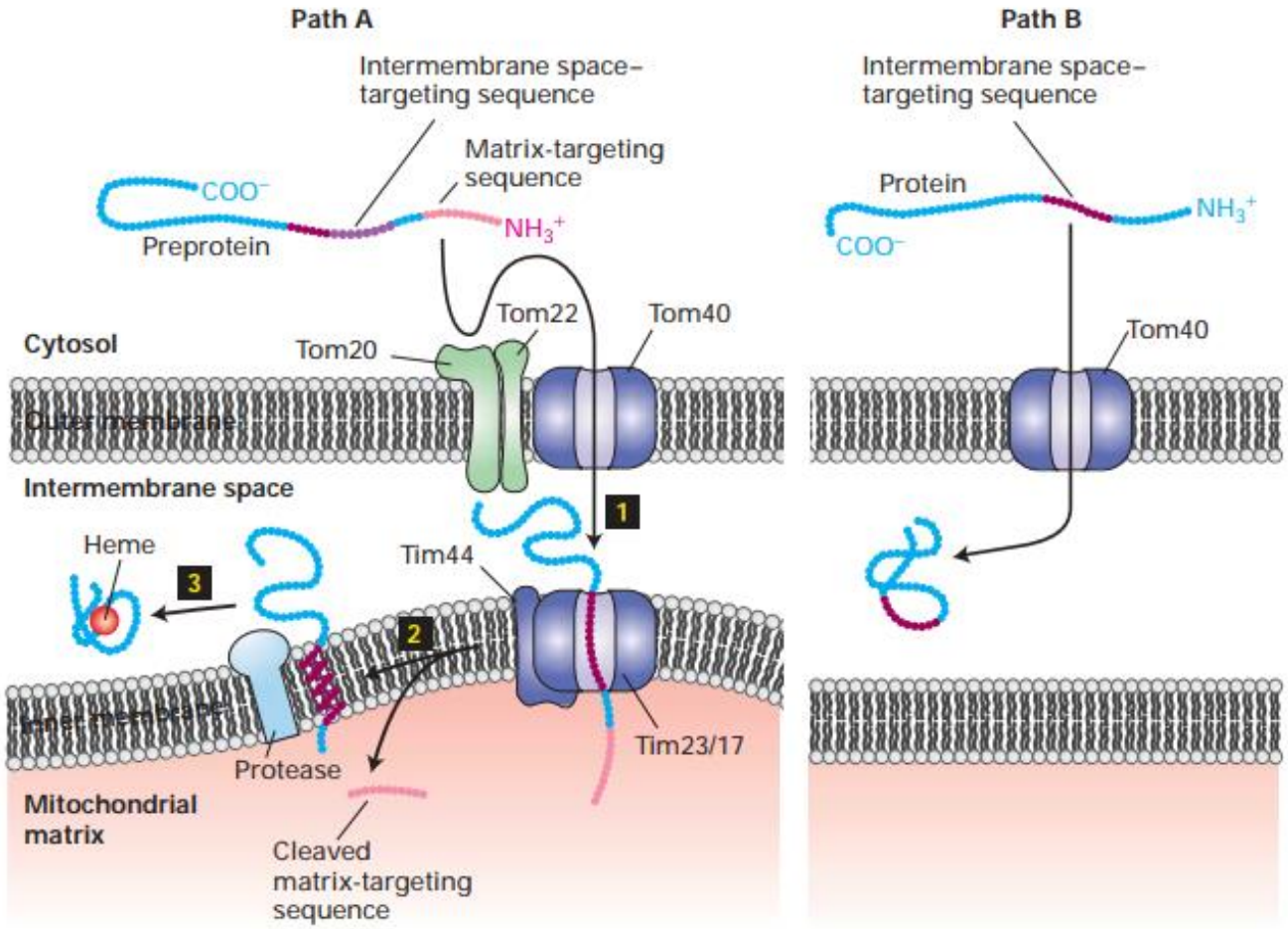
Three pathways for transporting proteins from the cytosol to the inner mitochondrial membrane. Proteins with different targeting sequences are directed to the inner membrane via different pathways. In all three pathways, proteins cross the outer membrane via the Tom40 general import pore. Proteins delivered by pathways A and B contain an N-terminal matrix-targeting sequence that is recognized by the Tom20/22 import receptor in the outer membrane. Although both these pathways use the Tim23/17 inner-membrane channel, they differ in that the entire precursor protein enters the matrix and then is redirected to the inner membrane in pathway B. Matrix Hsc70 plays a role similar its role in the import of soluble matrix proteins (see Figure 16-26). Proteins delivered by pathway C contain internal sequences that are recognized by the Tom70 import receptor. A different inner-membrane translocation channel (Tim22/54) is used in this pathway. Two intermembrane proteins (Tim9 and Tim10) facilitate transfer between the outer and inner channels. See the text for discussion. [See R. E. Dalbey and A. Kuhn, 2000, Ann. Rev. Cell Devel. Biol. 16:51, and N. Pfanner and A. Geissler, 2001, Nature Rev. Mol. Cell Biol. 2:339.]



▲ FIGURE 16-30 Two pathways for transporting proteins from the cytosol to the mitochondrial intermembrane space. Pathway A, the major one for delivery to the intermembrane space, is similar to pathway A for delivery to the inner membrane (see Figure 16-29). The major difference is that the internal targeting sequence in proteins such as cytochrome b_2 destined for the intermembrane space is recognized by an inner-membrane protease, which cleaves the protein on the intermembrane-space side of the membrane. The released protein then folds and binds to its heme cofactor within the intermembrane space. Pathway B involves direct delivery to the intermembrane space through the Tom40 general import pore in the outer membrane. See the text for further discussion. [See R. E. Dalbey and A. Kuhn, 2000, *Ann. Rev. Cell Devel. Biol.* **16**:51; N. Pfanner and A. Geissler, 2001, *Nature Rev. Mol. Cell Biol.* **2**:339; and K. Diekert et al., 1999, *Proc. Nat'l. Acad. Sci. USA* **96**:11752.]

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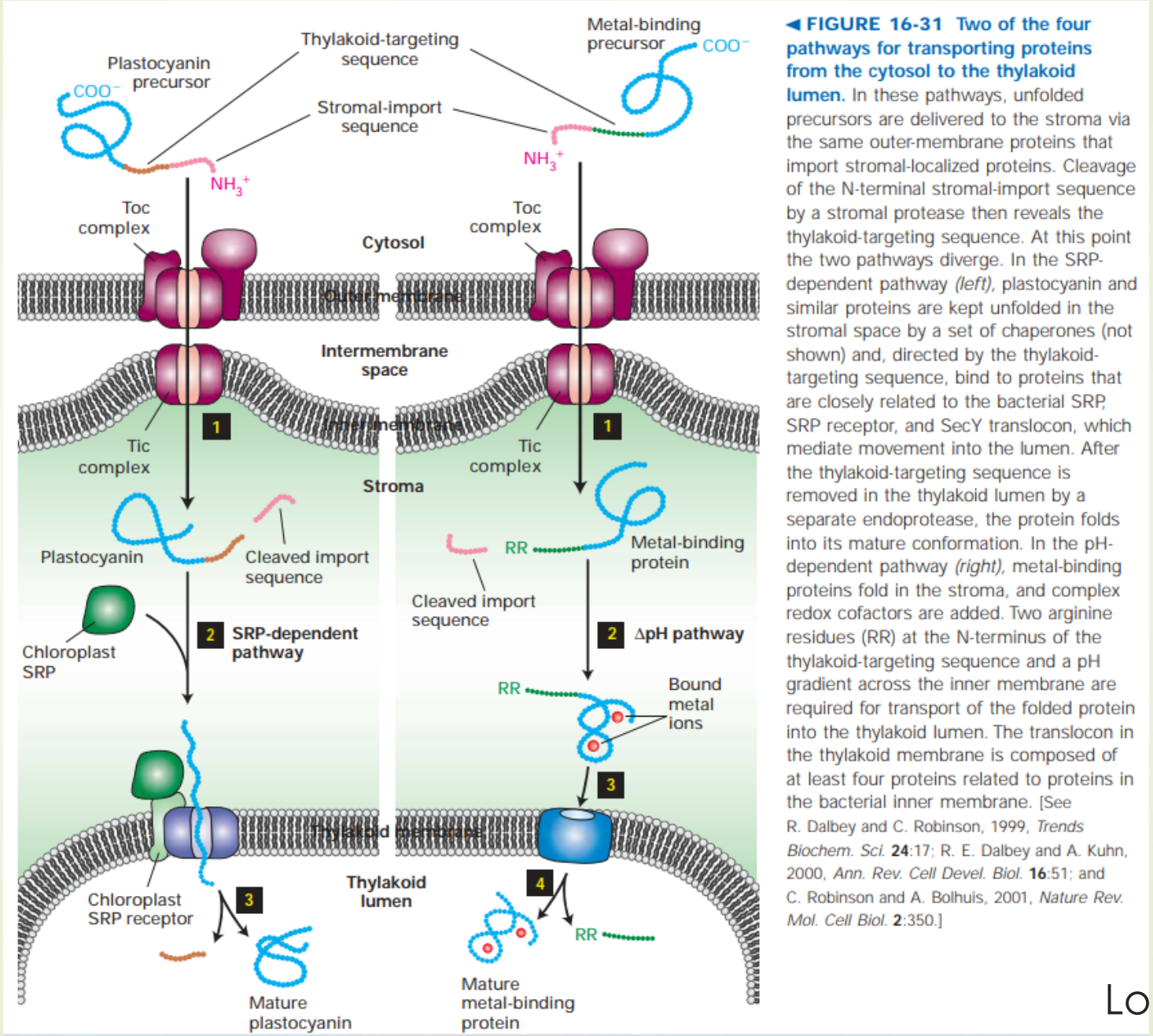

◀ FIGURE 16-31 Two of the four pathways for transporting proteins from the cytosol to the thylakoid lumen. In these pathways, unfolded precursors are delivered to the stroma via the same outer-membrane proteins that import stromal-localized proteins. Cleavage of the N-terminal stromal-import sequence by a stromal protease then reveals the thylakoid-targeting sequence. At this point the two pathways diverge. In the SRP-dependent pathway *(left)*, plastocyanin and similar proteins are kept unfolded in the stromal space by a set of chaperones (not shown) and, directed by the thylakoid-targeting sequence, bind to proteins that are closely related to the bacterial SRP, SRP receptor, and SecY translocon, which mediate movement into the lumen. After the thylakoid-targeting sequence is removed in the thylakoid lumen by a separate endoprotease, the protein folds into its mature conformation. In the pH-dependent pathway *(right)*, metal-binding proteins fold in the stroma, and complex redox cofactors are added. Two arginine residues (RR) at the N-terminus of the thylakoid-targeting sequence and a pH gradient across the inner membrane are required for transport of the folded protein into the thylakoid lumen. The translocon in the thylakoid membrane is composed of at least four proteins related to proteins in the bacterial inner membrane. [See R. Dalbey and C. Robinson, 1999, *Trends Biochem. Sci.* **24**:17; R. E. Dalbey and A. Kuhn, 2000, *Ann. Rev. Cell Devel. Biol.* **16**:51; and C. Robinson and A. Bolhuis, 2001, *Nature Rev. Mol. Cell Biol.* **2**:350.]

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