13.5 Targeting of Peroxisomal Proteins

Peroxisomes are small organelles bounded by a single membrane which surrounds a luminal space known as the *peroxisomal matrix*. Although peroxisomes have a simple structure, the biogenesis of peroxisomes is similar in fundamental outline to the biogenesis of the more complicated mitochondria and chloroplasts. Peroxisomal proteins, encoded by nuclear genes and synthesized on free ribosomes in the cytosol, are recognized by specific peroxisomal-targeting sequences. These sequences bind to a peroxisome-specific receptor protein and are then delivered either to the peroxisomal matrix or to the peroxisomal membrane by an energy-dependent targeting process.

The size and enzyme content of peroxisomes vary considerably among different kinds of cells. However, all peroxisomes contain enzymes that use molecular oxygen to oxidize various substrates such as fatty acids, breaking them down into smaller components for use in biosynthetic pathways. The hydrogen peroxide (H_2O_2) generated by these oxidation reactions is extremely reactive and potentially harmful to cellular components; however, the peroxisome contains other enzymes, such as catalase, that efficiently convert H_2O_2 into H_2O. In mammals, peroxisomes are most abundant in liver cells, where they constitute about 1–2 percent of the cell volume.

A Cytosolic Receptor Targets Proteins with an SKL Sequence at the C-Terminus to the Peroxisomal Matrix

Peroxisomal-targeting sequences were first identified by testing of peroxisomal proteins with deletions for a specific defect in peroxisomal targeting. In one early study, the gene for firefly luciferase was expressed in cultured insect cells, and the resulting protein was shown to be properly targeted to the peroxisome. However, expression of a gene missing a small portion of the sequence encoding the C-terminus of the protein led to luciferase that failed to be targeted to the peroxisome and remained in the cytoplasm. By testing various mutant luciferase proteins in this system, researchers discovered that the sequence Ser-Lys-Leu (SKL in one-letter code) or a related sequence at the C-terminus is necessary for peroxisomal targeting. Furthermore, addition of the SKL sequence to the C-terminus of a normally cytosolic protein leads to uptake of the altered protein by peroxisomes in cultured cells. All but a few of the many different peroxisomal proteins bear a sequence of this type, known as *peroxisomal-targeting sequence 1*, or simply *PTS1*.

The pathway for the import of catalase and other PTS1-bearing proteins into the peroxisomal matrix is depicted in Figure 13-30. In the cytosol, PTS1 binds to a receptor called *Pex5*. Pex5 has the remarkable property of being able to switch from a monomeric soluble form to an oligomeric form embedded in the peroxisomal membrane in a complex with the membrane protein Pex14. In a manner that is not well understood, the

PTS1-bearing protein is released from the oligomeric form of Pex5 into the interior of the peroxisome. This peroxisome import machinery, unlike most systems that mediate protein import into the ER, mitochondria, and chloroplasts, can translocate folded proteins across the membrane. For example, catalase assumes a folded conformation and binds to heme in the cytoplasm before traversing the peroxisomal membrane. Cell-free studies have shown that the peroxisome import machinery can transport large macromolecular objects, including gold particles about 9 nm in diameter, as long as they have a PTS1 tag attached to them. There is evidence that the size of oligomers of Pex5 bound to PTS1-bearing cargo molecules and Pex14 adjusts according to the size of the PTS1-bearing cargo molecules. The dynamic formation of oligomers is apparently the key mechanism by which PTS1-bearing cargo molecules can be accommodated without the formation of large stable pores that would disrupt the integrity of the peroxisomal membrane.

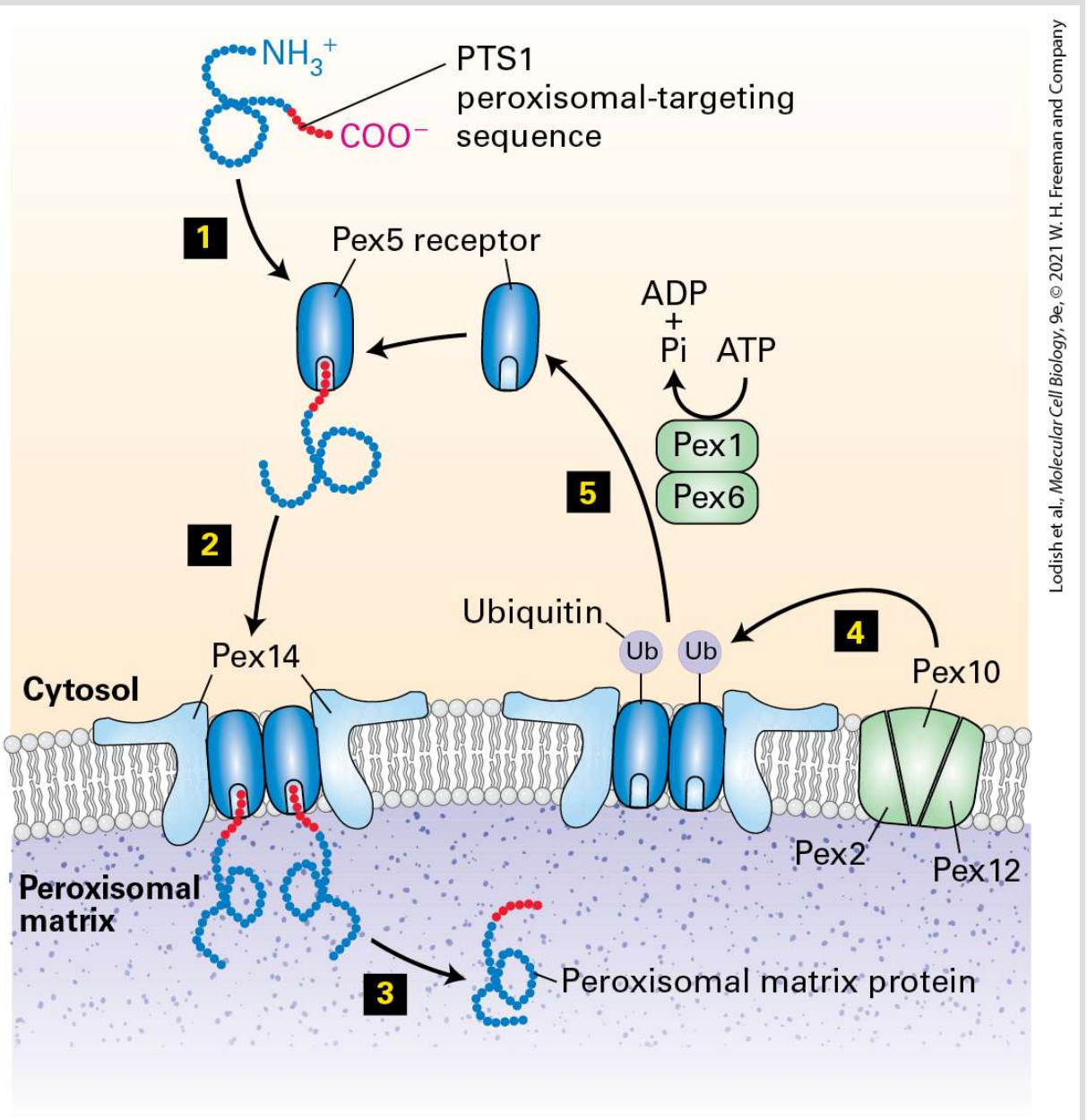


FIGURE 13-30 PTS1-directed import of peroxisomal matrix proteins. Step 1: Most peroxisomal matrix proteins contain a C-terminal PTS1 targeting sequence (red), which binds to the cytosolic receptor Pex5. Step 2: Pex5 with the bound matrix protein forms a multimeric complex with the Pex14 receptor located on the peroxisomal membrane. Step 3: After assembly of the matrix protein-Pex5-Pex14 complex, the matrix protein dissociates from Pex5 and is released into the peroxisomal matrix. Steps 4 and 5: Pex5 is then returned to the cytosol by a process that involves ubiquitinylation by the membrane proteins Pex2, Pex10, and Pex12, followed by ATP-dependent removal from the membrane by the AAA-ATPase proteins Pex1 and Pex6. Note that folded proteins can be imported into

peroxisomes and that the targeting sequence is not removed in the matrix. See P. E. Purdue and P. B. Lazarow, 2001, *Annu. Rev. Cell Dev. Biol.* **17**:701; S. Subramani et al., 2000, *Annu. Rev. Biochem.* **69**:399; and V. Dammai and S. Subramani, 2001, *Cell* **105**:187.

Description
The illustration shows a peroxisomal membrane separating cytosol from peroxisomal matrix. Step 1: The P T S 1 peroxisomal-targeting sequence at C-terminal of a protein chain binds in a pocket of a Pex 5 receptor. Step 2: Two such complexes get embedded between two Pex 14 in the peroxisomal membrane with protein chain inside the matrix. Step 3: Peroxisomal matrix protein detaches from the Pex 5 receptor in to the matrix. Step 4: A Pex 2, Pex 10, and Pex 12 complex embedded in the peroxisomal membrane transfers Ubiquitin to two embedded Pex 5 receptors. Step 5: A Pex 1-Pex 6 complex hydrolysis A T P to A D P and P i causing the detachment of Pex 5 receptors into cytosol. Steps repeat.

Once the PTS1-bearing cargo molecule is released into the interior of the peroxisome, the oligomeric complex of Pex5 and Pex14 is actively disassembled, thus releasing Pex5 back into the cytoplasm in a soluble state. Pex5 recycling involves modification of membrane-bound Pex5 by ubiquitinylation. A complex of the peroxisomal membrane proteins Pex10, Pex12, and Pex2 transfers a ubiquitin moiety to Pex5. The AAA ATPases Pex1 and Pex6, anchored to the peroxisomal membrane by Pex15, recognize ubiquitinylated Pex5 and use the energy from ATP hydrolysis to remove it from the oligomeric complex with Pex14 and release it into the cytosol. After removal of the ubiquitin modification, cytosolic Pex5 is ready to carry out another cycle of binding to a PTS1-bearing protein.

Peroxisomal import studies with purified components have shown that the binding of Pex5 to a PTS1-bearing protein, the assembly of an oligomeric

complex of Pex5 and Pex14, and release of the PTS1-bearing protein into the interior of the peroxisome can all occur spontaneously without a source of chemical energy such as ATP. In contrast, both ubiquitin modification of Pex5 and the recycling of Pex5 by the AAA-ATPase require ATP hydrolysis. Evidently, in the process by which Pex5 delivers PTS1-bearing proteins to the peroxisome matrix by cycling between the cytosol and the peroxisomal membrane, it is the recycling step that uses energy to power unidirectional translocation of cargo molecules into the peroxisomal matrix.

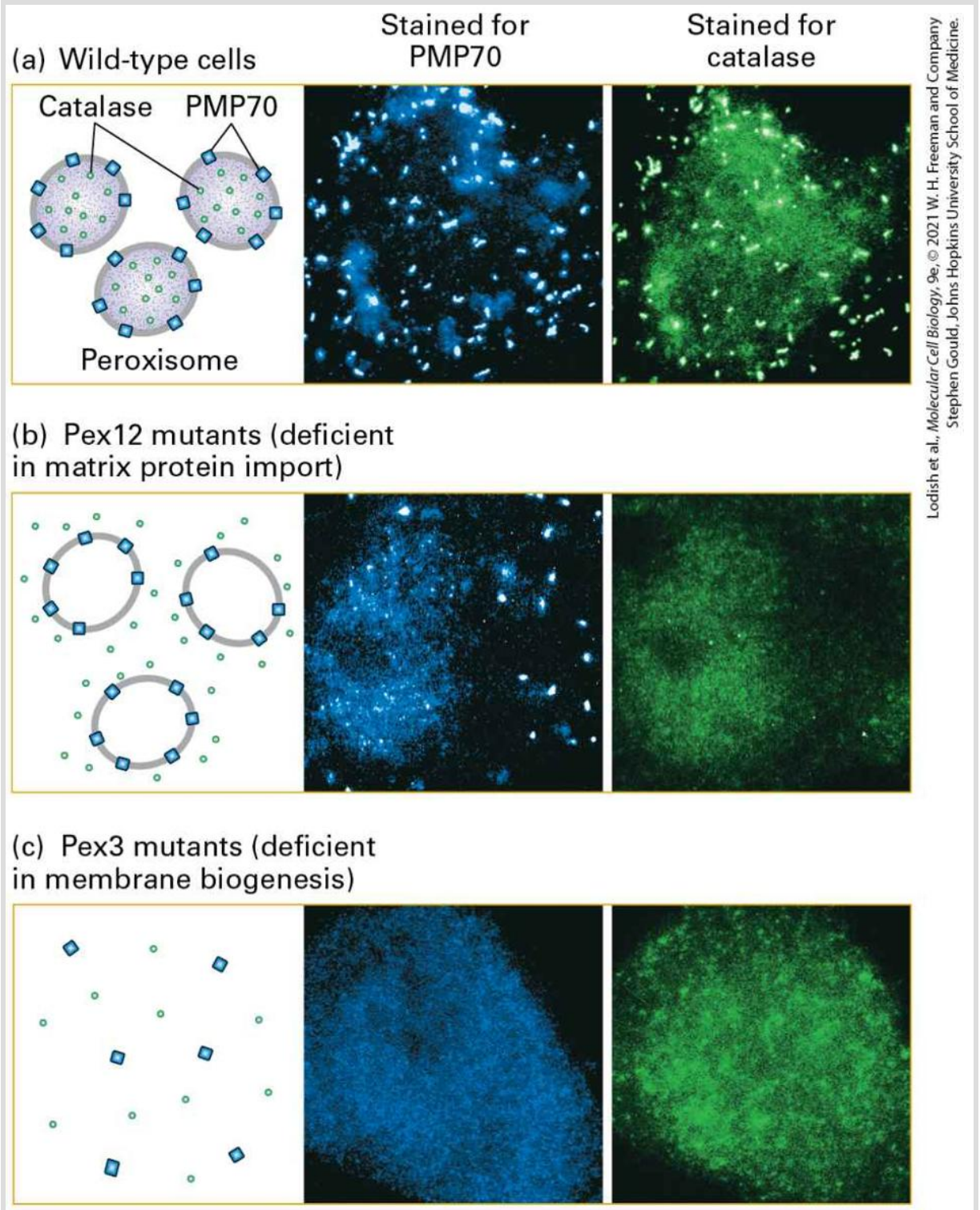
A few peroxisomal matrix proteins, such as thiolase, are synthesized as precursors with an N-terminal targeting sequence known as *PTS2*. These proteins bind to a different cytosolic receptor protein, but otherwise their import is thought to occur by the same mechanism as for PTS1-containing proteins.

Peroxisomal Membrane and Matrix Proteins Are Incorporated by Different Pathways

Autosomal recessive mutations that cause defective peroxisome assembly occur naturally in the human population. Such mutations can lead to severe developmental defects often associated with craniofacial abnormalities. In *Zellweger syndrome* and related disorders, for example, the transport of many or all proteins into the peroxisomal matrix is

impaired: newly synthesized peroxisomal enzymes remain in the cytosol and are eventually degraded. Genetic analyses of cultured cells from patients with Zellweger syndrome and of yeast cells carrying similar mutations have identified more than 20 genes that are required for peroxisome biogenesis. ■

Studies with peroxisome-assembly mutants have shown that different pathways are used for importing peroxisomal matrix proteins and for inserting proteins into the peroxisomal membrane (Figure 13-31). For example, analysis of cells from some patients with Zellweger syndrome led to the identification of the gene encoding Pex5 as well as many of the Pex genes needed for recycling of Pex5. Mutant cells that are defective in any one of these proteins cannot incorporate matrix proteins into peroxisomes; nonetheless, the cells contain empty peroxisomes that have a normal complement of peroxisomal membrane proteins (Figure 13-31b). Mutations in any one of three other genes were found to block insertion of peroxisomal membrane proteins as well as import of matrix proteins (Figure 13-31c). These findings demonstrate that one set of proteins translocates soluble proteins into the peroxisomal matrix, but a different set is required for insertion of proteins into the peroxisomal membrane. This situation differs markedly from that of the ER, mitochondrion, and chloroplast, whose membrane proteins and soluble proteins share many of the same components for their import into these organelles.



EXPERIMENTAL FIGURE 13-31 Studies reveal different pathways for incorporation of peroxisomal membrane and matrix proteins. Cells were stained with fluorescent antibodies to PMP70, a peroxisomal membrane protein, or with fluorescent antibodies to catalase, a peroxisomal matrix protein, then viewed in a fluorescence microscope. (a) In

wild-type cells, both peroxisomal membrane and matrix proteins are visible as bright foci in numerous peroxisomal bodies. (b) In cells from a Pex12-deficient patient, PMP70 is localized normally to peroxisomal bodies, but catalase is not delivered to peroxisomes and is distributed uniformly throughout the cytosol with no bright foci, whereas (c) in cells from a Pex3-deficient patient, peroxisomal membranes cannot assemble, and as a consequence, peroxisomal bodies do not form. Thus both catalase and PMP70 are mislocalized to the cytosol.

Description

The part (a) shows wild-type cells. The diagram shows three spherical peroxisomes with catalase inside and P M P 70 on the surface. First micrograph stained for P M P 70 shows blue fluorescence with numerous white speckles. Second micrograph stained for catalase shows green fluorescence with numerous white speckles.

The part (b) shows Pex 12 mutants (deficient in matrix protein import). The diagram shows three ring-shaped peroxisomes with P M P 70 on the surface and catalase in cytoplasm. First micrograph stained for P M P 70 shows blue fluorescence with few white speckles. Second micrograph stained for catalase shows green fluorescence with no white speckle.

The part (c) shows Pex 3 mutants (deficient in membrane biogenesis). The diagram shows P M P 70 and catalase in cytoplasm and no peroxisome. First micrograph stained for P M P 70 shows blue fluorescence and second micrograph stained for catalase shows green fluorescence without any white speckle.

KEY CONCEPTS OF SECTION 13.5

Targeting of Peroxisomal Proteins

- All luminal peroxisomal proteins are synthesized on free cytosolic ribosomes and incorporated into the organelle post-translationally.
- Most peroxisomal matrix proteins contain a C-terminal targeting sequence known as *PTS1*; a few have an N-terminal PTS2 targeting sequence. Neither targeting sequence is cleaved after import.

- All proteins destined for the peroxisomal matrix bind to a cytosolic receptor protein, which is Pex5 for PTS1-bearing proteins, and then are directed to common translocation machinery on the peroxisomal membrane (see Figure 13-30).
- Translocation of matrix proteins across the peroxisomal membrane depends on ATP hydrolysis, which is coupled to recycling of Pex5 from the peroxisomal membrane back to the cytosol.
- Unlike proteins imported to the ER, mitochondrion, or chloroplast, many peroxisomal matrix proteins fold in the cytosol and traverse the membrane in a folded conformation.
- Proteins destined for the peroxisomal membrane contain different targeting sequences than peroxisomal matrix proteins and are imported by a different pathway.