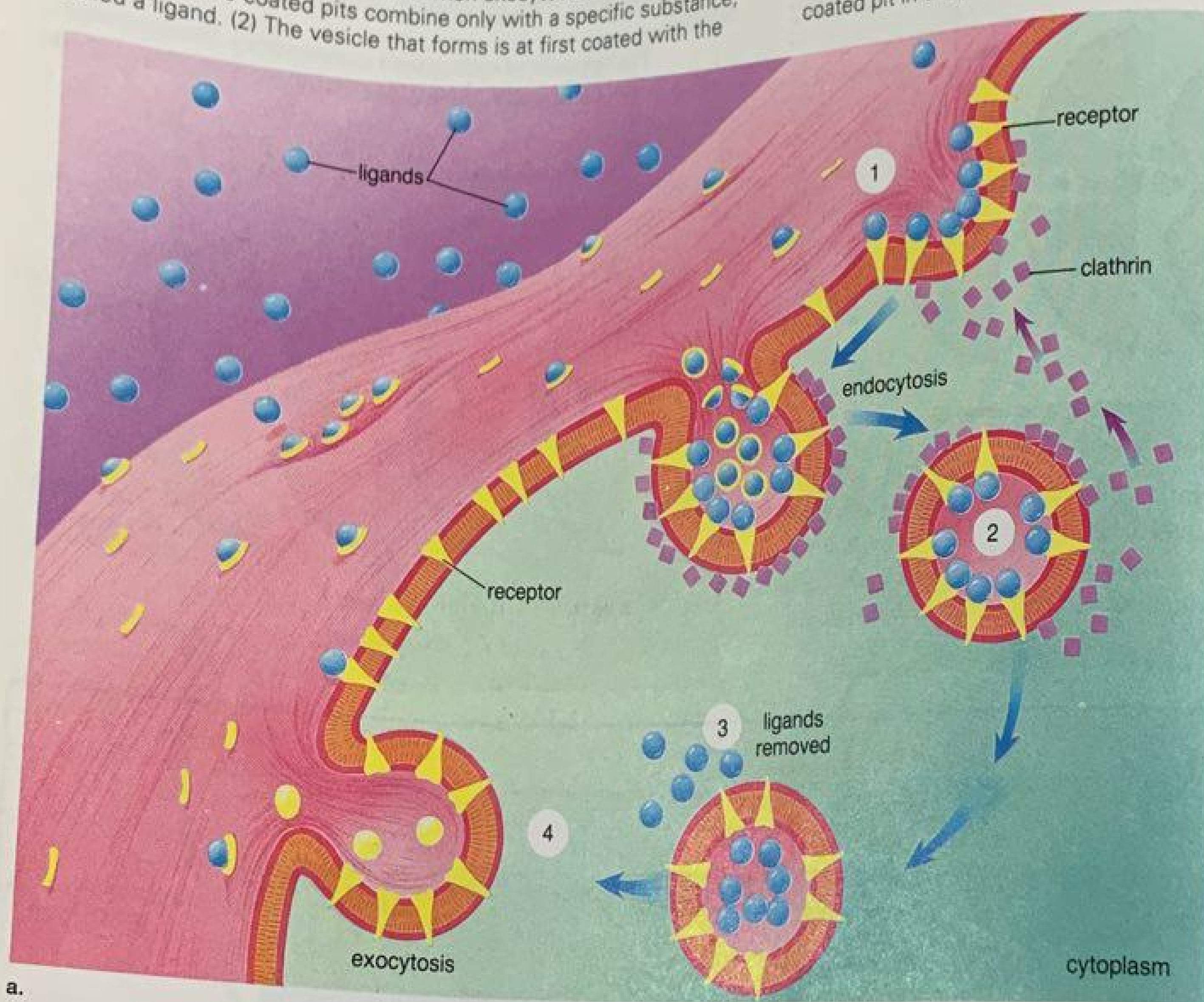
Figure 6.14
Receptor-mediated endocytic cycle takes up membrane when endocytosis occurs and returns it when exocytosis occurs. ***a.*** (1) The receptors in the coated pits combine only with a specific substance, called a ligand. (2) The vesicle that forms is at first coated with the structural protein clathrin but soon the vesicle loses its coat. Ligands leave the vesicle. (4) When exocytosis occurs memb returned to the plasma membrane. ***b.*** Electron micrographs of coated pit in the process of forming a vesicle.



As noted in chapter 5, vesicles formed at the Golgi apparatus secrete cell products in this manner. Exocytosis adds plasma membrane to a cell, and endocytosis takes it away. It can be reasoned that the amount of plasma membrane lost by endocytosis must be replaced by exocytosis unless the cell is growing. It is possible in that case that exocytosis would occur simply for the purpose of adding on more plasma membrane.

Receptor-Mediated Endocytic Cycle

Receptor-mediated endocytosis is a form of pinocytosis that is very specific because it involves the use of plasma membrane receptors. A macromolecule that binds to a receptor is called a ligand (fig. 6.14). The binding of ligands to specific receptor sites causes the receptors to gather at one location before endocytosis occurs. This location is called a coated pit because there is a layer of fibrous protein, called clathrin, on the cytoplasmic side. Clathrin is a protein designed to form lattices around membranous vesicles. When a vesicle forms, it also is coated, but soon it loses its coat. At this point the ligands can directly enter the cell or else end lysosomes, which digest them to smaller molecules, which the cell. In any case, the receptors return to the plasma memb and exocytosis occurs.

The importance of receptor-mediated endocytosis is emplified by the occurrence of a genetic disease. Normally take up cholesterol, which is carried in the blood by a lipopro called low density lipoprotein (LDL). When cells need cholesterol for membrane production they produce receptor LDL. After LDL molecules bind to receptors, receptor-medi endocytosis occurs. Later, the receptors are returned to the pla membrane and lysosomes disengage cholesterol from LDL. whole process goes awry in individuals who lack a gene or in a faulty gene for the LDL receptor. Because cholesterol is un to enter their cells, it builds up and forms plaque on blood ve walls leading to cardiovascular disease and heart attacks. Chil with this genetic disorder have been known to have heart a even as early as 6 years old.

MEDICAL APPLICATION

A large number of disorders arise from defective peroxisomal proteins, because this organelle is involved in several metabolic pathways. Probably the most common peroxisomal disorder is X-chromosome-linked adrenoleukodystrophy, caused by a defective integral membrane protein that participates in transporting very long-chain fatty acids into the peroxisome for β-oxidation. Accumulation of these fatty acids in body fluids destroys the myelin sheaths in nerve tissue, causing severe neurologic symptoms. Deficiency in peroxisomal enzymes causes the fatal Zellweger syndrome, with severe muscular impairment, liver and kidney lesions, and disorganization of the central and peripheral nervous systems. Electron microscopy reveals empty peroxisomes in liver and kidney cells of these patients.

THE CYTOSKELETON

The cytoplasmic cytoskeleton is a complex network of (1) microtubules, (2) microfilaments (actin filaments), and (3) intermediate filaments. These protein structures determine the shape of cells, play an important role in the movements of organelles and cytoplasmic vesicles, and also allow the movement of entire cells.

Table 2–2. **Examples of diseases caused by lysosomal enzyme failure and accumulation of undigested material in different cell types.**

Disease	Faulty Enzyme	Main Organs Affected
Hurler	α-L-Iduronidase	Skeleton and nervous system
Sanfilippo syndrome A	Heparan sulfate sulfamidase	Skeleton and nervous system
Tay-Sachs	Hexosaminidase-A	Nervous system
Gaucher	β-D-glycosidase	Liver and spleen
I-cell disease	Phosphotransferase	Skeleton and nervous system

Microtubules

Within the cytoplasmic matrix of eukaryotic, cells are fine tubular structures known as **microtubules** (Figures 2–28 and 2–29). Microtubules are also found in cytoplasmic processes called cilia (Figure 2–30) and flagella. They have an outer diameter of 24 nm, with a dense wall 5 nm thick and a hollow lumen. Microtubules are variable in length, but they can become many micrometers long. Occasionally, two or more microtubules are linked by protein arms or bridges, which are particularly important in cilia and flagella (Figure 2–31).

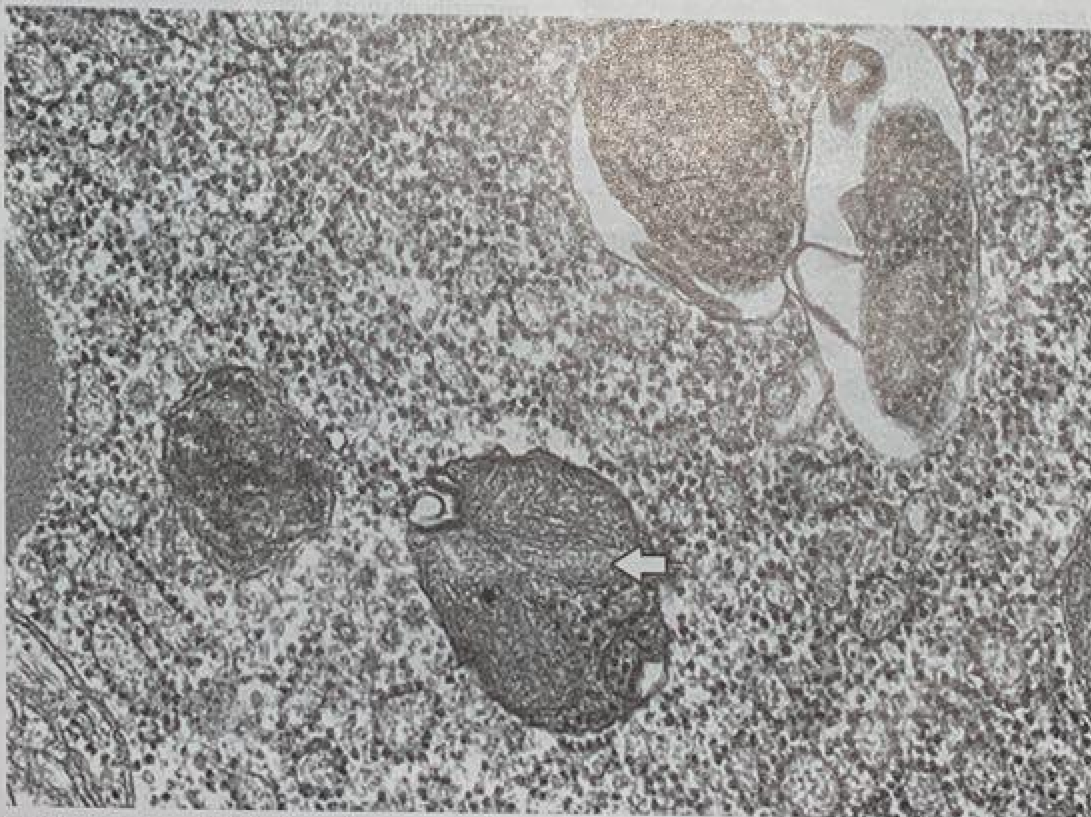


Figure 2–26. **Autophagosomes. Autophagy** is a process in which the cell uses lysosomes to dispose of obsolete or non-functioning organelles or membranes. Details of the process are highly regulated but not well-understood. Membrane of unknown origin encloses the organelles to be destroyed, forming an autophagosome which then fuses with a lysosome for digestion of the contents. In the TEM autophagosomes can sometimes be recognized by their contents, as shown here. **Upper right:** Two autophagosomes containing portions of the RER that are slightly more electron-dense than neighboring normal RER. **Center:** An autophagosome containing what may be mitochondrial membranes (arrow) plus RER. **Left:** A vesicle that may represent a residual body with indigestible material. X20,000.

The protein subunit of a microtubule is a heterodimer composed of α and β **tubulin** molecules of closely related amino acid composition, each with a molecular mass of about 50 kDa.

Under appropriate conditions (*in vivo* or *in vitro*), tubulin heterodimers polymerize to form microtubules, which have a slight spiral organization visible with special EM preparations. A total of 13 units is present in one complete turn of the spiral (Figure 2–28). Longitudinally aligned subunits make up protofilaments and 13 parallel protofilaments constitute a microtubule.

Polymerization of tubulins to form microtubules *in vivo* is directed by **microtubule organizing centers (MTOCs)**, which contain γ-tubulin ring complexes that act as nucleating sites for polymerization. MTOCs include centrosomes and the basal bodies of cilia. Microtubules are polarized structures and growth, via tubulin polymerization, occurs more rapidly at one end of existing microtubules (Figure 2-31a). This end is referred to as the plus (+) end, and the other is the minus (−) end. Microtubules show dynamic instability, with tubulin polymerization and depolymerization dependent on concentrations of Ca^{2+}, Mg^{2+}, GTP and specific **microtubule-associated proteins (MAPs).** Microtubule stability is variable; for example, microtubules of cilia are very stable, whereas microtubules of the mitotic spindle have a short duration. The antimitotic alkaloid colchicine binds specifically to tubulin, and when the complex tubulin-colchicine binds to microtubules, it prevents the addition of more tubulin in the plus (+) extremity. Mitotic microtubules are broken down because the depolymerization continues, mainly at the minus (−) end, and the lost tubulin units are not replaced.

MEDICAL APPLICATION

The antimitotic alkaloids are useful tools in cell biology (eg, colchicine is used to arrest chromosomes in metaphase and to prepare karyotypes) and in cancer chemotherapy (eg, vinblastine, vincristine, and taxol are used to arrest cell proliferation in tumors). Because tumor cells proliferate rapidly, they are more affected by antimitotic drugs than are normal cells. However, chemotherapy has many undesirable consequences. For example, some normal blood-forming cells and the epithelial cells that cover the digestive tract also show a high rate of proliferation and are adversely affected by chemotherapy.

Cytoplasmic microtubules are stiff structures that play a significant role in the formation and maintenance of cell shape. Procedures that disrupt microtubules result in the loss of cellular asymmetry.

Complex microtubule networks also participate in the intracellular transport of organelles and vesicles. Examples include axoplasmic transport in neurons, melanin transport in pigment cells, chromosome movements by the mitotic spindle, and vesicle movements among different cell compartments. In each of these examples, movement is suspended if microtubules are disrupted. Transport along microtubules is under the control of special MAPs called **motor proteins,** which use ATP to move molecules and vesicles. **Kinesins** carry organelles away from the MTOC toward the plus end of microtubules; **cytoplasmic dyneins** carry vesicles in the opposite direction.

Microtubules provide the basis for several complex cytoplasmic components, including centrioles, basal bodies, cilia, and flagella (Figure 2–31b and c). **Centrioles** are cylindrical structures (0.15 μm in diameter and 0.3–0.5 μm in length) composed primarily of short, highly organized microtubules (Figure 2–31c). Each centriole has nine microtubular triplets and adjacent microtubules share some protofilaments. A pair of centrioles surrounded by a matrix of tubulin subunits close to the nucleus of nondividing cells constitutes a **centrosome** (Figure 2–32).

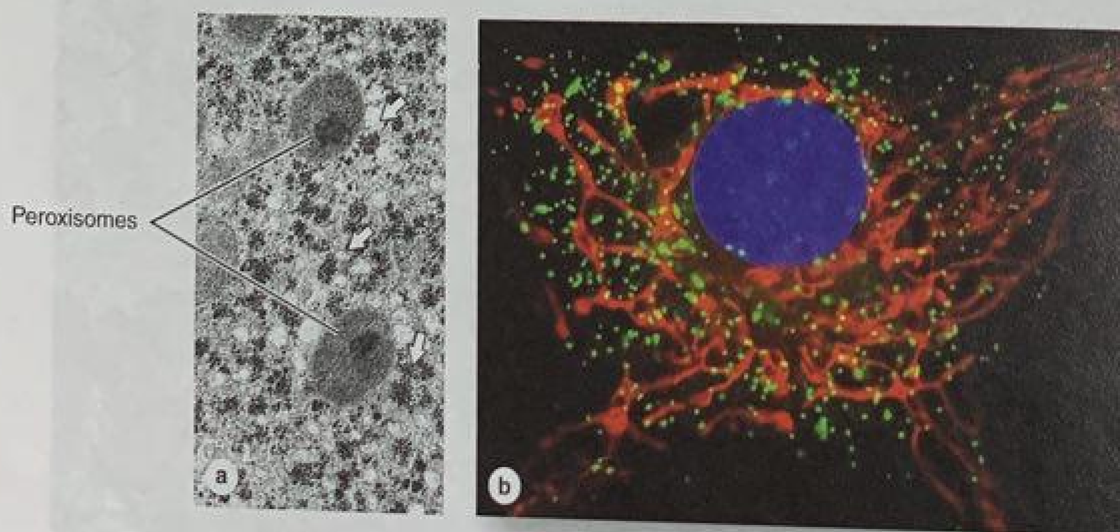


Figure 2–27. **Peroxisomes. Peroxisomes** (or microbodies) are small spherical, membranous organelles, containing enzymes that use O_2 to remove hydrogen atoms from substrates, typically fatty acids, in a reaction that produces hydrogen peroxide (H_2O_2) which must be broken down to water and O_2 by another enzyme, **catalase. (a):** By TEM peroxisomes generally show a homogenous matrix of moderate electron-density, but may include darker crystalloid internal structures representing very dense concentrations of enzymes. The arrows indicate small aggregates of glycogen. (x30,000) **(b):** A cultured endothelial cell processed by immunocytochemistry shows many peroxisomes (green) distributed throughout the cytoplasm among the vitally stained elongate mitochondria (red) around the DAPI-stained nucleus (blue). Peroxisomes shown here were specifically stained using an antibody against the membrane protein PMP70. (Figure 2–27b, with permission, from Invitrogen.)

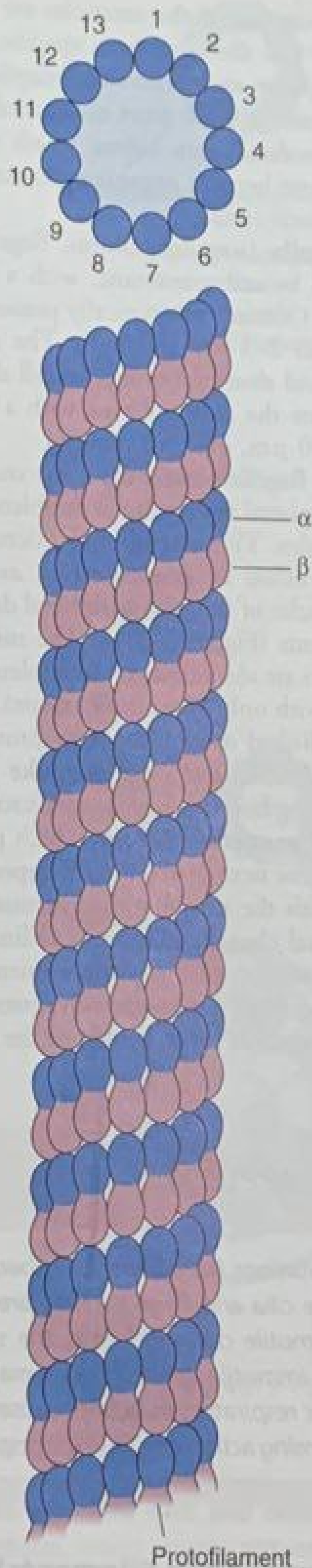


Figure 2–28. **Molecular organization of a microtubule. Microtubules** are rigid structures which assemble from heterodimers of α and β **tubulin**. Microtubules have an outer diameter of 24 nm and a hollow lumen 14 nm wide. Tubulin molecules are arranged to form 13 **protofilaments**, as seen in the cross section in the upper part of the drawing. The specific orientation of the tubulin dimers results in structural polarity of the microtubule. Microtubules elongate or rapidly shorten by the addition or removal of tubulin at the ends of individual protofilaments. The lengths and locations of cytoplasmic microtubules vary greatly during different phases of cell activity, with assembly dependent on shifting balances between polymerized and unpolymerized tubulin and other factors in "dynamic instability."

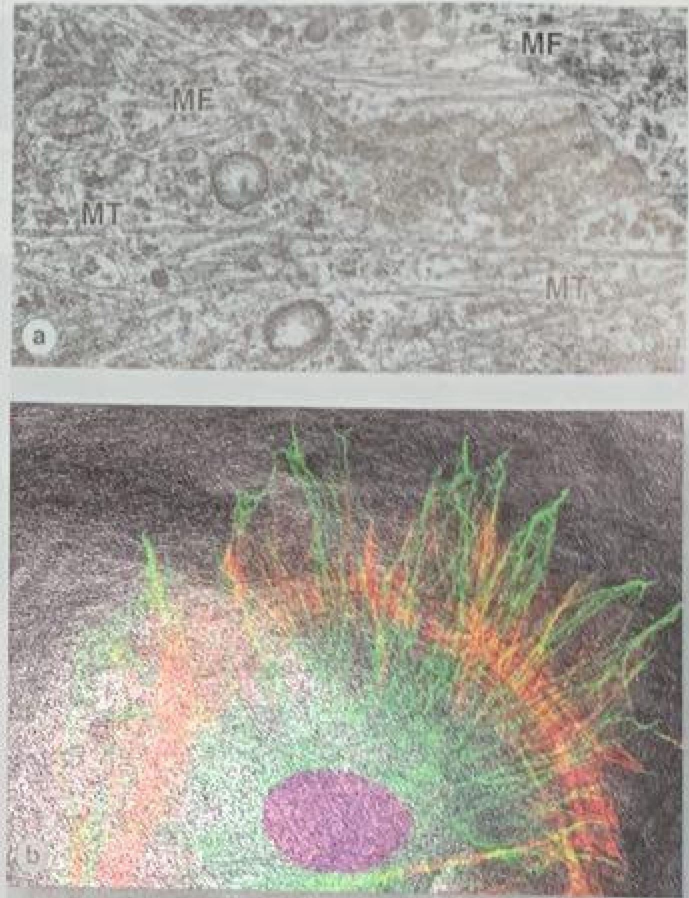


Figure 2–29. **Microtubules and actin filaments in cytoplasm.** (a): Actin micro filaments (MF) and **microtubules** (MT) can both be clearly distinguished in this TEM photo of fibroblast cytoplasm. The image also provides a good comparison of the relative diameters of these two cytoskeletal components. X60,000.

(b): The ultrastructural view can be compared to the appearance of microfilaments and microtubules in a cultured cell stained by immunocytochemistry. Actin filaments (red) are most concentrated at the cell periphery, forming prominent circumferential bundles from which finer filaments project into the transient cellular extensions at the edge of the cell and push against the cell membrane. Such an arrangement of actin filaments forms a dynamic network important for cell shape changes such as those during cell division, locomotion, and formation of cellular processes, folds, pseudopodia, lamellipodia, veils, microvilli, etc. which serve to change a cell's surface area or give direction to a cell's crawling movements.

Microtubules (green/yellow) are present throughout the cytoplasm and are oriented in arrays which generally extend from the area around the nucleus into the most peripheral extensions. Besides serving to stabilize cell shape, microtubules form the tracks for kinesin-based transport of vesicles and organelles into the cell periphery and dynein-based transport toward the cell nucleus. Variations of these arrangements of microfilaments and microtubules can be seen in Figure 2–20c and Figure 2–11b, respectively. (Figure 2–29b, with permission, from Albert Tousson, University of Alabama—Birmingham High Resolution Imaging Facility.)