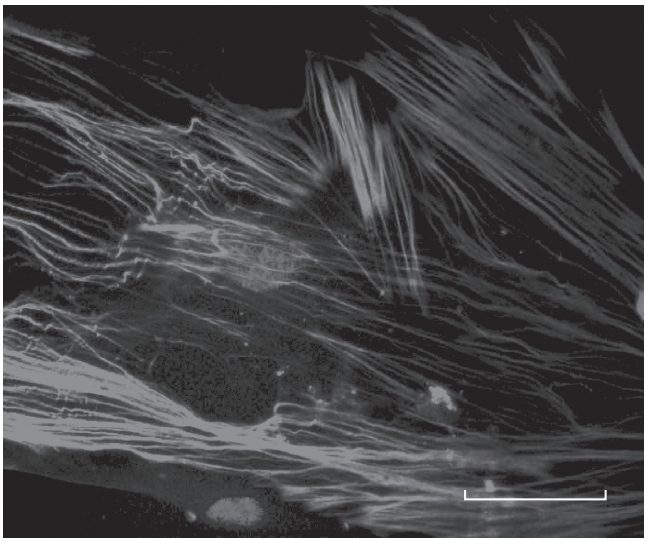
3

STRUCTURE AND FUNCTION OF CELLULAR COMPONENTS



α-Actin filaments in vascular smooth muscle cells derived from the mouse aorta. Smooth muscle cells were collected from the medial layer of the mouse aorta and cultured for 10 days. The α-actin filaments were labeled with an anti-smooth-muscle α actin antibody (red in color) and observed by fluorescence microscopy. Cell nuclei were labeled with Hoechst 33258 (blue in color). Scale bar: 5 μm. See color insert.

Bioregenerative Engineering: Principles and Applications, by Shu Q. Liu

A mammalian cell is composed of numbers of subcellular organelles, including the cell membrane, cytoskeleton, smooth and rough endoplasmic reticulum, Golgi apparatus, lysosomes, peroxisomes, mitochondria, and nucleus. A *cell membrane* is a phospholipid bilayer, which encloses cell contents and separates a cell into different compartments. The *cytoskeleton* is constituted with three distinct elements, including actin filaments, microtubules, and intermediate filaments, which not only give a cell shape, strength, and elasticity but also regulate various cellular functions. *Endoplasmic reticulum* is the site where proteins and phospholipids are synthesized. The *Golgi apparatus* is an organelle in which proteins are processed and modulated. *Lysosomes* contain digestive enzymes, participating in the degradation of engulfed molecules or microorganisms. *Peroxisomes* contain enzymes for the mediation of oxidative reactions. *Mitochondria* are machineries that generate and store energy in the form of ATP. The *nucleus* contains chromosomes and is the center for the storage and processing of genetic information. It becomes clear that each cellular organelle possesses distinct structure and function, yet all cell organelles work together in a highly coordinated manner, ensuring appropriate regulation of cellular activities and functions. In this chapter, the structure, organization, and function of major cellular organelles and compartments are briefly reviewed.

CELL MEMBRANE [3.1]

The cell membrane is composed of lipids and proteins. As discussed in Chapter 1, lipids are amphipathic in nature (i.e., each molecule contains a polar hydrophilic and a nonpolar hydrophobic end) and can spontaneously form bilayers when mixed with an aqueous solution. The most abundant lipids are phospholipids in the cell membrane. Each phospholipid molecule contains a polar hydrophilic head and two nonpolar hydrophobic tails. In addition, cholesterol molecules can be found in a cell membrane. The membrane of a mammalian cell contains about 1×10^9 lipid molecules. Lipid molecules constitute about half of the membrane mass, while the remaining half is primarily proteins. The lipid composition is asymmetric between the two lipid layers of the cell membrane. For instance, glycosphingolipids are found primarily in the external layer, whereas phosphatidylserine is in the internal layer. The primary functions of cell membranes are to separate cellular contents from the extracellular space, create a suitable internal environment for intracellular activities, and establish subcellular compartments for various metabolic and signaling processes.

A lipid bilayer is a fluid-like structure. Lipid molecules can move laterally or diffuse within a lipid monolayer, but cannot change the molecular polarity or flip from one lipid layer to the other. The fluid-like feature of lipid bilayers is dependent on the composition of the cell membrane. For instance, cholesterol molecules reduce the fluidity of cell membranes, and thus enhance the membrane rigidity. The fluidity of a cell membrane ensures dynamic movement of membrane components, including not only lipids but also proteins. The movement of membrane molecules is critical to the function of these molecules as well as the cell. For instance, integrins move toward the leading edge of cell migration and participate in the construction of focal adhesion contacts, regulating cell attachment to the substrate (Fig. 3.1). Growth factor receptors move dynamically, resulting in the redistribution of the receptors to regions that require increased signal inputs from growth factors.

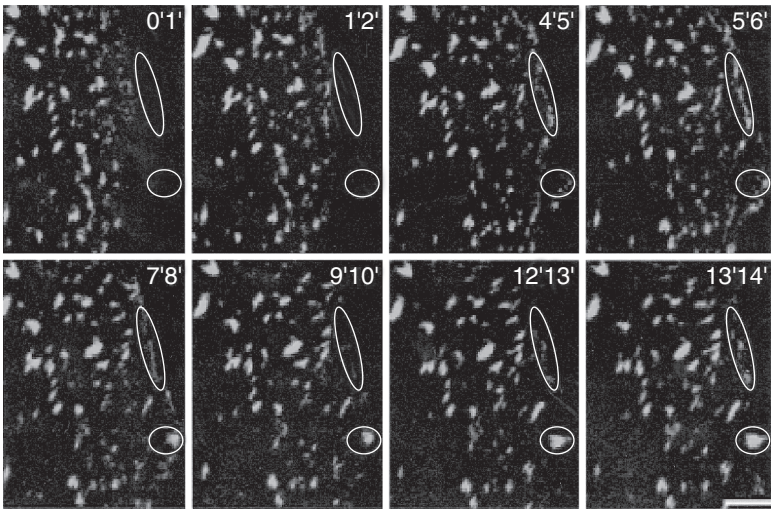


Figure 3.1. Dynamic formation of β3 integrin complexes in porcine arterial endothelial cells. Endothelial cells were transfected with a GFP-β3 integrin gene and cultured to confluence. Cell wound was created by mechanical scraping, which induces cell migration. The images were taken from migrating endothelial cells. Note that new integrin aggregates form at the leading edge of the migrating cells (within the ovals). The times of the sequential images are indicated at the upper right corners. Scale bar: 5 μm. (Reprinted from Zaidel-Bar R et al: *J Cell Sci* 116:4605–13, 2003 by permission of The Company of Biologists Ltd.)

The cell membrane contains various types and amounts of proteins, depending on the type and function of the cell. For instance, a myelin membrane, which encloses and protects the nerve axon, contains proteins about 25% of the membrane mass, whereas a cell membrane that is involved in extensive molecular transport and ligand–receptor interaction may contain up to 75% proteins. Cell membrane proteins may serve as ligand receptors, ion pumps, water and ion channels, or molecule carriers. Membrane proteins can be divided into several classes based on the structure and relationship with the lipid bilayer. One type is transmembrane proteins, which pass through the cell membrane and consist of three domains: the extracellular, transmembrane, and intracellular domains. The extracellular and intracellular domains are usually hydrophilic, whereas the transmembrane domain is hydrophobic. The hydrophilic domains can interact with water-soluble proteins, while the hydrophobic domain interacts with the fatty acid tails of membrane lipids via covalent bonds, serving as an anchoring structure for the protein. The second type of membrane protein is found at the external surface of a cell membrane. These proteins attach to the lipid bilayer via the linkage of oligosaccharides. The third type of protein attaches to the intracellular side of the cell membrane via covalent bonds with fatty acids. In addition, some proteins attach to membrane proteins via noncovalent bonds. The structural relationship between a protein and the cell membrane usually determines the protein function. For instance, transmembrane proteins are responsible for molecular transport across the cell membrane and signal transduction from extracellular ligands to intracellular signaling pathways. Proteins attached to the cytosolic side of the cell membrane usually serve as signaling molecules, which relay signals from transmembrane protein receptors.

CYTOSKELETON

The cell contains a filamentous framework, known as the *cytoskeleton*. There are three cytoskeletal elements: the actin filaments, intermediate filaments, and microtubules. These filaments not only determine the shape and mechanical strength but also participate in the regulation of cellular activities, such as cell adhesion, division, migration, and apoptosis. The structure and function of these filaments are briefly discussed here.

Actin Filaments

Structure and Organization of Actin Filaments [3.2]. An *actin filament* is a helical structure of 8 nm in diameter and is established via polymerization of actin monomers. Each actin monomer contains about 375 amino acid residues with a molecular size about 43 kDa. In mammalian cells, there exist several isoforms of actin (see examples listed in Table 3.1), including the α and β isoforms in muscular cells and β and γ isoforms in nonmuscular cells. The α type of actin constitutes the contractile actin filaments in skeletal, cardiac, and smooth muscle cells. The β and γ types of actin participate in the constitution of the cytoskeleton. Actin filaments with various actin isoforms are localized to different compartments in both muscular and nonmuscular cells. For instance, in nonmuscular cells, β-actin is primarily found near the edge of the cell membrane, whereas γ-actin constitutes stress fibers, which are distributed more uniformly. An actin filament is a polarized structure. When an actin filament is bound with myosin molecules, an array of asymmetric arrowhead-like structures appears under an electron microscope. The end of an actin filament consistent with the arrowhead is defined as the pointed end, whereas the other end is defined the barbed end.

Actin monomers can be self-assembled or polymerized into actin filaments through biochemical reactions (Fig. 3.2). *Actin polymerization* is accomplished in several steps, including actin nucleation, filament growth, and ATP hydrolysis. *Actin nucleation* is a process that induces the formation of actin trimers. These trimeric actin structures, known as *actin nuclei*, serve as initiators for actin polymerization or filament growth. In addition, actin polymerization can be initiated from the barbed end of grown actin filaments or random sites along the side of actin filaments (Fig. 3.2). The addition of an ATP-actin to an actin nucleus or an actin filament triggers hydrolysis of ATP into ADP and phosphate. The phosphate group dissociates from the actin, leaving a newly added actin molecule with a tightly bound ADP.

An actin filament can be simultaneously polymerized and depolymerized at both ends. Under a steady physiological condition, the addition of actin subunits to the barbed end of an actin filament is counterbalanced by the dissociation of actin subunits from the pointed end, resulting in a relatively constant density for actin monomers and filaments. However, the rate of polymerization and depolymerization may change in response to environmental alterations. For instance, an increase in the concentration of ATP and the presence of cations lower the critical level of actin monomers, enhancing actin polymerization. Actin monomers above a critical concentration can be all assembled into actin filaments.

Actin-Binding Proteins. Actin polymerization and depolymerization are regulated by numbers of actin-binding proteins. These proteins are classified into various groups on the basis of their functions, including actin monomer-binding proteins, actin filament-

TABLE 3.1. Characteristics of Selected Actin Isoforms*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Actin α, cardiac	Smooth muscle actin, cardiac actin α, actin α	377	42	Cardiomyocytes and smooth muscle cells	Forming contractile actin filaments in cardiomyocytes and smooth muscle cells
Actin α, skeletal 1	Actin α1	377	42	Skeletal muscle	Forming contractile actin filaments in skeletal muscle cells
Actin α2	Vascular smooth muscle actin, vascular smooth muscle actin α, vascular smooth muscle actin α2, actin 2α	377	42	Vascular smooth muscle cells	Forming actin contractile filaments in vascular smooth muscle cells
Actin β	Cytoskeletal actin β	375	42	Primarily nonmuscular cells	Constituting the cytoskeleton of nonmuscular cells, regulating the motility of nonmuscular cells
Actin γ1	Cytoskeletal actin γ	375	42	Primarily nonmuscular cells	Cytoplasmic actin found in nonmuscular cells, constituting cytoskeleton, and mediating cell motility
Actin γ2 enteric smooth muscle	Actin α3, smooth muscle actin γ	376	42	Intestinal smooth muscle cells	Constituting the cytoskeleton of intestinal smooth muscle cells

*Based on bibliography 3.2.

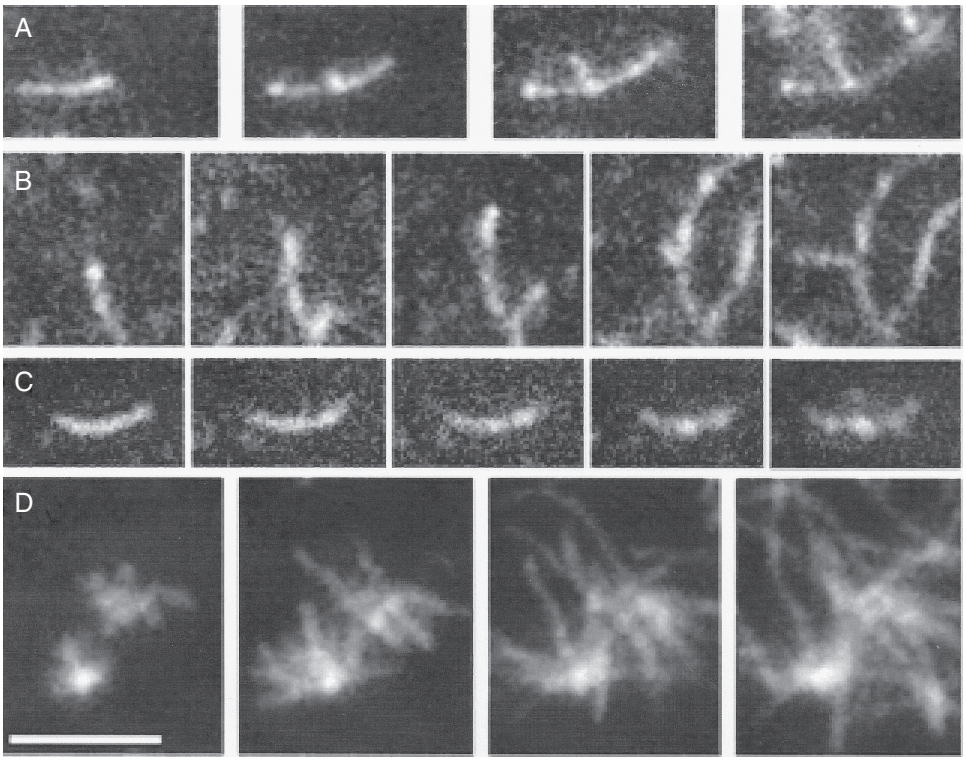


Figure 3.2. Actin filament polymerization and branching. Monomer actin molecules were prepared from rabbit skeletal muscle and labeled on Cys-374 with rhodamine. Actin polymerization was induced in the presence of 20% rhodamine-actin and observed by total internal reflection fluorescence microscopy (TIRFM). The images were subsequently captured at 100, 130, 170, and 210 s after initiating actin polymerization. Scale bar: 4 μm. (Reprinted by permission from Amann KJ et al: *Proc Natl Acad Sci USA* 98:15009–13, copyright 2001, National Academy of Sciences, USA.)

capping proteins, actin filament-binding proteins, actin filament-severing proteins, and actin filament crosslinking proteins.

Actin Monomer-Binding Proteins [3.3]. The family of *actin monomer-binding proteins* (see Table 3.2) includes several molecules, including β-thymosins, cofilins, profilins, and formins, which bind actin monomers and regulate the activities of the actin molecules. *β-Thymosins* are molecules that primarily bind to and sequester ATP-actin monomers, and thus inhibit actin polymerization. *Cofilins* bind ADP-actin with high affinity and destabilize actin filaments. However, a controversial role of cofilins has been observed. *Profilins* bind to ADP- and ATP-free actin monomers and play a role in sequestration of actin monomers. Profilins also inhibit nucleation and elongation at the pointed end of an actin filament, but do not influence the nucleation and elongation at the barbed end. *Formin* is a homodimer composed of formin homology 1 (FH1) and formin homology 2 (FH2) domains. The FH2 domain can bind to monomer actin and induce the nucleation and polymerization of actin filaments. Furthermore, The FH2 domain can bind to the barbed

TABLE 3.2. Characteristics of Selected Actin Monomer-Binding Proteins*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Cofilin 1	CFL1	166	19	Ubiquitous	Binding and depolymerizing filamentous F-actin, inhibiting the polymerization of monomeric G-actin; however, the role of cofilins is controversial
Cofilin 2	CFL2, muscle cofilin	166	19	Skeletal muscle, heart, brain, lung, liver, pancreas, kidney	Same as those for cofilins 1
Profilin	PFN1	140	15	Ubiquitous	Binding to ADP- and ATP-free actin monomers, and sequestering actin monomers
β-Thymosin	Thymosin β4 X chromosome	44	5	Ubiquitous	Sequestering actin monomers, inhibiting actin polymerization, enhancing cardiac cell survival, migration, and regeneration
Formin		844	95	Ubiquitous	Promoting nucleation and polymerization of actin filaments

*Based on bibliography 3.3.

end of actin filaments and promote the elongation of the filaments. The FH1 domain can bind to the actin-binding protein profilin. This process enhances the elongation of actin filaments.

Actin Filament-Capping Proteins [3.4]. Actin filament-capping proteins (see Table 3.3) are molecules that bind either the pointed or the barbed end of actin filaments and prevent actin polymerization or depolymerization. This family of proteins includes gelsolins, heterodimeric capping proteins, the actin-related protein (Arp)2/3 complex, tropomyosin, nebulin, and tropomodulin. *Gelsolins* are capable of binding to the barbed end and the side of actin filaments and inhibiting actin polymerization. *Heterodimeric capping proteins* bind and cap the barbed end of actin filaments, and impose effects similar to those of gelsolins. *Arp 2/3* is a complex of Arp 2 and Arp 3, which binds and caps the pointed end of an actin filament and promotes the attachment of the capped end to a different actin filament and the formation of actin filament branches. It has been shown that this process is regulated by the ρ family GTPases. ρ GTPases activate a protein known as the *Wiskott–Aldrich syndrome protein* (WASP), which in turn activates the Arp2/3 complex. Other actin filament-binding proteins, including tropomyosin, nebulin, capZ, and tropomodulin, bind to the side or ends of actin filaments and contribute to the stability of the filaments. *Tropomyosin* binds the side of actin filaments, induces an increase in the stiffness of the filaments, and stimulates the interaction of actin filaments with myosin. *Nebulin* is found in skeletal muscle cells and plays a role in the control of the length of actin filaments. *Tropomodulin* binds to the pointed end and enhances the stability of actin filaments.

Actin Filament-Severing Proteins. Actin filament-severing proteins include gelsolins, fragmin/severin, and cofilins. These molecules are able to sever actin filaments into short fragments and promote actin filament depolymerization. *Gelsolins* are also capping molecules for the barbed end of actin filaments. *Cofilins* can also bind to actin monomers.

Actin Filament-Crosslinking Proteins [3.5]. Actin filament crosslinking proteins (Table 3.4) include α-actinin, fimbrin, villin, and filamin. These molecules can bind simultaneously to multiple actin filaments and induce crosslink of actin filaments. α-*Actinin* is associated with actin stress fibers and the Z-disk of striated muscular actin fibers. In addition, α-actinin is a constituent of focal adhesion contacts, structures that mediate cell attachment and migration. This molecule possesses multiple functions. *Fimbrin* can bind and crosslink actin filaments in microvilli. *Villin* has a similar function as fimbrin. *Filamin* can not only crosslink actin filaments but also anchor actin filaments to integrins, major constituents of focal adhesion contacts. All these actin filament crosslinking molecules enhance the stability of actin filaments.

Regulation of Actin Assembly and Disassembly [3.6]. In mammalian cells, actin filaments undergo a dynamic turnover process, or simultaneous assembly and disassembly, under physiological conditions. The rate of turnover is dependent on cell types. Nonmuscular cells exhibit actin filament turnover at a timescale of minutes, while muscular cells demonstrate actin filament turnover at a scale of days. Actin polymerization (assembly) and depolymerization (disassembly) can be observed in living cells with fluorescent marker-tagged actin monomers. The fluorescent markers can be incorporated into actin filaments. Following photobleaching of fluorescent actin filaments, the bleached region

TABLE 3.3. Characteristics of Selected Actin Filament-Capping Proteins*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Gelsolin	Actin depolymerizing factor (ADF), Brevin	782	86	Ubiquitous	Inhibiting actin polymerization, severing actin filaments, and promoting actin filament depolymerization
Actin capping protein α1	Muscle Z-line actin filament capping protein α1, CAPZA1, F-actin capping protein α1 subunit	286	33	Skeletal muscle, red blood cells, placenta	Found at the Z line of muscular cells, binding to barbed end of actin filaments, and inhibiting actin polymerization
Actin-related protein 2	ARP2, actin-like protein 2	394	45	Ubiquitous	Constituting the ARP2/3 complex and participating in regulation of cell shape and motility via actin assembly and protrusion
Actin-related protein 3	ARP3, actin-like protein 3	418	47	Ubiquitous	Constituting the ARP2/3 complex and regulating actin assembly
Tropomyosin 1	Tropomyosin skeletal muscle α, tropomyosin 1α chain, α tropomyosin	284	33	Skeletal muscle	Binding to actin filaments in striated muscle cells, stabilizing actin filaments, and regulating calcium-dependent interaction of actin filaments with myosin molecules during muscle contraction
Nebulin	NEB	6669	773	Skeletal muscle cells	Coexisting with thick and thin filaments within sarcomeres of skeletal muscle and playing a critical role in both integrity and stability of contractile filaments
Tropomodulin	Tropomodulin 1, erythrocyte tropomodulin, E-tropomodulin	359	41	Skeletal muscle, heart, brain, lung, liver, kidney, pancreas	Binding to the pointed end and enhancing stability of actin filaments

*Based on bibliography 3.4.

TABLE 3.4. Characteristics of Selected Actin Filament Crosslinking Proteins*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Actinin α1	ACTN1	892	103	Nonmuscular cells	Interacting with actin filaments and regulating the assembly of actin filaments and focal adhesion contacts
Actinin α2	ACTN2, α actinin skeletal muscle isoform 2, F-actin-crosslinking protein	894	104	Skeletal muscle, cardiomyocytes	A muscle-specific α actinin that anchors actin filaments to the Z disks
Fimbrin	Intestine-specific plastin, I-plastin, plastin 1, accumentin	629	70	Intestine, lung, kidney, leukocytes	Binding to and crosslinking actin filaments
Villin	Villin 1	827	93	Intestine, kidney	Inducing crosslinking of actin filaments
Filamin A	Filamin α, filamin 1 (FLN1), actin-binding protein 280 (ABP280), nonmuscle filamin, α-filamin, endothelial actin-binding protein	2647	281	Primarily nonmuscular cells	Inducing crosslinking of actin filaments and regulating the organization and remodeling of actin cytoskeleton by interacting with integrins and transmembrane receptors
Filamin B	β filamin, filamin 1 (actin-binding protein-280)-like, actin-binding-like protein, truncated actin-binding protein Actin-binding protein 276/278, ABP276/278 truncated actin-binding protein	2602	278	Heart, skeletal muscle, brain, lung, liver, kidney, pancreas, uterus, ovary	Inducing actin filament crosslink in muscular and nonmuscular cells and regulating the organization of actin filaments

*Based on bibliography 3.5.

can be replaced with fluorescent actin filaments, suggesting dynamic reassembly of actin filaments. In nonmuscular cells, there exists a relatively high concentration of unpolymerized actin monomers (50–100 μM). Such a concentration allows rapid actin polymerization in response to stimulations that initiate cell adhesion and migration. Indeed, the concentration of actin monomers is a critical factor that controls actin filament assembly and disassembly.

The dynamics of actin assembly–disassembly is regulated by actin regulatory and binding proteins. Sequestration of actin monomers and the capping of actin filaments at the ends are two mechanisms that control the rate of actin filament assembly. As discussed above, profilin and thymosin can bind and sequester actin monomers and reduce the concentration of free actin monomers, suppressing the polymerization of actin filaments. Profilin- or thymosin-bound actin monomers have reduced capability of initiating nucleation. An increase in the activity of actin filament-capping proteins promotes actin polymerization.

Actin filaments are found in all mammalian cells and are organized into various patterns and structures. For instance, actin filaments form a network in the cortical region of the cell, while forming fiber bundles within filopodia or microvilli. The pattern formation of actin filaments is a process that may be regulated by the Rho family of GTPases, which includes Rho, Rac, and Cdc42 (see Table 3.5). These molecules have been shown to regulate distinct processes of actin assembly. Activated *Cdc42* stimulates the formation of filopodia, *Rho* enhances the formation of actin "stress fibers," while *Rac* promotes the formation of cortical network of actin filaments (Fig. 3.3). Although the signaling path-

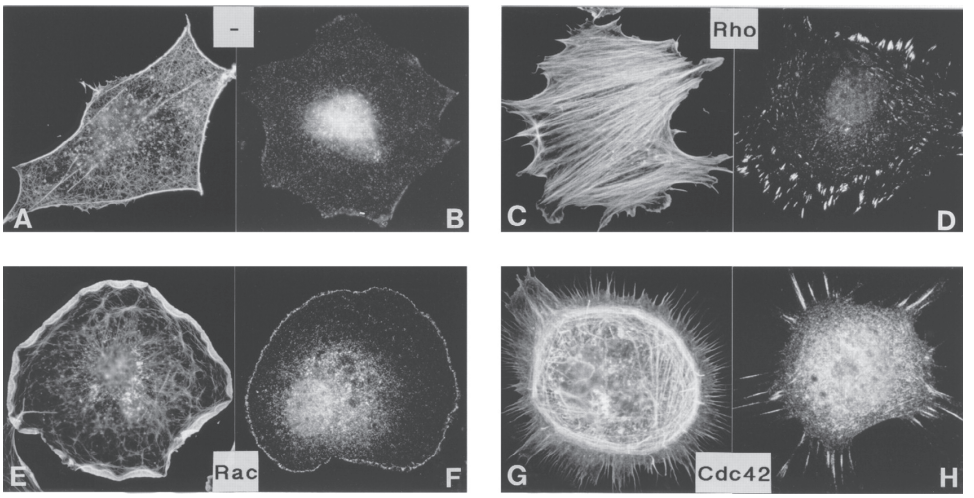


Figure 3.3. Influence of Rho, Rac, and Cdc42 on the organization of actin filaments and morphology of cells: (A,B) quiescent serum-starved Swiss 3T3 fibroblasts labeled for actin filaments and vinculin; (C,D) treatment of cells with lysophosphatidic acid, a growth stimulator, which activates Rho, leading to the formation of organized actin filaments or stress fibers (C) and focal adhesion contacts (D); (E,F) microinjection of Rac induces the formation of lamellipodia (E) and focal adhesion contacts (F); (G,H) microinjection of FGD1, an exchange factor for Cdc42, leads to formation of filopodia (G) and the focal adhesion contacts (H). (Reprinted by permission from Hall A: *Science* 279:509–14, 1998.)

TABLE 3.5. Characteristics of Selected Factors that Regulate the Formation of Actin Filaments*

Proteins	Alternative names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
RhoA	RHOA, aplysia Ras-related homolog 12, oncogene RhoH12, RHOH12, RHO12, transforming protein RhoA, Ras homolog gene family member A	193	22	Leukocytes, platelets	Enhancing the formation of actin stress fibers, regulating cell migration, polarity, and protrusion
RhoB	Oncogene RhoH6, RhoH6, aplysia Ras-related homolog 6	196	22	Nervous system, macrophage, lung	Regulating the formation of actin filaments and assembly of focal adhesion contacts, promoting cell adhesion, vesicle trafficking, MAPK signaling, and immunity
RhoC	Ras homolog gene family member C, aplysia Ras-related homolog 9, oncogene RhoH9, transforming protein RhoC	193	22	Leukocytes, lung, breast, carcinoma cells	Enhancing the formation of actin filaments, regulating cell motility, and mediating tumorigenesis
RAC1	p21-Rac1, Ras-related C3 botulinum toxin substrate 1, Rho family small GTP-binding protein RAC1, Ras-like protein TC25, TC-25	211	23	Ubiquitous	A small Ras GTP-binding protein that regulates cell survival, growth, cytoskeletal reorganization, and the activation of protein kinases
Cdc42	Cell division cycle 42, G25K, GTP-binding protein 25 kDa	191	21		A small ρ GTPase that regulates cell morphology, migration, endocytosis, polarity, and cell cycle progression; also regulates actin polymerization via interaction with neural Wiskott–Aldrich syndrome protein (N-WASP), which subsequently activates the Arp2/3 protein complex

*Based on bibliography 3.6.

ways for these molecules remain poorly understood, these observations provide insights into the mechanisms by which actin filaments form distinct patterns.

Actin assembly and disassembly are regulated by extracellular factors. For instance, *growth factors* and *cytokines* stimulate cell attachment and migration, which are associated with increased actin assembly. These observations suggest a role for growth factors and cytokines in the regulation of actin polymerization or depolymerization. However, exact mechanisms remain poorly understood. In addition, fluid shear stress has been shown to influence actin assembly in vascular endothelial cells. In cell culture models, the introduction of fluid shear stress to endothelial cells enhances actin filament assembly, forming actin "stress fibers." Shear stress-induced deformation of cell membrane receptors or other cell structures may play a role in the initiation of such a process. However, the signaling pathways that transduce shear stress signals remain to be identified.

Function of Actin Filaments [3.7]. Actin filaments participate in a number of functions, including cell contraction, migration, and division. In contractile cells, including skeletal, cardiac, and smooth muscle cells, actin filaments interact with myosin molecules, causing filament sliding and cell contraction, a fundamental process for force generation. In noncontractile cells, directed actin polymerization contributes to regional extension of cell membrane, a primary step in cell migration. The interaction of actin filaments and myosin molecules provide forces that induce cell traction and movement. During cell division, actin filaments form a ring-shaped structure between two premature daughter cells, known as the *contractile ring*, underneath the plasma membrane. Contraction of the ring is initiated following cell mitosis. Such an activity separates the mother cytoplasm into two daughter compartments. While chromosome separation is defined as *mitosis*, cytoplasmic separation is defined as *cytokenesis.*

BIBLIOGRAPHY

3.1. Cell Membrane

Mineo C, Gill GN, Anderson RG: Regulated migration of epidermal growth factor receptor from caveolae, *J Biol Chem* 274:30636–43, 1999.

Bretscher M: The molecules of the cell membrane, *Sci Am* 253:100–8, 1985.

Dowham W: Molecular basis for membrane phospholipid diversity: Why are there so many lipids? *Annu Rev Biochem* 66:199–212, 1997.

Englund PT: The structure and biosynthesis of glycosyl phosphatidylinositol protein anchors, *Annu Rev Biochem* 62:121–38, 1993.

Farazi TA, Waksman G, Gordon J: The biology and enzymology of protein N-myristoylation, *Annu Rev Biochem* 276:39501–64, 2001.

Petty HR: *Molewlar Biology of Membrmles: Structure and Function*, Plenum Press, New York, 1993.

Singer SJ: The structure and insertion of integral proteins in membranes, *Annu Rev Cell Biol* 6:247–96, 1990.

Singer SJ, Nicolson GL: The fluid mosaic model of the structure of cell membranes, *Science* 175:720–31, 1972.

Tamm LK, Arora A, Kleinschmidt JH: Structure and assembly of beta-barrel membrane proteins, *J Biol Chem* 276:32399–402, 2001.

Towler DA, Gordon J, Adams SP, Glaser L: The biology and enzymology of eukaryotic protein acylation, *Annu Rev Biochem* 57:69–99, 1988.

White SH, Ladokhin AS, Jayasinghe S, Hristoya K: How membranes shape protein structure, *J Biol Chem* 276:32395–8, 2001.

Yeagle PL: *The Membranes of Cells*, 2nd ed, Academic Press, San Diego, 1993.

Zhang FL, Casey PJ: Protein prenylation: Molecular mechanisms and functional consequences, *Annu Rev Biochem* 65:241–69, 1996.

Simons K, Vaz WL: Model systems, lipid rafts, and cell membranes, *Annu Rev Biophys Biomol Struct* 33:269–95, 2004.

Vereb G, Szollosi J, Matko J, Nagy P, Farkas T et al: Dynamic, yet structured: The cell membrane three decades after the Singer-Nicolson model, *Proc Natl Acad Sci USA* 100:8053–8, 2003.

Edidin M: The state of lipid rafts: From model membranes to cells, *Annu Rev Biophys Biomol Struct* 32:257–83, 2003.

Lipowsky R: The conformation of membranes, *Nature* 349:475–81, 1991.

3.2. Structure and Organization of Actin Filaments

α-Actin, Cardiac

Gunning P, Ponte P, Kedes L, Eddy R, Shows T: Chromosomal location of the co-expressed human skeletal and cardiac actin genes, *Proc Natl Acad Sci USA* 81:1813–7, 1984.

Hamada H, Petrino MG, Kakunaga T: Molecular structure and evolutionary origin of human cardiac muscle actin gene, *Proc Natl Acad Sci USA* 79:5901–5, 1982.

Humphries SE, Whittall R, Minty A, Buckingham M, Williamson R: There are approximately 20 actin genes in the human genome, *Nucleic Acids Res* 9:4895–908, 1981.

Mogensen J, Klausen IC, Pedersen AK, Egeblad H, Bross P et al: Alpha-cardiac actin is a novel disease gene in familial hypertrophic cardiomyopathy, *J Clin Invest* 103:R39–43, 1999.

Olson TM, Michels VV, Thibodeau SN, Tai YS, Keating MT: Actin mutations in dilated cardiomyopathy, a heritable form of heart failure, *Science* 280:750–2, 1998.

Schwartz K, de la Bastie D, Bouveret P, Oliviero P, Alonso S et al: Alpha-skeletal muscle actin mRNAs accumulate in hypertrophied adult rat hearts, *Circ Res* 59:551–5, 1986.

Takai E, Akita H, Shiga N, Kanazawa K, Yamada S et al: Mutational analysis of the cardiac actin gene in familial and sporadic dilated cardiomyopathy, *Am J Med Genet* 86:325–7, 1999.

Dunwoodie SL, Joya JE, Arkell RM, Hardeman EC: Multiple regions of the human cardiac actin gene are necessary for maturation-based expression in striated muscle, *J Biol Chem* 269:12212–9, 1994.

Skeletal α-Actin

Agrawal PB, Strickland CD, Midgett C, Morales A, Newburger DE et al: Heterogeneity of nemaline myopathy cases with skeletal muscle alpha-actin gene mutations, *Annu Neurol* 56:86–96, 2004.

Akkari PA, Eyre HJ, Wilton SD, Callen DF, Lane SA et al: Assignment of the human skeletal muscle alpha actin gene (ACTA1) to 1q42 by fluorescence in situ hybridization, *Cytogenet Cell Genet* 65:265–7, 1994.

Crawford K, Flick R, Close L, Shelly D, Paul R et al: Mice lacking skeletal muscle actin show reduced muscle strength and growth deficits and die during the neonatal period, *Mol Cell Biol* 22:5887–96, 2002.

Gunning P, Ponte P, Kedes L, Eddy R, Shows T: Chromosomal location of the co-expressed human skeletal and cardiac actin genes, *Proc Natl Acad Sci USA* 81:1813–7, 1984.

Gunning P, Ponte P, Okayama H, Engel J, Blau H et al: Isolation and characterization of full-length cDNA clones for human alpha-, beta-, and gamma-actin mRNAs: Skeletal but not cytoplasmic actins have an amino-terminal cysteine that is subsequently removed, *Mol Cell Biol* 3:787–95, 1983.

Hanauer A, Levin M, Heilig R, Daegelen D, Kahn A et al: Isolation and characterization of cDNA clones for human skeletal muscle alpha actin, *Nucleic Acids Res* 11:3503–16, 1983.

lkovski B, Cooper ST, Nowak K, Ryan MM, Yang N et al: Nemaline myopathy caused by mutations in the muscle alpha-skeletal-actin gene, *Am J Hum Genet* 68:1333–43, 2001.

Laing NG, Clarke NF, Dye DE, Liyanage K, Walker KR et al: Actin mutations are one cause of congenital fibre type disproportion, *Annu Neurol* 56:689–94, 2004.

Nowak KJ, Wattanasirichaigoon D, Goebel HH, Wilce M, Pelin K et al (and 20 others): Mutations in the skeletal muscle alpha-actin gene in patients with actin myopathy and nemaline myopathy, *Nature Genet* 23:208–12, 1999.

Taylor A, Erba HP, Muscat GEO, Kedes L: Nucleotide sequence and expression of the human skeletal alpha-actin gene: evolution of functional regulatory domains, *Genomics* 3:323–36, 1988.

Vascular SMC α-Actin

Kumar MS, Hendrix JA, Johnson AD, Owens GK: Smooth muscle alpha-actin gene requires two E-boxes for proper expression in vivo and is a target of class I basic helix-loop-helix proteins, *Circ Res* 92:840–7, 2003.

Ueyama H, Bruns G, Kanda N: Assignment of the vascular smooth muscle actin gene ACTSA to human chromosome 10, *Jpn J Hum Genet* 35:145–50, 1990.

Ueyama H, Hamada H, Battula N, Kakunaga T: Structure of a human smooth muscle actin gene (aortic type) with a unique intron site, *Mol Cell Biol* 4:1073–8, 1984.

β-Actin

Erba HP, Eddy R, Shows T, Kedes L, Gunning P: Structure, chromosome location, and expression of the human gamma-actin gene: Differential evolution, location, and expression of the cytoskeletal beta- and gamma-actin genes, *Mol Cell Biol* 8:1775–89, 1988.

Habets GGM, van der Kammen RA, Willemsen V, Balemans M, Wiegant J et al: Sublocalization of an invasion-inducing locus and other genes on human chromosome 7, *Cytogenet Cell Genet* 60:200–5, 1992.

Kedes L, Ng SY, Lin CS, Gunning P, Eddy R et al: The human beta-actin multigene family, *Trans Assoc Am Phys* 98:42–6, 1985.

Leavitt J, Bushar G, Kakunaga T, Hamada H, Hirakawa T et al: Variations in expression of mutant beta-actin accompanying incremental increases in human fibroblast tumorigenicity, *Cell* 28:259–68, 1982.

Nakajima-Iijima S, Hamada H, Reddy P, Kakunaga T: Molecular structure of the human cytoplasmic beta-actin gene; interspecies homology of sequences in the introns, *Proc Natl Acad Sci* 82:6133–7, 1985.

Ng SY, Gunning P, Eddy R, Ponte P, Leavitt J et al: Evolution of the functional human beta-actin gene and its multi-pseudogene family: Conservation of the noncoding regions and chromosomal dispersion of pseudogenes, *Mol Cell Biol* 5:2720–32, 1985.

Toyama S, Toyama S: A variant form of beta-actin in a mutant of KB cells resistant to cytochalasin B, *Cell* 37:609–14, 1984.

Ueyama H, Inazawa J, Nishino H, Ohkubo I, Miwa T: FISH localization of human cytoplasmic actin genes ACTB to 7p22 and ACTG1 to 17q25 and characterization of related pseudogenes, *Cytogenet Cell Genet* 74:221–4, 1996.

γ-Actin

Erba HP, Eddy R, Shows T, Kedes L, Gunning P: Structure, chromosome location, and expression of the human gamma-actin gene: differential evolution, location, and expression of the cytoskeletal beta- and gamma-actin genes, *Mol Cell Biol* 8:1775–89, 1988.

Erba HP, Gunning P, Kedes L: Nucleotide sequence of the human gamma cytoskeletal actin mRNA: Anomalous evolution of vertebrate non-muscle actin genes, *Nucleic Acids Res* 14:5275–94, 1986.

Leisel TP, Boujemaa R, Pantaloni D, Carlier MF: Reconstitution of actin-based motility of Listeria and Shigella using pure proteins, *Nature* 401:613–6, 1999.

Otterbein LR, Graceffa P, Dominguez R: The crystal structure of uncomplexed actin in the ADP state, *Science* 293:708–11, 2001.

Ueyama H, Inazawa J, Nishino H, Ohkubo I, Miwa T: FISH localization of human cytoplasmic actin genes ACTB to 7p22 and ACTG1 to 17q25 and characterization of related pseudogenes, *Cytogenet Cell Genet* 74:221–4, 1996.

van Wijk E, Krieger E, Kemperman MH, De Leenheer EMR, Huygen PLM et al: A mutation in the gamma actin 1 (ACTG1) gene causes autosomal dominant hearing loss (DFNA20/26), *J Med Genet* 40:879–84, 2003.

Zhu M, Yang T, Wei S, DeWan AT, Morell RJ et al: Mutations in the gamma-actin gene (ACTG1) are associated with dominant progressive deafness (DFNA20/26), *Am J Hum Genet* 73:1082–91, 2003.

Miwa T, Manabe Y, Kurokawa K, Kamada S, Kanda N et al: Structure, chromosome location, and expression of the human smooth muscle (enteric type) gamma-actin gene: Evolution of six human actin genes, *Mol Cell Biol* 11:3296–306, 1991.

Szucsik JC, Lessard JL: Cloning and sequence analysis of the mouse smooth muscle gamma-enteric actin gene, *Genomics* 28:154–62, 1995.

Ueyama H, Inazawa J, Nishino H, Han-Xiang D, Ochiai Y et al: Chromosomal mapping of the human smooth muscle actin gene (enteric type, ACTA3) to 2p13.1 and molecular nature of the HindIII polymorphism, *Genomics* 25:720–3, 1995.

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

3.3. Actin Monomer-Binding Proteins

Cofilin

Ghosh M, Song X, Mouneimne G, Sidani M, Lawrence DS et al: Cofilin promotes actin polymerization and defines the direction of cell motility, *Science* 304:743–6, 2004.

Gillett GT, Fox MF, Rowe PSN, Casimir CM, Povey S: Mapping of human non-muscle type cofilin (CFL1) to chromosome 11q13 and muscle-type cofilin (CFL2) to chromosome 14, *Annu Hum Genet* 60:201–11, 1996.

Kuhn TB, Meberg PJ, Brown MD, Bernstein BW, Minamide LS et al: Regulating actin dynamics in neuronal growth cones by ADF/cofilin and Rho family GTPases, *J Neurobiol* 44:126–44, 2000.

Maekawa M, Ishizaki T, Boku S, Watanabe N, Fujita A et al: Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase, *Science* 285:895–8, 1999.

Ono S, Minami N, Abe H, Obinata T: Characterization of a novel cofilin isoform that is predominantly expressed in mammalian skeletal muscle, *J Biol Chem* 269:15280–6, 1994.

Profilin

Kovar DR, Harris ES, Mahaffy R, Higgs HN, Pollard TD: Control of the assembly of ATP- and ADP-actin by formins and profilin, *Cell* 124:423–35, 2006.

Ampe C, Markey F, Lindberg U, Vandekerckhove J: The primary structure of human platelet profilin: reinvestigation of the calf spleen profilin sequence, *FEBS Lett* 228:17–21, 1988.

Goldschmidt-Clermont PJ, Janmey PA: Profilin, a weak CAP for actin and RAS, *Cell* 66:419–21, 1991.

Kwiatkowski DJ, Aklog L, Ledbetter DH, Morton CC: Identification of the functional profilin gene, its localization to chromosome subband 17p13.3, and demonstration of its deletion in some patients with Miller-Dieker syndrome, *Am J Hum Genet* 46:559–67, 1990.

Kwiatkowski DJ, Bruns GAP: Human profilin: Molecular cloning, sequence comparison, and chromosomal analysis, *J Biol Chem* 263:5910–5, 1988.

Theriot JA, Mitchison TJ: The three faces of profilin, *Cell* 75:835–8, 1993.

Vojtek A, Haarer B, Field J, Gerst J, Pollard TD et al: Evidence for a functional link between profilin and CAP in the yeast S. cerevisiae, *Cell* 66:497–505, 1991.

Witke W, Sutherland JD, Sharpe A, Arai M, Kwiatkowski DJ: Profilin I is essential for cell survival and cell division in early mouse development, *Proc Natl Acad Sci USA* 98:3832–6, 2001.

β-Thymosin

Bock-Marquette I, Saxena A, White MD, DiMaio JM, Srivastava D: Thymosin beta-4 activates integrin-linked kinase and promotes cardiac cell migration, survival and cardiac repair, *Nature* 432:466–72, 2004.

Clark EA, Golub TR, Lander ES, Hynes RO: Genomic analysis of metastasis reveals an essential role for RhoC, *Nature* 406:532–5, 2000.

Clauss IM, Wathelet MG, Szpirer J, Islam MQ, Levan G et al: Human thymosin-beta-4/6-26 gene is part of a multigene family composed of seven members located on seven different chromosomes, *Genomics* 9:174–80, 1991.

Gondo H, Kudo J, White JW, Barr C, Selvanayagam P et al: Differential expression of the human thymosin-beta(4) gene in lymphocytes, macrophages, and granulocytes, *J Immunol* 139:3840–8, 1987.

Li X, Zimmerman A, Copeland NG, Gilbert DJ, Jenkins NA et al: The mouse thymosin beta-4 gene: structure, promoter identification, and chromosome localization, *Genomics* 32:388–94, 1996.

Formin

Chang F, Drubin D, Nurse P: cdc12p, a protein required for cytokinesis in fission yeast, is a component of the cell division ring and interacts with profilin, *J Cell Biol* 137:169–82, 1997.

Kovar DR, Kuhn JR, Tichy AL, Pollard TD: The fission yeast cytokinesis formin Cdc12p is a barbed end actin filament capping protein gated by profilin, *J Cell Biol* 161:875–87, 2003.

Kovar DR, Pollard TD: Insertional assembly of actin filament barbed ends in association with formins produces piconewton forces, *Proc Natl Acad Sci USA* 101:14725–30, 2004.

Pruyne D, Evangelista M, Yang C, Bi E, Zigmond S et al: Role of formins in actin assembly: nucleation and barbed-end association, *Science* 297:612–5, 2002.

Romero S, Le Clainche C, Didry D, Egile C, Pantaloni D et al: Formin is a processive motor that requires profilin to accelerate actin assembly and associated ATP hydrolysis, *Cell* 119:419–29, 2004.

Sagot I, Rodal AA, Moseley J, Goode BL, Pellman D: An actin nucleation mechanism mediated by Bni1 and profilin, *Nature Cell Biol* 4:626–31, 2002.

Otomo T, Tomchick DR, Otomo C, Panchal SC, Machius M et al: Structural basis of actin filament nucleation and processive capping by a formin homology 2 domain, *Nature* 433:488–94, 2005.

Li F, Higgs HN: The mouse Formin mDia1 is a potent actin nucleation factor regulated by autoinhibition, *Curr Biol* 13:1335–40, 2003.

Kovar DR, Wu JQ, Pollard TD: Profilin-mediated competition between capping protein and formin Cdc12p during cytokinesis in fission yeast, *Mol Biol Cell* 16:2313–24, 2005.

Kovar DR, Pollard TD: Insertional assembly of actin filament barbed ends in association with formins produces piconewton forces, *Proc Natl Acad Sci USA* 101:14725–30, 2004.

Kobielak A, Pasolli HA, Fuchs E: Mammalian formin-1 participates in adherens junctions and polymerization of linear actin cables, *Nature Cell Biol* 6:21–30, 2004.

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

3.4. Actin Filament-Capping Proteins

Gelsolin

Kwiatkowski DJ, Ozelius L, Schuback D, Gusella J, Breakefield XO: The gelsolin (GSN) cDNA clone, from 9q32-34, identifies BclI and StuI RFLPs, *Nucleic Acids Res* 17:4425 (only), 1989.

Kwiatkowski DJ, Stossel TP, Orkin SH, Mole JE, Colten HR et al: Plasma and cytoplasmic gelsolins are encoded by a single gene and contain a duplicated actin-binding domain, *Nature* 323:455–8, 1986.

Lee WM, Galbraith RM: The extracellular actin-scavenger system and actin toxicity, *N Engl J Med* 326:1335–41, 1992.

Vasconcellos CA, Allen PG, Wohl ME, Drazen JM, Janmey PA et al: Reduction in viscosity of cystic fibrosis sputum in vitro by gelsolin, *Science* 263:969–71, 1994.

Witke W, Sharpe AH, Hartwig JH, Azuma T, Stossel TP, Kwiatkowski DJ: Hemostatic, inflammatory, and fibroblast responses are blunted in mice lacking gelsolin, *Cell* 81:41–51, 1995.

Capping Protein α1

Barron-Casella EA, Torres MA, Scherer SW, Heng HHQ, Tsui, LC et al: Sequence analysis and chromosomal localization of human Cap Z: Conserved residues within the actin-binding domain may link Cap Z to gelsolin/severin and profilin protein families, *J Biol Chem* 270:21472–9, 1995.

Hart MC, Korshunova YO, Cooper JA: Mapping of the mouse actin capping protein alpha subunit genes and pseudogenes, *Genomics* 39:264–70, 1997.

ARP2

Leisel TP, Boujemaa R, Pantaloni D, Carlier, MF: Reconstitution of actin-based motility of Listeria and Shigella using pure proteins, *Nature* 401:613–6, 1999.

Machesky LM, Reeves E, Wientjes F, Mattheyse FJ, Grogan A et al: Mammalian actin-related protein 2/3 complex localizes to regions of lamellipodial protrusion and is composed of evolutionarily conserved proteins, *Biochem J* 328:105–12, 1997.

Marchand JB, Kaiser DA, Pollard TD, Higgs HN: Interaction of WASP/Scar proteins with actin and vertebrate Arp2/3 complex, *Nature Cell Biol* 3:76–82, 2001.

Prehoda KE, Scott JA, Mullins RD, Lim WA: Integration of multiple signals through cooperative regulation of the N-WASP-Arp2/3 complex, *Science* 290:801–6, 2000.

Robinson RC, Turbedsky K, Kaiser DA, Marchand JB, Higgs HN et al: Crystal structure of Arp2/3 complex, *Science* 294:1679–84, 2001.

Volkmann N, Amann KJ, Stoilova-McPhie S, Egile C, Winter DC et al: Structure of Arp2/3 complex in its activated state and in actin filament branch junctions, *Science* 293:2456–9, 2001.

Welch MD, DePace AH, Verma S, Iwamatsu A, Mitchison TJ: The human Arp2/3 complex is composed of evolutionarily conserved subunits and is localized to cellular regions of dynamic actin filament assembly, *J Cell Biol* 138:375–84, 1997.

Welch MD, Iwamatsu A, Mitchison TJ: Actin polymerization is induced by Arp2/3 protein complex at the surface of Listeria monocytogenes, *Nature* 385:265–9, 1997.

ARP3

Machesky LM, Reeves E, Wientjes F, Mattheyse FJ, Grogan A et al: Mammalian actin-related protein 2/3 complex localizes to regions of lamellipodial protrusion and is composed of evolutionarily conserved proteins, *Biochem J* 328:105–12, 1997.

Robinson RC, Turbedsky K, Kaiser DA, Marchand JB, Higgs HN et al: Crystal structure of Arp2/3 complex, *Science* 294:1679–84, 2001.

Volkmann N, Amann KJ, Stoilova-McPhie S, Egile C, Winter DC et al: Structure of Arp2/3 complex in its activated state and in actin filament branch junctions, *Science* 293:2456–9, 2001.

Welch MD, DePace AH, Verma S, Iwamatsu A, Mitchison TJ: The human Arp2/3 complex is composed of evolutionarily conserved subunits and is localized to cellular regions of dynamic actin filament assembly, *J Cell Biol* 138:375–84, 1997.

Welch MD, Iwamatsu A, Mitchison TJ: Actin polymerization is induced by Arp2/3 protein complex at the surface of Listeria monocytogenes, *Nature* 385:265–9, 1997.

Tropomyosin 1

Brown JH, Kim KH, Jun G, Greenfield NJ, Dominguez R et al: Deciphering the design of the tropomyosin molecule, *Proc Natl Acad Sci USA* 98:8496–501, 2001.

Eyre H, Akkari PA, Wilton SD, Callen DC, Baker E et al: Assignment of the human skeletal muscle alpha-tropomyosin gene (TPM1) to band 15q22 by fluorescence in situ hybridization, *Cytogenet Cell Genet* 69:15–7, 1995.

Lees-Miller JP, Helfman DM: The molecular basis for tropomyosin isoform diversity, *BioEssays* 13:429–37, 1991.

Schleef M, Werner K, Satzger U, Kaupmann K, Jockusch H: Chromosomal localization and genomic cloning of the mouse alpha-tropomyosin gene Tpm-1, *Genomics* 17:519–21, 1993.

Thierfelder L, Watkins H, MacRae C, Lamas R, McKenna W et al: Alpha-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: A disease of the sarcomere, *Cell* 77:701–12, 1994.

Tiso N, Rampoldi L, Pallavicini A, Zimbello R, Pandolfo D et al: Fine mapping of five human skeletal muscle genes: Alpha-tropomyosin, beta-tropomyosin, troponin-I slow-twitch, troponin-I fast-twitch, and troponin-C fast, *Biochem Biophys Res Commun* 230:347–50, 1997.

Watkins H, McKenna WJ, Thierfelder L, Suk HJ, Anan R et al: Mutations in the genes for cardiac troponin T and alpha-tropomyosin in hypertrophic cardiomyopathy, *N Engl J Med* 332:1058–64, 1995.

Nebulin

Donner K, Sandbacka M, Lehtokari VL, Wallgren-Pettersson C et al: Complete genomic structure of the human nebulin gene and identification of alternatively spliced transcripts, *Eur J Hum Genet* 12:744–51, 2004.

Labeit S, Kolmerer B: The complete primary structure of human nebulin and its correlation to muscle structure, *J Mol Biol* 248:308–15, 1995.

Limongi MZ, Pelliccia F, Rocchi A: Assignment of the human nebulin gene (NEB) to chromosome band 2q24.2 and the alpha-1 (III) collagen gene (COL3A1) to chromosome band 2q32.2 by in situ hybridization: the FRA2G common fragile site lies between the two genes in the 2q31 band, *Cytogenet Cell Genet* 77:259–60, 1997.

Pelin K, Hilpela P, Donner K, Sewry C, Akkari PA et al: Mutations in the nebulin gene associated with autosomal recessive nemaline myopathy, *Proc Natl Acad Sci USA* 96:2305–10, 1999.

Schurr E, Skamene E, Gros P: Mapping of the gene coding for the muscle protein nebulin (Neb) to the proximal region of mouse chromosome 2, *Cytogenet Cell Genet* 57:214–6, 1991.

Stedman H, Browning K, Oliver N, Oronzi-Scott M, Fischbeck K et al: Nebulin cDNAs detect a 25-kilobase transcript in skeletal muscle and localize to human chromosome 2, *Genomics* 2:1–7, 1988.

Wang K, Knipfer M, Huang QQ, van Heerden A, Hsu LCL et al: Human skeletal muscle nebulin sequence encodes a blueprint for thin filament architecture: Sequence motifs and affinity profiles of tandem repeats and terminal SH3, *J Biol Chem* 271:4304–14, 1996.

Tropomodulin

Chu X, Thompson D, Yee LJ, Sung LA: Genomic organization of mouse and human erythrocyte tropomodulin genes encoding the pointed end capping protein for the actin filaments, *Gene* 256:271–81, 2000.

Conley CA: Leiomodin and tropomodulin in smooth muscle, *Am J Physiol Cell Physiol* 280: C1645–56, 2001.

Fowler VM, Sussmann MA, Miller PG, Flucher BE, Daniels MP: Tropomodulin is associated with the free (pointed) ends of the thin filaments in rat skeletal muscle, *J Cell Biol* 120:411–20, 1993.

Lench NJ, Telford EA, Andersen SE, Moynihan TP, Robinson PA et al: An EST and STS-based YAC contig map of human chromosome 9q22.3, *Genomics* 38:199–205, 1996.

Sung LA, Fan YS, Lin CC: Gene assignment, expression, and homology of human tropomodulin, *Genomics* 34:92–6, 1996.

Sung LA, Fowler VM, Lambert K, Sussman MA, Karr D et al: Molecular cloning and characterization of human fetal liver tropomodulin: a tropomyosin-binding protein, *J Biol Chem* 267:2616–21, 1992

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

3.5. Actin Filament Crosslinking Proteins

α-Actinin

Youssoufian H, McAfee M, Kwiatkowski DJ: Cloning and chromosomal localization of the human cytoskeletal alpha-actinin gene reveals linkage to the beta-spectrin gene, *Am J Hum Genet* 47:62–72, 1990.

Beggs AH, Byers TJ, Knoll JHM, Boyce FM, Bruns GAP et al: Cloning and characterization of two human skeletal muscle alpha-actinin genes located on chromosomes 1 and 11, *J Biol Chem* 267:9281–8, 1992.

Beggs AH, Phillips HA, Kozman H, Mulley JC, Wilton SD et al: A (CA)n repeat polymorphism for the human skeletal muscle alpha-actinin gene ACTN2 and its localization on the linkage map of chromosome 1, *Genomics* 13:1314–5, 1992.

Harper SQ, Crawford RW, DellRusso C, Chamberlain JS: Spectrin-like repeats from dystrophin and alpha-actinin-2 are not functionally interchangeable, *Hum Mol Genet* 11:1807–15, 2002.

Mills MA, Yang N, Weinberger RP, Vander Woude DL, Beggs AH et al: Differential expression of the actin-binding proteins, alpha-actinin-2 and -3, in different species: implications for the evolution of functional redundancy, *Hum Mol Genet* 10:1335–46, 2001.

Fimbrin

Lin CS, Shen W, Chen ZP, Tu YH, Matsudaira P: Identification of I-plastin, a human fimbrin isoform expressed in intestine and kidney, *Mol Cell Biol* 14:2457–67, 1994.

Villin

Phillips MJ, Azuma T, Meredith SLM, Squire JA, Ackerley CA et al: Abnormalities in villin gene expression and canalicular microvillus structure in progressive cholestatic liver disease of childhood, *Lancet* 362:1112–9, 2003.

Pringault E, Arpin M, Garcia A, Finidori J, Louvard D: A human villin cDNA clone to investigate the differentiation of intestinal and kidney cells in vivo and in culture, *EMBO J* 5:3119–24, 1986.

Pringault E, Robine S, Louvard D: Structure of the human villin gene, *Proc Natl Acad Sci USA* 88:10811–5, 1991.

Rousseau-Merck MF, Simon-Chazottes D, Arpin M, Pringault E, Louvard D et al: Localization of the villin gene on human chromosome 2q35-q36 and on mouse chromosome 1, *Hum Genet* 78:130–3, 1988.

Schurr E, Skamene E, Morgan K, Chu ML, Gros P: Mapping of Col3a1 and Col6a3 to proximal murine chromosome 1 identifies conserved linkage of structural protein genes between murine chromosome 1 and human chromosome 2q, *Genomics* 8:477–86, 1990.

Filamin A

Chakarova C, Wehnert MS, Uhl K, Sakthivel S, Vosberg HP et al: Genomic structure and fine mapping of the two human filamin gene paralogues FLNB and FLNC and comparative analysis of the filamin gene family, *Hum Genet* 107:597–611, 2000.

Gariboldi M, Maestrini E, Canzian F, Manenti G, De Gregorio L et al: Comparative mapping of the actin-binding protein 280 genes in human and mouse, *Genomics* 21:428–30, 1994.

Gorlin JB, Yamin R, Egan S, Stewart M, Stossel TP et al: Human endothelial actin-binding protein (ABP-280, nonmuscle filamin): A molecular leaf spring, *J Cell Biol* 111:1089–105, 1990.

Loy CJ, Sim KS, Yong EL: Filamin—a fragment localizes to the nucleus to regulate androgen receptor and coactivator functions, *Proc Natl Acad Sci USA* 100:4562–7, 2003.

Maestrini E, Patrosso C, Mancini M, Rivella S, Rocchi M et al: Mapping of two genes encoding isoforms of the actin binding protein ABP-280, a dystrophin like protein, to Xq28 and to chromosome 7, *Hum Mol Genet* 2:761–6, 1993.

Robertson SP, Twigg SRF, Sutherland-Smith AJ, Biancalana V, Gorlin RJ et al: Localized mutations in the gene encoding the cytoskeletal protein filamin A cause diverse malformations in humans, *Nature Genet* 33:487–91, 2003.

Sheen VL, Feng Y, Graham D, Takafuta T, Shapiro SS et al: Filamin A and filamin B are co-expressed within neurons during periods of neuronal migration and can physically interact, *Hum Mol Genet* 11:2845–54, 2002.

Vadlamudi RK, Li F, Adam L, Nguyen D, Ohta Y, Stossel TP et al: Filamin is essential in actin cytoskeletal assembly mediated by p21-activated kinase 1, *Nature Cell Biol* 4:681–90, 2002.

Filamin B

Brocker F, Bardenheuer W, Vieten L, Julicher K, Werner N et al: Assignment of human filamin gene FLNB to human chromosome band 3p14.3 and identification of YACs containing the complete FLNB transcribed region, *Cytogenet Cell Genet* 85:267–8, 1999.

Chakarova C, Wehnert MS, Uhl K, Sakthivel S, Vosberg HP et al: Genomic structure and fine mapping of the two human filamin gene paralogues FLNB and FLNC and comparative analysis of the filamin gene family, *Hum Genet* 107:597–611, 2000.

Krakow D, Robertson SP, King LM, Morgan T, Sebald ET et al (and 20 others): Mutations in the gene encoding filamin B disrupt vertebral segmentation, joint formation and skeletogenesis, *Nature Genet* 36:405–10, 2004.

Sheen VL, Feng Y, Graham D, Takafuta T, Shapiro SS et al: Filamin A and filamin B are co-expressed within neurons during periods of neuronal migration and can physically interact, *Hum Mol Genet* 11:2845–54, 2002.

Takafuta T, Wu G, Murphy GF, Shapiro SS: Human beta-filamin is a new protein that interacts with the cytoplasmic tail of glycoprotein Ib-alpha, *J Biol Chem* 273:17531–8, 1998.

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

3.6. Regulation of Actin Assembly and Disassembly

RhoA

Cannizzaro LA, Madaule P, Hecht F, Axel R, Croce CM et al: Chromosome localization of human ARH genes, a ras-related gene family, *Genomics* 6:197–203, 1990.

Kiss C, Li J, Szeles A, Gizatullin RZ, Kashuba VI, Lushnikova T et al: Assignment of the ARHA and GPX1 genes to human chromosome bands 3p21.3 by in situ hybridization and with somatic cell hybrids, *Cytogenet Cell Genet* 79:228–30, 1997.

Maekawa M, Ishizaki T, Boku S, Watanabe N, Fujita A et al: Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase, *Science* 285:895–8, 1999.

Nakamura M, Nagano T, Chikama T, Nishida T: Role of the small GTP-binding protein Rho in epithelial cell migration in the rabbit cornea, *Invest Ophthalm Vis Sci* 42:941–7, 2001.

Sin WC, Haas K, Ruthazer ES, Cline HT: Dendrite growth increased by visual activity requires NMDA receptor and Rho GTPases, *Nature* 419:475–80, 2002.

Wang HR, Zhang Y, Ozdamar B, Ogunjimi AA, Alexandrova E et al: Regulation of cell polarity and protrusion formation by targeting RhoA for degradation, *Science* 302:1775–9, 2003.

Wu KY, Hengst U, Cox LJ, Macosko EZ, Jeromin A et al: Local translation of RhoA regulates growth cone collapse [letter], *Nature* 436:1020–4, 2005.

RhoB

Liu AX, Cerniglia GJ, Bernhard EJ, Prendergast GC: RhoB is required to mediate apoptosis in neoplastically transformed cells after DNA damage, *Proc Natl Acad Sci USA* 98:6192–7, 2001.

Zhang J, Zhu J, Bu X, Cushion M, Kinane TB et al: Cdc42 and RhoB activation are required for mannose receptor-mediated phagocytosis by human alveolar macrophages, *Mol Biol Cell* 16:824–34, 2005.

Cannizzaro LA, Madaule P, Hecht F, Axel R, Croce CM et al: Chromosome localization of human ARH genes, a ras-related gene family, *Genomics* 6:197–203, 1990.

Chardin P, Madaule P, Tavitian A: Coding sequence of human rho cDNAs clone 6 and clone 9, *Nucleic Acid Res* 16:2717 (only), 1988.

Madaule P, Axel R: A novel ras-related gene family, *Cell* 41:31–40, 1985.

Maekawa M, Ishizaki T, Boku S, Watanabe N, Fujita A et al: Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase, *Science* 285:895–8, 1999.

Ridley AJ, Hall A: The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors, *Cell* 70:389–99, 1992.

Sandilands E, Cans C, Fincham VJ, Brunton VG, Mellor H et al: RhoB and actin polymerization coordinate Src activation with endosome-mediated delivery to the membrane, *Dev Cell* 7:855–69, 2004.

RhoC

Cannizzaro LA, Madaule P, Hecht F, Axel R, Croce CM, Huebner K: Chromosome localization of human ARH genes, a ras-related gene family, *Genomics* 6:197–203, 1990.

Chardin P, Madaule P, Tavitian A: Coding sequence of human rho cDNAs clone 6 and clone 9, *Nucleic Acid Res* 16:2717 (only), 1988.

Clark EA, Golub TR, Lander ES, Hynes RO: Genomic analysis of metastasis reveals an essential role for RhoC, *Nature* 406:532–5, 2000.

Morris SW, Valentine MB, Kirstein MN, Huebner K: Reassignment of the human ARH9 RAS-related gene to chromosome 1p13-p21, *Genomics* 15:677–9, 1993.

Rose R, Weyand M, Lammers M, Ishizaki T, Ahmadian MR et al: Structural and mechanistic insights into the interaction between Rho and mammalian Dia [letter], *Nature* 435:513–8, 2005.

Rac1

Benvenuti F, Hugues S, Walmsley M, Ruf S, Fetler L et al: Requirement of Rac1 and Rac2 expression by mature dendritic cells for T cell priming, *Science* 305:1150–3, 2004.

Chang HY, Ready DF: Rescue of photoreceptor degeneration in rhodopsin-null Drosophila mutants by activated Rac1, *Science* 290:1978–80, 2000.

Eden S, Rohatgi R, Podtelejnikov AV, Mann M, Kirschner MW: Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck, *Nature* 418:790–3, 2002.

Gu Y, Filippi MD, Cancelas JA, Siefring JE, Williams EP et al: Hematopoietic cell regulation by Rac1 and Rac2 guanosine triphosphatases, *Science* 302:445–9, 2003.

Joneson T, McDonough M, Bar-Sagi D, Van Aelst L: RAC regulation of actin polymerization and proliferation by a pathway distinct from Jun kinase, *Science* 274:1374–6, 1996.

Jordan P, Brazao R, Boavida MG, Gespach C, Chastre E: Cloning of a novel human Rac1b splice variant with increased expression in colorectal tumors, *Oncogene* 18:6835–9, 1999.

Katoh H, Negishi M: RhoG activates Rac1 by direct interaction with the Dock180-binding protein Elmo, *Nature* 424:461–4, 2003.

Kheradmand F, Werner E, Tremble P, Symons M, Werb Z: Role of Rac1 and oxygen radicals in collagenase-1 expression induced by cell shape change, *Science* 280:898–902, 1998.

Kissil JL, Johnson KC, Eckman MS, Jacks T: Merlin phosphorylation by p21-activated kinase 2 and effects of phosphorylation on merlin localization, *J Biol Chem* 277:10394–9, 2002.

Lanzetti L, Rybin V, Malabarba MG, Christoforidis S, Scita G et al: The Eps8 protein coordinates EGF receptor signalling through Rac and trafficking through Rab5, *Nature* 408:374–7, 2000.

Malecz N, McCabe PC, Spaargaren C, Qiu RG, Chuang Y et al: Synaptojanin 2, a novel Rac1 effector that regulates clathrin-mediated endocytosis, *Curr Biol* 10:1383–6, 2000.

Manser E, Leung T, Salihuddin H, Zhao ZS, Lim L: A brain serine/threonine protein kinase activated by Cdc42 and Rac1, *Nature* 367:40–6, 1994.

Miki H, Yamaguchi H, Suetsugu S, Takenawa T: IRSp53 is an essential intermediate between Rac and WAVE in the regulation of membrane ruffling, *Nature* 408:732–5, 2000.

Nakaya Y, Kuroda S, Katagiri YT, Kaibuchi K, Takahashi Y: Mesenchymal-epithelial transition during somitic segmentation is regulated by differential roles of Cdc42 and Rac1, *Dev Cell* 7:425–38, 2004.

Radisky DC, Levy DD, Littlepage LE, Liu H, Nelson CM et al: Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability, *Nature* 436:123–7, 2005.

Simon AR, Vikis HG, Stewart S, Fanburg BL, Cochran BH et al: Regulation of STAT3 by direct binding to the Rac1 GTPase, *Science* 290:144–7, 2000.

Sin WC, Haas K, Ruthazer ES, Cline HT: Dendrite growth increased by visual activity requires NMDA receptor and Rho GTPases, *Nature* 419:475–80, 2002.

Walmsley MJ, Ooi SKT, Reynolds LF, Smith SH, Ruf S et al: Critical roles for Rac1 and Rac2 GTPases in B cell development and signaling, *Science* 302:459–62, 2003.

Xiao GH, Beeser A, Chernoff J, Testa JR: p21-activated kinase links Rac/Cdc42 signaling to merlin, *J Biol Chem* 277:883–6, 2002.

Cdc42

Deloukas P, Schuler GD, Gyapay G, Beasley EM, Soderlund C et al (and 60 others): A physical map of 30,000 human genes, *Science* 282:744–6, 1998.

Erickson JW, Zhang C, Kahn RA, Evans T, Cerione RA: Mammalian cdc42 is a brefeldin A-sensitive component of the Golgi apparatus, *J Biol Chem* 271:26850–4, 1996.

Etienne-Manneville S, Hall A: Cdc42 regulates GSK-3-beta and adenomatous polyposis coli to control cell polarity, *Nature* 421:753–6, 2003.

Garrett WS, Chen LM, Kroschewski R, Ebersold M et al: Developmental control of endocytosis in dendritic cells by Cdc42, *Cell* 102:325–34, 2000.

Irie F, Yamaguchi Y: EphB receptors regulate dendritic spine development via intersectin, Cdc42 and N-WASP, *Nature Neurosci* 5:1117–8, 2002.

Kim AS, Kakalis LT, Abdul-Manan N, Liu GA, Rosen MK: Autoinhibition and activation mechanisms of the Wiskott-Aldrich syndrome protein, *Nature* 404:151–8, 2000.

Manser E, Leung T, Salihuddin H, Tan L, Lim L: A non-receptor tyrosine kinase that inhibits the GTPase activity of p21cdc42, *Nature* 363:364–7, 1993.

Munemitsu S, Innis MA, Clark R, McCormick F, Ullrich A et al: Molecular cloning and expression of a G25K cDNA, the human homolog of the yeast cell cycle gene CDC42, *Mol Cell Biol* 10:5977–82, 1990.

Nakaya Y, Kuroda S, Katagiri YT, Kaibuchi K, Takahashi Y: Mesenchymal-epithelial transition during somitic segmentation is regulated by differential roles of Cdc42 and Rac1, *Dev Cell* 7:425–38, 2004.

Nalbant P, Hodgson L, Kraynov V, Toutchkine A, Hahn KM: Activation of endogenous Cdc42 visualized in living cells, *Science* 305:1615–9, 2004.

Nicole S, White PS, Topaloglu H, Beigthon P, Salih M et al: The human CDC42 gene: Genomic organization, evidence for the existence of a putative pseudogene and exclusion as a SJS1 candidate gene, *Hum Genet* 105:98–103, 1999.

Shinjo K, Koland JG, Hart MJ, Narasimhan V, Johnson DI et al: Molecular cloning of the gene for the human placental GTP-binding protein G(p) (G25K): identification of this GTP-binding protein as the human homolog of the yeast cell-division-cycle protein CDC42, *Proc Natl Acad Sci USA* 87:9853–7, 1990.

Sin WC, Haas K, Ruthazer ES, Cline HT: Dendrite growth increased by visual activity requires NMDA receptor and Rho GTPases, *Nature* 419:475–80, 2002.

Wu WJ, Erickson JW, Lin R, Cerione RA: The gamma-subunit of the coatomer complex binds Cdc42 to mediate transformation, *Nature* 405:800–4, 2000.

Yasuda S, Oceguera-Yanez F, Kato T, Okamoto M et al: Cdc42 and mDia3 regulate microtubule attachment to kinetochores, *Nature* 428:767–71, 2004.

Zheng Y, Fischer DJ, Santos MF, Tigyi G, Pasteris NG et al: The faciogenital dysplasia gene product FGD1 functions as a Cdc42Hs-specific guanine-nucleotide exchange factor, *J Biol Chem* 271:33169–72, 1996.

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

3.7. Function of Actin Filaments

Bamburg JR, Wiggan OP: ADF/cofilin and actin dynamics in disease, *Trends Cell Biol* 12:598–605, 2002.

Campbell KP: Three muscular dystrophies: Loss of cytoskeleton-extracellular matrix linkage, *Cell* 80:675–9, 1995.

Holmes KC, Popp D, Gebhard W, Kabsch W: Atomic model of the actin filament, *Nature* 347:44–9, 1990.

Luna EJ, Hitt AL: Cytoskeleton—plasma membrane interactions, *Science* 258:955–64, 1992.

Schmidt A, Hall MN: Signaling to the actin cytoskeleton, *Annu Rev Cell Dev Biol* 14:305–38, 1998.

Winder SJ: Structural insights into actin-binding, branching and bundling proteins, *Curr Opin Cell Biol* 15:14–22, 2003.

Finer JT, Simmons RM, Spudich JA: Single myosin molecule mechanics: piconewton forces and nanometre steps, *Nature* 368:113–9, 1994.

Geeves MA, Holmes KC: Structural mechanism of muscle contraction, *Annu Rev Biochem* 68:687–728, 1999.

Goldman YE: Wag the tail: Structural dynamics of actomyosin, *Cell* 93:1–4, 1998.

Huxley HE: The mechanism of muscular contraction, *Science* 164:1356–65, 1969.

Pantaloni D, Le Clainche C, Carlier MF: Mechanism of actin-based motility, *Science* 292:1502–6, 2001.

Rayment I, Smith C, Yount RG: The active site of myosin, *Annu Rev Physiol* 58:671–702, 1996.

Schroder RR, Manstein DJ, Jahn W, Holden H, Rayment I et al: Three-dimensional atomic model of F-actin decorated with Dictyostelium myosin S1, *Nature* 364:171–4, 1993.

Welch MD, Mallavarapu A, Rosenblatt J, Mitchison TJ: Actin dynamics in vivo, *Curr Opin Cell Biol* 9:54–61, 1997.

Rayment I, Holden HM, Whittaker M, Yohn CB, Lorenz M et al: Structure of the actin-myosin complex and its implications for muscle contraction, *Science* 261:58–65, 1993.

Rayment I, Rypniewski WR, Schmidt-Base K, Smith R, Tomchick DR et al: Three-dimensional structure of myosin subfragment-1: A molecular motor, *Science* 261:50–58, 1993.

Ruppel KM, Spudich JA: Structure-function analysis of the motor domain of myosin, *Annu Rev Cell Dev Biol* 12:543–73, 1996.

Small JV, Glotzer M: Cell structure and dynamics, *Curr Opin Cell Biol* 18:1–3, 2006.

Tan JL, Ravid S, Spudich JA: Control of nonmuscle myosins by phosphorylation, *Annu Rev Biochem* 61:721–59, 1992.

Cramer LP, Mitchison TJ, Theriot JA: Actin-dependent motile forces and cell motility, *Curr Opin Cell Biol* 6:82–6, 1994.

Theriot JA, Mitchison TJ: Actin microfilament dynamics in locomoting cells, *Nature* 352:126–31, 1991.

Microtubules

Structure and Organization of Microtubules [3.8]. *Microtubules* are hollow polymeric microcylinders about 20 nm in diameter and up to 20 μm in length. Microtubules are composed of dimeric tubulins. There are three types of tubulin: α, β, and γ (see Table 3.6). The α- and β-tubulins are the primary constituents of microtubules, whereas the γ-tubulin regulates the nucleation of microtubule assembly. Each tubulin molecule used for constructing the microtubules is a heterodimer of α- and β-tubulin. In mammalian cells, there are several isoforms for α- as well as for β-tubulin. These isoforms have similar structures, but are originated from different genes. All tubulin isoforms can be polymerized into microtubules. Tubulin can be found in all mammalian cells. However, the distribution of tubulin varies in different cell types. For instance, the nerve cells exhibit a higher concentration of tubulin than do other cell types. The tubulin genes are highly conserved among different species.

In microtubules, α- and β-tubulin dimers are uniformly aligned along the axis of the microtubule, forming parallel protofilaments. In each protofilament, the α- or β-tubulin subunits are always arranged in the same direction, giving a polarity to microtubules with a plus and minus end. Each microtubule is composed of 13 protofilaments (Fig. 3.4). In

TABLE 3.6. Characteristics of Selected Tubulin Isoforms*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Tubulin α1	TUBA1, Tubulin α testis-specific	448	50	Nervous system, testis	Constituting microtubules
Tubulin α2	TUBA2	450	50	Thymus, leukocytes, intestine, overy, testis	Constituting microtubules
Tubulin α3	Tubulin α brain-specific, B α1	451	50	Brain	Constituting microtubules in central nervous system
Tubulin α, ubiquitous	K-α1	451	50	Ubiquitous	Constituting microtubules
Tubulin β	TUBB	445	50	Brain	Constituting microtubules
Tubulin γ	TUBG1, TUBG, tubulin γ1 chain, γ1 tubulin, γ tubulin complex component 1, tubulin γ polypeptide	451	51	Heart, lung, liver, kidney, intestine, ovary, skeletal muscle	Regulating the nucleation of microtubule assembly

*Based on bibliography 3.8.

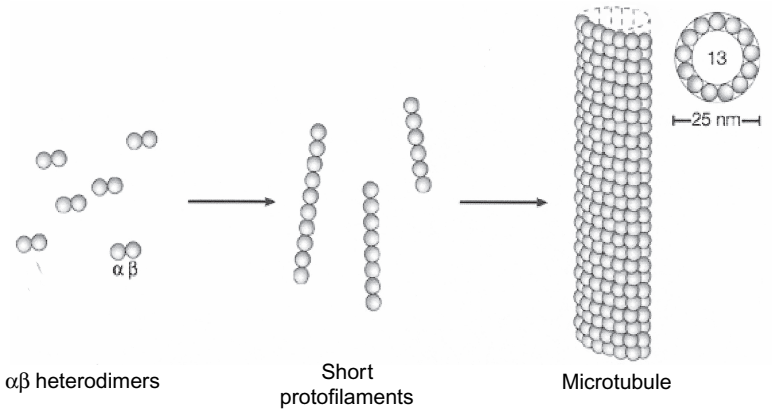


Figure 3.4. Formation of a microtubule from tubulin molecules. A microtubule is formed via several steps: (1) an α, β-tubulin monomer aggregates to form a tubulin heterodimer; (2) the tubulin heterodimers form short linear protofilaments; (3) 13 protofilaments are joined together laterally to organize into a microtubule. (Adapted by permission from Macmillan Publishers Ltd.: Westermann S, Weber K: *Nature Rev Mol Cell Biol* 4:938–48, copyright 2003.)

an interphase cell, microtubules are distributed in the radial direction with the minus end attached to the centrosome and the plus end toward the cell periphery.

It is important to note that several substances, including colchicine, colcemid, and taxol, are commonly used to modulate the assembly, structure, stability, and function of microtubules. *Colchicine* is an alkaloid extracted from meadow saffron. Colchicine can bind to tubulin and suppress tubulin polymerization or microtubule assembly. Since microtubules undergo continuously depolymerization, a treatment with colchicine facilitates the disassembly of microtubules. Once tubulin molecules are polymerized into microtubules, colchicine can no longer bind to tubulin. *Colcemid* is a substance similar to colchicine in function. Since the disassemble of microtubules interrupt cell mitosis, colchicine and colcemid are used to treat cancer. *Taxol* is derived from yew trees and can bind to polymerized microtubules. The binding of taxol enhances the stability of microtubules, inhibiting tubulin depolymerization. Such an effect induces cell arrest during mitosis. Taxol is also used as a drug for the treatment of cancer.

Microtubule Assembly and Disassembly [3.9]. Microtubule assembly is accomplished via tubulin polymerization, whereas its disassembly is via tubulin depolymerization. There are two critical processes, which are involved in microtubule assembly: nucleation and elongation. *Nucleation* is the formation of short tubulin protofilaments or oligomers, which further form a short initiating microtubule (Fig. 3.4). *Elongation* is the growth of microtubules based on the initial microtubule segment. Microtubule assembly can be simulated in vitro with tubulins in the presence of Mg^{2+} and GTP. The initial nucleation from tubulin heteodimers is a more difficult process than elongation. Thus nucleation is usually a slower process than elongation. While a microtubule is elongating via tubulin polymerization, there also exists simultaneous tubulin depolymerization. The rate of tubulin polymerization and depolymerization is dependent on the concentration of free tubulins. At a critical concentration of free tubulin, the rate of tubulin polymerization is counterbalanced by that of depolymerization, and microtubules cease growing.

Microtubules are connected at their minus end to a central structure within the cell, known as the *centrosome*, which is located in the nucleus during the interphase. The centrosome is considered the origin where microtubules grow from. The relationship of microtubules with the centrosome can be verified by observing the growth of degraded microtubules. A treatment with colcemid induces the degradation of microtubules. In the presence of fluorescent marker-tagged tubulins, it can be found that new microtubules grow from the centrosome following the removal of colcemid. These microtubules continuously elongate toward the cell periphery until a complete microtubule network is reestablished. Each centrosome contains two cylindrical structures perpendicular to each other, known as *centrioles*. During the interphase, the centrosome can be split into two daughter centrosomes, which move to opposite sides of the nucleus during the early stage of cell mitosis, serving as two poles for anchoring microtubule spindles (Fig. 3.5).

A microtubule undergoes rapid assembly and disassembly. The tubulins within a microtubule could be completely replaced with new tubulins within a period as short as 20 min. Such a process can be detected by injecting fluorescent marker-tagged tubulins into a living cell and observed by fluorescence microscopy. It is interesting to note that microtubules undergo alternating growth and retraction, resulting in a dynamic change in the length of microtubules. These dynamic changes are critical for the redistribution of microtubules within a cell.

Microtubule dynamics requires the presence of GTPs, which produce energy by hydrolysis. Each α- and β-tubulin is bound with a GTP molecule, which is required for tubulin polymerization. On the polymerization of a tubulin heterodimer to a microtubule, the GTP molecule associated with the β-tubulin can be hydrolyzed to produce energy, whereas the GTP molecule associated with the α-tubulin serves as a constituent of the tubulin and cannot be hydrolyzed. The energy produced by the hydrolysis of the β-tubulin-associated GTP is used for microtubule depolymerization, but not for polymerization. This can be

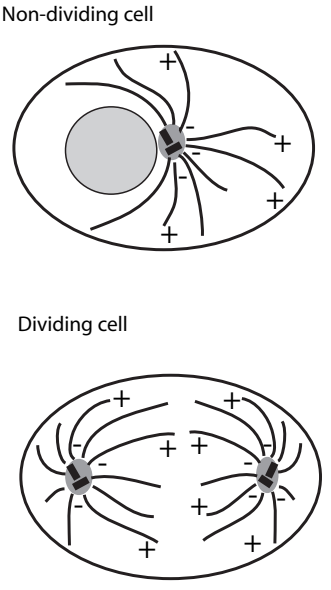


Figure 3.5. Centrosomes and microtubules in nondividing and dividing cells (based on bibliography 3.9).

verified by using GTP analogs that cannot be hydrolyzed. Tubulins associated with GTP analogs can be polymerized. However, once incorporated into a microtubule, these tubulin molecules cannot be depolymerized, suggesting that GTP hydrolysis is critical to the depolymerization of microtubules.

A microtubule can be assembled at the plus and minus ends, but exhibits different assembly rate at these ends under a given condition. The assembly of microtubules can be observed by using in vitro experiments. Isolated microtubules from cells can grow in the presence of free tubulins. The plus end of a microtubule grows about 3 times faster than the minus end. Since microtubules are aligned in the radial direction of a cell with the plus ends pointing at the periphery, microtubules often grow from the cell center to the periphery.

Regulation of Microtubule Dynamics [3.9]. The assembly and disassembly of microtubules are processes regulated by microtubule-associated proteins (Table 3.7). Two major types of microtubule-associated proteins have been identified in nerve cells: the high-molecular-weight proteins and the τ proteins. The *high-molecular-weight proteins* include microtubule-associated proteins 1 and 2 with molecular weights 200 and 300 kDa, respectively. The τ *proteins* have molecular weights ranging from 55 to 62 kDa. Each of these microtubule-associated proteins contains two domains; the first domain is capable of binding to microtubules, and the second domain binds to other types of intracellular structures. The binding of microtubule-associated proteins to microtubules prevents microtubules from depolymerization and enhances the stability of the microtubules. The exact regulatory mechanisms, however, remain to be investigated.

Function of Microtubules [3.10]. One of the primary functions of microtubules is the control of cell polarity. Microtubules exhibit nonuniform tubulin polymerization and depolymerization through the cell. Such a nonuniform feature is critical to the controlled distribution of microtubules, potentially contributing to cell polarization. At a given time, some microtubules may undergo predominant polymerization, while others may experience depolymerization. Fast-growing microtubules may be capped or protected by capping molecules, yielding stabilized microtubules in a specified direction. Meanwhile, uncapped microtubules are not stable and cannot grow as rapidly as the capped microtubules. The rapid growth of the capped microtubules causes regional extension of the cell membrane, leading to the formation of cell polarity.

Microtubules play a critical role in the transport of intracellular organelles and vesicles, which are required for a variety of metabolic and signaling activities. The transport function is accomplished by coordinated interactions of motor proteins, including kinesin and dynein, with microtubules. Each motor molecule is composed of two heavy chains and several light chains. Each heavy chain contains a globular head and a tail. The head interacts directly with microtubules and induces the sliding of the motor protein along a microtubule, a process dependent on ATPs, whereas the tail binds to an intracellular component to be moved. The light chains also play a role in the regulation of motor protein movement.

The motor proteins *kinesin* and *dynein* (Table 3.8) are both involved in the transport of intracellular organelles and chromosome separation during mitosis. However, kinesin and dynein move in opposite directions along a microtubule. Kinesin can only move intracellular organelles from the centrosome or the minus end of the microtubule to the cell periphery or the plus end of the microtubule, whereas dynein moves toward the cen-

TABLE 3.7. Characteristics of Selected Microtubule-Associated Proteins*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Microtubule -associated protein 1A	Microtubule-associated protein 1-like, MTAP1A	2805	306	Central nerveous system	Regulating microtubule assembly
Microtubule -associated protein 1B	MAP1B, MAP1 light chain LC1	2468	271	Central nerveous system	Regulating microtubule assembly
Microtubule-associated protein-2	MAP2, dendrite-specific MAP	1858	203	Central nerveous system	Regulating microtubule assembly
τ	Microtubule-associated protein τ, MAPT, MTBT1, neurofibrillary tangle protein, paired helical filament τ	758	79	Central nerveous system	Regulating microtubule assembly, playing a critical role in both integrity and functionality of neurons[a]

[a]Note that τ mutation induces the formation of neurofibrillary tangles and causes neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, frontotemporal dementia, and supranuclear palsy.
*Based on bibliography 3.9.

TABLE 3.8. Characteristics of the Motor Proteins Kinesin and Dynein*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Kinesin heavy chain 2	HK2	679	77	Nervous system, lung, spleen, stomach, testis, placenta	A constituent for the microtubule-associated motor protein kinesin, which mediates movement of cytoplasmic structures such as chromosomes and vesicles[a]
Kinesin light chain	Kinesin 2	569	65	Nervous system	A constituent for the microtubule-associated motor protein kinesin (see above for function)
Dynein cytoplasmic heavy chain 1	Dynein cytoplasmic heavy polypeptide 1	4646	532	Brain, heart, lung, pancreas, liver, testis	A constituent for the microtubule-associated motor protein dynein, which move organelles and vesicles from cell periphery or plus end of microtubule to centrosome or minus end of microtubule
Dynein cytoplasmic intermediate chain 1	Cytoplasmic dynein intermediate chain 1	645	73	Brain heart, lung, pancreas, liver, testis	A constituent for the microtubule-associated motor protein dynein (see above for dynein function)
Dynein cytoplasmic light chain 1	8-kDa dynein light chain, cytoplasmic dynein light polypeptide	89	10	Brain, heart, lung, pancreas, liver, testis	A constituent for the microtubule-associated motor protein dynein (see above for dynein function)

[a]Note that kinesins move organelles from the centrosome or the minus end of the microtubule to the cell periphery or the plus end of the microtubule.
*Based on bibliography 3.10.

trosome. A *kinesin* molecule is a tetramer composed of two heavy and two light chains. The heavy chain is located at the *N*-terminus of the molecule, and the light chains are at the *C*-terminus. The *N*-terminal heavy chains form the motor domains with the microtubule-binding regions, which mediate the sliding motion of the kinesin molecule along the microtubule. The *N*-terminal heavy chain also possesses an ATP-binding site, which serves as an ATPase and interacts with ATP molecules to provide energy for kinesin movement. The movement caused by kinesin molecules can be readily verified by using in vitro assays with purified motor proteins and microtubules. When microtubules are mixed with kinesin-coated polystyrene beads, the beads move toward the plus end of microtubules.

Dyneins are a family of motor proteins that are divided into two groups: the axonal and cytoplasmic dyneins. The axonal dynein molecules are responsible for organelle transport within the neuronal axon. Cytoplasmic dyneins mediate intracellular motility, protein sorting, and movement of intracellular organelles such as endosomes and lysosomes. A dynein molecule is comprised of two force-generating heavy chains and several intermediate and light chains. The heavy chains contain ATPases, which interact with ATP molecules and generate energy for mechanical movement. The motility of dynein molecules can be observed by using in vitro assays with purified dynein molecules and microtubules. Dynein molecules can move intracellular organelles from the cell periphery or the plus end of the microtubule to the centrosome or the minus end of the microtubule.

Microtubules are well known for their role in regulating cell mitosis or the segregation of chromosomes. Microtubules and associated proteins constitute a key structure for cell mitosis, known as the *mitotic spindle*, which plays a critical role in the alignment and separation of chromosomes. During the early stage of mitosis or prophase, the centrosome is separated into two daughter centrosomes, which move toward the two opposite poles. A mitotic spindle is initiated from the two centrosomes and gradually forms a polar structure. During metaphase, chromosomes are attached to the spindle microtubules. The shortening of the microtubules induces the movement of separated daughter chromosomes from the cell center toward the two centrosome poles. The destruction of microtubules by a treatment with colchicine interrupts cell mitosis.

Intermediate Filaments

Structure and Organization of Intermediate Filaments [3.11]. *Intermediate filaments* are one of the three types of filamentous structures that constitute the cytoskeleton. The term "intermediate" is derived from the fact that the diameter of intermediate filaments (~10 nm) is between the other two types of cytoskeletal filaments (Fig. 3.6), specifically, actin filaments (~8 nm) and microtubules (~25 nm). Intermediate filaments are composed of various molecules, including keratin, vimentin, neurofilament protein, and nuclear lamin. The constituent molecules of intermediate filaments are fibrous in shape. To form an intermediate filament, two molecules are organized into a parallel dimer with the amino termini at one end and the carboxyl termini at the other end. For most types of intermediate filaments, the two dimers in turn form an antiparallel tetramer bundle with the amino termini of one dimer arranged with the carboxyl termini of the other dimer at each end of the tetramer bundle (Fig. 3.7). The tetramers are the basic units that are assembled into helical intermediate filaments via bundle–lateral interactions. Because of the antiparallel feature of the tetramer bundles, intermediate filaments do not exhibit polarity.

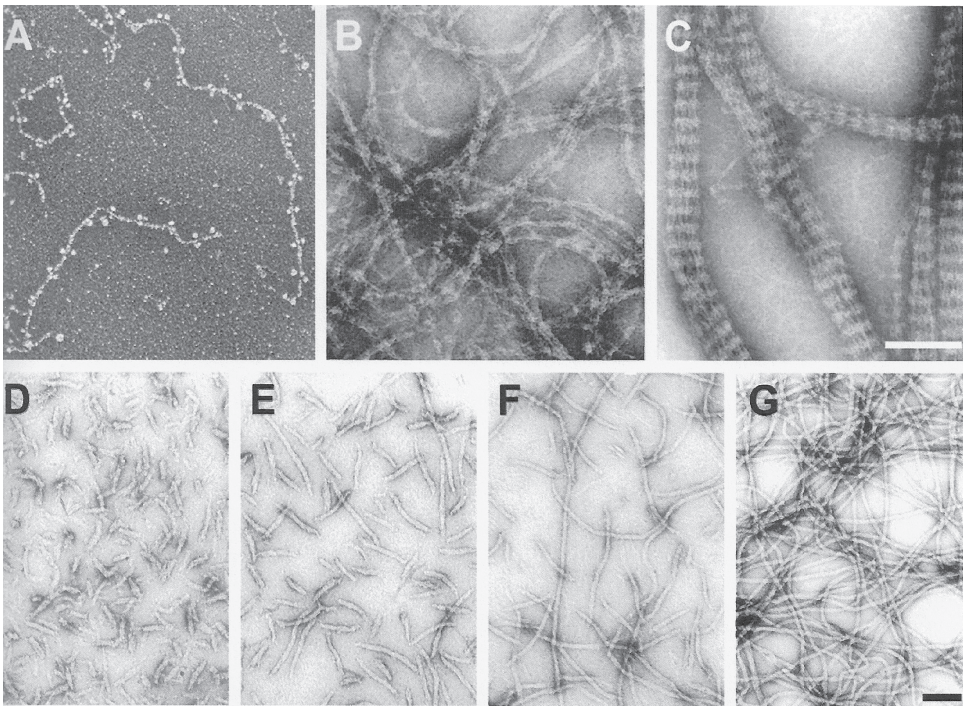


Figure 3.6. Electron micrographs of intermediate filaments at different assembly stages. (A–C) Lamin A/C. Lamin filaments can be dialyzed in pH 6.5/150 mM NaCl buffer, generating linear head-to-tail fibers (panel A). In the presence of Ca^{2+}, lamin filaments can be dialyzed into beaded long filaments (panel B). Panel C shows assembled lamin filaments. (D–G) Assembly of recombinant human vimentin. Vimentin filament assembly was initiated by adding filament buffer and fixed with 0.1% glutaraldehyde at 10 s (panel D), 1 min (panel E), 5 min, (panel F), and 1 h (panel G). Scale bar: 100 nm. (Reprinted by permission from Herrmann H, Aebi U: *Annu Rev Biochem*, 73:749–89, copyright 2004 by *Annual Reviews*, www.annualreviews.org.)

On the basis of constituents, intermediate filaments are classified into several subtypes, including keratin filaments, vimentin filaments, neurofilaments, and lamin filaments (see list in Table 3.9), which are found in different cell types. *Keratin filaments* are composed of various types of keratin and are present in epithelial cells, the hair, and the nails. Individual keratin molecules are different in structure and can be grouped into to subfamilies, including types I and II keratins, based on the properties of amino acids. Type I keratins are acidic with a molecular weight 40–70 kDa, whereas type II keratins are basic or neutral with a similar molecular weight. Both type I and type II keratins are required for the constitution of keratin filaments. In a typical epithelial cell, keratin filaments are connected at the end to *desmosomes*, a cell junction structure that joins two neighboring cells. In addition, keratin filaments anchor to hemidesmosomes, a structure that mediates cell attachment to the basal lamina.

Vimentin filaments are present in fibroblasts, endothelial cells, and leukocytes, and contain a single type of molecule: vimentin. In addition, there exist vimentin-related filaments, which exhibit structure and properties similar to those of vimentin filaments. One type is *desmin filaments*, which are composed of desmin and are present primarily in

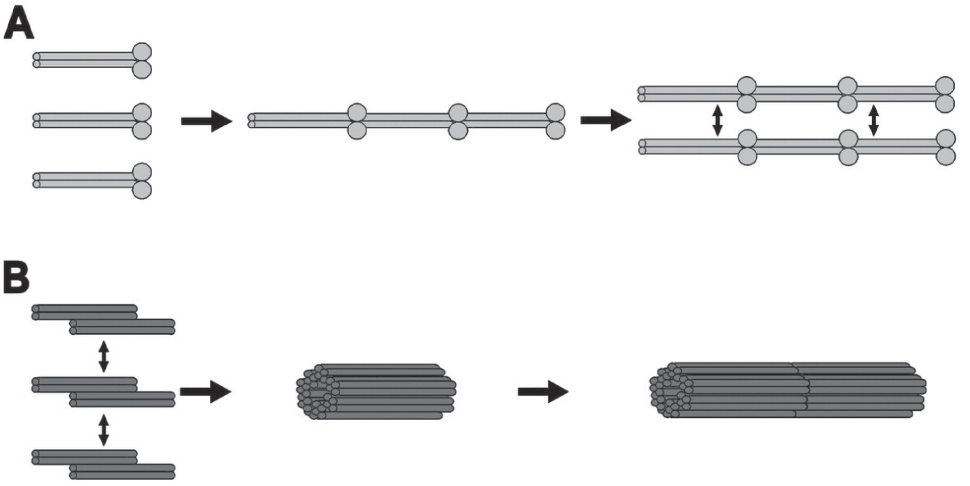


Figure 3.7. Schematic representation of intermediate filament assembly. (A) Lamin filament assembly. Lamin dimers are first associated into head-to-tail filaments, which are further associated laterally into complete filaments. (B) Vimentin filament assembly. Vimentin molecules first form antiparallel half-staggered double dimers (or tetramers), which form complete vimentin filaments. (Reprinted by permission from Herrmann H, Aebi U: *Annu Rev Biochem*, 73:749–89, copyright 2004 by *Annual Reviews*, www.annualreviews.org.)

muscle cells, including smooth, skeletal, and cardiac muscle cells. Desmin filaments often anchor to cell junctions. Another type is *glial filaments* composed of glial fibrillary acidic proteins. This type of intermediate filament is found in astrocytes of the central nervous system and Schwann cells of the peripheral nervous system. It is important to note that vimentin and vimentin-related proteins can be crosslinked together, but these proteins cannot be crosslinked with keratin-based intermediate filaments.

Neurofilaments are present in neurons, arranged primarily along the axon. There are three types of neurofilament proteins, including neurofilament-L, -M, and -H, based on low, medium, and high molecular weights, respectively. These molecular types can be found within all neurofilaments. In a typical neuronal axon, neurofilaments are uniformly spaced with a high density. These filaments are laterally crosslinked, providing mechanical strength to the axon.

Lamin filaments are found in the nuclear lamina, which is a ~20-nm membrane lining the internal surface of the nuclear membrane. Lamin filaments are composed of two types of lamin: lamin A (or A/C) and lamin B. In structure, lamin is similar to other intermediate filament proteins. However, lamin contains signaling structures that direct lamin transport from the cytosol to nucleus. The lamin filaments undergo dynamic disassembly during early mitosis and reassembly during the late mitosis in coordination with chromosome reorganization and separation. In interphase cells, lamin filaments are organized into a dense lattice network. The network is interrupted at nuclear pores, which allow the transport of molecules from and to the nucleus.

Function of Intermediate Filaments [3.12]. A major function of intermediate filaments is to provide mechanical strength to cells and tissues. Such a function is supported by observations from transgenic keratin-deficient animal models. In transgenic mice with a

TABLE 3.9. Characteristics of Selected Molecules that Constitute Intermediate Filaments*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Keratin	Cytokeratin, cytokeratin 1, keratin type II cytoskeletal 1, hair α protein, 67-kDa cytokeratin	644	66	Keratinocytes	Constituting keratin filaments in epithelial cells, hair, and nails
Vimentin	Vim	467	57	Epithelial cells, fibroblasts, muscular cells	Constituting vimentin intermediate filaments
Neurofilament protein heavy polypeptide	Neurofilament heavy polypeptide, neurofilament triplet H protein, 200-kDa neurofilament protein	1026	112	Nervous system	Constituting neurofilaments in neurons
Neurofilament protein light polypeptide	Neurofilament protein light chain, neurofilament triplet L protein, 68-kDa neurofilament protein, neurofilament protein	544	62	Nervous system	Constituting neurofilaments in neurons
Lamin A/C	Lamin A, lamin C, 70-kDa lamin	702	79	Ubiquitous	A constituent for nuclear lamina that regulates nuclear stability, chromatin structure, and gene expression
Lamin B	LMNB1, LMNB	586	66	Ubiquitous	A constituent for nuclear lamina

*Based on bibliography 3.11.

mutant keratin gene that lacks the amino/carboxyl-terminal domains, the mechanical strength of epidermis reduces significantly, resulting in cell injury in response to mechanical impacts that are harmless to normal cells. In human genetic diseases with mutation in the keratin gene, epidermal cells and tissues demonstrate a similar phenomenon, leading to skin blistering. In the human or animal skin, there exists a layer of keratin filaments that are highly crosslinked. Such a keratin layer serves as a protective structure for internal tissues.

The function of intermediate filaments is not limited to the enhancement of mechanical strength. Various types of intermediate filaments are bound to other cytoskeletal filaments. For instance, desmin filaments are linked to actin filaments in muscular cells, suggesting a role for the desmin filaments in regulating the interaction of contractile filaments. In addition, desmin filaments are attached to cell junctions, suggesting a role for these filaments in regulating cell-to-cell interactions.

ENDOPLASMIC RETICULUM [3.13]

Endoplasmic reticulum (ER) is a cytosolic membrane system consisting of lipid bilayers and is involved in the synthesis of proteins and lipids as well as in the sequestration and release of calcium. There is a rich network of interconnected tubular branches or sheets in the ER, forming a continuous membrane system in each cell. The ER membrane constitutes about 50% of the total cell lipid membrane. The ER tubular structures occupy about 10% of the total volume of the cell. There are two types of ER: rough and smooth. *Rough ER* is defined as ER with attached ribosomes on the cytosolic surface, whereas *smooth ER* is that without ribosomes.

ER is involved in the synthesis of proteins as well as lipids. Ribosomes bound to the ER are sites for protein translation. Proteins translated by ribosomes are transported to the rough ER for further processing before being released into the cytosol. In the lumen of the rough ER, proteins are modified by ER resident protein enzymes, a process critical in protein folding and assembly. An important enzyme for protein modification is protein disulfide isomerase in the rough ER. This enzyme catalyzes the formation of disulfide (S—S) bonds between cysteines, a proces critical in the formation of a three-dimensional protein structure. Another function of rough ER is to add sugar residues to proteins, a process known as *glycosylation*, which results in the formation of glycoproteins. The addition of sugar residues to proteins is catalyzed by enzymes present in the rough ER. A typical enzyme is oligosaccharyl transferase, which is localized to the ER membrane. This enzyme catalyzes the addition of a preformed oligosaccharide, composed of *N*-acetylglucosamine, mannose, and glucose, to the side NH_2 group of asparagines. The original oligosaccharide chain is trimmed or processed to remove certain sugar residues while the glycoproteins are still in the ER. Glycoproteins will be further processed when the molecules are transported into the Golgi apparatus (see the following section). Glycoproteins serve as cell membrane receptors. The sugar residues play a critical role in the recognition of and interaction with extracellular ligands.

The *smooth ER* constitutes a small fraction of the ER system in most cells and is connected to the rough ER. Rough ER segments are often found in smooth ER-dominant regions. A primary function of the smooth ER is to transport proteins from the ER to the Golgi apparatus. In addition, smooth ER is involved in the synthesis of lipids. Almost all lipid bilayers are assembled within the ER system. The ER system of hepatocytes is

involved in the synthesis of lipoproteins. These molecules are released into blood and serve as lipid carriers between various tissues and organs. Cells for the synthesis of steroid hormones are rich in smooth ER.

The ER system also plays a critical role in the storage and controlled release of calcium. In an inactive state, calcium is stored in the ER, where calcium-binding proteins sequester calcium. In response to stimulation for intracellular signaling processes that require calcium, the calcium channels of the ER are open, resulting in the release of calcium. Calcium mediates a variety of molecular processes, ranging from actin–myosin interaction to activation of signaling protein kinases.

GOLGI APPARATUS [3.14]

The *Golgi apparatus* is a stack of lipid membrane cisternae and tubular networks and is involved in the synthesis of carbohydrates and in the modification and sorting of proteins transported from the ER. The Golgi apparatus is located near the cell nucleus and centrosome. There exist several subsystems in the Golgi apparatus, including the *cis*-Golgi network, *cis*-cisterna, medial cisterna, *trans*-Golgi cisterna, and *trans*-Golgi network (Fig. 3.8). The *cis*-Golgi network is a membrane tubular network, which is connected to the *cis*-*cis*terna and serves as the entrance for protein-containing vesicles transported from the ER. Proteins are transported from the *cis*-Golgi network to the *cis*-cisterna. The *cis*-cisterna is adjacent, but not connected to the medial cisterna. Proteins are transported from the *cis*-cisterna to the medial cisterna via vesicular carriers. Similarly, the medial cisterna is not connected to the *trans*-cisterna. Vesicular transport is required for the movement of proteins from the medial cisterna to the *trans*-cisterna. The *trans*-cisterna is connected to the *trans* network, which serves as an exit for processed proteins. The exiting proteins are carried by vesicles to cellular compartments, including cell membranes, secretary vesicles, and lysosomes, where proteins are used for various purposes.

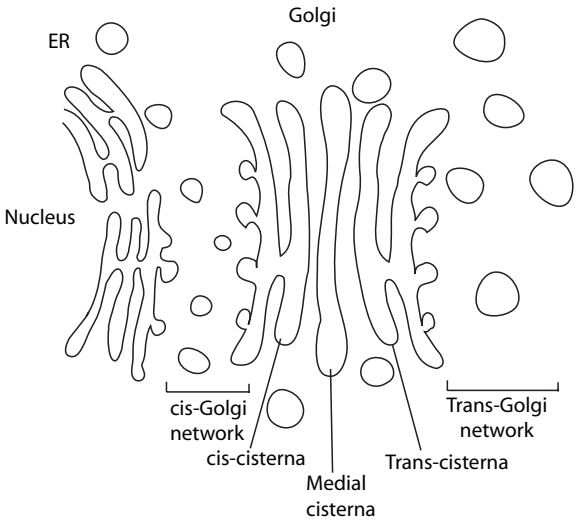


Figure 3.8. Schematic representation of Golgi apparatus (based on bibliography 3.14).

Major functions of the Golgi apparatus are to *modify proteins* and *synthesize carbohydrates*. Proteins are preliminarily modified in the ER by the addition of oligosaccharides. When transported to the Golgi apparatus, the proteins are further processed by glycosylation, or the addition of complex oligosaccharides and high-mannose-content oligosaccharides. The glycosylation process, which occurs through the Golgi cisternae, is critical to the formation of glycolproteins. In addition, the Golgi apparatus assembles *proteoglycans*, a process involving the polymerization of glycosaminoglycans (GAG) and the linkage of GAG chains to core proteins. Proteoglycans are deployed to the extracellular space and serve as ground substance. It is important to note that lipid vesicles can bud from the Golgi network and cisternae. These vesicles play a critical role for the transport of proteins between the Golgi subsystems and from the Golgi apparatus to destination compartments.

ENDOSOMES AND LYSOSOMES [3.15]

Endosomes are lipid vesicles that form by budding from cell membranes during *endocytosis*, a process by which cells ingest macromolecules and cell debris. Endocytosis is initiated when a stimulating macromolecule contacts the cell membrane. In response to such a contact, the stimulated region of the cell membrane invaginates, pinches off from the cell membrane, encloses the stimulating macromolecule, and forms an endosome. Most cells are capable of ingesting fluids, solutes, and small molecules, while phagocytic cells, such as macrophages and neutrophils, can take up large particles with a diameter in the order of μm (micrometers), such as bacteria and cell debris. Endosomes in phagocytic cells are also known as *phagosomes*. Endocytosis in phagocytic cells plays a critical role in protecting cells from bacterial infection and in scavenging debris from damaged and dead cells. Endosomes or phagosomes are eventually transformed to lysosomes, where ingested contents are degraded by enzymes.

Lysosomes are lipid membrane vesicles in which ingested molecules or particles are digested or degraded. All mammalian cells contain lysosomes. A typical lysosome contains numbers of hydrolytic enzymes, including proteases, lipases, phospholipases, and glycosidases, which degrade a variety of molecules. These digestive enzymes are synthesized by ribosomes in the rough ER, processed in the ER and Golgi apparatus, and delivered to lysosomes by Golgi vesicles. The internal environment of lysosomes is highly acidic with a pH value of ~5, which is advantageous for the activation of the hydrolytic enzymes. The internal H^+ concentration is maintained by H^+ pumps in the lysosomal membrane at the expense of energy from ATP molecules. The final products of the digestion, including saccharides, amino acids, and nucleotides, are transported across the lysosomal membrane to the cytosol, where these products are recycled.

In addition to the endosomes formed by endocytosis, there is another route that delivers materials to lysosomes for digestion. This route is used for the destruction and disposal of intracellular obsolete structures and organelles, a process known as *autophagy*. An obsolete organelle is usually enclosed by an ER membrane, forming an autophagosome. The autophagosome is then fused with a lysosome or endosome, where the enclosed organelle is degraded and disposed. Thus, endosomes and lysosomes play a critical role in the destruction and clearance of externally ingested materials as well as internally obsolete subcellular organelles.

MITOCHONDRIA [3.16]

Structure and Organization

Mitochondria are intracellular lipid membrane organelles that generate, store, and dispatch energy necessary for molecular activities. There are two types of specialized membrane for each mitochondrion: the internal and external membrane. These membranes divide a mitochondrion into two compartments: the *internal matrix space* and the *intermembrane space*. While the external membrane appears smooth, the internal membrane forms numbers of protrusions into the internal matrix space, known as *cristae*. The protrusions greatly increase the surface area of the internal membrane, which is necessary for membrane-related energy-generating processes. Each mitochondrial compartment and membrane contains distinct proteins that are developed for specialized functions as discussed below.

The external layer of mitochondria is composed of a large number of porins, proteins that form channels across the membrane. The porin channels allow the transport of water, salts, small proteins, and other molecules with a molecular weight <~5 kDa. Most of these molecules, however, cannot pass through the internal membrane. Because of the high permeability of the external membrane, electrolytes, water, and small molecules are equilibrated between the intermembrane space and the cytosol.

The internal membrane of the mitochondria is different from the external membrane. It is composed of a high density of cadiolipin, a phospholipid molecule containing four fatty acids. The presence of this lipid molecule renders the internal membrane highly impermeable to ions. The internal membrane contains a variety of specialized transport proteins, which exhibit selective permeability to molecules necessary for intramitochondrial activities. Because of the selective permeability of the internal membrane, the environment in the internal matrix space is different from that of the intermembrane space. Most importantly, the internal membrane consists of enzymes of the intracellular respiratory chain, forming an enzymatic cascade responsible for oxidation reactions and energy generation. One enzyme, known as *ATP synthase*, catalyzes the formation of ATP molecules.

The internal matrix space of mitochondria contains enzymes that metabolize pyruvate and fatty acids, generating acetyl CoA. This space also contains enzymes that oxidize acetyl CoA. The end products of these enzymatic reactions include nicotine adenine dinucleotide hydride (NADH) and CO_2. NADH is a form of nicotine adenine dinucleotide (NAD) with the addition of two electrons and is a major carrier and source of electrons for energy generation in the mitochondria. CO_2 is a waste product, which is released into the blood and removed from the lung and kidney. The internal matrix also contains mitochondrial DNA, ribosomes, tRNA, and enzymes necessary for regulating the expression of mitochondrial genes.

ATP Generation

The primary function of mitochondria is generation of energy in the form of ATP for molecular and cellular activities. Sources for mitochondrial energy generation are fatty acids and glycogens, or glucose polymers. Fatty acids are a more efficient form than glycogen for energy generation. The oxidation of fatty acids can generate energy 6 times as much as that of an equal amount of glycogen. Fatty acids are mainly stored in fat cells,

whereas glycogens are stored in liver and muscle cells. It is important to note that glucose can be converted to fatty acids, but fatty acids cannot be converted to glucose.

For fatty acid oxidation, fatty acid molecules are transported through the external and internal membranes of the mitochondria to the internal matrix. Each fatty acid is processed through a four-enzyme oxidation cycle, which catalyzes the oxidation of fatty acids. Each cycle reduces a fatty acid by two carbons, giving an acetyl CoA and two distinct high-energy electron carriers: NADH and $FADH_2$ (flavin adenine dinucleotide hydride). The acetyl CoA molecule is further oxidized in the citric cycle, and NADH and $FADH_2$ are used for electron transfer in energy generation.

For glycogen metabolism, cells first break down glycogen into glucose 1-phosphate, which occurs in the cytosol. Each glucose 1-phosphate is further catalyzed into two pyruvate molecules, which are transported from the cytosol into the mitochondrial internal matrix. The pyruvate molecules are catalyzed by a complex of enzymes and coenzymes into acetyl CoA and CO_2. The acetyl CoA molecule is further oxidized for energy generation through the citric cycle.

The *citric cycle*, also known as the Krebs cycle or tricarboxylic acid cycle, is the principal process that oxidizes fatty acids and pyruvates. About 60% of carbohydrates are processed by the citric cycle. Such a process produces CO_2 as a waste and high-energy electrons, which are carried by NADH and $FADH_2$ and used for the generation of ATP molecules. The citric cycle is a sequence of enzymatic events, starting with the formation of citric acid from acetyl CoA or pyruvate. Each cycle produces 2 CO_2, 2 H_2O, 1 $FADH_2$, 3 NADH with 3 H^+, and 1 GTP. The GTP molecule is converted to ATP by direct transfer of a high-energy phosphate group.

In the citric cycle, most energy from the oxidation of carbohydrates is saved in the form of high-energy electrons, which are carried by NADH and $FADH_2$. These electrons are transferred through the respiratory chain to oxygen, providing energy for the formation of ATP molecules. Such a process is referred to as *oxidative phosphorylation*. It has been hypothesized that oxidative phosphorylation is dependent on a chemiosmotic process. In such a process, chemically generated high-energy electrons from the hydrogen of NADH and $FADH_2$ are transported through the electron-carrying molecules of the respiratory chain localized to the mitochondrial internal membrane (note that each hydrogen atom gives a proton H^+ and an electron e^-). The energy released from the electron transfer is used to pump H^+ from the matrix side to the intermembrane side of the internal membrane, establishing a proton gradient across the internal membrane. This gradient drives H^+ flow in the opposite direction, providing energy for the synthesis of ATPs from ADPs and phosphates by ATP synthase.

CELL NUCLEI [3.17]

The cell nucleus is an organelle that contains the hereditary molecules—DNAs. The nucleus is enclosed with a nuclear envelope, which contains two lipid membranes: the outer and inner membranes. The outer membrane is a continuation of the adjacent ER membrane, and the intermembrane space is connected to the ER. The nucleus membranes are supported by an internal layer and an external layer of intermediate filaments. The internal supporting layer is a relatively dense structure composed of nuclear lamin and is defined as the *nuclear lamina*. The external supporting layer is composed of loosely organized intermediate filaments. These intermediate filament-containing layers protect

the nucleus from mechanical impacts and injury. Across the nucleus membrane and dense nuclear lamina, there exist pores, which allow the transport of selected molecules between the cytosol and nucleus. The nucleus contains chromosomes. The structure and function of chromosomes are discussed in Chapter 1.

BIBLIOGRAPHY

3.8. Structure and Organization of Microtubules

α-Tubulin

Ravelli RBG, Gigant B, Curmi PA, Jourdain I, Lachkar S et al: Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain, *Nature* 428:198–202, 2004.

Villasante A, Wang D, Dobner P, Dolph P, Lewis SA et al: Six mouse alpha-tubulin mRNAs encode five distinct isotypes: Testis-specific expression of two sister genes, *Mol Cell Biol* 6:2409–19, 1986.

Wilde CD, Chow LT, Wefald FC, Cowan NJ. Structure of two human alpha-tubulin genes, *Proc Natl Acad Sci USA* 79:96–100, 1982.

Dode C, Weil D, Levilliers J, Crozet F, Chaib H et al: Sequence characterization of a newly identified human alpha-tubulin gene (TUBA2), *Genomics* 47:125–30, 1998.

Hall JL, Cowan NJ: Structural features and restricted expression of a human alpha-tubulin gene, *Nucleic Acids Res* 13:207–23, 1985.

Miller FD, Naus CCG, Durand M, Bloom FE, Milner RJ: Isotypes of alpha-tubulin are differentially regulated during neuronal maturation, *J Cell Biol* 105:3065–73, 1987.

Watts NR, Sackett DL, Ward RD, Miller MW, Wingfield PT et al: HIV-1 rev depolymerizes microtubules to form stable bilayered rings, *J Cell Biol* 150:349–60, 2000.

Cowan NJ, Dobner PR, Fuchs EV, Cleveland DW: Expression of human alpha-tubulin genes: Interspecies conservation of 3-prime untranslated regions, *Mol Cell Biol* 3:1738–45, 1983.

β-Tubulin

Cleveland DW, Lopata MA, MacDonald RJ, Cowan NJ, Rutter WJ et al: Number and evolutionary conservation of alpha- and beta-tubulin and cytoplasmic beta- and gamma-actin genes using specific cloned cDNA probes, *Cell* 20:95–105, 1980.

Cleveland DW, Sullivan KF: Molecular biology and genetics of tubulin, *Annu Rev Biochem* 54:331–65, 1985.

Ravelli RBG, Gigant B, Curmi PA, Jourdain I, Lachkar S et al: Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain, *Nature* 428:198–202, 2004.

Wang HW, Nogales E: Nucleotide-dependent bending flexibility of tubulin regulates microtubule assembly, *Nature* 435:911–5, 2005.

Yen TJ, Machlin PS, Cleveland DW: Autoregulated instability of beta-tubulin mRNAs by recognition of the nascent amino terminus of beta-tubulin, *Nature* 334:580–5, 1988.

γ-Tubulin

Aldaz H, Rice LM, Stearns T, Agard DA: Insights into microtubule nucleation from the crystal structure of human gamma-tubulin, *Nature* 435:523–7, 2005.

Oakley CE, Oakley BR: Identification of gamma-tubulin, a new member of the tubulin superfamily encoded by mipA gene of Aspergillus nidulans, *Nature* 338:662–4, 1989.

Rommens JM, Durocher F, McArthur J, Tonin P, LeBlanc, JF et al: Generation of a transcription map at the HSD17B locus centromeric to BRCA1 at 17q21, *Genomics* 28:530–42, 1995.

Stearns T, Evans L, Kirschner M: Gamma-tubulin is a highly conserved component of the centrosome, *Cell* 65:825–36, 1991.

Wise DO, Krahe R, Oakley BR: The gamma-tubulin gene family in humans, *Genomics* 67:164–70, 2000.

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

3.9. Microtubule Assembly and Disassembly

Microtubule-Associated Protein 1A

Fink JK, Jones SM, Esposito C, Wilkowski J: Human microtubule-associated protein 1a (MAP1A) gene: genomic organization, cDNA sequence, and developmental- and tissue-specific expression, *Genomics* 35:577–85, 1996.

Hammarback JA, Obar RA, Hughes SM, Vallee RB: MAP1B is encoded as a polyprotein that is processed to form a complex N-terminal microtubule-binding domain, *Neuron* 7:129–39, 1991.

Ikeda A, Zheng QY, Zuberi AR, Johnson KR et al: Microtubule-associated protein 1A is a modifier of tubby hearing (moth1), *Nature Genet* 30:401–5, 2002.

Langkopf A, Hammarback JA, Muller R, Vallee RB, Garner CC et al: Microtubule-associated proteins 1A and LC2: Two proteins encoded in one messenger RNA, *J Biol Chem* 267:16561–6, 1992.

Lien LL, Feener CA, Fischbach N, Kunkel LM: Cloning of human microtubule-associated protein 1B and the identification of a related gene on chromosome 15, *Genomics* 22:273–80, 1994.

Microtubule-Associated Protein 1B

Allen E, Ding J, Wang W, Pramanik S, Chou J et al: Gigaxonin-controlled degradation of MAP1B light chain is critical to neuronal survival, *Nature* 438:224–8, 2005.

Edelmann W, Zervas M, Costello P, Roback L, Fischer I et al: Neuronal abnormalities in microtubule-associated protein 1B mutant mice, *Proc Natl Acad Sci USA* 93:1270–5, 1996.

Lien LL, Boyce FM, Kleyn P, Brzustowicz LM, Menninger J et al: Mapping of human microtubule-associated protein 1B in proximity to the spinal muscular atrophy locus at 5q13, *Proc Natl Acad Sci USA* 88:7873–6, 1991.

Lien LL, Feener CA, Fischbach N, Kunkel LM: Cloning of human microtubule-associated protein 1B and the identification of a related gene on chromosome 15, *Genomics* 22:273–80, 1994.

Wirth B, Voosen B, Rohrig D, Knapp M, Piechaczek B et al: Fine mapping and narrowing of the genetic interval of the spinal muscular atrophy region by linkage studies, *Genomics* 15:113–8, 1993.

Zhang YQ, Bailey AM, Matthies HJG, Renden RB, Smith MA et al: Drosophila fragile X-related gene regulates the MAP1B homolog Futsch to control synaptic structure and function, *Cell* 107:591–603, 2001.

MAP2

Garner CC, Tucker RP, Matus A: Selective localization of messenger RNA for cytoskeletal protein MAP2 in dendrites, *Nature* 336:674–7, 1988.

Kalcheva N, Albala J, O'Guin K, Rubino H, Garner C et al: Genomic structure of human microtubule-associated protein 2 (MAP-2) and characterization of additional MAP-2 isoforms, *Proc Natl Acad Sci USA* 92:10894–8, 1995.

Marsden KM, Doll T, Ferralli J, Botteri F, Matus A: Transgenic expression of embryonic MAP2 in adult mouse brain: Implications for neuronal polarization, *J Neurosci* 16:3265–73, 1996.

τ Proteins

Abel KJ, Boehnke M, Prahalad M, Ho P, Flejter WL et al: A radiation hybrid map of the BRCA1 region of chromosome 17q12-q21, *Genomics* 17:632–41, 1993.

Alonso ADC, Grundke-Iqbal I, Iqbal K: Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules, *Nature Med* 2:783–7, 1996.

Andreadis A, Brown WM, Kosik KS: Structure and novel exons of the human tau gene, *Biochemistry* 31:10626–33, 1992.

Clark LN, Poorkaj P, Wszolek Z, Geschwind DH, Nasreddine ZS et al: Pathogenic implications of mutations in the tau gene in pallido-ponto-nigral degeneration and related neurodegenerative disorders linked to chromosome 17, *Proc Natl Acad Sci* 95:13103–7, 1998.

Conrad C, Andreadis A, Trojanowski JQ, Dickson DW, Kang D et al: Genetic evidence for the involvement of tau in progressive supranuclear palsy, *Ann Neurol* 41:277–81, 1997.

Giasson BI, Forman MS, Higuchi M, Golbe LI, Graves CL, Kotzbauer PT et al: Initiation and synergistic fibrillization of tau and alpha-synuclein, *Science* 300:636–40, 2003.

Goedert M, Spillantini MG, Potier MC, Ulrich J, Crowther RA: Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain, *EMBO J* 8:393–9, 1989.

Goedert M, Wischik CM, Crowther RA, Walker JE, Klug A: Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: Identification as the microtubule-associated protein tau, *Proc Natl Acad Sci USA* 85:4051–5, 1988.

Gotz J, Chen F, van Dorpe J, Nitsch RM: Formation of neurofibrillary tangles in P301L tau transgenic mice induced by A-beta42 fibrils, *Science* 293:1491–5, 2001.

Holzer M, Craxton M, Jakes R, Arendt T, Goedert M: Tau gene (MAPT) sequence variation among primates, *Gene* 341:313–22, 2004.

Hong M, Zhukareva V, Vogelsberg-Ragaglia V, Wszolek Z, Reed L et al: Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17, *Science* 282:1914–7, 1998.

Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S et al: Association of missense and 5-prime-splice-site mutations in tau with the inherited dementia FTDP-17, *Nature* 393:702–5, 1998.

Lewis J, Dickson DW, Lin WL, Chisholm L, Corral A et al: Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP, *Science* 293:1487–91, 2001.

Lewis J, McGowan E, Rockwood J, Melrose H, Nacharaju P et al: Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein, *Nature Genet* 25:402–5, 2000.

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

3.10. Function of Microtubules

Kinesin Heavy Chain 2

Debernardi S, Fontanella E, De Gregorio L, Pierotti MA, Delia D: Identification of a novel human kinesin-related gene (HK2) by the cDNA differential display technique, *Genomics* 42:67–73, 1997.

Homma N, Takei Y, Tanaka Y, Nakata T, Terada S et al: Kinesin superfamily protein 2A (KIF2A) functions in suppression of collateral branch extension, *Cell* 114:229–39, 2003.

Kinesin Light Chain

Cabeza-Arvelaiz Y, Shih LCN, Hardman N, Asselbergs F, Bilbe G et al: Cloning and genetic organization of the human kinesin light-chain (KLC) gene, *DNA Cell Biol* 12:881–92, 1993.

Chernajovsky Y, Brown A, Clark J: Human kinesin light (beta) chain gene: DNA sequence and functional characterization of its promoter and first exon, *DNA Cell Biol* 15:965–74, 1996.

Goedert M, Marsh S, Carter N: Localization of the human kinesin light chain gene (KNS2) to chromosome 14q32.3 by fluorescence in situ hybridization, *Genomics* 32:173–5, 1996.

Kamal A, Stokin GB, Yang Z, Xia C, Goldstein LS: Axonal transport of amyloid precursor protein is mediated by direct binding to the kinesin light chain subunit of kinesin-I, *Neuron* 28:449–59, 2000.

Dynein

Criswell PS, Ostrowski LE, Asai DJ: A novel cytoplasmic dynein heavy chain: Expression of DHC1b in mammalian ciliated epithelial cells, *J Cell Sci* 109:1891–8, 1996.

Hafezparast M, Klocke R, Ruhrberg C, Marquardt A, Ahmad-Annuar A et al: Mutations in dynein link motor neuron degeneration to defects in retrograde transport, *Science* 300:808–12, 2003.

Harada A, Takei Y, Kanai Y, Tanaka Y, Nonaka S et al: Golgi vesiculation and lysosome dispersion in cells lacking cytoplasmic dynein, *J Cell Biol* 141:51–9, 1998.

Kural C, Kim H, Syed S, Goshima G, Gelfand VI et al: Kinesin and dynein move a peroxisome in vivo: A tug-of-war or coordinated movement? *Science* 308:1469–72, 2005.

Mikami A, Paschal BM, Vallee RB: Molecular cloning of retrograde transport motor cytoplasmic dynein, *Neuron* 10:788–96, 1993.

Narayan D, Desai T, Banks A, Patanjali SR, Ravikumar TS et al: Localization of the human cytoplasmic dynein heavy chain (DNECL) to 14qter by fluorescence in situ hybridization, *Genomics* 22:660–1, 1994.

Vaisberg EA, Grissom PM, McIntosh JR: Mammalian cells express three distinct dynein heavy chains that are localized to different cytoplasmic organelles, *J Cell Biol* 133:831–42, 1996.

Vaisberg EA, Koonce MP, McIntosh JR: Cytoplasmic dynein plays a role in mammalian mitotic spindle formation, *J Cell Biol* 123:849–58, 1993.

Cytoplasmic Dynein Intermediate Chain 1

Crackower MA, Sinasac DS, Xia J, Motoyama J, Prochazka M et al: Cloning and characterization of two cytoplasmic dynein intermediate chain genes in mouse and human, *Genomics* 55:257–67, 1999.

Horikawa I, Parker ES, Solomon GG, Barrett JC: Upregulation of the gene encoding a cytoplasmic dynein intermediate chain in senescent human cells, *J Cell Biochem* 82:415–21, 2001.

Dynein Light Chain

Dick T, Ray K, Salz HK, Chia W: Cytoplasmic dynein (ddlc1) mutations cause morphogenetic defects and apoptotic cell death in Drosophila melanogaster, *Mol Cell Biol* 16:1966–77, 1996.

Fuhrmann JC, Kins S, Rostaing P, El Far O, Kirsch J et al: Gephyrin interacts with dynein light chains 1 and 2, components of motor protein complexes, *J Neurosci* 22:5393–402, 2002.

Jaffrey SR, Snyder SH: PIN: An associated protein inhibitor of neuronal nitric oxide synthase, *Science* 274:774–7, 1996.

Kaiser FJ, Tavassoli K, Van den Bemd GJ, Chang GTG, Horsthemke B et al: Nuclear interaction of the dynein light chain LC8a with the TRPS1 transcription factor suppresses the transcriptional repression activity of TRPS1, *Hum Mol Genet* 12:1349–58, 2003.

Bornens M: Centrosome composition and microtubule anchoring mechanisms, *Curr Opin Cell Biol* 14:25–34, 2002.

Desai A, Mitchison TJ: Microtubule polymerization dynamics, *Annu Rev Cell Dev Biol* 13:83–117, 1997.

Hays T, Li M: Kinesin transport: Driving kinesin in the neuron, *Curr Biol* 11:R136–9, 2001.

Job D, Valiron O, Oakley B: Microtubule nucleation, *Curr Opin Cell Biol* 15:111–7, 2003.

Karsenti E, Vernos I: The mitotic spindle: A self-made machine, *Science* 294:543–7, 2001.

Surrey T, Nedelec F, Leibler S, Karsenti E: Physical properties determining self-organization of motors and microtubules, *Science* 292:1167–71, 2001.

Carazo-Salas RE, Gruss OJ, Mattaj IW, Karsenti E: Ran-GTP coordinates regulation of microtubule nucleation and dynamics during mitotic-spindle assembly, *Nature Cell Biol* 3:228–34, 2001.

Mitchison T, Kirschner M: Dynamic instability of microtubule growth, *Nature* 312:237–42, 1984.

Nogales E, Whittaker M, Milligan RA, Downing KH: High-resolution model of the microtubule, *Cell* 96:79–88, 1999.

Rieder CL, Salmon ED: The vertebrate cell kinetochore and its roles during mitosis, *Trends Cell Biol* 8:310–8, 1998.

Yildiz A, Tomishige M, Vale RD, Selvin PR: Kinesin walks hand-over-hand, *Science* 303:676–8, 2004.

Rogers GC, Rogers SL, Schwimmer TA, Ems-McClung SC, Walczak CE et al: Two mitotic kinesins cooperate to drive sister chromatid separation during anaphase, *Nature* 427:364–70, 2004.

Vale RD: The molecular motor toolbox for intracellular transport, *Cell* 112:467–80, 2003.

Vallee RB, Stehman SA: How dynein helps the cell find its center: A servomechanical model, *Trends Cell Biol* 15:288–94, 2005.

Vallee RB, Sheetz MP: Targeting of motor proteins, *Science* 271:1539–44, 1996.

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

3.11. Structure and Organization of Intermediate Filaments

Keratin

Chipev CC, Korge BP, Markova N, Bale SJ, DiGiovanna JJ et al: A leucine-to-proline mutation in the H1 subdomain of keratin 1 causes epidermolytic hyperkeratosis, *Cell* 70:821–8, 1992.

Compton JG: Epidermal disease: Faulty keratin filaments take their toll, *Nature Genet* 6:6–7, 1994.

Compton JG, DiGiovanna JJ, Santucci SK, Kearns KS, Amos CI et al: Linkage of epidermolytic hyperkeratosis to the type II keratin gene cluster on chromosome 12q, *Nature Genet* 1:301–5, 1992.

Rothnagel JA, Dominey AM, Dempsey LD, Longley MA, Greenhalgh DA et al: Mutations in the rod domains of keratins 1 and 10 in epidermolytic hyperkeratosis, *Science* 257:1128–30, 1992.

Schimkat M, Baur MP, Henke J: Inheritance of some electrophoretic phenotypes of human hair, *Hum Genet* 85:311–4, 1990.

Sprecher E, Yosipovitch G, Bergman R, Ciubutaro D, Indelman M et al: Epidermolytic hyperkeratosis and epidermolysis bullosa simplex caused by frameshift mutations altering the V2 tail domains of keratin 1 and keratin 5, *J Invest Dermatol* 120:623–6, 2003.

Syder AJ, Yu QC, Paller AS, Giudice G, Pearson R et al: Genetic mutations in the K1 and K10 genes of patients with epidermolytic hyperkeratosis: Correlation between location and disease severity, *J Clin Invest* 93:1533–42, 1994.

Neurofilament Heavy Chain

Bucan M, Gatalica B, Nolan P, Chung A, Leroux A et al: Comparative mapping of 9 human chromosome 22q loci in the laboratory mouse, *Hum Mol Genet* 2:1245–52, 1993.

Collard JF, Cote F, Julien JP: Defective axonal transport in a transgenic mouse model of amyotrophic lateral sclerosis, *Nature* 375:61–4, 1995.

Elder GA, Friedrich VL Jr, Kang C, Bosco P, Gourov A et al: Requirement of heavy neurofilament subunit in the development of axons with large calibers, *J Cell Biol* 143:195–205, 1998.

Hirokawa N, Takeda S: Gene targeting studies begin to reveal the function of neurofilament proteins, *J Cell Biol* 143:1–4, 1998.

Lees JF, Shneidman PS, Skuntz SF, Carden MJ, Lazzarini RA: The structure and organization of the human heavy neurofilament subunit (NF-H) and the gene encoding it, *EMBO J* 7:1947–55, 1988.

Mattei MG, Dautigny A, Pham-Dinh D, Passage E, Mattei JF et al: The gene encoding the large human neurofilament subunit (NF-H) maps to the q121-q131 region on human chromosome 22, *Hum Genet* 80:293–5, 1988.

Rao MV, Houseweart MK, Williamson TL, Crawford TO, Folmer J et al: Neurofilament-dependent radial growth of motor axons and axonal organization of neurofilaments does not require the neurofilament heavy subunit (NF-H) or its phosphorylation, *J Cell Biol* 143:171–81, 1998.

Rouleau GA, Merel P, Lutchman M, Sanson M, Zucman J et al: Alteration in a new gene encoding a putative membrane-organizing protein causes neuro-fibromatosis type 2, *Nature* 363:515–21, 1993.

Watson CJ, Gaunt L, Evans G, Patel K, Harris R et al: A disease-associated germline deletion maps the type 2 neurofibromatosis (NF2) gene between the Ewing sarcoma region and the leukaemia inhibitory factor locus, *Hum Mol Genet* 2:701–4, 1993.

Zhu Q, Lindenbaum M, Levavasseur F, Jacomy H, Julien JP: Disruption of the NF-H gene increases axonal microtubule content and velocity of neurofilament transport: Relief of axonopathy resulting from the toxin beta,beta-prime-iminodipropionitrile, *J Cell Biol* 143:183–93, 1998.

Vimentin

Geisler N, Plessmann U, Weber K: Amino acid sequence characterization of mammalian vimentin, the mesenchymal intermediate filament protein, *FEBS Lett* 163:22–4, 1983.

Colucci-Guyon E, Portier MM, Dunia I, Paulin D, Pournin S et al: Mice lacking vimentin develop and reproduce without an obvious phenotype, *Cell* 79:679–94, 1994.

Ferrari S, Battini R, Kaczmarek L, Rittling S, Calabretta B et al: Coding sequence and growth regulation of the human vimentin gene, *Mol Cell Biol* 6:3614–20, 1986.

Ferrari S, Cannizzaro LA, Battini R, Huebner K, Baserga R: The gene encoding human vimentin is located on the short arm of chromosome 10, *Am J Hum Genet* 41:616–26, 1987.

Gieser L, Swaroop A: Expressed sequence tags and chromosomal localization of cDNA clones from a subtracted retinal pigment epithelium library, *Genomics* 13:873–6, 1992.

Mathew CG, Wakeling W, Jones E, Easton D, Fisher R et al: Regional localization of polymorphic markers on chromosome 10 by physical and genetic mapping, *Ann Hum Genet* 54:121–9, 1990.

Mor-Vaknin N, Punturieri A, Sitwala K, Markovitz DM: Vimentin is secreted by activated macrophages, *Nature Cell Biol* 5:59–63, 2003.

Perreau J, Lilienbaum A, Vasseur M, Paulin D: Nucleotide sequence of the human vimentin gene and regulation of its transcription in tissues and cultured cells, *Gene* 62:7–16, 1988.

Zhang X, Diab IH, Zehner ZE: ZBP-89 represses vimentin gene transcription by interacting with the transcriptional activator Sp1, *Nucleic Acids Res* 31:2900–14, 2003.

Neurofilament Light Chain

Fabrizi GM, Cavallaro T, Angiari C, Bertolasi L et al: Giant axon and neurofilament accumulation in Charcot-Marie-Tooth disease type 2E, *Neurology* 62:1429–31, 2004.

Hirokawa N, Takeda S: Gene targeting studies begin to reveal the function of neurofilament proteins, *J Cell Biol* 143:1–4, 1998.

Hurst J, Flavell D, Julien JP, Meijer D, Mushynski W et al: The human neurofilament gene (NEFL) is located on the short arm of chromosome 8, *Cytogenet Cell Genet* 45:30–2, 1987.

Nguyen MD, Lariviere RC, Julien JP: Deregulation of Cdk5 in a mouse model of ALS: toxicity alleviated by perikaryal neurofilament inclusions, *Neuron* 30:135–47, 2001.

Previtali SC, Zerega B, Sherman DL, Brophy PJ, Dina G et al: Myotubularin-related 2 protein phosphatase and neurofilament light chain protein, both mutated in CMT neuropathies, interact in peripheral nerve, *Hum Mol Genet* 12:1713–23, 2003.

Zhu Q, Couillard-Despres S, Julien JP: Delayed maturation of regenerating myelinated axons in mice lacking neurofilaments, *Exp Neurol* 148:299–316, 1997.

Lamin A/C

Bonne G, Di Barletta MR, Varnous S, Becane HM, Hammouda EH et al: Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy, *Nature Genet* 21:285–8, 1999.

De Sandre-Giovannoli A, Bernard R, Cau P, Navarro C, Amiel J et al: Lamin A truncation in Hutchinson-Gilford progeria, *Science* 300:2055, 2003.

Eriksson M, Brown WT, Gordon LB, Glynn MW, Singer J et al: Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome, *Nature* 423:293–8, 2003.

Fatkin D, MacRae C, Sasaki T, Wolff MR, Porcu M, et al: Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease, *New Engl J Med* 341:1715–24, 1999.

Fisher DZ, Chaudhary N, Blobel G: cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins, *Proc Natl Acad Sci USA* 83:6450–4, 1986.

Goldman RD, Shumaker DK, Erdos MR, Eriksson M, Goldman AE et al: Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome, *Proc Natl Acad Sci USA* 101:8963–8, 2004.

Lin F, Worman HJ: Structural organization of the human gene encoding nuclear lamin A and nuclear lamin C, *J Biol Chem* 268:16321–6, 1993.

Lloyd DJ, Trembath RC, Shackleton S: A novel interaction between lamin A and SREBP1: Implications for partial lipodystrophy and other laminopathies, *Hum Mol Genet* 11:769–77, 2002.

McKeon FD, Kirschner MW, Caput D: Homologies in both primary and secondary structure between nuclear envelope and intermediate filament proteins, *Nature* 319:463–8, 1986.

Mounkes LC, Kozlov S, Hernandez L, Sullivan T, Stewart CL: A progeroid syndrome in mice is caused by defects in A-type lamins, *Nature* 423:298–301, 2003.

Scaffidi P, Misteli T: Reversal of the cellular phenotype in the premature aging disease Hutchinson-Gilford progeria syndrome, *Nature Med* 11:440–5, 2005.

Shackleton S, Lloyd DJ, Jackson SNJ, Evans R, Niermeijer MF et al: LMNA, encoding lamin A/C, is mutated in partial lipodystrophy, *Nature Genet* 24:153–6, 2000.

Lamin B

Furukawa K, Hotta Y: cDNA cloning of a germ cell specific lamin B3 from mouse spermatocytes and analysis of its function by ectopic expression in somatic cells, *EMBO J* 12:97–106, 1993.

Justice MJ, Gilbert DJ, Kinzler KW, Vogelstein B, Buchberg AM et al: A molecular genetic linkage map of mouse chromosome 18 reveals extensive linkage conservation with human chromosomes 5 and 18, *Genomics* 13:1281–8, 1992.

Lin F, Worman HJ: Structural organization of the human gene (LMNB1) encoding nuclear lamin B1, *Genomics* 27:230–6, 1995.

Maeno H, Sugimoto K, Nakajima N: Genomic structure of the mouse gene (Lmnb1) encoding nuclear lamin B1, *Genomics* 30:342–6, 1995.

Vergnes L, Peterfy M, Bergo MO, Young SG, Reue K: Lamin B1 is required for mouse development and nuclear integrity, *Proc Natl Acad Sci USA* 101:10428–33, 2004.

Wydner KL, McNeil JA, Lin F, Worman HJ, Lawrence JB: Chromosomal assignment of human nuclear envelope protein genes LMNA, LMNB1, and LBR by fluorescence in situ hybridization, *Genomics* 32:474–8, 1996.

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

3.12. Function of Intermediate Filaments

Fuchs E, Cleveland DW: A structural scaffolding of intermediate filaments in health and disease, *Science* 279:514–9, 1998.

Getsios S, Huen AC, Green KJ: Working out the strength and flexibility of desmosomes, *Nat Rev Mol Cell Biol* 5:271–81, 2004.

Helfand BT, Chang L, Goldman RD: The dynamic and motile properties of intermediate filaments, *Annu Rev Cell Dev Biol* 19:445–67, 2003.

Worman HJ, Courvalin JC: The nuclear lamina and inherited disease, *Trends Cell Biol* 12:591–8, 2002.

Hutchison CJ: Lamins: Building blocks or regulators of gene expression? *Nat Rev Mol Cell Biol* 3:848–58, 2002.

Clarke EJ, Allan V: Intermediate filaments: Vimentin moves in, *Curr Biol* 12:R596–8, 2002.

Green KJ, Gaudry CA: Are desmosomes more than tethers for intermediate filaments? *Nat Rev Mol Cell Biol* 1:208–16, 2000.

Chou YH, Helfand BT, Goldman RD: New horizons in cytoskeletal dynamics: Transport of intermediate filaments along microtubule tracks, *Curr Opin Cell Biol* 13:106–9, 2001.

Coulombe PA, Bousquet O, Ma L, Yamada S, Wirtz D: The "ins" and "outs" of intermediate filament organization, *Trends Cell Biol* 10:420–8, 2000.

Herrmann H, Aebi U: Intermediate filaments and their associates: Multi-talented structural elements specifying cytoarchitecture and cytodynamics, *Curr Opin Cell Biol* 12:79–90, 2000.

Stuurman N, Heins S, Aebi U: Nuclear lamins: Their structure, assembly, and interactions, *J Struct Biol* 122:42–66, 1998.

Herrmann H, Aebi U: Intermediate filament assembly: Fibrillogenesis is driven by decisive dimer-dimer interactions, *Curr Opin Struct Biol* 8:177–85, 1998.

3.13. Endoplasmic Reticulum

Voelker DR: Bridging gaps in phospholipid transport, *Trends Biochem Sci* 30:396–404, 2005.

Hebert DN, Garman SC, Molinari M: The glycan code of the endoplasmic reticulum: Asparagine-linked carbohydrates as protein maturation and quality-control tags, *Trends Cell Biol* 15:364–70, 2005.

Rapoport TA, Goder V, Heinrich SU, Matlack KE: Membrane-protein integration and the role of the translocation channel, *Trends Cell Biol* 14:568–75, 2004.

Watanabe R, Riezman H: Differential ER exit in yeast and mammalian cells, *Curr Opin Cell Biol* 16:350–5, 2004.

Toyoshima C, Inesi G: Structural basis of ion pumping by Ca2+-ATPase of the sarcoplasmic reticulum, *Annu Rev Biochem* 73:269–92, 2004.

Pfeffer S: Membrane domains in the secretory and endocytic pathways, *Cell* 112:507–17, 2003.

Venkatachalam K, van Rossum DB, Patterson RL, Ma HT, Gill DL: The cellular and molecular basis of store-operated calcium entry, *Nature Cell Biol* 4:E263–72, 2002.

Holthuis JC, Pomorski T, Raggers RJ, Sprong H, Van Meer G: The organizing potential of sphingolipids in intracellular membrane transport, *Physiol Rev* 81:1689–723, 2001.

Hirschberg CB, Robbins PW, Abeijon C: Transporters of nucleotide sugars, ATP, and nucleotide sulfate in the endoplasmic reticulum and Golgi apparatus, *Annu Rev Biochem* 67:49–69, 1998.

3.14. Golgi Apparatus

Machesky LM, Bornens M: Cell structure and dynamics, *Curr Opin Cell Biol* 15:2–5, 2003.

Puthenveedu MA, Linstedt AD: Subcompartmentalizing the Golgi apparatus, *Curr Opin Cell Biol* 17:369–75, 2005.

Rios RM, Bornens M: The Golgi apparatus at the cell centre, *Curr Opin Cell Biol* 15:60–6, 2003.

Pfeffer SR: Constructing a Golgi complex, *J Cell Biol* 155:873–5, 2001.

Glick BS: Organization of the Golgi apparatus, *Curr Opin Cell Biol* 12:450–6, 2000.

Bannykh SI, Nishimura N, Balch WE: Getting into the Golgi, *Trends Cell Biol* 8:21–5, 1998.

Munro S: The Golgi apparatus: defining the identity of Golgi membranes, *Curr Opin Cell Biol* 17:395–401, 2005.

Lee MC, Miller EA, Goldberg J, Orci L, Schekman R: Bi-directional protein transport between the ER and Golgi, *Annu Rev Cell Dev Biol* 20:87–123, 2004.

Altan-Bonnet N, Sougrat R, Lippincott-Schwartz J: Molecular basis for Golgi maintenance and biogenesis, *Curr Opin Cell Biol* 16:364–72, 2004.

de Graffenried CL, Bertozzi CR: The roles of enzyme localisation and complex formation in glycan assembly within the Golgi apparatus, *Curr Opin Cell Biol* 16:356–63, 2004.

Graham TR: Membrane targeting: Getting Arl to the Golgi, *Curr Biol* 14:R483–5, 2004.

Palmer KJ, Stephens DJ: Biogenesis of ER-to-Golgi transport carriers: Complex roles of COPII in ER export, *Trends Cell Biol* 14:57–61, 2004.

3.15. Endosomes and Lysosomes

Piper RC, Luzio JP: CUPpling calcium to lysosomal biogenesis, *Trends Cell Biol* 14:471–3, 2004.

Fevrier B, Raposo G: Exosomes: Endosomal-derived vesicles shipping extracellular messages, *Curr Opin Cell Biol* 16:415–21, 2004.

Miaczynska M, Pelkmans L, Zerial M: Not just a sink: Endosomes in control of signal transduction, *Curr Opin Cell Biol* 16:400–6, 2004.

Gruenberg J, Stenmark H: The biogenesis of multivesicular endosomes, *Nat Rev Mol Cell Biol* 5:317–23, 2004.

Piddini E, Vincent JP: Modulation of developmental signals by endocytosis: Different means and many ends, *Curr Opin Cell Biol* 15:474–81, 2003.

Raiborg C, Rusten TE, Stenmark H: Protein sorting into multivesicular endosomes, *Curr Opin Cell Biol* 15:446–55, 2003.

Bonifacino JS, Traub LM: Signals for sorting of transmembrane proteins to endosomes and lysosomes, *Annu Rev Biochem* 72:395–447, 2003.

Sandvig K, van Deurs B: Endocytosis, intracellular transport, and cytotoxic action of Shiga toxin and ricin, *Physiol Rev* 76:949–66, 1996.

Nixon RA, Cataldo AM: The endosomal-lysosomal system of neurons: New roles, *Trends Neurosci* 18:489–96, 1995.

Bomsel M, Mostov K: Sorting of plasma membrane proteins in epithelial cells, *Curr Opin Cell Biol* 3:647–53, 1991.

3.16. Mitochondria

Chen XJ, Butow RA: The organization and inheritance of the mitochondrial genome, *Nature Rev Genet* 6:815–25, 2005.

Frey TG, Mannella CA: The internal structure of mitochondria, *Trends Biochem Sci* 25:319–24, 2000.

Bonen L: The mitochondrial genome: So simple yet so complex, *Curr Opin Genet Dev* 1:515–22, 1991.

3.17. Cell Nuclei

Grunstein M: Histones as regulators of genes, *Sci Am* 267(4): 68–74B, 1992.

Heck MMS: Condensins, cohesins, and chromosome architecture: How to make and break a mitotic chromosome, *Cell* 91:5–8, 1997.

Kornberg RD: Chromatin structure: A repeating unit of histones and DNA, *Science* 184:868–71, 1974.

Koshland D, Strunnikov A: Mitotic chromosome condensation, *Annu Rev Cell Biol* 12:305–33, 1996.

Luger K, Mader AW, Richmond RK, Sargent DE, Richmond TJ: Crystal structure of the nucleosome core particle at 2.8 A resolution, *Nature* 389:251–60, 1997.

Paulson JR, Laemmli UK: The structure of histone-depleted metaphase chromosomes, *Cell* 12:817–28, 1977.

Richmond TJ, Finch JT, Rushton B, Rhodes D, Klug A: Structure of the nucleosome core particle at 7A resolution, *Nature* 311:532–7, 1984.

Saitoh Y, Laemmli UK: Metaphase chromosome structure: Bands arise from a differential folding path of the highly AT-rich scaffold, *Cell* 76:609–22, 1994.

Van Holde KE, Zlatanovai J: Chromatin higher order structure: Chasing a mirage, *J Biol Chem* 270:8373–6, 1995.