The cytoskeleton provides dynamic organization of the cytoplasm. The cytoskeleton is a network of protein filaments that provides a scaffold for cell shape and for positions of organelles, and which causes cell movement including motility and cell division. Protein filaments are linked to organelles and to the plasma membrane by accessory proteins.

**Student Learning Outcomes:**

1*. Explain the structure/function relationships of three different types of filaments of the cytoskeleton: actin, intermediate filaments and microtubules.
2. Describe the different monomers that comprise each type, and associated proteins utilized for functional filaments.
3*. Briefly explain the filaments involved in cell movement, plus the additional proteins and energy requirements.
4. Explain briefly how modern microscopic techniques, studies of mutant proteins, and studies with inhibitor molecules have probed the cytoskeleton.
5. State some examples of human diseases due to defects in cytoskeleton

**12.1* Actin filaments (microfilaments, 7 nm diameter)**

Are thin fibers, major cytoskeletal proteins;
Reversibly organized into higher-order bundles and networks
Actin is 375 aa (43 kD); abundant (5-10% of cell protein)
Yeast have 1 actin gene; mammals 6 different genes

Actin has pointed end, barbed end (Fig. 2*)
Head-to-tail polymerization of monomers:

**globular [G] actin monomers** form
helical actin filaments ([F] actin).
Reversible polymerization reaction (Fig. 3):
Association depends on monomer concentration
Polymerization requires ATP (Fig. 4), but not hydrolysis
**Treadmilling** reflects two ends of filament
growing at different rates: actin-ATP adds
to barbed end (ATP is hydrolyzed later)
while actin-ADP dissociates from pointed end.

Drugs useful in cell biology affect polymerization:
**Cytocholasins** bind barbed end, block elongation;
Block cell division after mitosis
**Phalloidin** prevents dissociation of filaments:
Fluorescent phalloidin reveals filaments

**Actin-binding proteins** regulate assembly and disassembly of filaments (Table 1; Fig. 5*)
bind filaments to stabilize; cap ends,
cross-link filaments; modulate ATP/ADP of monomers

*Activities of actin-binding proteins are controlled
by cell signal mechanisms (Chapt. 15) to quickly
remodel cytoskeleton for movement and shape.
Formin (140 kd) nucleates filaments to start chain (Fig. 6) -stays at barbed end
Arp2/3 complex initiates branches (Fig. 7)
ADF/cofilin is actin depolymerizer, severs chain
Profilin stimulates exchange of ADP of monomer to ATP
Tropomyosins stabilize filament, binds lengthwise

Some actin-binding proteins cross-link actin filaments to form bundles or 3-D networks (Fig. 9).
They have two actin-binding domains (ABD) and Ca^{++} binding domains (Figs. 10*, 11)
Fimbrin is a small actin bundling protein (68 kD)
   2 ABD domains hold filaments 14-nm apart
α-actinin is large (102 kD); for contractile bundles
   with myosin; dimer holds filaments farther apart (40 nm)
Filamin is very large (280 kD); V-shaped dimer;
   3-D cross-links filaments

Erythrocyte (red blood cell) model system Fig. 14*): (erythrocytes lack nucleus and organelles, and in humans, also lack microtubules, intermediate filaments)
Actin filaments associate with plasma membrane via proteins spectrin, ankyrin, protein 4.1, Band 3.

Actin filaments and Actin binding proteins form a network called cell cortex
Spectrin is large with has 2α, 2β chains (220-240 kD each) Fig. 13), 2 ABD domains
Ankyrin links spectrin to transmembrane protein Band 3
Protein 4.1 links spectrin/actin network to the Transmembrane protein glycophorin (Fig. 14*)

Related proteins link actin to plasma membrane in other cells.

Dystrophin, a 427 kD member of the large calponin family (including α-actinin, spectrin, filamin) is absent or mutated in Duchenne’s and Becker’s (X-linked) muscular dystrophies: Progressive degeneration of skeletal muscle.
Dystrophin has ABD at one end and membrane binding at other end; links actin filaments to transmembrane protein to extracellular matrix to stabilize muscle cells.

Actin bundles attach to plasma membrane through transmembrane proteins to anchor cells
at regions of cell-cell and cell-substratum contact:
Focal adhesions (Figs. 16) are sites of attachment of cells to extracellular matrix material of culture dishes:
occurs with transmembrane integrin proteins binding bundles of actin filaments (stress fibers).
vinculin and talin help.
Adherens junctions (Fig. 17) (adhesion belt) (see Chapt. 14) uses transmembrane cadherins plus cytoplasmic catenins to attach actin filaments; vinculin helps.

Actin filaments support protrusions of cell surface:
Microvilli (Fig. 18,19) on epithelia cells are fingerlike extensions; involved in absorption
Specialized microvilli of auditory cells detect sound
Villin is main actin bundling protein (95 kD), plus fimbrin, myosin I and calmodulin (Ca++ binding)

Other surface protrusions are transient:
Pseudopodia are 3-D actin filament network extensions;
Responsible for phagocytosis and cell locomotion (Fig. 20)

2. Actin, myosin and cell movement
Actin and myosin are responsible for many cell movements, uses energy of ATP.

Muscle contraction uses myosin II as the motor protein with energy from ATP hydrolysis to generate force and movement.

Skeletal muscles have muscle fibers: (Fig. 21)
Each is bundle of single large multinucleate cells
Each muscle fiber has myofibrils (bundles of thick myosin and thin actin)

Myosin. Actin and myosin filaments slide past each other in sarcomeres, using repeated cycles of interaction of myosin and actin (movement of myosin head group along actin) (Fig. 22-27); Fig. 25*, 27*

Structure of sarcomeres: Z disc at ends (Fig. 22)
Dark bands (myosin) alternate with light (actin)
Filaments overlap in peripheral region

Actin attaches at barb end to z disc; uses α-actinin (Fig. 23)
Myosin attaches at M line.
Huge titin protein (3000 kD) extends from M to Z like long spring
Nebulin attaches to actin and helps stabilize structure

Sliding filament model of contraction (Fig. 24):
Actin slides past myosin towards middle -> shortens sarcomere, not filaments.

Myosin II is 500 kD (2 heavy chains and 2 light):
Globular head and helical tail of Heavy 1 light chain is regulatory (Fig. 25*)
Myosins assemble in parallel staggered array of tails; head binds actin (Fig. 26);
** Fig. 27** Model of muscle contraction:
- Binding of ATP dissociates myosin from actin;
- Conformation change of myosin head ->
- Head binds new position on actin filament;
- Myosin head returning to original shape drags actin filament

** Nerve impulse causes contraction by release of Ca\(^{++}\) from special muscle ER (sarcoplasmic reticulum) ->
- Increased Ca\(^{++}\) signals contraction (Fig. 28) via
  - Actin binding proteins tropomyosin and troponin.
  - Tropomyosin all along actin filament + troponins
  - Normally block binding site for myosin.
- Ca\(^{++}\) -> complex shifts and exposes myosin site.

Non-muscle cells have contractile assemblies of actin and myosin (Fig. 29*).
**Myosin II** bipolar filaments (15-25 ea)
Also tropomyosin on actin filaments.
Ex. stress fibers, adhesion belts.
Ex. cytokinesis (Fig. 30):
  - Uses contractile ring of actin/myosin II.

Regulation of contraction in non-muscle cells involves
Phosphorylation of regulatory light chain myosin (Fig. 31*). Phosphorylation ->
  - Increased assembly of Myosin filaments ->
  - Increased myosin catalytic activity for contraction.
- Enzyme doing phosphorylation is MLCK
  (myosin light chain kinase)
  - Which itself is regulated by Ca\(^{++}\) binding calmodulin.
- Increased Ca\(^{++}\) in cytosol -> indirect activation of myosin in nonmuscle cells for contraction.

Non-muscle myosins are not involved in contraction
Many types in addition to myosin II.
No tails on other myosins, so not form coil-coils.
Other movements include: transport of vesicles and organelles along actin filaments;
Phagocytosis, pseudopodia

**Myosin I** (Fig. 32) globular head and short tail.
- Binds to vesicle with tail, to actin with head.
- Moves vesicle towards barbed end of actin.
- Myosin I also links actin bundles in microvilli
  - To plasma membrane

12 other non-muscle myosins (III – XIV)
Myosin V is 2 headed (Fig. 33); moves organelles to barbed end
Myosin III involved with vision
Myosins VI, VII involved with hearing
Protrusions and cell movement (Fig. 34). Ex. crawling amoebas attach, extend, attach, retract. Ex. wound healing (Fig. 35) cells at edge move to cover. WASP/Scar complex activates actin to plasma membrane.

3. Intermediate filaments (8-11 nm diameter)

NOT directly involved in cell movement
Structural role – mechanical strength
Polymers of >65 different proteins - expressed in different cells

6 groups of proteins based on sequence (Table 2)

I. (acidic) and II. (basic) keratins 1 of each copolymerize
   Hard keratins -> hair, nails, horn
   Soft keratins -> cytoplasm of epithelial cells
   Different ones in differentiated cells

III. Vimentin – fibroblasts, smooth muscles
   Network from nucleus to periphery

IV. Neurofilament (NF) – neurons, esp. axons

V. Nuclear lamins – underneath nuclear membrane

VI. Nestin (stem cell) – embryonic

Common structure of intermediate filaments
   N-terminal head domain; C-terminal tail (Fig. 36);
   central alpha-helical rod (310-350 aa)
   Dimers form parallel strands (helices)
   Tetramer forms with antiparallel dimmers (Fig. 37).
   Then protofilament and rope-like filament –
   both ends are equivalent

Specific interactions of different intermediate filaments:
   Keratin always one type I and one type II heterodimer

Rather stable filaments –
   phosphorylation can regulate assembly/disassembly
   ex. nuclear lamins: phosphorylation -> breakdown in mitosis

Intracellular organization: Network of keratin and vimentin
   extends from nucleus to plasma membrane
Epithelial cells have keratin filaments anchored to plasma membrane at
desmosomes and hemi-desmosomes (cell contacts; Fig. 39).
   Anchored to dense plaques by other proteins
   of plakin family (related to cadherin).

Abnormal keratins and neurofilaments are associated with disease
(Fig. 41; Elaine Fuchs). Transgenic mice with truncated keratin 14 (type II) die after birth:
epithelial lysis, disordered epidermis: human epidermolysis bullosa simplex
ALS (Lou Gehrig disease) has abnormal neurofilament type IV affect neurons (NF-L, NF-H)
4. Microtubules are rigid hollow rods (25 nm diameter)
Dynamic structures: assembly and disassembly
Control cell shape and cell movements, some locomotion, transport of organelles,
  Separation of chromosomes in mitosis

Structure and dynamic organization
Monomer is tubulin (55 kD): α-tubulin, β-tubulin
Small family of related genes; (γ-tubulin special role in centrosomes)

Tubulin dimers can polymerize in vitro (Fig. 42)
  13 protofilaments, hollow core; Head-to-tail arrays, parallel
Polar structure: fast-growing + end, slow – end
  Determines direction of movement
Depolymerization /polymerization is rapid:
  Both subunits can bind GTP
  GTP bound to β-tubulin is hydrolyzed to GDP
  after polymerization -> weakens affinity for other
dimmers and favors depolymerization (Figs. 12, 43, 44)
Treadmilling of microtubules as for actin
  High GTP tubulin favors growth; low GTP disassembly
Drugs affect microtubule assembly – colchicines, vincristine, taxol

Assembly of microtubules in animal cells.
They extend from centrosome (near nucleus in interphase)
Centrosome duplicates at mitosis, migrates, and
  Microtubules form spindle (Fig. 45)
Centrosome binds – end of microtubules; + end grows out
  γ-tubulin ring complex is seed for microtubule growth

Centrosome = 2 centrioles perpendicular (Fig. 48).
  9 triplets of microtubules (like flagella basal bodies)
  Plus pericentriolar material; Organizing role,
  Centrioles are complex and polar, with special proteins
  [plant cells not have centrosome; microtubules attach to nucleus]

Microtubule associated proteins (MAPs)
Regulate microtubule assembly, disassembly
Some MAPs stabilize (cap ends), others sever, depolymerize, track + end
Different MAPs in different cells, or parts of cells:
  Neurons have MAP-1, MAP-2, tau (Fig. 49)
  Tau is found Alzheimer’s plaques
  But difference dendrites to axons
MAPs are regulated by phosphorylation, or other.

5. Microtubule motors and movement
Intracellular transport, position of vesicles and organelles, separation of chromosomes,
  Beating of cilia and flagella
Motor proteins use energy of ATP hydrolysis
Two families of proteins:
Kinesins mostly move to + end of microtubules
  45 different ones in humans – Specialized for cargoes
**Dyneins** move to – end of microtubules
Several different ones – different cargoes

**Kinesin I** first purified 1985 from squid axon;
Takes vesicles away from cell body.
380 kD (2 heavy, 2 light chains):
Globular head binds ATP and microtubule; tail binds vesicles and organelles
Evolutionary relationship of kinesin and myosin.

**Cytoplasmic Dynein** (2000 kD): 2 heavy chains, + light
Globular ATP binding motor domain
Basal part binds organelles and others

**Cargo transport and intracellular organization:** (fig. 12.51)
Microtubules position organelles, transport macromolecules and vesicles
Esp. important for nerve cell axons which are long:
(Ribosomes only in cell body and dendrites, so
Secretory vesicles go out from cell body;
Endocytic vesicles come back to cell body)
Ex. Kinesin II transport mRNAs to cell cortex of *Xenopus* oocyte

**Cilia and flagella** are **microtubule-based projections of plasma membrane**
[bacterial flagella are very different = pure protein filament].
Diameter 250 nm; cilia short; flagella long
**Axoneme** = microtubules + associated proteins (Fig. 12.54)
9 + 2 pairs of microtubules: A has 13, B has 10 subunits;
exin linker, - end of microtubules in basal body.
2 dynein arms provide bending (Fig. 12.56):
Attached to A tubule; moving towards – end of B

**Microtubules reorganize in mitosis to form spindle.** (Fig. 12.58)
Radiating microtubules from centrioles and centrosome:
**Kinetochore** microtubules attach to centromeres of chromosomes
**Chromosomal** microtubules connect to ends of chromosomes
**Polar** microtubules overlap in center of cell, stabilize
**Astral** microtubules go to cell periphery.
Kinetochore microtubules shorten to move chromosomes
Movement of astral and polar separates