I. Non-muscle Actin: **Bundles and Networks**

1. Actin bundles: (“Plus” ends are attached to membranes)
   a) “Tight bundles”: bundled by **fascin** [fibroblasts], or **villin, fimbrin** [microvilli of intestinal epithelial cells; *fig 17-4, p716*]. (Protein structures listed in figure 17-18, p 729)

   Examples: fibroblasts: *lamellipodia, microspikes (10 µm), filopodia (= long microspikes: 50µm)*
   epithelial cells: *Microvilli [ fig 17-4, p716]*

   b) “Loose bundles” (contractile): bundled by **α-actinin** [leaves room in between parallel actin filaments for the insertion of myosin I or II]

   Examples: (i) fibroblast “stress fibers” (*fig 19-32, p832*)
   (⇒ focal adhesions/“3D” adhesions) (*fig 19-33, p834; fibronectin (Fn) Fn receptor (an Integrin) Focal adhesion kinase (*fak*) Src kinase vinculin, talin, pp. 10-12 of handout)
   (ii) contractile ring in mitotic cells (*fig 17-34, p742*)
   (iii) adherens belt in epithelial cells (*fig 17-4, p716*)

2. Actin networks: **filamin** [crosslinks f-actin, leading to gel formation] (*fig 17-18, p 729*), versus **gelsolin** [Ca++-activated severing of f-actin, leading to sol formation]

   Examples: cell cortex; *gel-sol conversions* in amoeba pseudopod (Tom Stossel, *American Scientist*, 78, 408-423 [1990]: *pp 17a,17b of handout*)

3. Actin filaments are linked to membrane proteins

   Examples: dystrophin; the actin-spectrin network underlying the red blood cell membrane


1. Critical concentration for polymerization (**C_c**)
2. Plus and minus ends
3. Significance of ATP hydrolysis: **C_c** for plus end can be different from **C_c** for minus end
4. Treadmilling
5. Drugs affecting polymerization/depolymerization of actin: **cytochalasin** (destabilizes) & **phalloidin** (stabilizes)
arrowhead decoration permits us to conclude that the more rapidly elongating (+) end corresponds to the barbed end of a filament and the more slowly growing (−) end corresponds to the pointed end of a filament.

The difference in elongation rates at the opposite ends of a filament is caused by a difference in Cₗₘ₀ values at the filament ends. This difference can be measured by blocking one or the other end with proteins that ‘cap’ the ends of actin filaments. (We will discuss (+) end and (−) end capping proteins in a following section.) Shown in the schematic in Figure 22-10b, a capping protein on the (+) end of actin causes the filament to elongate from its (−) end. Conversely, elongation occurs at the (+) end when the (−) end of a actin filament is blocked. In either case, the Cₗₘ₀ values for each end of a filament are measured in actin polymerization assays (Figure 22-10c) – 0.1 μM for addition at the (+) end of the filament and 0.8 μM for addition at the (−) end. Based on the Cₗₘ₀ values for the (+) and (−) ends of a filament, we would observe that: (1) no filament growth at G-actin concentrations below 0.1 μM, (2) growth only from the (+) end at G-actin concentrations between 0.1 and 0.8 μM, and (3) growth on both ends at G-actin concentrations above 0.8 μM [remember, growth will still be faster at the (+) end than at the (−) end].

A FIGURE 22.8 The three phases of actin polymerization. (a) During the polymerization of G-actin in vitro, the mass of actin filaments increases after an initial lag period and eventually reaches a steady state. In the first phase, G-actin monomers bind with ATP (pink subunits), form nuclei—stable complexes of actin (purple subunits) which in the second phase are elongated by the addition of subunits to both ends of the filament. In the third phase of assembly, steady state, the ends of actin filaments are in equilibrium with monomeric ATP-G-actin. After their incorporation into a filament, subunits slowly hydrolyze ATP and become stable ADP-F-actin (white subunits). (b) Polymerization can be speeded by adding actin filament fragments (nuclei) at the start of assembly. The lag period is thus bypassed because the actin nuclei promote immediate elongation into filaments.

This difference in the Cₗₘ₀ of filament ends leads to an interesting phenomenon called treadmilling (Figure 22-10d). At G-actin concentrations intermediate between the Cₗₘ₀ for the (+) and (−) ends, subunits dissociate from the (−) end but add to the (+) end. The newly added subunits would travel through the filament, as if on a treadmill, until they reach the (−) end, where they dissociate. A treadmill is interesting because it can perform work; theoretically, objects attached to the filaments could be carried along by the transit of subunits through the filament. In the actual cell, actin filaments probably do not treadmilk, because their ends are usually bound by capping proteins; as we shall see, these proteins block the addition or loss of G-actin monomers. A similar process of treadmilling occurs during microtubule assembly and disassembly.

ATP is the controlling parameter of actin polymerization because it affects the Cₗₘ₀ for polymerization. This role of ATP is demonstrated by an experiment in which actin filaments are polymerized with ADP-actin monomers. The result is that both ends of a filament elongate at the slower rate characteristic of the (−) end. Thus, despite the inherent structural polarity of a filament, the Cₗₘ₀ of the (+) end becomes equal to the (−) end of the filament when ADP-actin monomers are incorporated into filaments.
The way it would be if actin did not bind ATP and hydrolyze it after polymerization:

Figure 7. Plot of the rate of polymerization vs. monomer concentration for elongation of a polar polymer showing bidirectional growth. $C_c$ denotes the critical concentration, which is the equilibrium monomer concentration. Below $C_c$, each end of the filament shortens, as indicated by the negative rates of polymerization.

The way it actually is (because $A$ binds and hydrolysis):

Figure 8. Plot of the rate of polymerization vs. monomer concentration for a polymer showing treadmilling. $C_+^*$ denotes the critical concentration for the plus end, and $C_-^*$ denotes the critical concentration for the minus end. $C_s$ corresponds to the steady-state monomer concentration, where the overall rate (the sum of the rates off the plus end and minus end) is zero. At this concentration, treadmilling takes place because there is a net flux of subunits off the minus end and a corresponding net flux onto the plus end. The shaded area denotes the concentration range in which elongation can proceed from the plus end, but where free filaments are unstable.
(+ end growing faster than (-) end and is falling apart.

\( a' > b' \)

\[ a = -b \]

(Treadmill with no change in length of filament)

Rate of Polymerization

Monomer Concentration

\( C^+ \)

\( C^- \)

\( C_c \)
A FIGURE 22.10 Uneven growth at the two ends of an actin filament. The two ends of a filament have different critical concentrations. (a) When short myosin-decorated filaments are the nuclei for actin polymerization, ATP-G-actin monomers add unequally to the two ends. Because of the polarity of the myosin heads, the barbed, or (+), end of the filament grows measurably faster than the pointed, or (−), end. The (−) end corresponds to the clefted (top) end of the actin filament model shown in Figure 22-2c. (b) Simple experiments blocking the (+) or (−) ends of a filament with actin-capping proteins permit growth only at the opposite

end. Under these conditions, the critical concentration is determined by the sole growing end. (c) The critical concentration (C_c) is the concentration of monomers which support actin polymerization. Below the C_c, actin remains monomeric, while at concentrations greater than C_c, actin filaments assemble and the monomer concentration remains invariant. The C_c for the (+) end (blue trace) is less than the C_c for the (−) end (red trace). (d) At concentrations intermediate between the C_cS for the (−) and (+) ends, actin subunits can flow through the filaments by attaching preferentially to the (+) end (follow the colored subunits) and dissociating preferentially from the (−) end of the filament, a phenomenon known as "treadmilling." The oldest subunits in a treadmilling filament lie at the C − D end. [Part (a) courtesy of T. Pollard.]
Two well-known drugs that affect actin filaments are Cytochalasin and Phalloidin

Cytochalasin D is a fungal alkaloid. It depolymerizes actin filaments by binding to the (+) end of filaments, thereby “poisoning” the (+) end; i.e., inhibiting any further polymerization at the (+) end. Filaments then slowly depolymerize from the (-) end.

![Cytochalasin D](image)

Phalloidin has the opposite effect: it hyper-stabilizes actin filaments, driving the equilibrium in the cell such that all actin is polymerized, and dynamic behavior is lost. Fluorescently-labeled phalloidin binds only to f-actin, and is widely used to stain actin filaments for light microscopy.

![Phalloidin](image)

Angel of Death mushroom (also known as “Deathcap”)

![Angel of Death mushroom](image)
III. Regulation of actin polymerization/depolymerization, *in vivo*

*Based on the intracellular concentration of actin (0.5mM) and ionic conditions *in vivo*, one would predict that nearly all cellular actin should exist as filaments. Actual measurements, however, show that as much as 40% of actin is unpolymerized. What’s going on?

1. Inhibition of f-actin assembly by *thymosin β4* [forms 1:1 complex with g-actin (ATP form of g-actin); covers up ATP binding site]; *profilin* also forms 1:1 complex with g-actin, competes with thymosin β4 for binding to g-actin. Profilin binds on + side of g-actin, leaving the ATP-binding site on the other side (-) open and accessible (fig 17-11, p.722).

2. Promotion of f-actin assembly by *profilin* [lower affinity for g-actin than thymosin β4, and permits ADP/ATP exchange; binds membranes via PIP2] (fig 17-11, p.722).

3. After actin polymerizes, it quickly (few seconds) hydrolyzes ATP, but ADP + Pi remains in binding site. *Cofilin* promotes release of Pi, and severing of f-actin, leading to depolymerization. (fig 17-11, p.722) (Handout, pp 35, 36)

4. Two classes of cytosolic proteins nucleate actin filament polymerization in vivo: formins and Arp2/3 complexes

   (a) *Formins*: formins nucleate the polymerization of linear actin filaments that form the contractile actomyosin stress fibers which connect focal adhesion structures; many formins are regulated by Rho family G-proteins. (Figs 17-13a, 17-14) (Notes, pp 32, 33)

   (b) *Arp2/3 complexes* associate WASp family proteins (eg, N-WASp, WAVE) to nucleate actin filament polymerization. (Fig 17-16)

   - When partnering with a WASp family member such as N-WASp, the Arp 2/3 complex initiates the polymerization of linear actin filaments, which then bundle to form filopodia at the leading edge of moving cells. (Notes p. 34a)

   - When acting with a WAVE partner (eg, WAVE2), which is activated via the small G-protein Rac, the Arp 2/3 complex initiates the formation of branched actin filaments, which can form lamellipodia at the leading edge of a moving cell. (Notes p. 34b)

5. The Cdc42 G-protein (WASp activation) and Rac G-protein (WAVE activation) are found at the leading edge of a moving fibroblast where they generate lamellipodia and filopodia; Rho is confined to the rear of the cell, where it initiates formation of actin filaments that interact with myosin contractile bundles in both stress fibers and the cell cortex. (Notes pp 39, 40)
Fig 18.14, p 744 (4th Ed.)
Complementary roles of profilin + Thymosin:

(a) Unactivated

(b) Activated

(c) Assembly

(d) Exchange

Fig 18.15 p. 715 (4th Ed.)

Note: 5th Ed.

25.
Complementary roles of profilin and thymosin in regulating actin polymerization:

Fig 19-10

Profilin promotes actin polymerization.
Thymosin inhibits actin polymerization.
Two classes of cytosolic proteins nucleate actin filament polymerization in vivo: formins and Arp/2/3 complexes.

(1) Formin family proteins nucleate polymerization of linear (unbranched) actin filaments. Many formin proteins are under the control of the Rho G-protein, and initiate actin filaments that will form the actomyosin contractile structures that connect focal adhesions.

Formin proteins contain an FH2 domain. FH2 domains fold to form a flexible dimer-like structure which serves to nucleate actin polymerization. The growing (+) end is “capped” by the FH2 domain, which continues to mediate ongoing elongation at the (+) end of the growing actin filament by a process termed “progressive capping”:

Activation of formin by Rho:

Inactive formin is folded up on itself. Active GTP-Rho binds to the RBD domain of formin, causing formin to assume its active, extended conformation. In this conformation, the FH1 domain of formin binds profilin-ATP-Actin. The ATP-Actin is thereby delivered to the adjacent FH2 domains which mediate polymerization of actin filaments:
Sidebar:

Before going on to discuss the second class of actin nucleating proteins, we need to know some general background information on the Rho family of small GTPases (also known as, “small G-proteins”) (Cdc42, Rac, Rho):

G-proteins are so named because they bind Guanine nucleotides. There are two broad categories of G-proteins: (1) “(large) Heterotrimeric G-proteins” and (2) “small G-proteins”. Members of the first category were discovered first, and play an important role in the intracellular signal transduction initiated by the “G-Protein Coupled hormone Receptors” (GPCRs). The small G-proteins were discovered later. Many small G-proteins play an important role in regulating the cytoskeleton and in growth-hormone signaling events. Many of the small G-proteins are members of the Rho family of small G-proteins, whose prototype members are Cdc42, Rac and Rho. All G-proteins have a slow GTPase activity. (This is why the term “small GTPase” is used synonymously with the term “small G-protein.”)

G-proteins in their GDP-bound form are inactive (they have a shape which is unable to bind to and activate a downstream target protein). An upstream activator (ie, hormone receptor) activates a GEF protein (“GEF” = “Guanine nucleotide Exchange Factor”). The activated GEF then binds the inactive GDP-G-protein, such as GDP-Rho in the figure above, and catalyzes the release of GDP and the acquisition of GTP. When G-proteins acquire a molecule of GTP, they assume a new “active” shape which permits them to bind to and activate one or more downstream target “effector” proteins (See GTP-Rho, above). The activated G-protein only stays active for a short while, because the intrinsic GTPase activity of the G-protein results in the cleavage of bound GTP to GDP + P_i. In the case of small G-proteins, the intrinsic GTPase activity is activated by the binding a “GTPase Activating Protein” (a GAP protein), as shown above. The GDP-G-Protein is thus now once again in its inactive state. It can be reactivated by a GEF if the hormone receptor is still in its active state. (Sometimes a GDI protein (“Guanine nucleotide Displacement Inhibitor”) sequesters the GDP-G-Protein. (Most large, heterotrimeric G-proteins have a built-in GAP domain.)
Two classes of cytosolic proteins nucleate actin filament polymerization in vivo: more detail on the second class, Arp/2/3 complexes.

(2) The Arp 2/3 complex consists of seven subunit proteins, two of which, Arp 2 and Arp 3, resemble actin (“Actin-related proteins”) and nucleate polymerization of either linear (unbranched) actin filaments or branched actin filaments. The Arp 2/3 complex works in conjunction with a partner protein of either the WASP or WAVE family of proteins. WASp proteins (“Wiskott-Aldrich Syndrome protein”) are activated by the small G-protein Cdc42 (in its GTP-form); WAVE proteins (“WASP family verprolin-homologous”) can be activated via the small G-protein Rac (in its GTP-form).

(2a) **When partnering with a WASP family member** such as N-WASP (activated by Cdc42), the Arp 2/3 complex initiates the polymerization of linear actin filaments, which then bundle to form **filopodia** at the leading edge of moving cells.

![Diagram](image-url)

Fig. 2. Mechanism of activation of N-WASP. In the inactive state, N-WASP is folded by an interaction between the GBD/CRIB domain and the coillin-homology (C) domain that masks the VCA region. Cooperative binding of PtdIns(4,5)P2 to the basic (B) region and of active Cdc42 to the GBD/CRIB domain exposes the VCA region, resulting in Arp2/3 complex activation. Alternatively, WISH binds to the proline-rich region of N-WASP and exposes the VCA region independently of PtdIns(4,5)P2 and Cdc42 binding. N-WASP activation stimulates formation of filopodia and appears to require additional factors that bundle and knit the actin filaments into straight filaments.

(Takenawa, T. and Miki, H., Journal of Cell Science 114, 1801-1809 (May, 2001))
**More detail on Arp/2/3 complexes:** partnering with the **WAVE protein** to initiate branched actin filaments.

**(2b)** When acting together with its **WAVE partner** (eg, WAVE2), which is activated via the small G-protein Rac (acting through the IRSp53 protein), the Arp 2/3 complex initiates the formation of **branched actin filaments**, which can form **lamellipodia** at the leading edge of a moving cell:

![Diagram of Arp2/3 complex activation](image)

*Fig. 1. Mechanism of activation of WAVE2. WAVE2 is probably inactive in the resting state, although WAVEs purified from a baculovirus system are already activated. In response to extracellular stimuli, Rac is activated. IRSp53 is recruited through its Rac-binding domain (RCB) and binds to the proline-rich region of WAVE2. As a result, the VCA region of WAVE2 is exposed and causes Arp2/3 complex activation. This leads to assembly of a meshed network of actin filaments and the formation of lamellipodia.*

Another model (more detail) on the partnering of Arp/2/3 complexes with a WAVE family protein (here designated “WASp”) to initiate branched actin filaments.

At the time the paper with this model was published role of WASP related WAVE proteins had not yet been distinguished, and so the essentially WAVE-like protein shown here is designated as “WASp.” But this model correctly shows the “WASp” WAVE protein recruiting Profilin-ATP:Actin complexes and delivering them to the Arp 2/3 complex, also bound to the WAVE protein. Then, (as also partly shown in your book in Fig 17-15), the Arp 2/3 complex with the nucleated actin filament jumps onto the side of an existing actin filament, and the second filament continues to grow at a 70º angle on the side of the first filament, with the ongoing assistance of the membrane-associated WAVE (“WASp”) protein.

The last panel (panel f) shows that the lower affinity of the Arp 2/3 complex for actin subunits in the ADP-actin state results in the release of actin filaments from the branched actin filament network, and also the role of Cofilin in disassembling ADP-actin filaments.
Fig. 1. Actin turnover. Actin filaments have polarity owing to the presence of barbed and pointed ends, fast- and slow-polymerizing, respectively. Profilin facilitates exchange of actin-bound ADP for ATP, forming a profilin–actin–ATP complex, which is recruited to and incorporated into the barbed end of F-actin. After hydrolysis of ATP, actin–ADP subunits within the filament can bind to cofilin (C), which increases their propensity to dissociate from the pointed ends. The cycle is completed by binding of profilin to ADP–actin, converting it to the ATP–actin form that is primed for polymerization.
How cofilin catalyzes depolymerization of actin filaments

1. ADF/cofilin promotes phosphate release from ADP-Pi actin filaments
2. ADF/cofilin binds and severs ADP-actin filaments creating a barbed end and a pointed end
3. ADP-containing pointed ends dissociate from Arp2/3 complex creating a new free pointed end

Potential sites of subunit dissociation

The role of actin filaments in cell migration:

Overview: Steps in cell movement

1. **Extension**: Movement begins with the extension of filopodia and lamellipodia from the leading edge of the cell. These extensions have adhesion molecules such as integrins at their tips.

2. **Adhesion**: Some of these lamellipodia and filopodia then adhere to the substratum by forming focal adhesions.

3. **Translocation**: Contraction of actomyosin bundles (“stress fibers”) at the back of the cell causes the bulk of the cytoplasm at the rear of the cell to move forward.

4. **De-adhesion**: The trailing edge of the cell remains attached to the substratum until the tail eventually detaches and retracts into the cell body. During this process, the endocytic cycle internalizes membrane and integrins at the rear of the cell and transports them via vesicle trafficking to the front of the cell for reuse in making new adhesions.
The role of actin filaments in cell migration:

More detail on the actin-based structures involved in locomotion

(1) **Extension:** Arp 2/3 and WASP/WAVE partners under the control of Cdc42 and Rac GTPases initiate the extension of filopodia and lamellipodia from the leading edge of the cell. These extensions have adhesion molecules such as integrins at their tips.

(2) **Adhesion and translocation:** Focal adhesion formed earlier and now located at the rear and middle of the cell associate with contractile bundles of actin and myosin. The actin filaments in these contractile bundles were initiated by formins, under the control of Rho GTPases. Contraction of the actomyosin bundles mediates translocation of the cell body forward.
A summary of the role of Rho family GTPases in cell migration:

Formin activation, leading to unbranched actin filaments; formation of bipolar myosin bundles, leading to formation contractile actomyosin fibers.