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 5-28, p173].
   
   Examples: **fibroblasts**: lamellipodia, microspikes (10 𝜇m), filopodia (= long microspikes: 50 𝜇m)
   
   epithelial cells: microvilli [fig 5-28, p173]
   
   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 6-26, p222)
   
   (⇒ focal adhesions/"3D" adhesions) (fig 6-27, p224;
   
   fibronectin (Fn)
   
   Fn receptor (an Integrin)
   
   Focal adhesion kinase (fak)
   
   Src kinase
   
   vinculin, talin,
   
   (ii) contractile ring in mitotic cells (fig 19-20, p796)
   
   (iii) adhesion belt in epithelial cells (fig 6-5, p203)

2. Actin networks: **filamin** [crosslinks f-actin, leading to gel formation], versus **gelsolin** [Ca^{++}-activated severing of f-actin, leading to sol formation]
   
   Examples: cell cortex (fig 19-15, p790); gel-sol conversions in amoeba pseudopod
   
   (Tom Stossel, American Scientist, 78, 408-423 [1990])

3. Actin filaments are linked to membrane proteins
   
   Examples: dystrophin; the actin-spectrin network underlying the red blood cell membrane

II. Polymerization Kinetics of Actin

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)
Actin filaments are linked to membrane proteins:

Role of dystrophin

Muscular Dystrophy is caused by a mutant Dystrophin protein.
cytoplasmic subcomplex, the COOH-terminal region of dystrophin is bound to syntrophin, whereas the amino-terminus of dystrophin binds F-actin.

Abnormalities of dystrophin are the most common cause of muscular dystrophy (27, 45), accounting for both the Duchenne and Becker phenotypes. Duchenne muscular dystrophy (DMD) is a devastating muscular dystrophy and the most common inherited childhood-letal disorder of humans worldwide (45). DMD is caused by mutations in the dystrophin gene that precludes the production of stable dystrophin molecules and results in sarcolemmal instability and contraction-induced myofiber necrosis. Dystrophin is highly con-served through vertebrate evolution, the dystrophin gene is X-linked in placental mammals, and dystrophin deficiency appears to be completely specific for DMD (46, 48). Thus any animal that manifests dystrophin deficiency as an X-linked inherited trait is a candidate model of DMD. Among other causes of muscular dystrophy are 1) Becker muscular dystrophy, a disorder known to be allelic (due to a different mutation in the same gene) to DMD (54), and 2) genetic defects of the SG subcomplex (α, β, γ, δ) of the myofiber cytoplasm (sarcoglycanopathies), a common cause of LGMD-2C, -2D, -2E, and -2F (1, 27, 37, 40, 86).

Identification of animal models for DMD and the sarcoglycanopathies has been instrumental in research on the pathogenesis, pathophysiology, and treatment of these disorders. Indeed, the past 15 yr have been witness to a virtual explosion of knowledge regarding 1e molecular pathology of DMD, Becker muscular dystrophy, and LGMD in humans and their mamma-

lilian counterparts (45). This knowledge, in turn, is being used to develop novel approaches to the treatment of these disorders, including gene therapy (47, 93, 95), myoblast transfer (71), and new pharmacological interventions (38). There remains, however, a gap between our knowledge of the molecular pathology of muscular dystrophy and downstream biomechanical events. Given that most muscular dystrophies are associated with primary defects of the muscle membrane cytoskeleton and characterized phenotypically by muscle weakness, a host of potential functional targets exist beyond those involved in the cascade leading to muscle cell death (45, 73). Targets include the metabolic pathways involved in intracellular calcium regulation (t tubule system, sarcoplasmic reticulum, and excitation-contraction coupling), myosin molecular motor function, and bioenergetics (mitochondria and phosphocreatine-creatine kinase energy shuttle), among others. An expanded knowledge of these secondary consequences of muscular dystrophy will enhance our understanding of these disorders and dystrophic skeletal muscle dysfunction. Moreover, the aforementioned novel therapeutic approaches will ultimately be judged by their ability to enhance dystrophic skeletal muscle function. To date, such therapies have been almost exclusively applied and tested in animal models. Below, we discuss our current understanding of the functional status of dystrophic skeletal muscle from selected animal models used in the study of muscular dystrophy with a specific focus on 1) the mdx mouse model of DMD, 2) the Bio 14.6 8-SG-deficient hamster model of LGMD, and 3) transgenic null mutant murine...
Short segments of actin plus tropomyosin act together with band 4.1 protein to anchor spectrin filaments to the transmembrane protein glycoporphin.

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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 $C_c$ 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 filament is blocked. In either case, the $C_c$ of each end of a filament is 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 $C_c$ 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].

This difference in the $C_c$ of filament ends leads to an interesting phenomenon called treadmill (Figure 22-10d). At G-actin concentrations intermediate between the $C_c$s 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 treadmill, 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 treadmill occurring during microtubule assembly and disassembly.

ATP is the controlling parameter of actin polymerization because it affects the $C_c$ 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 slowest rate characteristic of the (−) end. Thus, despite the inherent structural polarity of a filament, the $C_c$ 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:

\[ \text{Non-hydrolyzable ATP} \]

Kinetics for some "theoretical" polymer

Figure 7. Plot of the rate of polymerization vs. monomer concentration for elongation of a polar polymer showing bi-directional growth. \( C_0 \) denotes the critical concentration, which is the equilibrium monomer concentration. Below \( C_0 \), each end of the filament shortens, as indicated by the negative rates of polymerization.

The way it actually is (because of ATP binding and hydrolysis):

ATP hydrolysis means the \((-\) end can have a different \( C_0 \) from the \( +\) end. This means treadmilling can occur.

\[ \text{ATP:} \rightarrow \text{different} \ C_0 \text{ for} \ (\pm) \text{ ends of actin filament} \]

Figure 8. Plot of the rate of polymerization vs. monomer concentration for a polymer showing treadmilling. \( C^+_0 \) denotes the critical concentration for the plus end, and \( C^-_0 \) denotes the critical concentration for the minus end. \( C^+_0 \) 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.
In this range of monomer concentrations, actin filament is treadmilling, getting shorter as time goes by.

At this precise concentration ($C_c$), the filament is treadmilling, with no change in length of filament over time.

In this range of monomer concentrations, filament is treadmilling, but getting longer over time.

$(+)$ end growing faster than $(−)$ end and is falling apart.

$\alpha' = (a' > b')$

$\beta$ or $\beta'$

$(−)$ end grows faster than $(+)$ end and is falling apart.
The dynamics of actin assembly are illustrated in the image. The two ends of an actin 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, c) 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 \( C_c \)s 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; ie, 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”)
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 19-10, p.787).

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 19-10, p.787).

3. Actin filament assembly at the inner membrane surface (to produce filopodia, lamellipodia and focal adhesions/stress fibers) is initiated by certain proline-rich proteins such as Wasp ("Wiscott-Aldrich syndrome protein"), which bind the polymerization-nucleating Arp complex (Actin-related protein). The nucleation process is regulated by Rho family G-proteins (fig 19-29, p804).
Both thymosin and profilin bind g. actin on opposite sides.

Thymosin β4
(Thymosin covers the ATP cleft)

Actin
ATP binding cleft
ADP + Pi
Profilin

(Not in current edition)
Complementary roles of profilin and thymosin in regulating actin polymerization:

Fig 19-10

[Diagram showing the process of actin polymerization with profilin and thymosin]

**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 “**processive capping**”:

![Diagram of formin FH2 domain](image1)

**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:

![Diagram of formin activation by Rho](image2)
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 \textit{in vivo}: more detail on the second class, \textit{Arp/2/3 complexes}.

(2) The \textit{Arp 2/3} complex consists of seven subunit proteins, two of which, \textit{Arp 2} and \textit{Arp 3}, resemble actin ("Actin-related proteins") and nucleate polymerization of \textit{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) \textbf{When partnering with a WASP family member} such as N-WASP (activated by Cdc42), the Arp 2/3 complex initiates the polymerization of \textit{linear} actin filaments, which then bundle to form \textit{filopodia} at the leading edge of moving cells.

(In addition to activation of N-WASP via GTP-Cdc42, it can alternatively be activated via the WISH protein.)

\textbf{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)P$_2$ 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)P$_2$ 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.

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** (e.g., 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](image)

**Fig. 4.** 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.
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.

Contraction of myosin II filaments in both stress fibers and cell cortex.