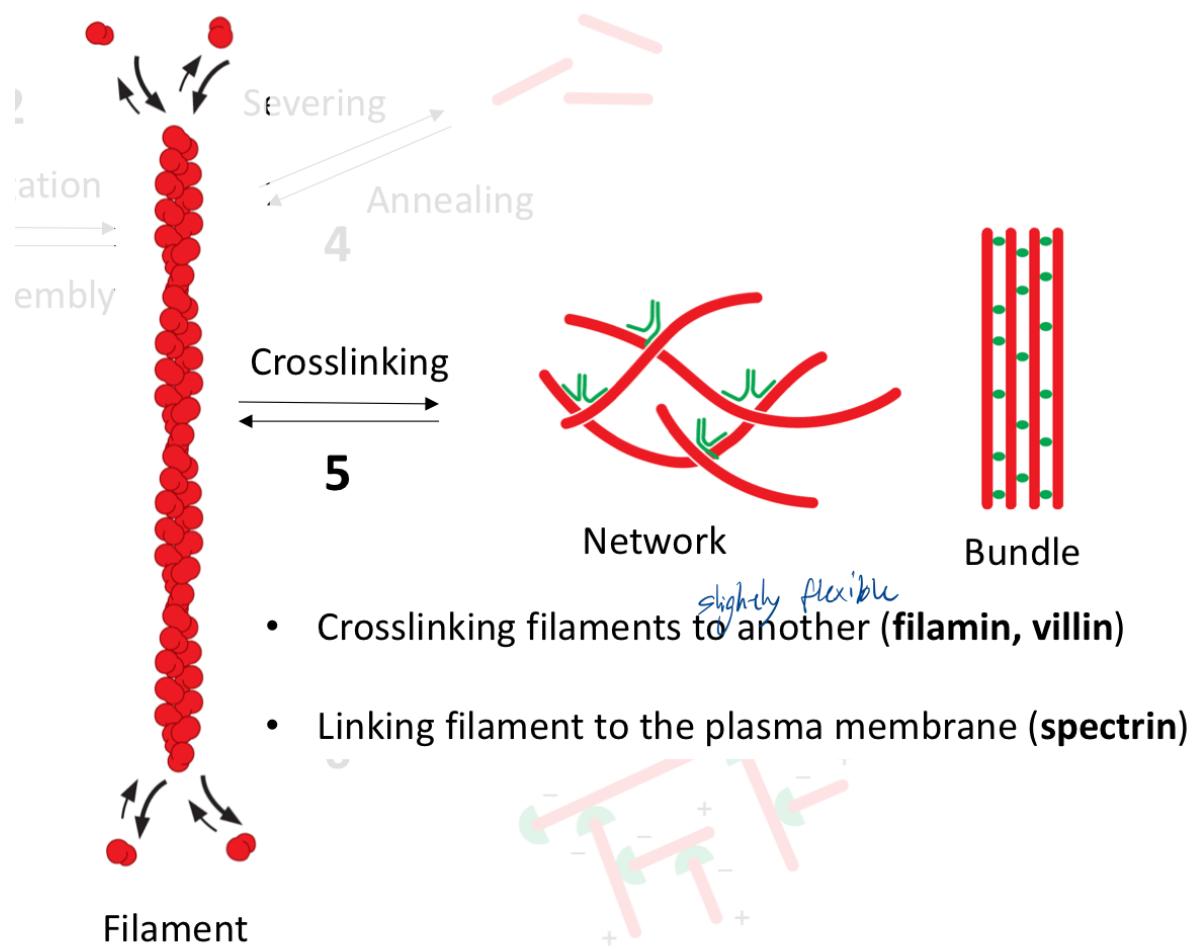
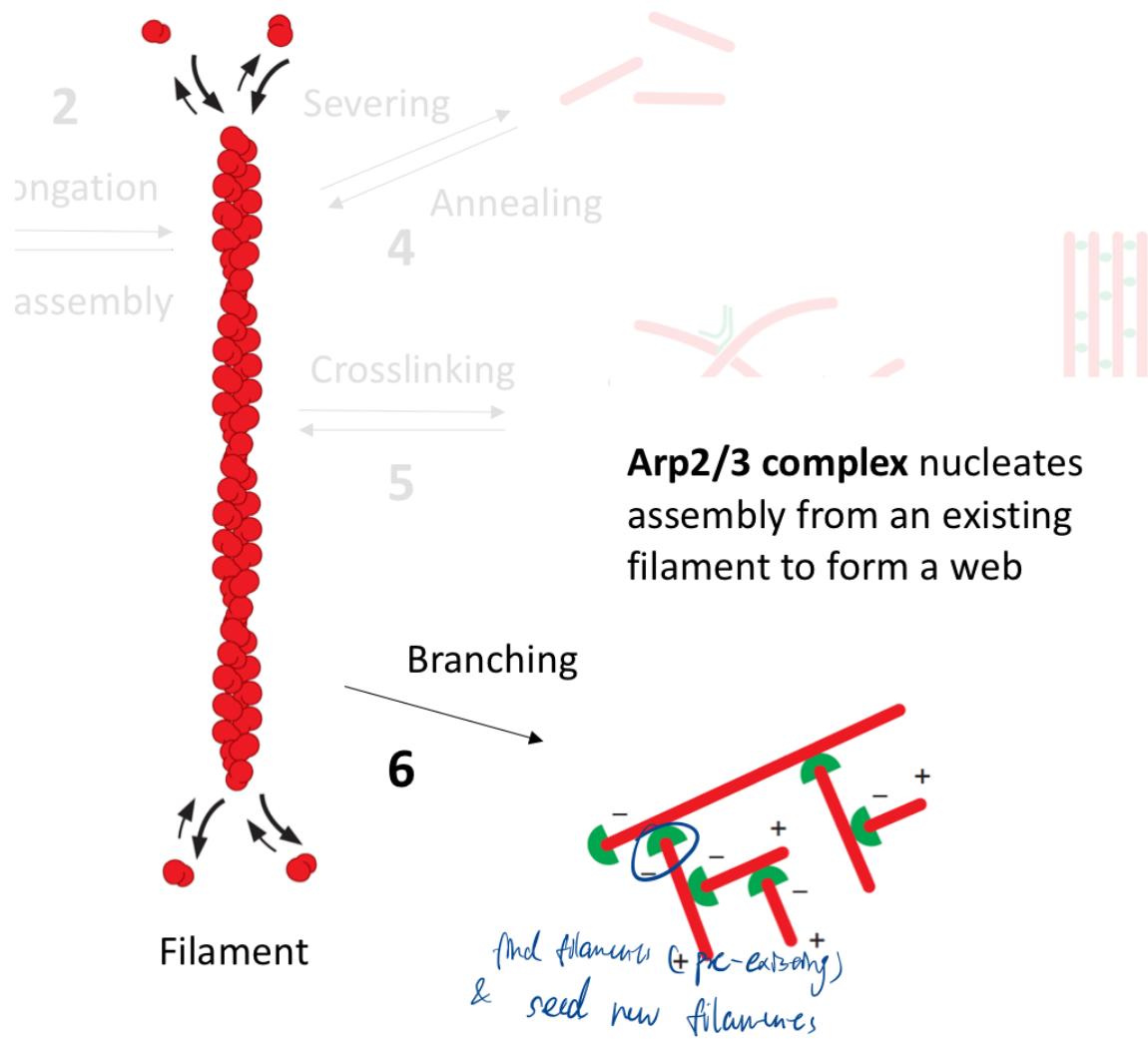


Breakage to form two minus ends → theoretically can depolymerise more efficiently

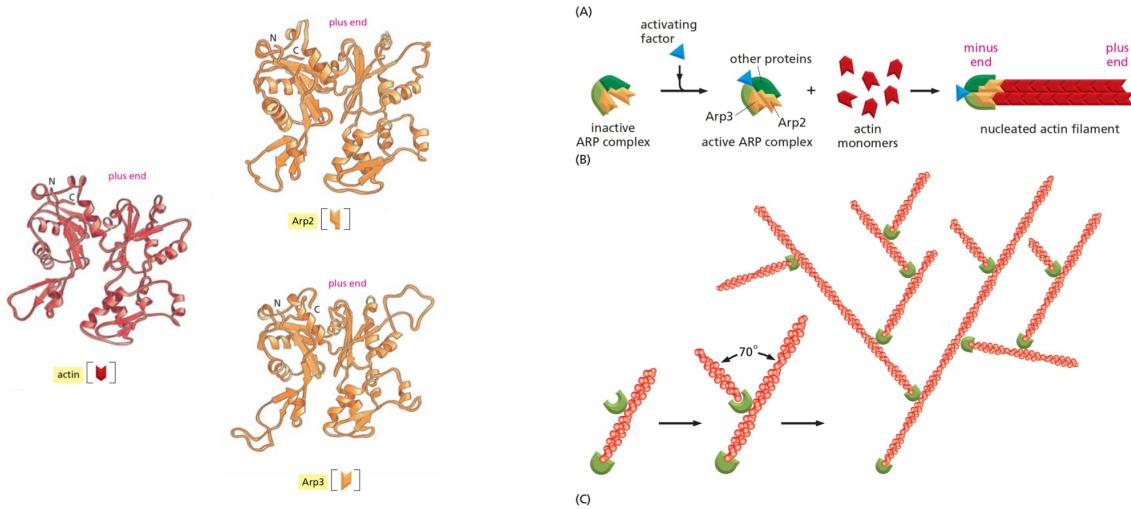
▼ For higher structure



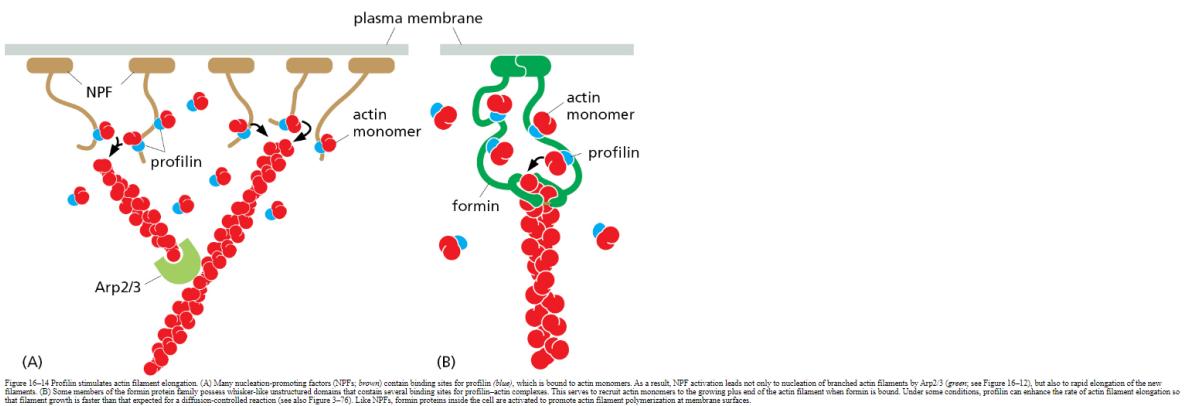
▼ Branching - Arp2/3 complex — lamelopodia



- ARP — Actin-related protein
- Arp2/3 complex **bound to the -end** nucleates actin filament growth and **allow rapid elongation at the +end**
- Arp2/3 activity requires binding of **other proteins** (e.g. nucleation-promoting factor (**NPF**)) — after activation, together with other proteins it recruits actin monomers and bind to the -end of the newly-formed actin filament → Arp2/3 complex is further stimulated when it attaches to the side of a preexisting actin filament
- The new filament branch out at **70° angle relative to the original filament**



- push the network towards greater dimentionality



▼ Motor proteins — Myosins

- various structures, but have commonalities
- The head domain is at N-terminus vs tail domain is at C-terminus

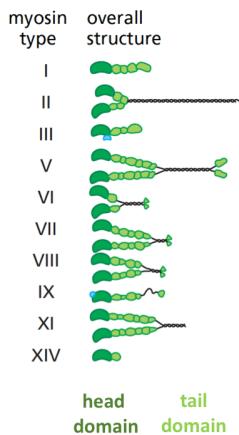
Myosin motors

conserved by species

conserved in hair, variation in tail domain

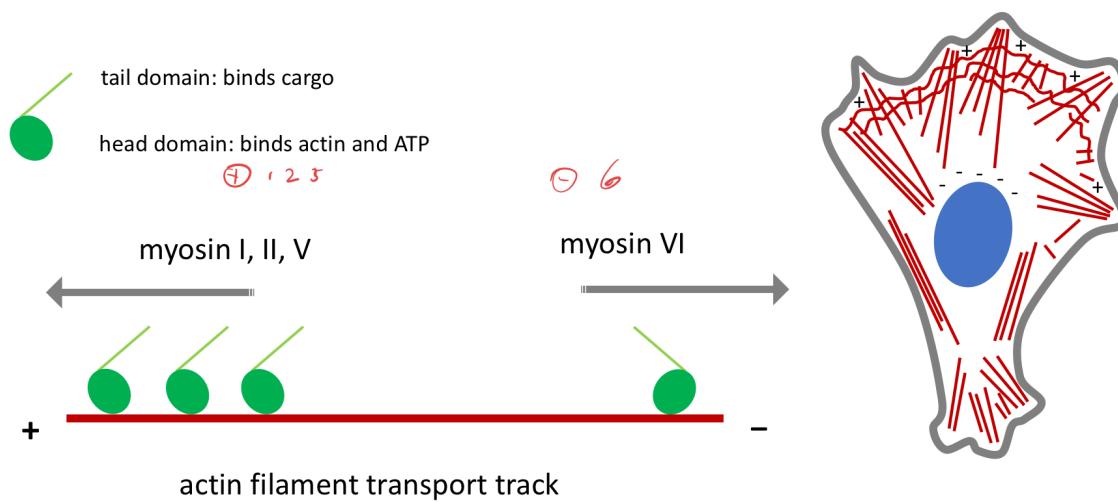


able to integrate various cargos



- All myosins have a head, neck and tail domain
- Different myosins act as monomers, dimers or bundles
- Some myosins (myosin I, V, VI) bind cargo via their tail domains for transport
- Myosin tail domains either bind cargo or another tail domain to form myosin bundles

Myosin motors can only move one way along its actin filament track



Actin filament function

- Non-contractile
 - form cell shape

- Involved in endo- & exocytosis → deliver cargo at specific time point to specific location
- microvilli
- Contractile (**myosin II**)
 - Muscle contraction
 - cytokinesis
- Combination of non-contractile & contractile
 - cell migration
 - filopodia
 - lamellipodia

▼ Dynamic actin filament behaviour primarily occurs at the cell cortex: branched networks,, parallel bundles, combinations of branched and straight filaments

- branched network is primarily nucleated by **Arp2/3 complex**
- the straight bundles are produced by **formins**

▼ Formation of filopodia & lamellipodia

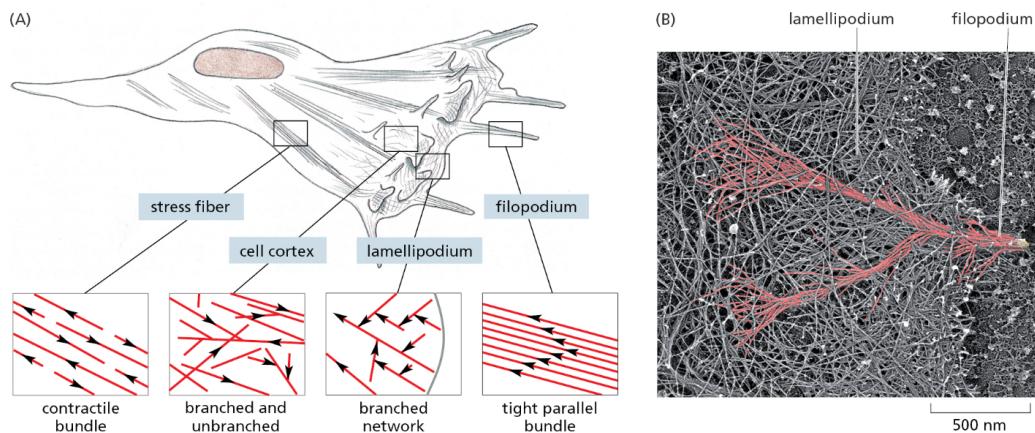
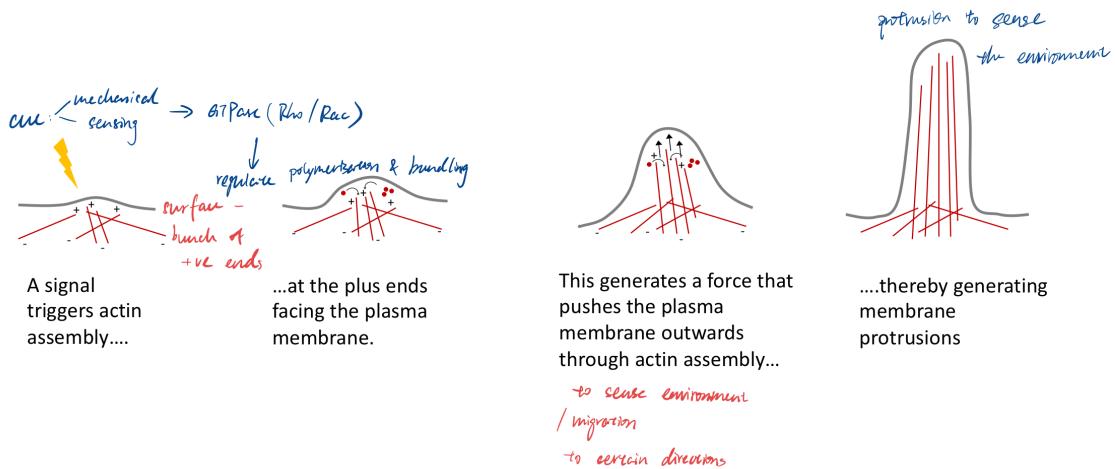


Figure 16-18 Actin arrays in a cell moving along a flat surface. (A) A schematic of a fibroblast migrating in a tissue-culture dish is shown with four areas enlarged to show the arrangement of actin filaments. The actin filaments are shown in red, with arrowheads pointing toward the minus end. Stress fibers are contractile and exert tension. The actin cortex underlies the plasma membrane and consists of actin networks that enable membrane protrusion at lamellipodia. Filopodia are spike-like projections of the plasma membrane that sense extracellular signals and allow a cell to explore its environment. (B) A migrating cell that was fixed, dried, and shadowed with platinum reveals a dense network of actin filaments at the leading edge (right side of image). The fast-growing plus ends are oriented toward the cell edge. Accessory proteins organize the filaments. Pseudo-colored in red are actin filaments in the process of becoming a filopodium. (B, courtesy of Tatjana Svitkina.)

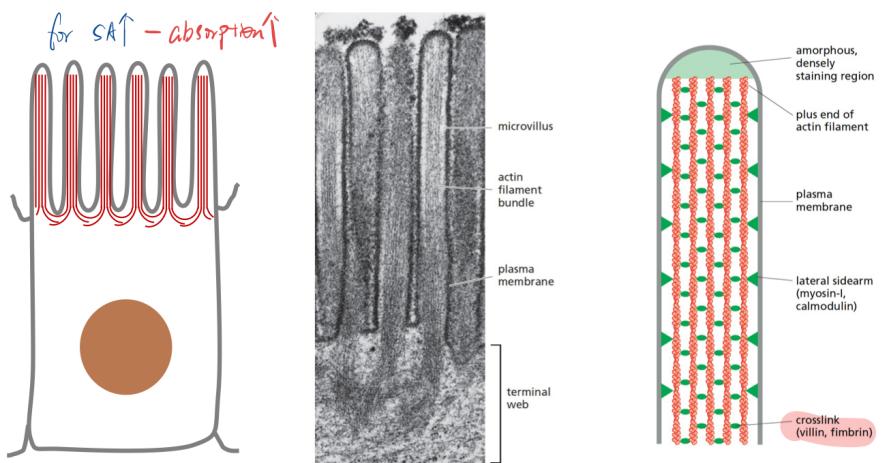
- Filopodia

- **one dimensional**; long, **bundled actin** filaments — thinner and longer than microvilli
- e.g. can extend from the leading edge, to **sense environmental cues** & also involved in migration
- Lamellipodia
 - **two-dimensional** & sheet-like
 - **cross-linked mesh**
 - drives the forward movement of the **leading edge of migrating cells**



▼ Non-contractile : microvilli

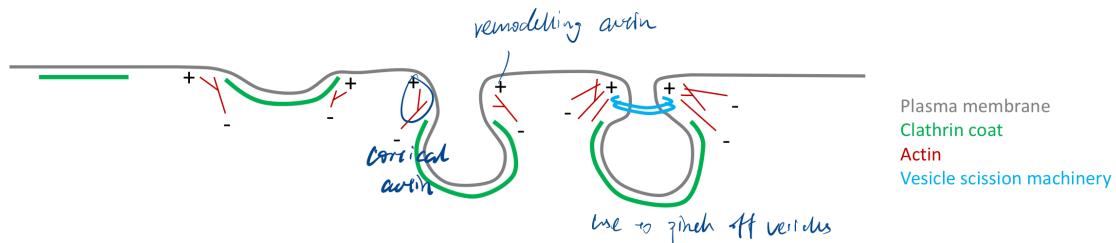
- extension from the cell surface → increased cell surface area → facilitate absorption
- bundling protein: villin & vimbrin



- Cell surface extension to maximize nutrient absorption of intestinal epithelial cells
- Created by a bundle of actin filaments cross-linked by actin-bundling proteins **villin** and **vimbrin**
- Lateral side arms composed of myosin I and calmodulin connect bundles to the plasma membrane

▼ Non-contractile: endo- & exo-cytosis

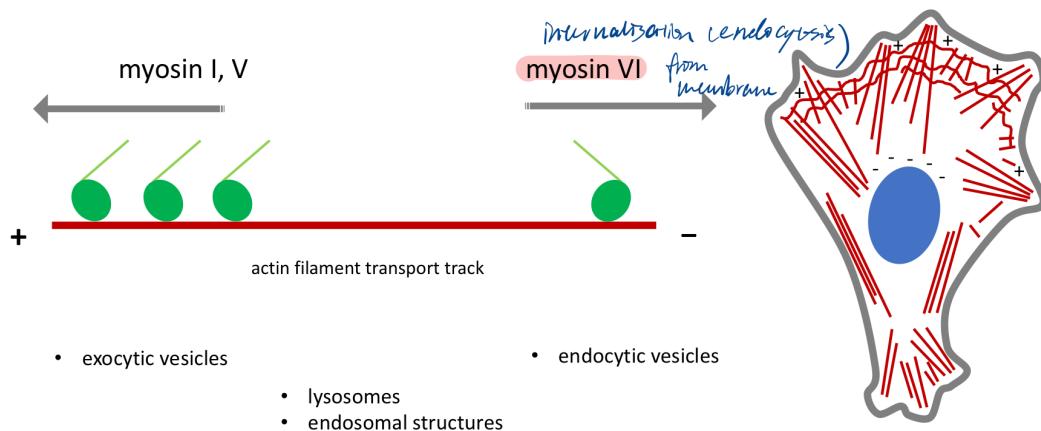
- assist clathrin-coated vesicle formation
- actin is more vital in yeast endocytosis



- In mammalian cells, actin may assist in clathrin-mediated vesicle formation as source of force for membrane deformation
- In yeast, actin is a vital component of the endocytosis machinery
- endocytosis transport is facilitated by the -ve end motor protein (VI myosin)

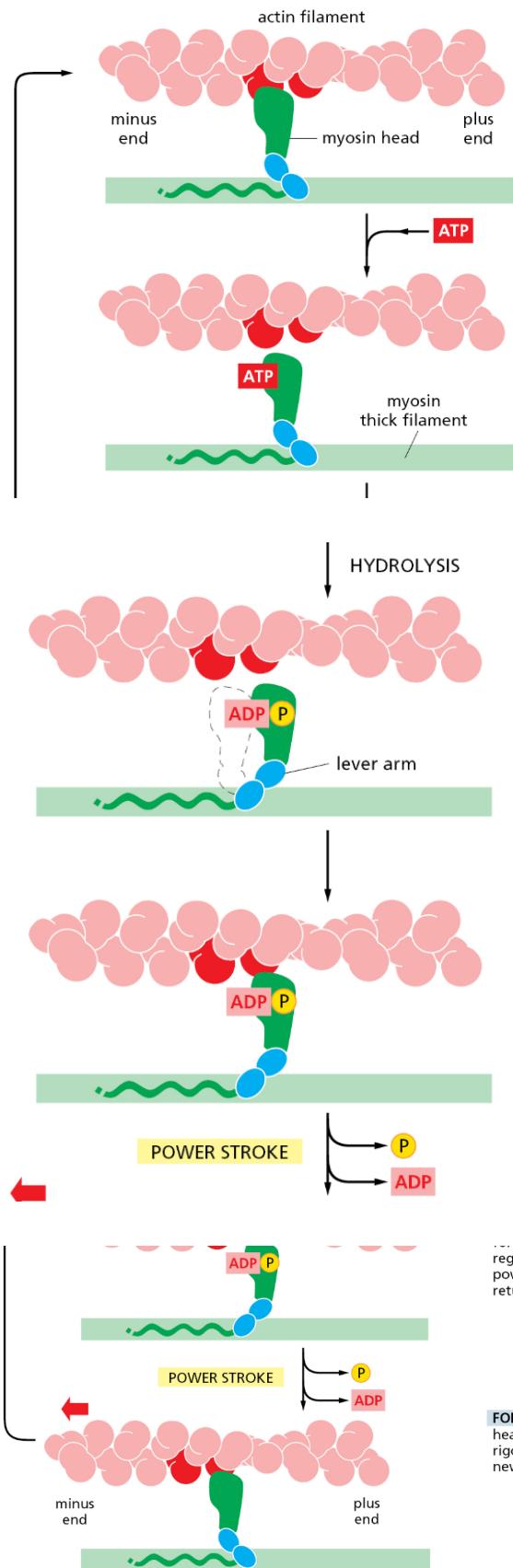
1) Non-contractile actin structures: Endo-and Exocytosis

by internalizing filament w/ organelles



▼ Contractile

- Myosin II filaments
- actin filament plus ends anchored at Z disc
- Z disc: CapZ & alpha-actinin; CapZ → capping the plus ends → prevent depolymerisation
- tropomodulin: cap the minus end
- tropomyosin - stabilise the actin filament along the way
- titin (largest protein?) : act as the molecular spring



ATTACHED At the start of the cycle shown in this figure, a myosin head lacking a bound nucleotide is locked tightly onto an actin filament in a *rigor* configuration (so named because it is responsible for *rigor mortis*, the rigidity of death). In an actively contracting muscle, this state is very short-lived, being rapidly terminated by the binding of a molecule of ATP.

RELEASED A molecule of ATP binds to the large cleft on the “back” of the head (that is, on the side furthest from the actin filament) and immediately causes a slight change in the conformation of the actin-binding site, reducing the affinity of the head for actin. (The space drawn here between the head and actin emphasizes this change, although in reality the head probably remains very close to the actin.)

COCKED ATP binding triggers a conformational change in the cleft that leads to a rotation in the converter domain, causing the lever arm to swing out and the head to be displaced along the filament by a distance of about 5 nm. Hydrolysis of ATP occurs, but the ADP and inorganic phosphate (P) remain tightly bound to the protein.

RE-BINDING AND POWER STROKE The myosin head binds weakly to a new site on the actin filament, causing release of the inorganic phosphate produced by ATP hydrolysis, concomitantly with the tight binding of the head to actin. This release triggers the power stroke—the force-generating change in shape during which the head regains its original conformation. In the course of the power stroke, the head loses its bound ADP, thereby returning to the start of a new cycle.

Force-generating change in shape during which the head regains its original conformation. In the course of the power stroke, the head loses its bound ADP, thereby returning to the start of a new cycle.

FORCE GENERATING At the end of the cycle, the myosin head is again locked tightly to the actin filament in a rigor configuration. Note that the head has moved to a new position on the actin filament.

Figure 16-24 The cycle of structural changes used by myosin II to walk along an actin filament. In the myosin II cycle, the head remains bound to the actin filament for only about 5% of the entire cycle time, allowing many myosins to work together to move a single actin filament. (Based on I. Rayment et al., *Science* 261:58–65, 1993.)

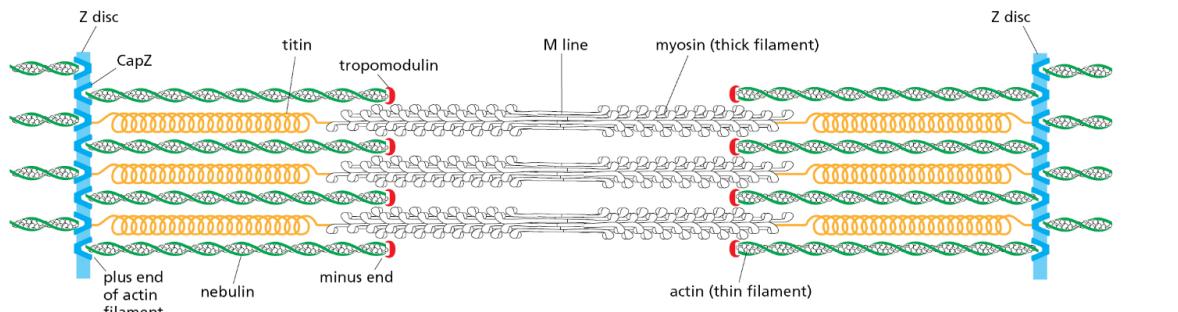
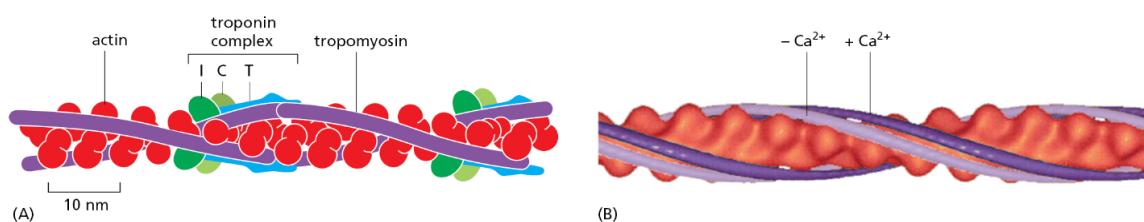
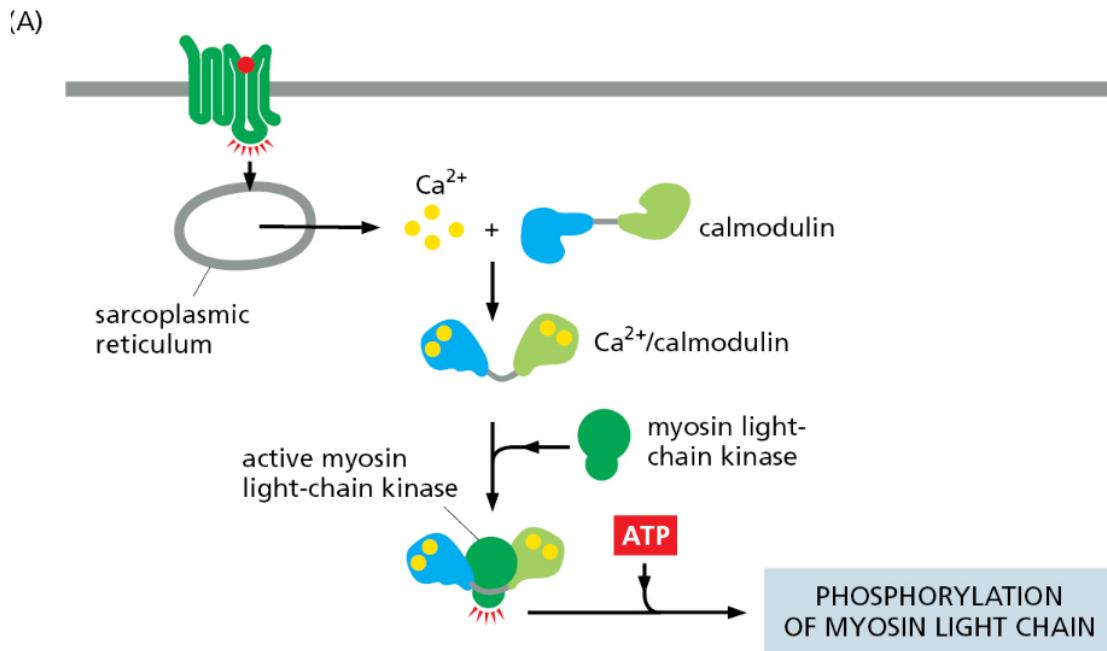


Figure 16–29 Organization of accessory proteins in a sarcomere. Each giant titin molecule extends from the Z disc to the M line—a distance of more than 1 μm . Part of each titin molecule is closely associated with a myosin thick filament (which switches polarity at the M line); the rest of the titin molecule is elastic and changes length as the sarcomere contracts and relaxes. Each nebulin molecule is exactly the length of a thin filament. The actin filaments are also coated with tropomyosin and bound intermittently by troponin (not shown; see Figure 16–31) and are capped at both ends. Tropomodulin caps the minus end of the actin filaments, and CapZ anchors the plus end at the Z disc, which also contains α -actinin (not shown).



The Ca^{2+} dependence of vertebrate skeletal muscle contraction, and hence its dependence on commands transmitted via nerves, is due entirely to a set of specialized accessory proteins that are closely associated with the actin thin filaments. One of these accessory proteins is a muscle form of **tropomyosin**, the elongated protein that binds along the groove of the actin filament helix. The other is **troponin**, a complex of three polypeptides, troponins T, I, and C (named for their tropomyosin-binding, inhibitory, and Ca^{2+} -binding activities, respectively). Troponin I binds to actin as well as to troponin T. In a resting muscle, **the troponin I-T complex pulls the tropomyosin out of its normal binding groove into a position along the actin filament that interferes with the binding of myosin heads**, thereby preventing any force-generating interaction. When the level of **Ca^{2+} is raised**, troponin C—which binds up to **four molecules of Ca^{2+}** —causes troponin I to release its hold on actin. This allows the **tropomyosin molecules to slip back into their normal position** so that the myosin heads can walk along the actin filaments (Figure 16–31). **Troponin C is closely related to the ubiquitous Ca^{2+} -binding protein calmodulin** (see Figure 15–34); it can be thought of as a specialized form of calmodulin that has acquired binding sites for troponin I and troponin T,

thereby ensuring that the myofibril responds extremely rapidly to an increase in Ca^{2+} concentration



In smooth muscle cells, so called because they lack the regular striations of skeletal muscle, contraction is also triggered by an influx of calcium ions, but the regulatory mechanism is different. **Smooth muscle forms the contractile portion of the stomach, intestine, and uterus, as well as the walls of arteries and many other structures requiring slow and sustained contractions.**

Smooth muscle is composed of sheets of highly elongated spindle-shaped cells, each with a single nucleus. Smooth muscle cells do not express the troponins. Instead, elevated intracellular Ca^{2+} levels regulate contraction by a mechanism that depends on calmodulin (Figure 16–32). **Ca^{2+} -bound calmodulin activates myosin light-chain kinase (MLCK)**, thereby inducing the **phosphorylation of smooth muscle myosin** on one of its two light chains. When the light chain is phosphorylated, the **myosin head can interact with actin filaments and cause contraction**; when it is dephosphorylated, the myosin head tends to dissociate from actin and becomes inactive.