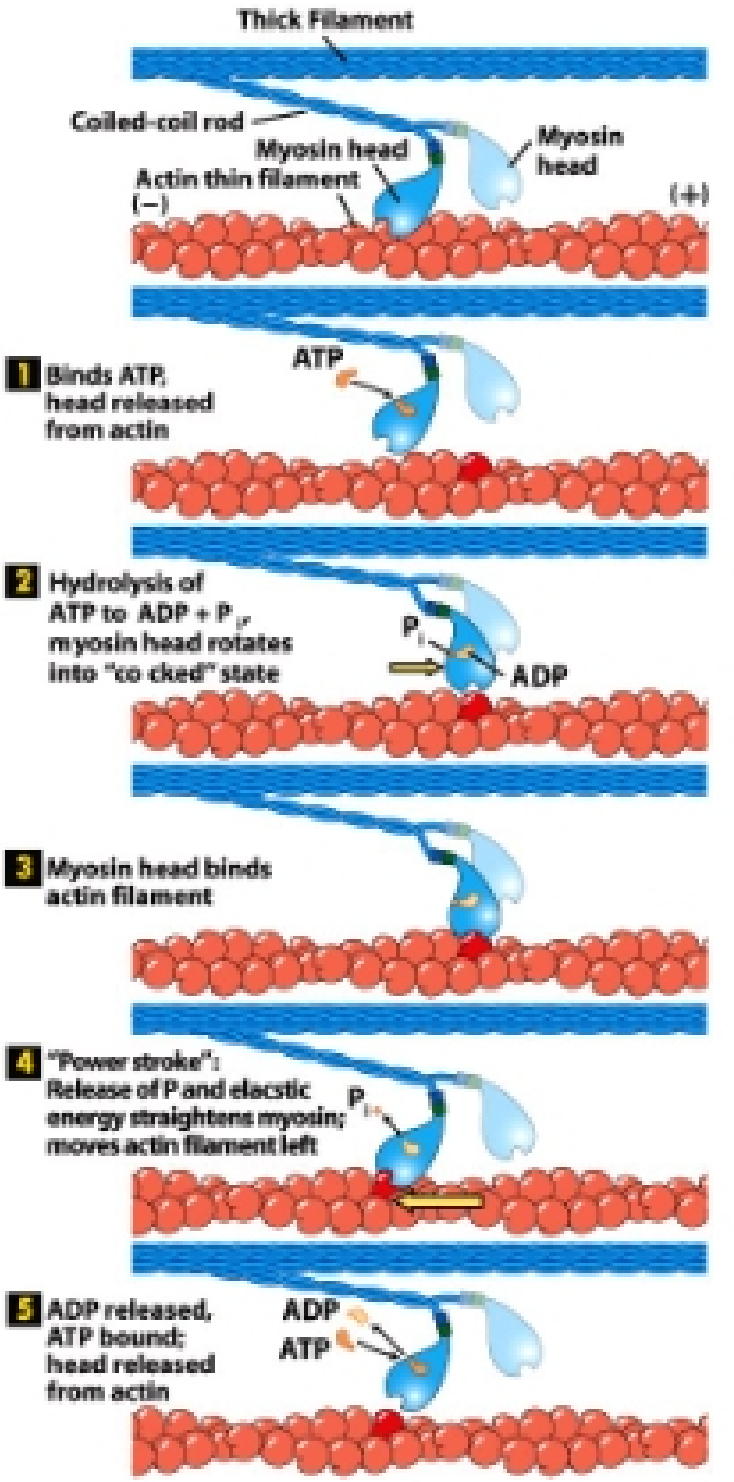
CBIO 3400 – Week 8 Study Questions

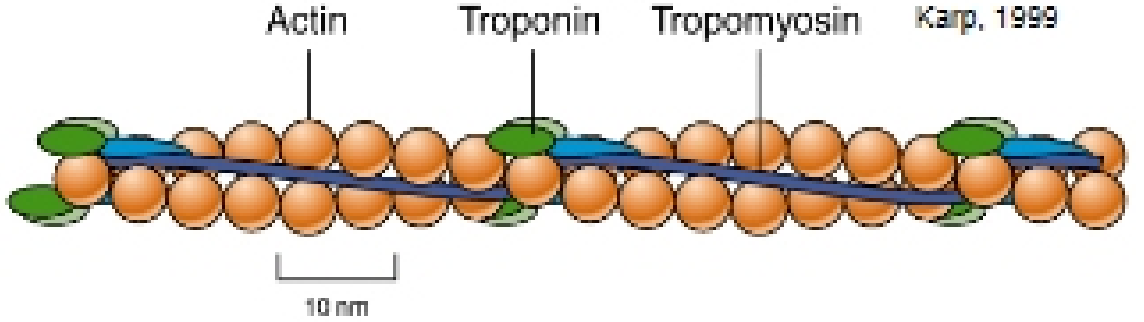
1. Explain the correlation between actin, myosin, and ATP.
   Actin has 2 subdomains connected by a cleft that binds ATP. Myosin is an actin-dependent motor that hydrolyzes ATP and converts chemical energy into movement along actin filaments. Myosin has 3 parts:
   - head = motor domain; binds actin and ATP
   - neck = lever arm; undergoes movements during force generation
   - tail = may contain dimerization and cargo, such as lipid-binding domains

   Small conformational changes in the motor domain caused by binding and hydrolysis of ATP are transferred into large movements of the neck (lever arm). In the absence of actin, myosin hydrolyzes ATP very slowly; the presence of actin stimulates binding to actin and the hydrolysis of ATP (i.e., myosin is an actin-dependent ATPase).

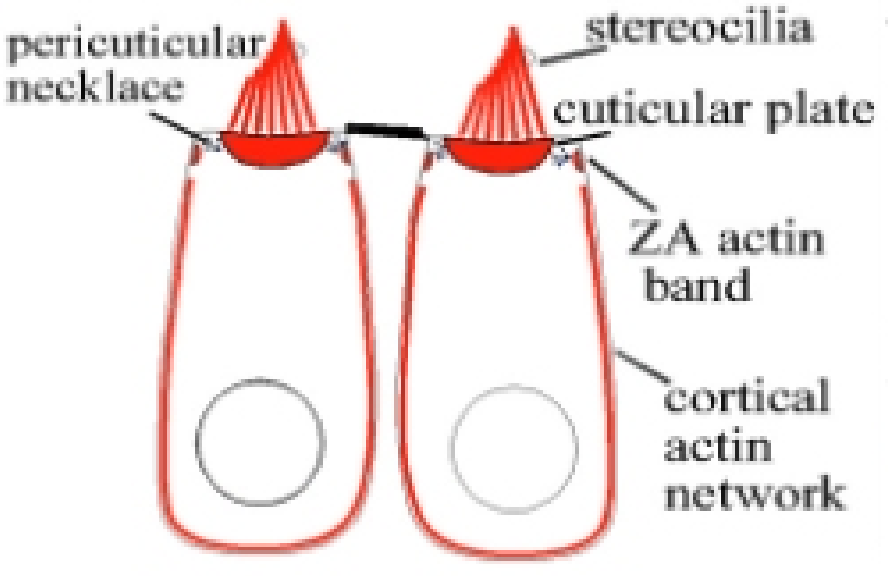


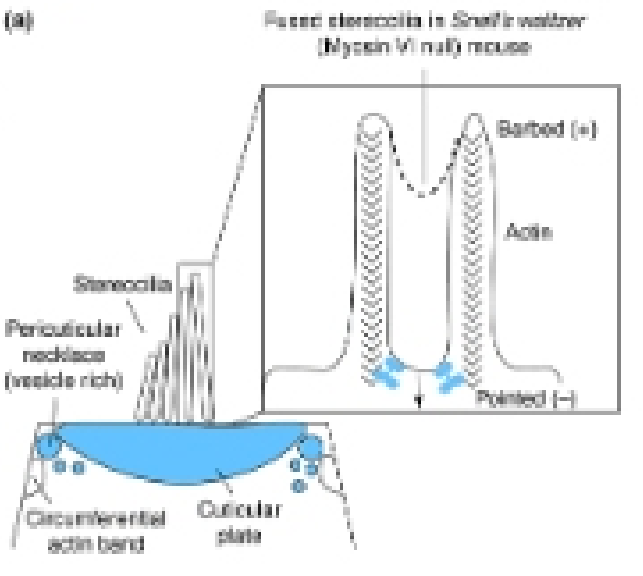
2. What is the function of Arp2/3? How does it work?
   Arp2/3 nucleates actin and forms branches. You can expect to find Arp2/3 near the leading edge of a membrane, much like actin. Arp2/3 is found at all branch points in actin filaments. ActA stimulates Arp2/3 because Arp2/3 alone has a relatively weak ability to stimulate actin assembly. ActA binds to Arp2/3 and enhances its ability to nucleate actin assembly (i.e., make new actin subunits). Thus, actin will polymerize preferentially near the surface of the pathogen.

3. Describe the mechanism of how troponin and tropomyosin affect the muscle contraction.
   Tropomyosin is an elongated protein associated with 7 actin monomers. It binds Ca^{2+} and undergoes a conformational change that allows tropomyosin to move along actin. Troponin is a globular complex with 40 nm spacing and in contact with both tropomyoisin and actin (i.e., 1 troponin for each tropomyosin). The mechanism associated with muscle contraction is as follows:
   1. Troponin (TN-C subunit) binds Ca^{2+}.
   2. The conformational change in troponin is transmitted to tropomyosin.
   3. Tropomyosin displacement allows myosin to bind actin.
   4. Ca^{2+} is released from ER in response to plasma membrane depolarization caused by an impulse from a nerve cell (sarcoplasmic reticulum).



4. What is the cause of hearing loss in Snell's Waltzer mouse? Why?
   Myosin-VI mutations are responsible for hearing loss and balance defects in the Snell's Waltzer mouse. Inner ear hair cells have stereocilia (microvilli). The motion of stereocilia in response to sound opens mechanosensitive channels, and this depolarizes the plasma membrane, leading to activation of exocytosis and release of neurotransmitters at the basal end. Neurotransmitters then diffuse to a nerve terminal. Within cells, myosin-VI is enriched at the base of the stereocilia. In the SW mouse, stereocilia fuse soon after birth, and myosin-VI (concentrated at the base of stereocilia) may be pulling the membrane down (toward the pointed end of the actin filament, near the base of stereocilia). The fused stereocilia prevent the appropriate movement of the microvilli in the ear upon the arrival of sound and thus cause hearing loss.



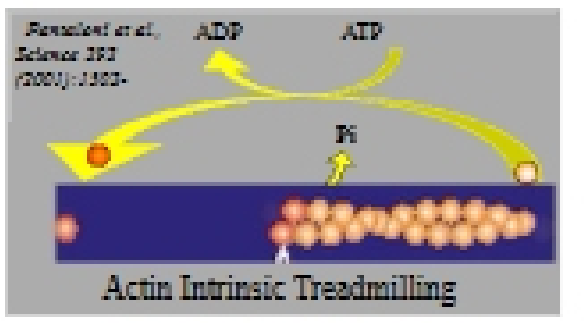


5. What role does "nucleation" play in actin/microtubule assembly?

Nucleation is completed by ActA and Arp2/3. ActA binds to Arp2/3 and enhances its ability to nucleate actin assembly (i.e., make new actin subunits). "Nucleation" refers to actin assembly or basically the polymerization of actin.

6. What is "treadmilling" in cytoskeleton assembly? When does it occur?

"Treadmilling" occurs at actin concentrations above Cc for the + end and below Cc for the – end. This is a very slow process *in vitro*. It is essentially a process where subunits are lost from one end (usually the – end) and added to the other end (usually the + end).



7. Explain the dynamic instability of microtubules.

The dynamic instability of microtubules is their alternation between phases of growth and shrinkage. Individual microtubules behave independently. Dynamic instability is most frequent in the steady state, when the depolymerization of dimers is near the Cc. GTP hydrolysis induces a conformational change inside the tubulin dimer that increases the curvature of microtubule filaments, making them more prone to depolymerization. Dynamic instability occurs when the rate of nucleotide hydrolysis is near the rate of subunit addition (i.e., near the Cc), resulting in frequent transitions between polymerization and depolymerization. Dynamic instability is the ground state of