Chapter 17. Cilia and Flagella

- What are cilia and flagella?
  - Eukaryotic flagella and cilia are basically the same thing.
  - Both are very different from bacterial flagella.
- Cilia and flagella contain stable MTs moved by dynein.

Chapter 17. Cilia and Flagella

- Where is the motor?
- What powers the motor?
- How does the motor result in beating?
  - The motor can generate sliding.
  - Sliding generates bending.

Chapter 17. Cilia and Flagella

- Evidence that dynein is the motor.
  - Dynein is an ATPase.
  - Dynein is in the right place.
  - Dynein-less mutants are not motile.
  - Dynein "concentration" is proportional to beat frequency.

Chapter 17. Actin Filaments

- A reminder: two things I said that we should keep an eye on for each of the components of the cytoskeleton:
  - The role of polymerization and depolymerization
  - The role of accessory proteins.

Chapter 17. Actin Filaments

- Structure of actin filaments (Fig. 17-30)
- Actin monomers cycle between making up the polymer and becoming free monomers.
Chapter 17. Actin Filaments

• Terminology
  - Polymer is often called f-actin (for filamentous actin), microfilament or thin filament (esp. in muscle)
  - Monomer is often called g-actin (for globular actin)

• Drugs can be used to experimentally change the ratio of polymer to monomer.
  - Cytochalasin binds to the plus end of the filament and prevents addition of monomers to that end -- leads to disassembly of actin filaments.
  - Phalloidin stabilizes the polymer -- leads to net assembly of actin filaments.

• Actin Filaments are functionally polar.
  - In vitro evidence.

• Actin Filaments are functionally polar.
  - In vitro evidence.

• The two ends of the microfilament are functionally different. (Fig. 17-26)
  - Reason for the names (plus end, minus end)
  - Monomer-polymer molecular binding constants are different for the plus and minus ends.
  - Actin-ATP and actin-ADP form the basis for these different behaviors.
Chapter 17. Actin Filaments

• Similarities and differences between the dynamic nature of the tubulin / MT system and the g-actin / f-actin system.
  - Incredibly similar:
    • Short half lives
    • Plus, minus ends
    • Role of NTP hydrolysis
    • Internal and (we will see) external capping proteins
  - Different evolutionary history
  - Similarities due to similar requirements

Chapter 17. Actin Filaments

• General characteristics of actin filaments
  - Actin filaments are thin and flexible.
  - Actin filaments usually occur in bundles (exception the red blood cell membrane).
  - Actin filaments are often associated with the membrane.
  - Actin filaments primarily serve as structural components. Even when involved in motility, they typically serve as ropes upon which force is generated, and do not generate force themselves.

Chapter 17. Actin Filaments

• The importance of actin binding proteins
  - There are a very large number of actin binding proteins in cells.
  - Much of our knowledge of actin binding proteins comes from biochemical studies of "actin rich extracts from cells"
  - We will consider in vitro interactions first and then see how they integrate with the suspected functions of actin in cells.

Chapter 17. Actin Filaments

• Proteins that bind g-actin (monomer sequestering proteins).
  - Example: Profilin. (Fig 17-32)

Chapter 17. Actin Filaments

• Proteins that bind g-actin.
  - A biological example of profilin function: the sea urchin acrosome.
    • The system
    • Effect of profilin ("profilactin")
    • Effect of the accessory protein "scruin"
Chapter 17. Actin Filaments

• Proteins that bind g-actin.
  - Nucleating proteins. (Fig 17-27)

Chapter 17. Actin Filaments

• Examples of the importance of such proteins:
  - Sea urchin acrosomal reaction (discussed previously)
  - Initiation of microfilament growth in filapodia, microvilli and "stereocilia" (to be discussed shortly)

Chapter 17. Actin Filaments

• Proteins that bind f-actin.
  - Tight bundling proteins that result in parallel microfilaments. (Fig 17-27)

Chapter 17. Actin Filaments

• Proteins that bind f-actin.
  - Biological examples of the importance of tight bundles: filapodia, microvilli and "stereocilia" (Figs 17-29, 17-34, 12-25)
Chapter 17. Actin Filaments

- Proteins that bind f-actin.
  - Gelation and solation proteins. (Fig 17-27)
  - Proteins that bind along the filament. (Fig 17-32)
  - Proteins that cap one or both ends. (Fig 17-27)

- Typically these stabilize the actin filament and make it long lived. An example of this is tropomyosin, found in the muscle and elsewhere.
  - They may also regulate the interaction of other proteins with actin. The most prominent example is troponin in muscle which regulated actin and myosin interactions.

- These also stabilize the actin filament against depolymerization. Probably important many places, but thought to play key roles in intracellular actin bundles (to be discussed later).
Chapter 17. Actin Filaments
• Proteins that bind f-actin.
  - Myosin a motor protein (Fig 17-32)

Chapter 17. Actin Filaments
• Myosin is an important motility protein, not only in muscle cells but in non-muscle cells as well.
• There are several types of myosin. (c.f. Fig 17-40)
• Structure of the myosin molecule

Chapter 17. Actin Filaments
• Roles for myosin in eukaryotic cells. (Fig 17-38)

Chapter 17. Actin Filaments
• An example of non-muscle myosin whose role is well understood: the contractile ring.

Chapter 17. Actin Filaments
• Other examples of non-muscle myosin may not be so well understood. An example:
  - Non-muscle myosin in present in the membrane of the microvillus.

Chapter 17. Actin Filaments
• Crawling motility.
  - A good example because not completely understood (like much of biology).
  - It is actin based and involves many of the molecules/processes we have talked about.
  - Therefore a good review as well as an introduction to a new topic.
  - Different cells may involve different combinations of mechanisms.
Chapter 17. Actin Filaments

• The importance and mechanism of new polymerization.
  - The leading edge (= lamellipodium)
    • Plus ends towards membrane.
    • New polymerization involves actin-related proteins (ARP).
    • New growth extends lamellipodia.
    • The back of the actin array depolymerizes.
    • The probable mechanism (next slide)

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Chapter 17. Actin Filaments

• ARC binds to side of filament
• Initiates branch
• Plus end capping proteins halt elongation of particular filaments.
• Depolymerization at minus end of array.

Fig 17.36

Chapter 17. Actin Filaments

• The above should extend the lamellipodium, but how does the cell move forward?
  - Actin bundles (stress fibers) attach to transmembrane proteins (integrins) that in turn attach to the matrix.

39  Fig 17.37  (A)  (B)  10 μm

Chapter 17. Actin Filaments

• These actin bundles are anti-parallel, and can therefore contract with myosin.
  - Presumably pull the cell forward.
  - Must be disassembled after use.

40  Fig 17.33

Chapter 17. Actin Filaments

• Myosin working with unordered (gel) actin filaments might cause contraction and thus move the cytosol (including new actin filaments) forward.
• Gel to sol and sol to gel transformations may be particularly important in amoeboid motility.

Chapter 17. Muscle Contraction

• Will not cover this in detail here except:
  - Actin-myosin based.
  - Very ordered cytoskeleton.
  - Cytoskeletal elements are very stable (unlike that in most non-muscle systems).
  - Involves many of the same molecules found in non-muscle cells.
  - First cytoskeletal system really understood.
• Review for GRE, Medcats etc.

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