iBiology.org Teaching Tools
Julie Theriot's Lecture Part 1:
Actin Based Cell Motility

Teaching Tools were prepared by David Halladin and Julie Theriot.

Contents

---

1. Keywords and Terms

cytoskeleton, actin, motility, force generation, *Listeria*, comet tail, neutrophil, keratocyte, Arp2/3 complex, filament dynamics, polymerization, nucleation, protrusion, branched network, Brownian ratchet

2. Lecture Notes

There are three major types of cytoskeletal filaments in eukaryotic cells: actin filaments, microtubules and intermediate filaments

The dynamic nature of filaments allows for rapid regulation of important cellular processes and the spatial distribution of filaments is essential for proper cellular organization.

Actin filaments, concentrated beneath the plasma membrane, are involved in cell shape and motility.

Microtubules direct intracellular traffic and the distribution of organelles as well as segregating chromosomes during mitosis.

Intermediate filaments provide mechanical strength to structures (e.g. nuclei, cells and epithelial layers).

Bacteria also have structurally homologous cytoskeletal proteins

Bacterial actin-like proteins are important in cell shape (e.g. MreB).

Tubulin-like proteins are essential for binary fission (e.g. FtsZ).

Intermediate filaments-like proteins are less common and are necessary for specific cell shapes (e.g. crescentin is required for the curved shape of *Caulobacter crescentus*).

Nucleation, polymerization and polarity of actin

Cytoskeletal filaments are made of many individual subunits that have the inherent property of self-assembly, which allows the cell to form larger structures that can span throughout the entire cell body. Cytoskeletal filaments such as microtubules and actin filaments are typically made up of multiple protofilaments. Protofilaments are single strings of subunits that are joined end-to-end. Actin filaments are made of two protofilaments, while microtubules have about 13 protofilaments. Cytoskeletal filaments must be able to maintain an appropriate degree of stability while preserving their dynamic properties. For example, tight binding between adjacent subunits increases filament stability but is detrimental to filament dynamics, while weak binding between subunits increases subunit exchange but results in less stable filaments.

Nucleation is the rate-limiting step of polymerization. Actin nucleation is stimulated by nucleating factors, such as the Arp2/3 complex, that promote the formation of a stable actin trimer that acts as the nucleus for polymerization. During actin polymerization, ATP-bound actin monomers (G-actin) add to existing filaments (F-actin). Nucleotide hydrolysis occurs shortly after the monomer assembles into the filament and produces ADP-F-actin, which is thermodynamically less stable than ATP-F-actin and therefore more likely to disassemble (i.e. ADP-F-actin favors filament depolymerization).

Actin filaments have a fast-growing end called the plus end (or barbed end) and a slow-growing end called the minus end (or pointed end). The polarity of filaments results from the structural asymmetry of actin subunits and the head-to-tail assembly of subunits into filaments.

Actin bundles and branching

Actin can form higher-order F-actin structures, such as filament bundles and dendritic branched networks, which have advantageous mechanical properties. Actin bundles are advantageous because they are stiffer than individual filaments, and therefore more resistant to bending and breaking, while allowing dynamical properties of individual filaments.

In addition to nucleating actin filament growth de novo, the Arp2/3 complex can also bind to the side of existing actin filaments to nucleate the growth of a new filament that branches off the preexisting filament. This process results in the formation of branched webs of actin filaments. The role of branched actin networks in cellular motility will be discussed later.

Introduction to cellular motility

Migration toward a chemical attractant is called chemotaxis. As an example, the neutrophil, shown in David Rogers' movie, is chemotaxing up a gradient of chemotactic peptides that are produced by the bacterium. In a chemoattractant gradient, neutrophils become polarized and actively move toward higher concentrations of the chemoattractant. Chemotaxing amoebae look morphologically similar to neutrophils and they share *many of the* underlying molecular mechanisms that drive neutrophil motility. In addition, there are motile cells, such as cells involved in wound healing, that have distinct morphology and motility characteristics but preserve many of the molecular

mechanisms important in neutrophil motility. This suggests that the molecular processes involved in cellular motility have been conserved over evolutionary time.

Steps in actin-based cell motility

1. Establish polarity, front (leading edge) to rear (uropod).
2. Protrusion of leading edge, resulting from the assembly of actin filaments.
3. Retraction of rear, driven by the actin-based motor protein myosin.

In order for the cell to move, these events must be coordinated with the formation and disassembly of cell-surface adhesions.

Nanometer proteins to micrometer cells

Typically, eukaryotic cells are on the order of tens of micrometers in size while the proteins these cells are built from are on the order of nanometers in size. This size discrepancy presents a problem of coordinating processes that are separated by distances that are many orders of magnitude larger than individual proteins. As a way to combat this problem, the cell uses filamentous structures that are assembled from many small subunits. In our discussion of cell motility, the cytoskeletal filaments of eukaryotic cells have three critical properties. First, filaments are able to assemble and disassemble rapidly, in order to change shape and orientation of a cell in response to changing signals from its environment. Second, filament assembly can function as a force generating mechanism that is important in protrusion of the leading edge. Third, cytoskeletal filaments can serve as directional tracks for molecular motor proteins (e.g. kinesin, dynein and myosin). This property of actin filaments is important for myosin-dependent retraction of the cell rear.

Cell Protrusion: leading edge moves forward

The protrusion of the leading edge does not rely on preassembled structures. Rather, the cell must spatially regulate the assembly and disassembly of filaments. At the leading edge, actin polymerizes to form networks of filaments that assemble into parallel bundles and branched meshes. These networks of actin filaments exert a force that pushes against the plasma membrane and thus drives the protrusion of the leading edge.

The process of assembly and disassembly of actin filaments is a highly dynamic process that is regulated by hundreds of actin-binding proteins. As a result, the activity and localization of actin-binding proteins must be carefully modulated to produce highly ordered outcomes that are observed during cellular motility. The dynamic nature of actin filaments is seen in filament turnover, where the average filament half-life is estimated to be approximately one minute.

Force generation during actin polymerization

Actin polymerization can be modeled using the following binding reaction.

Where P_1 is a single subunit, P_n is the filament length before subunit addition and P_{n+1} is the filament length after subunit addition. k_{on} is the on rate and k_{off} is the off rate.

In the cell, there is always an excess of monomers, thus the formation of filaments is energetically favorable. In the case of motility, the negative free energy produced during actin polymerization is coupled to drive the physical process of leading edge protrusion. This free energy is not free; remember, ATP is hydrolyzed after ATP-G-actin assembles into the filament.

Force generation by the addition of a single actin monomer

The maximum force generated by the addition of a single monomer can be estimated using the equation

Where *k* is Boltzmann's constant and *T* is the temperature in degrees Kelvin. At room temperature, *kT* ? 4.1 pN nm (picoNewton nanometers), this value represents the natural energy unit of a single molecule within a cell and is used as the natural energy scale in physical biology. The concentration of free actin monomers, defined as C, varies in different cell types but is typically on the order of a few tens of micromolar. C_{crit} is the equilibrium constant for polymerization (C_{crit} ?k_{off}/k_{on} ? 0.1 micromolar*).* (Note: C_{crit} is different for ATP-G-actin and ADP-G-actin, and can also differ at the two structurally distinct ends of the actin filament; the number given is for ATP-G-actin at the plus end of the filament. The distance the load must move forward for new monomer incorporation is expressed as *d (d* ? 2.5 nanometers: 5 nanometer per subunit/2 protofilaments per actin filament).

Then, plugging these values into the equation above: *Fmax* ? 5-10 pN.

Putting this into perspective, the force generated from the assembly of a single subunit of actin is equivalent to the force generated from the power stroke of myosin and during a step by kinesin. Given this force, it is easy to imagine how the localized polymerization of many thousands of actin filaments at the leading edge of the cell would push the plasma membrane forward.

The speed of network growth, generated by actin polymerization, is comparable with the rate of cell motility. Mathematical models predict the net polymerization rate to be on the order of one micrometer per second, which is similar to the speed of fast motile cells such as neutrophils.

The study of microbes can advance our understanding of basic cell biology

There are a number of intracellular microbial pathogens (i.e. disease-causing microorganisms living within a host cell) that use host actin polymerization to propel themselves through the cytosol of the host cell and into neighboring cells, resulting in increased cell-to-cell spread of the microbe. Actin-based motility allows the microbe to infect and replicate within the host while actively avoiding the antibody-mediated arm of the host immune response. Examples of microbes displaying actin-based motility include Vaccinia virus and the bacteria *Shigella* and *Listeria*. In all instances, microbial surface proteins stimulate the Arp2/3 complex (either directly or indirectly), promoting actin polymerization at the microbial surface. Actin dynamics at the bacterial surface resemble the dynamics observed at the leading edge of a moving cell. Therefore, it is not surprising that the study of actin-based motility of intracellular microbes has increased our understanding of the molecular processes involved in cell motility.

Putting actin-based motility of Listeria into perspective

Listeria is approximately 2 micrometers in length and travels through the cytosol at a speed of roughly 0.2 micrometers per second. Therefore, every ten seconds *Listeria* travels one cell length.

The Ohio Class SSBN submarine has similarities to *Listeria*; both travel through a liquid medium, are similar in shape, and travel through their environments at a comparable relative speed.

The submarine is 560 feet in length and travels at a speed of 30 feet per second. Therefore, in comparison, the relative speed of Listeria is approximately twice as fast as the submarine, which takes around 19 seconds to travel its length.

Biochemical events in actin-based motility of Listeria and Shigella

1. Expression of surface protein (*Listeria* ActA, *Shigella* IcsA).
2. Activation of the host Arp2/3 complex (ActA directly activates Arp2/3, IcsA activates Arp2/3 via host N-WASP protein). This is the only identified bacterial contribution to actin-based motility.
3. Arp2/3 binds the side of existing actin filament and nucleates the growth of a new filament.
4. Filaments grow by monomer addition at the barbed end, which faces the bacterial surface.
5. Growing filaments exert a force on the bacterium.
6. Capping proteins bind the barbed ends of filaments that are removed from surface (host proteins, CapZ and gelsolin). This restricts polymerization to the bacterium surface.
7. Disassembly of old filaments (host proteins, cofilin and ADF) to regenerate the monomer pool.
8. Return to step 2.

Questions to ponder

How much force is generated by actin polymerization *in vivo* (efficiency)?

How are multiple filaments coordinated to work together?

How are actin forces coordinated with other cellular forces in both space and time?

How are these processes regulated with the environment?

3. Recommended Reading

1. Molecular Biology of the Cell, Alberts et al.

   Chapter 16, pgs 965-1010 in the 5th edition (general overview of the cytoskeleton)
   Chapter 24, pgs 1514-1517 (actin-based motility of *Listeria*)

2. Physical Biology of the Cell, Phillips et al.

   Chapter 15, pgs 541-545 and pgs 566-586 (cytoskeletal protein dynamics)

   Chapter 15, pgs 629-643 (force generation by polymerization)

3. A. Mogilner and G. Oster (2003). Polymer motors: Pushing out the front and pulling up the back. Current Biology 13: R721-33.

4. Review Questions

1. The molecular processes of actin-based motility are highly conserved; list three examples of actin-based motility in eukaryotic cells.

2. During cell motility, why is it necessary to coordinate front protrusion and rear retraction with the formation of adhesive surface contacts?

3. Explain how nucleotide binding influences actin dynamics (ATP vs. ADP bound actin).

4. Why is Listeria movement through the host cytosol more impressive than a submarine moving through the ocean?

5. What are two models for opening space to allow actin monomer insertion between a filament tip and a load during actin-based force generation?

6. Given the information presented in the lecture, what effect would increasing or decreasing temperature have on force generation at the leading edge during actin-based motility?

7. Compare the molecular cytoskeletal events of leading edge protrusion with actin-based motility of Listeria.

8. How would you design an experiment to determine if Listeria ActA is the only bacterial protein necessary for actin-based motility?

5. Answers to Review Questions

1. There are many correct answers. Neutrophils, amoebae and keratocytes were mentioned in the lecture. The movement of microbial pathogens, which are not eukaryotic cells, was mentioned as well.

2. Adhesive contacts allow the cell to remain in contact with its substrate, without such contacts the cell would essentially be devoid of any directed movement that is characteristic of cellular motility. If the protrusion and retraction are not coordinated with adhesive contacts, then the process of cell motility would be significantly less efficient. The exact outcome would be dependent on the relationship between these events.

3. Actin monomers bound to ATP are more likely to polymerize (lower C_{crit}, higher k_{on}, lower k_{off}) than monomers bound to ADP, while ADP-actin subunits are more likely to depolymerize (higher C_{crit}, lower k_{on}, higher k_{off}) than actin subunits bound to ATP. The intrinsic properties of ATP and ADP-actin, along with actin-binding proteins, allow for rapid assembly and disassembly of actin filaments.

4. The bacterium must maneuver through a complex and dense environment, while the submarine is essentially travelling through a body of salt water. For example, the cytoplasm has a protein concentration of roughly 30%, which is approximately the concentration used when crystallizing proteins. In addition, the cytosol is filled with organelles that are larger in size than the bacterium.

5. In one model, the actin filament is fixed in space and the load undergoes thermal fluctuations in position, which allows monomers to assemble at the end of the filament. In this model, monomers ratchet the Brownian motion of the load to move the load one step forward. In the other model, the actin filament undergoes thermal fluctuations which cause the filament to bend away from the surface, allowing for monomer insertion. The growing filament generates a pushing force on the load to move the load forward.

6. The force generated by the addition of a single actin subunit was calculated using the equation. In this equation, the force is seen to increase as a linear function of absolute temperature (C_{crit} probably also changes with temperature but how temperature affects the critical concentration is not well understood; generally both k_{on} k_{off} should increase with increasing temperature, so the ratio between the two C_{crit} may remain fairly constant). Therefore, increasing the temperature would cause the actin network to generate more force and conversely, decreasing the temperature would cause the actin network to generate less force. Changes in temperature will affect many other cellular processes, such as the rate of filament assembly and disassembly, but these processes were not discussed in sufficient detail to predict changes in cell speed.

7. Similar proteins and dynamic processes are involved in actin network assembly in both cases. For Listeria movement, the bacterial protein ActA activates the Arp2/3 complex, which in turn nucleates actin growth. The critical role of ActA is illustrated by reconstituting actin-based motility using a polystyrene bead coated with purified ActA. For leading edge protrusion, intrinsic cell factors that are localized to the membrane at the leading edge perform a function similar to ActA. An important difference between leading edge protrusion and Listeria motility is that cell-substrate adhesions must be formed for productive leading edge protrusion.

8. One way to do this is using polystyrene beads. Polystyrene beads that are similar in size and shape to Listeria can be coated with purified ActA. Next, ActA-coated beads can be incubated with the appropriate host proteins (in vitro motility can be achieved with only four host proteins: actin, Arp2/3 complex, an actin depolymerizing protein, and capping protein). Finally, actin-based motility can be observed using video microscopy. Listeria can be used as a positive control and uncoated polystyrene beads can be used as a negative control.

6. Discussion Questions

1. Recently, it was shown that bacteria also have proteins that are structurally homologous to the cytoskeletal proteins of eukaryotes. What differences between bacteria and eukaryotes might have misled researchers to previously assume that bacteria don’t have cytoskeletal proteins?

2. There is in vitro and in vivo evidence that capping protein promotes network assembly and accelerates actin-based motility in a concentration-dependent manner. Given what you know about capping protein, why are these observations paradoxical and how might they be explained?

3. The dynamic nature of actin filaments allow the cell to quickly respond to changing environmental conditions. Give an example of a cell responding to environmental cues.

4. The delivery of actin monomers to the site of leading edge protrusion is too fast to be explained by diffusion alone (Zicha et al. (2003). Rapid actin transport during cell protrusion. Science. 300: 142-145). How are actin monomers delivered to sites of active protrusion?

7. Answers to Discussion Questions

1. The functions and processes of cytoskeletal elements in eukaryotes were thought to be unnecessary for small, simple prokaryotic cells. For example, cytoskeletal proteins are involved in directed transport, cell shape and intracellular compartmentalization, chromosome segregation and generating cell-cell junctions. It was thought that prokaryotes don't need cytoskeletal proteins because diffusion can be used instead of directed transport, peptidoglycan distribution determines cell shape, intracellular organization isn't needed in such simple cells, and they generally are unicellular so cell-cell junctions aren't necessary.

2. The most common role attributed to capping protein is the binding of barbed filament ends, which prevents filament elongation. Therefore, it would be logical to assume that capping protein inhibits network assembly and decrease the rate of actin-based motility by blocking filament elongation. The Actin Funneling Hypothesis was the most widely accepted explanation of experimental data. In this model, it is proposed that capping protein accelerates the rate of motility by increasing the pool of monomers that drive the polymerization of the remaining uncapped barbed ends. Recently, it has been suggested that faster motility is the result of capping protein promoting filament nucleation by the Arp2/3 complex.

3. There are many correct answers for this question.

a. Neutrophils travel to site of an infection by chemotaxing toward host and bacterial chemical attractants. Then, once at the site of an infection, the neutrophil must find and engulf the invading microbes (as seen in the movie).

b. Upon wounding, epithelial cell must migrate to repair the wounded area.

c. The motility of many eukaryotic cells is influenced by electric fields (some cell preferentially migrate toward the cathode, others the anode), where changing the direction of the electric field alters the direction of cell motility in a predictable manner.

4. The answer to this question is not known. Since diffusion is too slow it indicates that active transport is involved. Existing data suggest that the process is dependent on ATP and nonmuscle myosin II, while microtubules are not required. Therefore, myosin II dependent retraction of the rear may result in hydrodynamic flow of actin monomers to sites of membrane protrusion.

8. Explain or Teach These Concepts to a Friend

1. Why is *kT* considered the natural energy scale in cell biology?
2. Explain the importance of coordinating force generating mechanisms during cell motility.
3. Forces are generated in the eukaryotic cytoskeleton by two common mechanisms: stepping of molecular motor proteins (such as myosin) and assembly/disassembly of cytoskeletal filaments. Which kinds of cell movements use each of these two mechanisms?
4. Why are molecular motor proteins better suited to generate force for some cell movements and filament assembly better for others?

9. Research the Literature on Your Own

1. What experimental techniques are used to measure forces in the picoNewton to nanoNewton range? Describe the theory behind the technique and list at least two biological applications for each technique.
2. Describe in detail the events of actin-based motility in a eukaryotic cell or bacterium of your choosing. Next, use metaphors to describe each event in the process.
3. Actin polymerization drives the motility of *Listeria* and the protrusion of the leading edge of many motile cells. The speed of cell movement can vary over a wide range, from about 1 micron per second (for chemotaxing neutrophils) to about one micron per hour (for some fibroblasts). *Listeria* moves at a speed of about 0.2 microns per second in epithelial cells but up to five times faster in macrophages. What might cause the speed of actin-based motility to vary so much? How could you test your ideas?