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Ron Vale's Lecture Part 1:
Molecular Motor Proteins

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1. Keywords and Terms

Molecular motors, kinesin, myosin, dynein, microtubules, actin, filament polarity, cytoskeleton, processivity, power stroke, ATPase cycle, x-ray crystallography, electron microscopy, in vitro motility assays

2. Lecture Notes

There are three classes of cytoskeletal motor proteins: kinesins, dyneins, myosins

Kinesin and dynein move along microtubules

Myosin moves along actin

There are other types of motors that are not discussed in this lecture: for example linear motors that move along DNA (helicases and polymerases) and rotary motors, such as the F1-Fo ATPase that produces ATP in mitochondria and the bacterial flagellar rotary motor.

Motor proteins are enzymes.

These enzymes hydrolyze ATP to generate phosphate and ADP, which are released sequentially, allowing the enzyme to rebind ATP and start another cycle.

During one cycle of ATP hydrolysis/product release, the motor undergoes a conformational change that can produce force and unidirectional motion. Kinesin, for example, takes an 8 nm step (the distance between adjacent a/b tubulin dimers on the microtubule) for each ATPase cycle. Cytoskeletal motor can work at 60% efficiency (efficiency is the theoretical chemical energy released from ATP hydrolysis that gets converted into work). This is much better than a car!

Cytoskeletal motor proteins have two major types of domains:

- The Motor domain binds ATP and the cytoskeletal track. It is the “engine” that produces movement. The motor domain can be separated from the rest of the motor protein (by proteolysis, or by genetic engineering and expression) and it will produce movement in vitro (ie. in a test tube situation)
- The Tail domain is everything in the polypeptide other than the motor domain. Part of the tail domain is involved in “cargo binding”, i.e. attaching the motor to the correct object in the cell that needs to be transported. Other segments might be involved in regulating (turning on or off) the motor activity. Yet other parts of the tail can be involved in dimerization of two motor protein polypeptide chains (e.g. through a coiled coil interaction) or higher order structures (e.g. muscle myosin also will polymerize into filaments).

Polymer tracks for cytoskeletal motors- actin and microtubules

The Microtubule

- Composed of a/b tubulin heterodimer (different isoforms exist)
- A cylindrical, hollow polymer of 25 nm diameter
- Very stiff , comparable to plexiglass

Actin

- Composed of one protein- actin (although different isoforms exist)
- A helical polymer with an 8 nm diameter
- Much more flexible than a microtubule
- Discussed more in the lecture by Julie Theriot on iBioSeminars

Actin and microtubule polymers are polar. The polarity is due to the fact that the individual subunits are asymmetric and they polymerize in a head-to-tail manner.

Motors recognize the polarity of the filament and travel in only one direction.

- Kinesins travel towards the microtubule plus end (Although one class of kinesin (Kinesin 13) travels to the minus end)
- Dyneins travel towards the microtubule minus end
- Myosins travel towards the actin plus end (also called "barbed end") (Although the Myosin VI class moves towards the plus end)

Cells organize the location and polarity of cytoskeletal filaments

In most cells, actin is found at the periphery, nucleated near the membrane. The plus ends point towards the membrane. In muscle, actin is very well ordered, with the plus end at the muscle Z line (not discussed in this lecture but found in many textbooks).

In most animal cells, microtubules are nucleated from the "centrosome" which is positioned near the nucleus (exceptions exist). The microtubules are long and can

extend to cell periphery. The plus ends are located near the periphery and the minus ends are anchored at or near the centrosome. In a mitotic spindle, the microtubules' plus ends interact with the chromosomes.

The defined polarity of filament organization and the fact that motors recognize this polarity creates a "navigation" system in the cell. For example, imagine that you are a small vesicle in the middle of the cell and your destiny is to travel to and fuse with the plasma membrane. You cannot "see" the plasma membrane and embark on that journey, as humans transport themselves to a destination. But you might have instructions to bind a kinesin motor to your surface. Then you (the vesicle) hop on the nearest microtubule track and kinesin will recognize the MT polarity and transport you to the near the plasma membrane. Once you get close, you can get to the plasma membrane by diffusion or pick up a myosin that will transport you through the actin network.

Motor proteins execute many types of biological activities

The lecture highlighted several types of activities for kinesins (a partial list)

- Powering organelle transport
- Positioning/transport of large organelles (e.g. Golgi, ER, nucleus)
- Transport/localization of mRNAs
- Ciliary biogenesis (movement of protein building blocks into cilia/flagella)
- Cell division/mitosis
- Signal transduction

This same list largely pertains to dyneins and myosins. Dyneins also power the movement of cilia and flagella. Myosins are not involved in ciliary biogenesis but power muscle contraction (they are not involved in chromosome motion but pinch the dividing cell into two during the process of cytokinesis).

Motor proteins constitute "superfamilies," and the different members are specialized for different biological tasks.

Every organism has different numbers of kinesins, myosins and dyneins

There are 45 Kinesin genes in human

They can be recognized by their similar “motor” domains (~30% identical in amino acid sequence)

Each gene has a very different “tail” domain (virtually no identity)- this allows for different localization, different cargo binding, unique regulatory control.

A similar situation is true for myosin and dynein superfamily members

How do molecular motors work?

The myosin motility cycle

Myosin binds ATP, hydrolyses the b-g phosphate bond, releases phosphate, and then releases ADP. ATP can rebind to restart the cycle.

These chemical transitions produce conformational changes as seen in the movie.

1. ATP binding causes myosin to release from actin.
2. ATP hydrolysis is thought to “recock” the myosin lever arm.
3. Actin binding helps to dissociate phosphate.
4. Phosphate release causes myosin to bind very tightly to actin and then causes the rotation of a lever arm domain (~10 nm displacement- the “power stroke”).
5. ADP release is needed to reset the cycle (not shown in the movie but this step might be sensitive to tension- more tension slows down release of ADP so that you can produce force for a longer time).

Many myosins simultaneously producing 10 nm strokes cause sarcomeres to shorten and your muscle to contract. Muscle myosin is not processive- it takes a “stroke” and then detaches from actin for most of its ATPase cycle. This is good for contracting muscle, since attached myosins finished with their “stroke” are not producing a “drag”

for other stroking myosins (Other myosins in the cell, such as myosin V, are processive; see below).

The kinesin motility cycle

Kinesin is a processive motor. A single motor can move along a microtubule track for a hundred or more ATPase cycles without detaching. The two heads of kinesin move in a hand-over-hand manner, somewhat like walking across evenly spaced stepping stones placed across a pond.

These chemical transitions produce conformational changes as seen in the movie.

1. ATP binding causes a small peptide (the neck linker) to dock tightly to a complementary binding site in the enzymatic core. ATP is also in a tight microtubule binding state (opposite of myosin).
2. This docking causes the “hand-over-hand” movement of the two heads of the dimer. After neck linker docking, the rear kinesin head is positioned at the “front”.
3. The new front head can search around and bind to the next tubulin subunit, locking an 8 nm step in place.
4. To take the next step, the rear head has to dissociate. Its grip on the microtubule weakens after it hydrolyzes ATP and releases phosphate (the ADP state is a weak microtubule binding state for kinesin, the opposite of myosin).

How do you study the mechanism of a molecular motor?

X-ray crystallography:

This provides atomic detail; you know how every amino acid in the protein is positioned in space and this gives one insight into the ATPase cycle and the conformational change mechanism.

Looking at 3-D atomic structures derived from this technique revealed a surprise- kinesin and myosin and even G proteins have similar structural features, indicating that they all evolved from a common ancestral protein. The feature that they most closely

share is how they hydrolyze nucleotide and how they switch conformation after hydrolysis and phosphate release (ie between ATP and ADP states for motor proteins and GTP and GDP states for G proteins).

Even though kinesin and myosin are rather similar around their nucleotide binding regions, they have two major differences-

1. very different structural elements for binding polymers (kinesin binds microtubules and myosin binds actin)
2. different mechanical elements (kinesin has the small 'neck linker' and myosin has a much bigger 'lever arm'.

Electron microscopy (see techniques lecture by Eva Nogales)

No one has been able to obtain an X-ray crystal structure of a motor bound to its filamentous track. However, electron microscopy allows one to examine motor-track interactions, which is very informative, albeit at somewhat lower resolution than X-ray crystallography.

In vitro motility assays

These powerful methods allow one to study the dynamics of motor protein movement (EM and X-ray crystallography provide static views). These in vitro motility assays involve a purified motor, purified cytoskeletal filaments (ie polymerized from purified tubulin or actin), and ATP (which one can buy).

This lecture shows two motility assays-

1. Purified Kinesin bound (nonspecifically) to one micron plastic beads; kinesin carries these beads along the stationary microtubules bound to a glass coverslip.

2. A “gliding” assay. Kinesin is bound nonspecifically to the glass (so the motor is not moving) but it grabs hold of microtubules in solution and it moves the microtubules across the glass surface.

New types of in vitro motility assays allow one to study single motor proteins

Using a method called an optical trap (see lecture 2 by Vale)- one can measure individual steps taken by motors (kinesin takes 8 nm steps, as shown by Steve Block and colleagues) and measure very small forces produced by motors (few piconewtons).

Other microscopes (using a technique called total internal reflection illumination and very sensitive modern cameras) allow one to visualize the fluorescence emitted from a single dye molecule. By putting a fluorescent dye or the green fluorescent protein onto a motor protein like kinesin, one can follow a single motor protein moving along its track.

Genetic engineering of motors proteins allows one to test theories of how they work.

Using expression plasmids, one can express and purify motile kinesin from bacteria. (Myosin is generally made in insect cells by baculovirus expression, and dynein has been expressed in yeast and Dictyostelium). Thus, one is not restricted to purifying motors from native tissues or cells, where their abundance might be very low. With expression systems, one also can change the sequence of the motor gene to test ideas of how the motor works and what parts of the protein contribute to its motor activity.

Motors and medicine

The lecture ends by describing the relevance of motor proteins to medicine, a big topic that is only briefly covered. Several human hereditary diseases are caused by mutations in motor protein genes. Small molecules directed against motor proteins might also have therapeutic benefit. Described in this lecture is a small molecule that activates cardiac myosin and improves cardiac contractility. This drug is being tested in humans in phase II clinical trials for patients suffering from heart failure (with impaired ventricular contraction). In section 3 of this lecture, a small molecular inhibitor of a mitotic kinesin is described.

3. Recommended Reading

Molecular Biology of the Cell, Alberts et al.

In 5th Edition: Chapter 16, pgs 1010-1035 deal most specifically with molecular motors

(An equivalent chapter from a basic cell biology textbook will also be fine)

A general review comparing myosin and kinesin (somewhat dated but still largely accurate)

Vale, R.D. and Milligan, R.A. 2000. Science 288:88-95.

The way things move: looking under the hood of molecular motor proteins.

4. Review Questions

1. Name three types of molecular motors
2. What does kinesin and myosin use as their energy source?
3. What does “processive movement” mean? Is kinesin or muscle myosin processive?
4. Name four types of motility driven by molecular motors.
5. What types of material are being transported in a nerve cell?
6. Which of the following methods allows one to measure dynamic properties of motility?
    a. X-ray crystallography
    b. Optical trapping
    c. Electron Microscopy
    d. Single molecule fluorescence microscopy

7. Which of the above techniques provides the highest resolution information of a protein?
8. What has a higher work efficiency- a molecular motor protein or an automobile?
9. Why does a cell have so many different types of molecular motors?
10. To study molecular motors, one has to purify them from their native tissues or cells. True or false?

5. Answers to Review Questions

1. Myosin, dynein, kinesin (also mentioned are the F1 ATPase, bacterial flagellar motor, F1 ATPase, DNA and RNA polymerase)

2. Adenosine triphosphate (ATP)

3. The ability of a motor to take many steps along the microtubule without detaching. Kinesin is a processive motor but muscle myosin is not.

4. Lots of possibilities; discussed in this lecture- muscle contraction, movement of cilia and flagella, organelle/vesicle transport, chromosome movements in mitosis, mRNA/protein transport, movement of protein building blocks into cilia and flagella, movement of large organelle systems such as Golgi and endoplasmic reticulum.

5. Mitochondria and small membrane vesicles and organelles

6. b, d

7. a

8. The molecular motor protein by several fold.

9. Each motor is thought to be specialized for a particular transport task and there are lots of cargos to be transported in cells and other force-producing needs. Some motors also modulate microtubule dynamics (not discussed).

10. False. One can clone the gene for a motor and express the gene (or even just the motor domain part of the gene) in bacteria, yeast or certain types of insect cells. The motor can be expressed (often in much greater quantities than in their native cells), purified and it usually produces motility in vitro.

6. Discussion Questions

An in vitro motility assay allows a type of biological motion to occur outside of the cell and in a "test tube" environment. In this lecture, two in vitro motility assays are described. A purified motor is attached to an artificial bead (e.g. a 1 micron polystyrene bead) and the motor will transport the bead along a microtubule attached to a glass surface (microtubule made from purified tubulin) in the presence of ATP. In another assay, the motor is fixed onto the glass surface and the fixed motors push microtubules across the surface (like people standing in place and passing a pole from one to another). These assays are useful because you can study how these proteins work without the complications of the several thousand other proteins in the cell and one has complete control of the environment- one can change the amount of the fuel (ATP concentration), add regulatory proteins, or change the motor itself through genetic engineering.

1. In the *in vitro* gliding assay, kinesin is bound nonspecifically to the glass, facing in all directions. Why is it that the microtubules are moving continuously in one direction and are not in a tug-of-war? What end of the microtubule is "leading" (plus or minus end) as they travel across the glass?

2. What is the gravitational attractive force produced by you and your friend at arms length and how does it compare to the force produced by one molecular motor (let us say that it is 5 pN)?

3. How do muscle myosin and kinesin differ in their motility cycles?

4. Explain the different functions of the "motor domain" and the "tail domain"? Which one is most highly conserved within a motor superfamily (e.g. between all kinesins or between all myosins) and why?

5. Why is it important for the cell to organize its microtubules with uniform polarity (e.g. microtubule plus ends extending away from the centrosome?
6. Why is muscle myosin non-processive while conventional kinesin has evolved to be processive?

7. Answers to Discussion Questions

1. This answer is not discussed in the lecture and requires some thought and discussion. The microtubule is polar (all of the tubulin subunits are arranged in a head-to-tail manner). Kinesin recognizes the polarity of the microtubule, and will only bind in one orientation or direction. Consider kinesin molecules randomly bound to the glass surface. Kinesin facing the "wrong way" simply will not bind to the microtubule. Only kinesin aligned in the proper orientation to the microtubule will bind and produce force, which keeps the microtubule moving more or less straight on the glass. (Kinesin may have some flexibility, so if not perfectly aligned, it can pivot or twist and align its motor domains to the microtubule even its "base" is slightly off angle.) The minus end of the microtubule will be leading as the microtubule moves across the surface (stationary kinesins are trying to move towards plus end and thus sliding the microtubule in the opposite direction).

2. The equation for gravitational attraction is

   $F_{gravity} = (G \times m_1 \times m_2)/(d^2)$
   m is the mass in kg of the two objects (let us say 60 kg for you and your friend)
   d is the distance between the two of you in meters; let's say your arm is 1 m.
   G is the gravitational constant (6.67 x 10-11 Nm^2/kg^2) (N is Newtons)
   Plugging these values in we get, 2.4×10^{-7} N. This is about 50,000 times stronger than the force produced by a single motor protein!

3. ATP binding causes myosin to release from actin but causes tight binding of kinesin to microtubules. The "power stroke" in myosin is caused by phosphate release but is caused by ATP binding in kinesin. Muscle myosin is "non-processive" and kinesin is "processive."

4. The motor domain binds ATP and the track and will produce motility on its own. The "tail" domain refers to everything else in the polypeptide chain. The tail might be

involved in binding to cargo, dimerization (or even higher order oligomerization) of two motor polypeptides and motor regulation. The motor domain is conserved between kinesins or between myosins (the basic engine was preserved by evolution). The tail domain differs greatly between different motor genes. Evolution combined a conserved engine (with somewhat different properties of movement) to many different protein structures to allow for attachment to different cargos and to achieve differential regulation.

5. Well, let's consider the opposite scenario- microtubules organized randomly. In this case, it would be hard to transport cargo anywhere. Kinesin moving a cargo towards a microtubule plus would travel a micron or so in one direction then detach (this is about how far kinesin travels processively), bind to another microtubule with the opposite polarity and travel in the opposite direction- chaos!

6. Very few kinesin molecules are transporting cargo (perhaps 1-5 on a small vesicle for example). To undergo long distance transport, it is advantageous for the one or few kinesin motors to hold onto the cargo and not let go after taking a step. If the motor lets go for too long (even a few milliseconds), then the vesicle can diffuse away from the microtubule and transport will cease and the vesicle has to find the same or different microtubule (again by a diffusion-based process). In a muscles fiber, millions of myosin motors are interacting with actin filaments in a well organized structure where the two components cannot diffuse away from one another and where many molecules are contributing to movement. If myosin holds on after it produces its power stroke, then it provides a resistance or drag that other active myosin have to work against. In a rapidly contracting muscle (ie not working against a load), it makes more sense to bind, produce a stroke, and then detach from the actin or bind very weakly. Dyneins in cilia/flagella work in a similar way (while cytoplasmic dynein is processive like kinesin).

8. Explain or Teach These Concepts to a Friend

1. Explain what happens in the myosin motility cycle
2. How do you express a motor protein in bacteria and purify it? (This will require some background reading beyond the lecture).

3. Why do microtubule and actin filaments have a defined polarity and how they are nucleated/distributed in cells?

9. Research the Literature on Your Own

4. What is a genetic disease caused by a mutation in a molecular motor protein? What is the exact defect and is it known how the disease is produced?
5. Why are the class V myosins similar to the kinesin motor shown in this lecture?
6. What evidence do scientists have to show that the two kinesin motor domains move in a hand-over-hand manner?
7. A myosin II (same class as muscle myosin) is present in most eukaryotic cells. What are its similarities/differences to muscle myosin and what does it do?
8. Research and present a “story” of how a molecular motor can be regulated (muscle myosin, conventional kinesin (Kinesin 1), myosin V, and Kinesin 3 (also called Unc104) might be good and somewhat different case studies).
9. What are the similarities and differences between motors (kinesin/myosin) and G proteins?