Supplementary Reading List for Dynein, Cilia, and Flagella -- 2005


Review articles about dynein:


Selected research papers:


Biological Motors:
I. Most biological movement is carried out by motor enzymes that use ATP
dephosphorylation to provide energy for repeated cycles of movement along a cytoskeletal
substrate.

Three main types of motor enzymes in eukaryotic cells:

<table>
<thead>
<tr>
<th>Cytoskeletal substrate:</th>
<th>Myosins</th>
<th>Dyneins</th>
<th>Kinesins</th>
</tr>
</thead>
<tbody>
<tr>
<td>actin filaments</td>
<td>microtubules (“ropes”)</td>
<td>microtubules (“straws”)</td>
<td></td>
</tr>
<tr>
<td>microtubules</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Polarity of filament determines usual direction of movement towards the + (“barbed”) end – end + end (a few – end) (some – end)

The head of a myosin motor enzyme clamps on to the actin filament and then “swings its lever arm” to produce movement. In skeletal muscle, myosin has a low “duty ratio”, because the period of strong attachment to actin is a small fraction of the ATPase cycle time. Several myosins can therefore move an actin filament faster than one myosin, with this effect diminishing as the duty cycles start to overlap. Although muscle myosins are homodimers, the heads appear to act independently. In skeletal muscle, the “tails” of the myosins are combined to form the myosin filament.

Typical kinesins are also homodimers, and the heads appear to act cooperatively. The current model is: One head is attached to a microtubule 8 nm behind the other. The leading head executes a power stroke when the trailing head detaches. The trailing head swings around and reattaches 16 nm ahead of its former position, to become the leading head. Because at least one of the heads is always strongly attached, kinesin has a very high “duty ratio”, and one kinesin can move a microtubule at the same velocity as many kinesins, if there is no opposing load.

The “cargo” moved by a kinesin is attached to a chain between the heads.

There are axonemal dyneins and cytoplasmic dyneins. Cytoplasmic dyneins are homodimers with high duty ratio, which appear to function like kinesin (but move in opposite direction).

Axonemal dyneins are the motors of eukaryotic flagella and cilia. [Not like bacterial “flagella”]

The tail of a sea urchin spermatozoon is a prime example of a flagellum. Readily available source of flagella for examination. Easy to observe, using dark field microscopy and stroboscopic flash illumination.(short flashes, <100 µs, stop movement). Planar. Swim close to surfaces.

Diameter about 0.2 µm
Sea urchin; length ~ 40 µm. others can range from 5 to >1000 µm.
Frequency ~40 Hz; others range from <1 Hz to >100 Hz.

How do these bending movements cause forward propulsion of the spermatozoa?
The viscous drag on a thin cylinder moving perpendicularly to its length is about twice the drag of the same cylinder moving at the same velocity, but parallel to its length. For very thin wires, the ratio is 2.0. For sperm tails, it is probably about 1.7 to 1.8. It the ratio were 1.0, no forward propulsion would result.

Some flagella produce 3-dimensional, sometimes nearly helical, bending waves.
What do cilia do? Eukaryotic flagella and cilia are very similar; it would probably be better if there were a single term agreed upon for them. The ones that are called cilia are usually found in large, closely-packed arrays on the surfaces of cells, and this makes them difficult to photograph. They produce fluid movement parallel to the surface, because they have an asymmetric beat cycle.

The typical bending cycle involves an effective stroke in which the cilium extends away from the surface and sweeps through the fluid while it is relatively straight, and a recovery stroke in which it bends and returns to its starting position by a path that is closer to the surface. The net result is that it pushes fluid to one side, parallel to the surface.

Analysis is more complicated than for the symmetric bending waves of flagella, but there are two factors that are important for the net fluid movement parallel to the surface: 1: Shape -- cilium pushes more on fluid when extended, moving perpendicularly, than when it is bent and moving parallel to its length. 2: Surface drag -- it is difficult to move the fluid near the surface, which is held back by surface drag. So if the return stroke is very close to the surface, it causes little movement of the fluid, in contrast to during the effective stroke when the cilium is extended maximally away from the surface.

We know that spermatozoa are important. What about cilia?

In humans, cilia are especially important for clearing foreign particles out of the respiratory system. The particles are trapped on a thin film of mucous that covers the surfaces of the respiratory passages, and this film is moved by the tips of short cilia on the epithelial cells. There are a number of genetic diseases of humans, in which all of the cilia and flagella in the body are non-functional or poorly-functional. These individuals have a characteristic group of symptoms including a history of chronic respiratory disease because of poor clearance of foreign particles and mucous from the lungs and upper respiratory tract; ear infections, for the same reason; and male sterility, because the sperm flagella are non-motile. One groups of these is known as Kartagener-Afzelius Syndrome. Kartagener was an MD who noticed (1934) that in about 50% of cases, these symptoms were associated with a conditions known as situs inversus, in which the asymmetry of the internal organs is reversed, with “the heart on the wrong side of the chest”. Afzelius used electron microscopy to reveal structural deficiencies in the cilia of these patients, and suggested that situs inversus could be explained if at an early stage in development the asymmetry of the embryo is established by an asymmetric fluid current caused by ciliary movement. Without this movement the direction of the asymmetry is random. This suggestion has now been confirmed, at least in mice where there are mutants with situs inversus.

An interesting question is whether female sterility is associated with this syndrome. This might be expected, because the internal surfaces of the Fallopian tubes and oviduct are ciliated, and ciliary movements are believed to be involved in ovum transport. The evidence is that these affected females are not sterile, but have lower than normal fertility. Apparently there are redundant mechanisms to ensure ovum transport, and ciliary movement is not essential.

These important cilia are difficult to study, so that’s why we turn to other systems such as sea urchin sperm flagella and Chlamydomonas as material for experiments that try to find the details about how cilia work.

How do cilia and flagella work?

1. The cell body is not required, except as an anchor and a source of fuel (ATP).

2. A flagellum is normally covered by a membrane that is a rather typical extension of the cell membrane. The membrane can be removed with detergent, and normal movement can be obtained if a proper environment: salt concentration, pH, and MgATP, is provided. Removing the membrane exposes the internal machinery for a variety of experimental manipulations.
3. **Electron microscopy** has provided extremely important information about the **axoneme** -- which is the term used for the specialized **cytoskeleton** of cilia and flagella -- the structures that remain after removal of the membrane. Most flagella and cilia have axonemes of almost identical structure, called the “9+2” structure, because of the arrangement of **microtubules** that is seen in cross-sections of the axoneme.

In the 9+2 axoneme there are 9 **outer doublets**, with a complete A tubule and a partial B tubule, and there are two **central pair microtubules**. Axonemal microtubules are very stable microtubules, composed of 55 kD α and β tubulin subunits. About 50% of the protein of a typical flagellum is tubulin. Outer doublets are unusual-- contain tektin protofilaments also. Tubulins in A and B tubules differ, as a result of post-translational modifications. Making microtubules by polymerizing tubulin dimers is a standard laboratory procedure, but no one knows how to make these doublet microtubules in vitro.

In almost all cases, these microtubules extend straight along the flagellum, with little or no twist. In almost all respects, the structure of the axoneme is uniform along its entire length, except at the basal end and the tip.

The basal end of a flagellum is attached to a basal body that appears to be important for anchorage and initiation of flagellar growth, but not for motility. The basal body contains 9 triplet microtubules, and resembles a centriole. Some flagella have unusual axonemes: 3+0, 6+0, 9+0, 12+0, 14+0, 9+3, etc. Some flagella have other structures in addition to a 9+2 axoneme. For example, mammalian spermatozoa have 9 **outer dense fibers**: one attached to the outside of each outer doublet. 

4. What do the microtubules do when the flagellum bends?

   When the axonemal structure was first seen, it was suggested that the outer doublets caused bending by contracting and elongating. This is now known to be **wrong**.

   Bending of an axoneme involves sliding of the outer doublet microtubules, which do not change their lengths. This was first demonstrated by electron microscopy of dead cells, but more recently, it has been possible to demonstrate sliding during the normal bending movement of axonemes. This demonstration makes use of the reactivated movement of demembranated spermatozoa, and gold microbeads that stick to the exposed outer doublet microtubules to provide markers of positions on the microtubules.

   You can see a demo of this at: 

5. **Bending is caused by active sliding.**

   The first evidence for this was provided by Summers & Gibbons in 1971. They broke up demembranated sea urchin sperm to obtain short lengths of axonemes, which normally do not show any bending when supplied with ATP. Then they treated these axonemes briefly with trypsin, an enzyme that digests proteins. After trypsin treatment, addition of ATP caused the axonemes to disintegrate, and observation of this disintegration using dark-field microscopy showed that it occurred by sliding.

   More information was obtained by using electron microscopy to look at the results: In some cases, the microtubules slid out to produce a string of microtubules that was 5-7 times the length of the original fragment of axoneme. This observation was very important, because it showed that the microtubules were sliding against each other, rather than all sliding with respect to the matrix of structures attached to the central pair (which would at most give 3 times the original length.)

   Using electron microscopy to examine the results of this ATP-induced sliding also allowed the direction of sliding to be identified. It was shown that the A tubule of a doublet moved towards the basal end of the B tubule with which it was interacting. Velocities during axonemal disintegration are similar to or higher than those occurring during normal bending.
6. Active sliding in axonemes is caused by dynein. Electron microscopy in late 60’s identified “arms” attached to the A tubule and extending towards the B tubule. Inner and outer rows. Arms in the outer row have clear 24 nm periodicity (3 x tubulin periodicity). The inner arm complex has a complicated pattern with a 96 nm repeat.

Brief extraction with 0.5 to 0.6 M KCl or NaCl removes outer arms from sea urchin sperm, and the extract contains High MW proteins with ATPase activity. The name dynein was given to these proteins.

After extraction of outer arms by salt solutions, sea urchin spermatozoa beat with reduced frequency (about half). In some cases, frequency can be increased by adding back purified dynein. (Sea urchin, Chlamydomonas)

7. Dyneins are large molecular complexes with 1, 2 or 3 high molecular weight “heavy chain” polypeptides and 8-12 intermediate and low MW peptides. These heavy chains contain the ATPase enzyme activity and can function as motors. In the axoneme, dyneins attached to the A tubule push the B tubule tip-wards.

Sea urchin and other sperm: outer arm dyneins have 2 heavy chains and total MW of about 1.25 million Daltons. Sea urchin β heavy chain is 512 kDa, 4466 amino acids. Chlamydomonas and Tetrahymena outer arm dyneins have 3 heavy chains and total MW of about 1.8 million daltons.

In vitro motility assays demonstrate that purified dynein can cause translocation of any kind of microtubules. Translate towards minus end, push mts + end forwards. Velocities usually lower than those occurring during normal bending (??).

8. Similarities between dyneins and myosins

ATPase activity is activated by microtubules, but ratio is less than for myosin, kinesin.

In the absence of ATP (also called the apo state), a strong rigor state is produced in axonemes, presumably because the dynein forms strong cross-bridges between the A tubule and the opposite B tubule.

In solution, outer arm dynein binds to microtubules in absence of ATP, but there are species differences. Addition of ATP releases this binding. A relaxed state can be maintained with vanadate, which produces a very stable ADP Vi state that mimics the ADP Pi state that normally follows ATP hydrolysis.

Can measure stiffness of axonemes directly -- about 20 times greater in rigor.

9. Dyneins have a structure unlike myosin or kinesin

When the first dynein was sequenced, 4 short sequences with “P-loops” were identified as possible ATP binding sites. One, with the most normal P-loop, was identified as the important site of ATP hydrolysis, the others were incomplete. Now known that each P-loop is part of an AAA domain (AAA = ATPases Associated with diverse cellular Activities). There are actually 6 AAA domains in the head of the motor. 1-4 have P loops, with #1 being the primary ATP hydrolysis site; 5 and 6 have lost their P loops. Between 4 and 5 is a region containing the site of binding to the substrate microtubule, at the end of a coiled-coil stalk.

The N-terminal region of the motor forms a stem, which attaches the motor to the A tubule in the axoneme (or the “cargo” carried by cytoplasmic dynein).

When the stalk is visible in electron micrographs of an axoneme, it is always touching the B tubule - no distinct attached and detached states are seen.

Electron microscopy has provided evidence for different conformations of rigor and relaxed states of outer arm dyneins (Burgess, 1995) and a single-headed inner arm dynein (Burgess et al., 2004). This change may correspond to beginning and end of a “power stroke” that can cause sliding between the A and B tubules. However, this change occurs in the main mass of the dynein that is attached to the A tubule, and communication with the B tubule appears to involve the thin stalk, about 12 nm long.

This is not at all like the standard model for myosin.
Dyneins are so large that longitudinal interactions between dyneins in an axoneme are likely, and some evidence is provided by the Burgess 1995 micrographs.

10. How does active sliding produce bending?
Bending occurs between two points that have different sliding velocities.
In some flagella, such as sea urchin sperm, presence of normal basal end that does not allow sliding is essential for bending. A resistance to sliding at the basal end is important for conversion of sliding into bending.

11. What makes flagella oscillate and generate particular patterns of propagated bending waves?
This is a major unanswered question.
We expect that the answer will involve regulation of dynein activity. Uniform activation of dynein in an axoneme would not be expected to generate bending. For planar bending waves, we assume that when dyneins on one side of the axoneme are active, those on the other side are inactive. But this is only an assumption.
Dynein forces are required between bends to create and maintain bends (opposing the elasticity of the axoneme, which tries to straighten it out). Active sliding is required within bends to propagate the bends and perform work against the external viscous resistance. Therefore, the direction of active sliding should reverse at the trailing edge of an interbend region. However, there is little direct evidence that dynein is regulated in this manner. (But see Morita & Shingyoji, 2004)

12. A “curvature-controlled” computer model of flagella is remarkably successful
One simple model for oscillation and bend propagation involves “curvature control”. Computer simulations demonstrate that a simple control of the dynein activity by the curvature of the axoneme could lead to oscillation and bend propagation. Other models have been suggested in which shear or sliding velocity regulate dynein. There is no direct evidence to distinguish between these possibilities.
With original models, could not reproduce an important experimental result -- reduction to 1/2 frequency without change in bending pattern when outer arms are absent. Answer came from observations on Chlamydomonas mutants, which indicate that inner and outer arm dyneins have different functions. To accurately reproduce the effect of outer arm dynein removal, it is necessary to use different variations of a myosin model for inner and outer arm dyneins in the computer simulation model.

This is a tremendously oversimplified model. We really don’t know that dynein is controlled in this manner. There are a lot of things that we know about dyneins that are not incorporated into this model, and a lot of movements of flagella and cilia that have not been reproduced successfully by this model.

13. Why are there so many different kinds of dynein in an axoneme?
Sequence of β-heavy chain of outer arm dynein was used to search for similar sequences. Evidence for about 14 genes with similar sequence in several species. Two appear to be cytoplasmic dyneins, others are axonemal.
a). **outer arm dyneins** in an axoneme appear to be relatively homogeneous. Most *Chlamydomonas* outer arm mutants remove either all or none of the outer arms, not a particular subset. However, as already mentioned, some outer arm dyneins have two heavy chains (α & β), and some have three (α, β, & γ). These outer arm dynein heavy chains are not equivalent.
Sea urchin outer arm dynein can be enzymatically split into two fragments. The fragment containing the β heavy chain translocates microtubules in vitro at rate >= intact outer arm dynein, but does not bind to mts in absence of ATP. The fragment containing the α heavy chain binds to mts in absence of ATP, but does not translocate mts in vitro.
*Chlamydomonas* mutant *oda11*. Outer arm dynein is missing α heavy chain. Motility is slightly less than wild type, in contrast to *oda* mutants that completely lack outer arms and have less than half the normal beat frequency.
**Chlamydomonas** mutant *sup-1*. β heavy chain has a small deletion (about 7 amino acids). Beat frequency is reduced as much as in mutants that totally lack outer arms.

b). **inner arm dyneins** are diverse. Gels of Chlamydomonas oda mutants show 6-7 inner arm dynein heavy chains.

Dynein extracts from **Chlamydomonas** mutant missing outer arms separate into 7 distinct species by HPLC. Each species has one or two heavy chains, plus smaller peptides. 6 of these translocate microtubules in vitro. 5 of these also cause microtubule rotation in these assays (first found for an inner arm dynein from *Tetrahymena*). One of these, known as inner arm dynein C, has been particularly useful for detailed study. This is a single-headed dynein, which can function as a motor. Electron microscopy of isolated dyneins reveals distinct conformations corresponding to the rigor (no ATP) and relaxed (ATP + vanadate) states. The difference between these two conformations is a rotation that moves the stalk (which interacts with the B tubule) relative to the stem (which probably binds the molecule to the A tubule).

Mutants that cause loss of only a subset of inner dynein arms suggest 3 main subsets. EM localization of inner arm components shows a complicated pattern. Motility phenotypes of mutants with partial inner arm deficiency show reduced amplitude, in contrast to *oda* mutants that have normal amplitude and reduced frequency.

Mutant *pf23* with most inner arms missing is non-motile. No naturally occurring motile 9+2 axonemes without inner arms are known.

Inner arm dyneins appear to be necessary and sufficient for generating flagellar bending cycles, but when outer arm dyneins are present, they appear to act as amplifiers to increase the frequency of beating and generate most of the power that is required to overcome viscous resistance of the environment.

**14. What are the roles of other components?**

a). **Central pair of microtubules.**

Often a correlation between plane of beat and orientation of central pair. In flagella that normally generate planar bends, such as sea urchin sperm flagella, there also appears to be a permanent connection between doublets 5 and 6 (the “5-6 bridge”), rather than normal dynein arms. However, even these flagella can make helical bending waves under some conditions.

Some cilia do not have a central pair. Often these have helical beat instead of planar. In some cases, evidence for rotation of central pair. More information about central pair rotation at:

*www.wsu.edu/~omoto/essay/videoessay.html*

**Chlamydomonas** mutants without central pair can oscillate and propagate bends.

b). **Radial spokes.**

*Chlamydomonas* mutants without radial spokes can oscillate and propagate bends, but bending pattern does not have normal asymmetry.

Evidence for enzymes associated with radial spokes, which can modify sliding velocity.