1. Keywords and Terms

polymers, microtubules, microtubule dynamics, Xenopus cytoplasm extracts, total internal reflection microscopy (TIRF), catalyst, polymerase, secondary screen

2. Lecture Notes

Lecture 1 described a genome-wide RNAi screen for genes involved in cell division, and this lecture discusses a secondary screen and detailed follow-up on genes important for microtubule growth. Students will learn about the organization and dynamics of microtubules and about the different techniques and systems with which they are studied.
3. Review Questions

1. What are some of the biological roles for dynein?
2. Is dynein closely related to kinesin? Myosin? Neither?
3. What does cytoplasmic dynein do in yeast?
4. What does the “stall force” of a dynein mean?
5. Name a few fundamental differences between Dynein and Kinesin

4. Answers to Review Questions

1. Axonemal dyneins drive the beating of cilia and flagella

   Cytoplasmic dynein transports membrane organelles and vesicles (also viruses that invade cells) towards the minus ends of microtubules and also plays various roles in mitotic spindle assembly and kinetochore-microtubule interactions.

2. Answer is neither! It is most closely related to another superfamily called AAA (triple A) ATPases. Most AAA ATPases are involved in unfolding polypeptides for proteolysis or in dissociating protein-protein interactions.

3. Dynein is bound to the cortex in the bud (which becomes the daughter cell), grabs hold of microtubule growing from a dividing nucleus and then pulls the nucleus towards the bud. This ensures that the one nucleus will be segregated to the daughter cell during cell division.

4. If a motor protein is working against a load, its velocity progressively slows down with increasing load. At a critical load, the motor will “stall”, unable to move forward. This opposing load equals the maximum force (the stall force) that can be produced by the motor.

5. 
   a. The Dynein motor domain is much larger (about 5-fold larger) than kinesin’s.
   b. Dynein has multiple ATP binding domains (dynein has 4 and kinesin has 1).
c. Dynein moves towards the microtubule minus end whereas most kinesins move towards the plus end.

d. The microtubule binding domain is found at the end of a long stalk in dynein, whereas in kinesin, the microtubule binding domain is integrated in the main body of the enzyme core.

5. Discussion Questions

1. Why has dynein been harder to study than kinesin?

2. Why did the Vale lab choose yeast as a model system?

3. What type of experiment allowed the investigators to show that dynein was processive?

4. What is the difference in the stepping behavior of kinesin and dynein?

5. How did the Vale lab show that dynein is processive and requires two motor domains?

6. Why does yeast dynein need to be processive and produce large force?

7. What evidence do the investigators present that the two dynein motor domains shuffle past one another (like walking legs)?

6. Answers to Discussion Questions

1. A big barrier has been the size of the dynein polypeptide, one of the largest expressed by the genome of most organisms. The kinesin motor domain is ~45 kDa and can be easily expressed in bacteria. The minimal dynein motor domain is ~320 kDa, a size which precludes its expression in bacteria (it can be made in other somewhat more complicated expression systems such as yeast, the slime mold Dicystostelium or insect cells using baculovirus infection). The size makes other efforts such as X-ray crystallography much more difficult (the dynein motor domain has yet to be crystallized).

2. It is difficult to express large proteins like dynein in bacteria, the easiest system for protein expression. Proteins also can be expressed in eukaryotic cells; a gene is
cloned into a plasmid that can replicate or become integrated into the host eukaryotic cell, the gene is expressed, and then the expressed protein is purified (usually using a “tag” that will bind to an affinity column). However, the motor domain is large (~8 kb), which can make cloning and mutagenesis more difficult. However, the Vale lab chose to use the genomic copy of the dynein gene (instead of plasmids) in yeast and introduced mutations, tags, promoters by homologous recombination (a relatively easy procedure with S. cerevisiae). Dynein is non-essential in S. cerevisiae, so it is possible to propagate yeast strains that express mutant dynein genes that do not have biological activity.

3. The purified dynein protein was labeled with a single fluorescent dye. The fluorescently labeled dynein was combined with microtubules attached to a glass coverslip along with ATP. The sample was imaged by total internal reflection microscopy with a sensitive camera (an electron multiplying ccd camera), which can visualize the fluorescence from a single dye molecule. Using this method, the researchers can visualize the linear movement of single fluorescent “spots” along the microtubule.

4. Kinesin is a regular stepper- it takes regular 8 nm steps along a microtubule protofilament. The size of this step corresponds to the spacing between adjacent tubulin subunits (a/b dimers) along a one of the 13 protofilament tracks of the microtubules. Dynein, on the other hand, is a “variable” stepper. It takes 8, 16 nm and occasionally longer steps. It also takes occasional “backwards” steps, something that kinesin very rarely does in the absence of a load.

5. They first produced a monomeric dynein motor domain and showed that it was not processive (single fluorescently labeled dynein motor domains do not move along microtubules). However, the monomer was not “dead”; it had ATPase activity and it moved microtubules in a “gliding” assay (multiple dynein monomers bound to the glass surface and interacting simultaneously with a microtubule). They then joined two motor domains together with a chemical crosslinker (rapamycin) and showed that this is sufficient to induce processive movement.

6. We do not have a definitive answer to this question. In fact, we do not know or have good experimental evidence for why evolution has tuned motors to move at particular velocities, produce specific forces, or have specific “run lengths” (distance moved per encounter with a microtubule). These remain as interesting questions for future research. However, we can speculate and perhaps test these ideas in the
future. In yeast, dynein’s main biological role is to pull the nucleus, a very large structure, into the bud. Relatively few (<5) dyneins on the bud cortex are thought to bind to a microtubule and pull. Making dynein processive would allow these small numbers of dyneins to tenaciously hold onto the microtubule and not let go of the microtubule and the attached nucleus. Making dynein relatively strong (~ 6 pN, similar to kinesin) should allow it to move the large nucleus efficiently through the yeast cytoplasm, which might constitute a high viscosity environment (membrane organelles and actin cables also producing an effective “drag” on a nucleus moving through the cytoplasm). Of course, one could test whether processivity and high force are really needed, by mutating the dynein motor so that it has lower processivity or lower force (tested in vitro) and then replacing the endogenous dynein gene with the mutant gene (by homologous recombination) and seeing if the movement of the nucleus is affected and if there is a defect in nuclear segregation.

7. The investigators placed a fluorescent probe (a quantum dot) on a motor domain and found that it moved in ~16 nm steps, whereas they found shorter steps (8 nm) if placed in the middle between the two motor domains. From this data (and other information described in the Reck-Peterson et al. paper (see reading)), they inferred that a rear motor domain can swing past the center of mass and the partner head to bind to a forward microtubule subunit. However, a better experiment would be to follow the two motor domains separately and in the same experiment, by attached different colored fluorescent dyes on the two motor domains and following the motion of each motor domain. The two fluorescent dyes should alternate in position, one color being in front of the other and then two dyes switching as the motor takes a step (this has been done with myosin V). There is always a way of devising a better experiment to prove a hypothesis!

7. Explain or Teach These Concepts to a Friend

1. How does homologous recombination in yeast work and how can it be used for protein engineering (as in making new dynein proteins described in this lecture)?

2. What is a Q (quantum) dot and how does it differ from an organic fluorophore?

3. What is the resolution limit of conventional light microscopy? How do you track the movement of a fluorescent motor with a few nanometer resolution?
4. How does an optical trap work? Why can it capture a bead but not a single protein?

8. Research the Literature on Your Own

1. How does the ATPase ring communicate with the MT binding domain through the coiled coil stalk? (There is some new data on this subject in the literature since this lecture was filmed; see also Journal Club Papers described below).

2. What is dynactin and what are its biological roles?

9. Papers for Journal Club

The first two papers describe conformational changes that occur in dynein. This is not a primary topic of the iBioSeminars lecture, but these are nice papers that explore another aspect of the dynein motor. (These three papers also all appeared after the iBioSeminars lecture was taped). They also illustrate three different approaches/techniques. The Roberts et al. paper primarily uses electron microscopy to study conformational changes in the entire dynein motor domain. The Carter et al. paper uses primarily X-ray crystallography to study the microtubule binding domain (and also functional studies to look at the role of the microtubule binding domain). The Kon et al. paper is complementary to the Carter et al. study, as it uses crosslinking studies to test a model of how the affinity in the microtubule binding domain is regulated by the sliding of residues in an anti-parallel coiled coil.


These next two papers are the single molecule studies that are featured in the lecture. The Reck-Peterson et al. paper describes single molecule fluorescence as a tool to understand dynein processivity and step sizes. The Gennerich paper uses an optical trap to examine dynein force production and behavior of dynein stepping under load (in more depth than described in the iBioSeminar lecture).
