18 Cell Organization and Movement II: Microtubules and Intermediate Filaments

Review the Concepts

1. The basis of microtubule polarity is the head-to-tail assembly of αβ-tubulin heterodimers, which results in a crown of α-tubulin at the (–) end and a crown of β-tubulin at the (+) end. In nonpolarized animal cells, (–) ends are typically associated with MTOCs, and (+) ends may extend toward the cell periphery. Other arrangements occur in different types of cells, but the (–) ends are associated with a MTOC in most cases. Microtubule motors can "read" the polarity of microtubule, and a specific motor protein will transport its cargo toward either the (+) or the (–) end of the microtubule.

2. During dynamic instability, microtubules alternate between growth and shortening. The current model to account for dynamic instability is the GTP cap model. According to this model, GTP-tubulin (subunits with GTP bound to β-tubulin) can add to the end of a growing microtubule, but at some time after assembly the GTP will be hydrolyzed to GDP, leaving GDP-tubulin, which makes up the bulk of the microtubule. Thus GTP-tubulin is present only at the microtubule end, and as long as this situation holds, the microtubule will continue to grow since the cap stabilizes the entire microtubule. However, if GDP-tubulin becomes exposed at the end then the stabilizing cap is lost and the microtubule will begin to shorten. The microtubule will continue to shorten until it disappears or until GTP-tubulin returns to the end and a new GTP cap is formed.

3. The best understood proteins involved in regulating microtubule assembly are the stabilizing MAPs. These proteins bind to microtubules and promote assembly, increase microtubule stability, and, in some cases, cross-link microtubules into bundles. The other main group of MAPs functions to destabilize microtubules. This group includes proteins such as katinin, which severs microtubules, and Op18, which promotes the frequency of microtubule catastrophe.

4. Microtubule organizing centers (MTOCs), also known as centrosomes, are responsible for determining the arrangement of microtubules within a cell. The typical cell contains a single MTOC, although mitotic cells contain two (the spindle poles), and certain types of cells may contain several hundred. Microtubules are nucleated by γ-tubulin ring complexes, which are located in the pericentriolar material of an MTOC. The γ-tubulin provides a binding site for αβ-tubulin dimers, and the ring complex structure appears to provide a template for nucleating microtubule formation.

5. Microtubule/tubulin-binding drugs are used to treat a variety of diseases, including gout, certain skin and joint diseases, and cancer. Such drugs either prevent microtubule assembly (e.g., colchicine) or prevent microtubule disassembly (e.g., taxol). Although the effects are opposite, the results are the same: inhibition of cellular processes that depend on microtubules and the dynamic rearrangement of these polymers.

6. Kinesin I was first isolated from squid axons, which were manipulated to produce extruded cytoplasm, a cell-free system to study synaptic vesicle motility. Video microscopy was used to follow the ATP-dependent movement of synaptic vesicles along individual microtubules in the extruded cytoplasm, but when purified synaptic vesicles were added to purified microtubules, no movement was observed even in the presence of ATP. Subsequent addition of a squid cytosolic extract to the purified components restored ATP-dependent vesicle

movement along microtubules, indicating that a soluble protein in the cytosol was responsible for driving vesicle movement. To identify the soluble "motor" protein, researchers took advantage of previous experiments with extruded cytoplasm that demonstrated that a nonhydrolyzable ATP analog (AMPPNP) caused vesicles to bind so tightly to microtubules that movement was stopped. To purify the motor, AMPPNP was added to a mixture of cytosolic extract and microtubules with the goal of forcing the motor to bind microtubules. The microtubules and any bound proteins were then recovered by centrifugation and treated with ATP to release proteins that bound microtubules in an ATP-dependent manner (a fundamental property of microtubule-dependent motor proteins). The predominant protein released in this approach was kinesin I.

7. Although microtubule orientation is fixed by the MTOC (and any given motor moves only in one direction), some cargoes are able to move in both directions along a microtubule because they are able to interact with both (+)-end- and (–)-end-directed motor proteins. The direction that a given cargo moves along a microtubule appears to be controlled by swapping one motor protein for the other (it may also be possible to activate one motor and inactivate the other). Certain cargoes may move on both microtubules and actin filaments if the cargo contains binding sites for both microtubule and actin motor proteins.

8. The kinesin family motor (or head) domain contains the ATP binding site and the microtubule-binding site of the motor, while the neck is a flexible region connecting the motor domain to the central stalk domain. The kinesin motor domain is required to generate movement but does not appear to determine the direction a kinesin motor will move on a microtubule. Instead, the neck determines the direction of movement. These conclusions are based on experiments in which the motor domains of (+)- and (–)-end-directed motors were swapped with no effect on direction of motor movement, and on experiments in which mutations of the neck region caused a change from a (–)- to a (+)-end-directed motor.

9. The appendages (cilia and flagella) used for cell swimming contain a highly organized core of microtubules and associated proteins. This core, termed the *axoneme*, is typically made of nine outer doublet microtubules and two central pair microtubules (known as the 9 + 2 arrangement). Each outer doublet consists of a 13-protofilament microtubule and a 10-protofilament microtubule, while the central pair contains 13 protofilaments each. Cell movement depends on axoneme bending, which, in turn, depends on force generated by axonemal dyneins. These motor proteins act to slide outer doublet microtubules past each other, but this sliding motion is converted into bending because of restrictions imposed by cross-linking proteins in the axoneme, and perhaps by the action of inner arm dyneins.

10. The three types of microtubules that make up the spindle are kinetochore microtubules, polar microtubules and astral microtubules. The (–) ends of all three types associate with spindle poles. Kinetochore microtubules connect chromosomes, via the kinetochore attachment site, to the spindle poles. Polar microtubules from each pole overlap and are involved in holding the poles together and regulating pole-to-pole distance. Astral microtubules radiate from each spindle pole toward the cortex of the cell, where they help position the spindle and determine the plane of cytokinesis.

11. Inhibition of kinesin-5 would be expected to have a number of effects. First, the centrosomes duplicated in S phase would not move apart during prophase to make the mitotic asters. Second, if the drug were added after the centrosomes had separated and become spindle poles, the spindle poles would not separate during anaphase B. Inhibiting kinesin-13 would affect chromosome movement of captured chromosomes in prometaphase as well as anaphase A, as the shortening of microtubules in both these cases would be compromised. Inhibiting kinesin-4 — the kinesin that attaches to chromosome arms and

interacts with spindle microtubules to pull them towards the center of the spindle — would affect chromosome congression at prometaphase.

12. Proteins such as kinesin-13 at the kinetochore may allow this structure to hold onto shortening microtubules. It is not clear whether this activity requires ATP hydrolysis, since kinetochores have been shown to hold onto depolymerizing microtubules in vitro in the absence of ATP.

13. The separation of spindle poles during anaphase B is thought to depend on Kinesin-5 motors present on microtubules in the overlap zone between the poles, which act to push the spindles apart, as well as on cytosolic dynein motors on the inner surface of the cell membrane, which act to pull astral microtubules and hence the poles apart (different organisms may utilize pushing and pulling forces to differing degrees during anaphase B). In addition, elongation of microtubules in the overlap zone appears to increase the extent of pole separation.

14. In animal cells, the spindle determines the cleavage plane. Microtubules are therefore involved with determining the plane of cytokinesis while actin filaments, as components of the contractile ring, carry out the process of cytokinesis.

15. Unlike microfilaments and microtubules, intermediate filaments do not have an intrinsic polarity. Thus, it is not surprising that there are no motor proteins that use intermediate filaments as tracks.

Analyze the Data

a. Kinesin 1 has a globular, bulbous region at one end of the molecule and this region is enlarged when antibody that binds to the motor domain is present. Accordingly, these observations suggest that the globular region contains the kinesin motor domain. The two heavy chains appear to interact in parallel, as the motor domain is observed only at one end of the molecule. In contrast, kinesin 5 has two bulbous regions, each of which is enlarged when antibody to the motor domain is present. These data suggest that kinesin 5 has motor domains at each end of the molecule, i.e., it is a bipolar kinesin. Because it is a tetramer, rather than a dimer, it may be formed by two dimers interacting tail to tail in an antiparallel fashion.

b. The brighter end of each microtubule would represent the (–) end of the microtubule while the less bright region would represent the (+) end of the microtubule. On kinesin 5, the microtubules are observed to glide with their (–) ends leading (bright ends lead). Accordingly, because kinesin 5 is immobilized on the surface and cannot move but causes the microtubule to move towards its own (–) end, relative to the microtubule kinesin 5 is moving towards the (+) end. Therefore, kinesin 5 is a (+) end microtubule motor.

c. The two microtubules are oriented with their minus ends opposite each other. Thus, because these microtubules are cross-bridged by kinesin 5, these data suggest that kinesin 5 cross-bridges microtubules of opposite polarity. When ATP is added, the ends of the two microtubules move farther apart, suggesting that kinesin 5 causes the microtubules to slide apart. Because kinesin 5 is bipolar (see text figure on page 799, part a), can crossbridge microtubules, and is a (+) end microtubule motor (see text figure part b), one would predict that kinesin 5 would cause two cross-bridged microtubules to slide apart with their minus ends leading.

d. Loss of Eg5 can be observed in these data to result in lack of formation of a bipolar mitotic spindle. Therefore, Eg5 appears to be required to form a normal spindle. Because kinesin 5 can induce sliding apart of microtubules of opposite polarity (see text figure part c), Eg5 might be required to help the two centrosomes move apart to form the poles of a bipolar spindle. If the two poles (centrosomes) do not move apart in preparation for mitosis, then a bipolar spindle cannot form between them.