PEARSON NEW INTERNATIONAL EDITION

Principles of Animal Physiology

Christopher D. Moyes
Patricia M. Schulte

Second Edition

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Overview

Every physiological process, be it intracellular transport, changes in cell shape, cell motility, or muscle-dependent animal locomotion, depends in some way on movement. Regardless of the type of movement, the same intracellular machinery underlies each one: the *cytoskeleton* and its *motor proteins*. Recall that eukaryotic cells possess a cytoskeleton composed of microtubules, microfilaments, and intermediate filaments. Of these, only microtubules and microfilaments have important roles in cellular movement. Microtubules work in conjunction with the motor proteins kinesin and dynein. Myosin, in contrast, is the actin-dependent motor protein. The diversity in cellular movement is possible because these basic elements can be arranged and used in countless combinations.

There are three general ways that cells use these elements to move (Figure 1). Most commonly, cells use the cytoskeleton as a roadway, where motor proteins act as trucks carrying cargo over the complex cytoskeletal networks. Just as the highway route controls traffic, cells mediate intracellular traffic by controlling where the roads go, which vehicles ride the road, and the nature of the cargo. For example, the precision of cell signaling pathways depends on motor proteins being able to carry secretory vesicles from sites of synthesis to the plasma membrane for exocytosis. If a vesicle is carried to the wrong place or released at the wrong time, dangerous miscommunications can result.

A second class of movement is driven by active reorganization of the cytoskeletal network. Rather than acting as a road, in this case the cytoskeletal fibers act as bulldozers that push the cellular contents forward. This type of movement, often called amoeboid movement, is most common in protists. Many metazoan cell types, such as leukocytes and macrophages, also use amoeboid movement. Motor proteins may or may not be involved in the process. Cells regulate this type of movement by controlling the rate and direction of growth of cytoskeletal fibers.

The third type of movement is analogous to a group of people pulling a rope. In this case, the motor protein pulls on the cytoskeletal rope. Cells then organize the cytoskeleton in a way that translates this tugging action into movement. As you will see later in this chapter, these cytoskeletal superstructures are the foundation of cilia, flagella, and muscle. Cells primarily regulate this type of movement by controlling the activity of the motor protein.

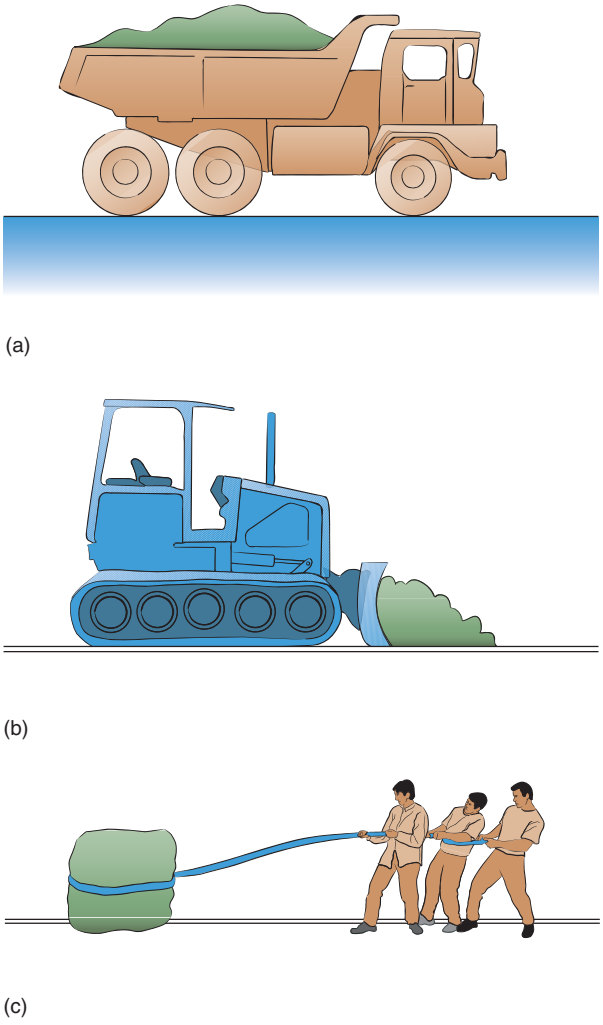


Figure 1 Three ways to use the cytoskeleton for movement **(a)** Cells can use their cytoskeleton as a road on which motor proteins move, often carrying intracellular cargo. **(b)** Some cells move by pushing the cytoskeleton forward, much like a bulldozer pushes earth ahead. **(c)** Movement sometimes resembles a tug-of-war, where motor proteins, depicted as people, can pull the cytoskeleton, symbolized by the rope.

Cytoskeleton and Motor Proteins

The cytoskeleton and motor proteins work in conjunction to enable animals to mediate intracellular trafficking, changes in cell shape, and cellular movement. Three general explanations exist for

the variations seen in the cellular movement in animal cells. First, most animals possess multiple isoforms of critical cytoskeletal and motor proteins. This arsenal of genetic variation allows metazoans to build specialized types of cells. Second, animal cells can use a single set of building blocks to organize the cytoskeleton in different ways. Third, animals can regulate an existing suite of proteins in real time; hormones bind to receptors, triggering regulatory cascades that alter enzyme activity that modifies the properties of the cytoskeleton and motor proteins. These three aspects of diversity account for the distinct ways animal cells build and use the cytoskeleton and motor proteins for movement. The capacity to be different at a cellular level is central to the animals' ability to generate specific types of cells, as well as to adapt to evolutionary challenges. As we proceed through this textbook, you will see that these cellular processes underlie many important physiological systems.

Microtubules

Cells can organize microtubules in many arrangements. Most cells gather the ends of microtubules near the nucleus of the cell at the **microtubule-organizing center (MTOC)** (Figure 2). The microtubules radiate from the MTOC like spokes of a wheel that extend to all margins of the cell. The outward ends of microtubules are anchored to integral proteins embedded within the plasma membrane. This microtubule network is vital to intracellular traffic, as motor proteins can move either toward the central MTOC or to the periphery of the cell.

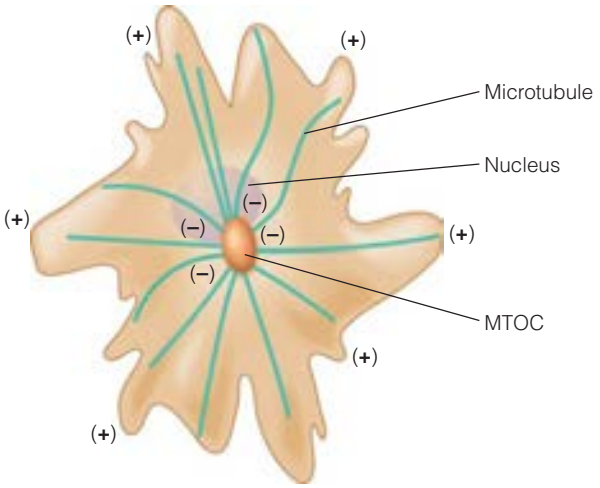


Figure 2 Microtubule network of cells Many cells organize microtubules into a network, with the minus ends gathered near the center of the cell at the microtubule-organizing center (MTOC).

Cells use their microtubule network to control the movement of subcellular components, such as vesicles and organelles. Microtubule systems also mediate the rapid changes in skin color seen in some animals that use cryptic coloration, such as the African claw-toed frog, *Xenopus laevis* (Figure 3). Skin color is determined by the distribution of dark pigment granules within cells called *melanophores*. When the pigment granules are concentrated near the MTOC, the skin is pale in color. When the granules are dispersed throughout the cell, the skin darkens. Changes in the directional movement of pigment granules along microtubule tracks within the melanophore, controlled and triggered by hormones, create adaptive coloration in animals. A closer look at how microtubules are built will lay the foundation for understanding the role they play in vesicle traffic, pigment dispersal, and other types of intracellular and cellular movements that are central to physiological function.

Microtubules are composed of α-tubulin and β-tubulin

Microtubules, so named because of their tubelike appearance, are composed of long strings of the protein tubulin, itself a dimer of two closely related proteins: α-tubulin and β-tubulin. The evolutionary history of tubulin is intriguing and rich in paradoxes. For example, tubulin genes have changed very little since the earliest eukaryotes. The α-tubulin of yeast is very similar to your own; even α-tubulin and β-tubulin are nearly 40% identical in most species. Many animals have multiple tubulin isoforms that are expressed in different tissues. Because of the similarity in the structures of different isoforms, they were believed to be interchangeable: for example, one α-tubulin isoform could be replaced with another α-tubulin isoform without obvious consequences. The importance of the subtle differences in tubulin structure between species, as well as within a species, has only recently been appreciated. In one instance, when nematodes (*C. elegans*) were genetically modified to express a different isoform of β-tubulin in their touch neurons, the mutant worms had sensory dysfunction. These studies showed that even subtle differences in the structure of tubulin isoforms have important consequences for cellular function.

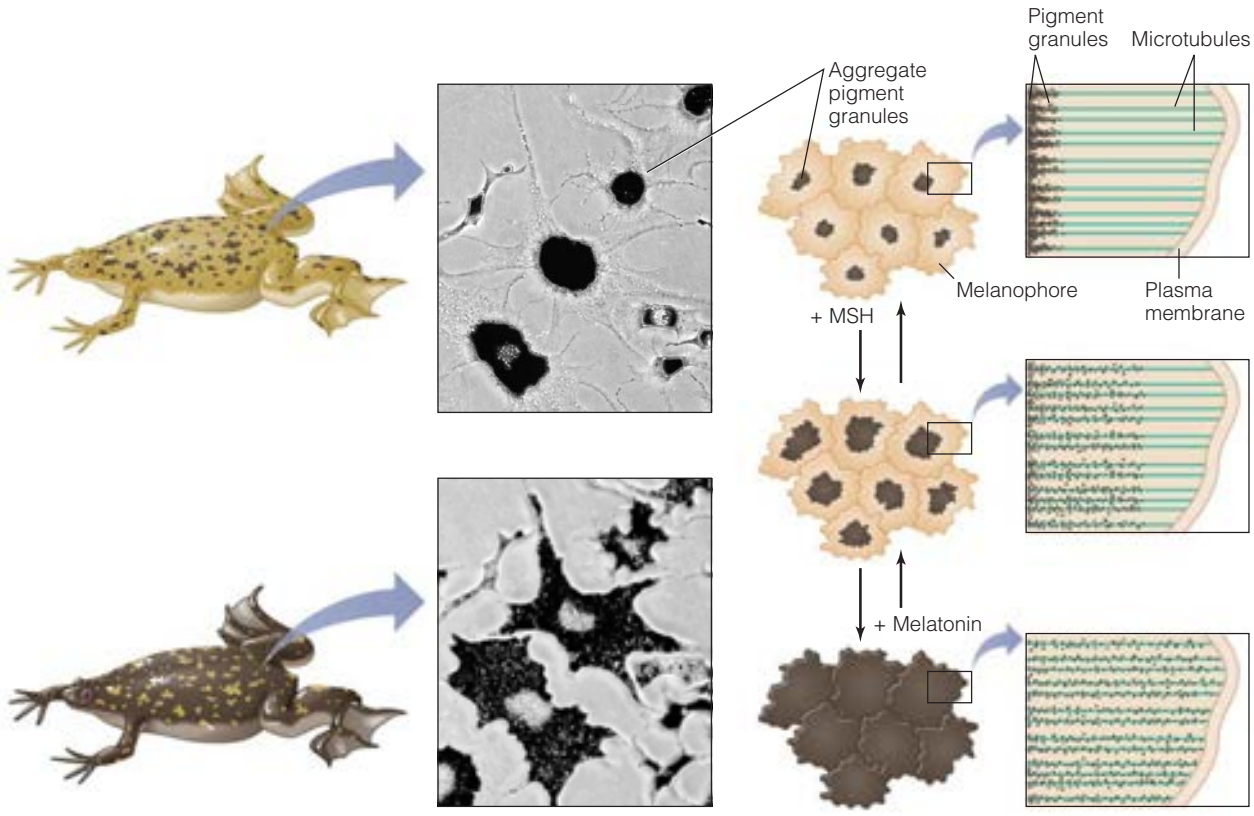


Figure 3 Movement of pigment granules Melanophores from the African clawtoed frog *Xenopus* allow rapid changes in color. Arrays of microtubules radiating from the central MTOC carry pigment granules throughout the cell. Actin filaments, not shown here, also play a role in controlling local pigment distribution. Pigment granules aggregate in response to the hormone melatonin, and disperse in response to melanophore stimulating hormone, MSH. (Micrographs courtesy of V. Gelfand, University of Illinois)

Unlike many large, complex proteins, microtubules form spontaneously within cells, a feature that is central to microtubule function. The first step of assembly (Figure 4) occurs when α-tubulin and β-tubulin combine to form tubulin. Prior to dimerization, both subunits bind to a single molecule of GTP. When tubulin forms, the GTP bound by β-tubulin may be hydrolyzed into GDP and phosphate. In contrast, the GTP bound by α-tubulin remains intact and bound within the tubulin structure. The α-tubulin, with its GTP intact, is on one end of the dimer; the β-tubulin, with its hydrolyzed GTP, is on the other end of the dimer. The difference between the two monomers creates structural asymmetries within tubulin, known as *polarity*. The α-tubulin subunit is at the so-called minus end (−) of the tubulin dimer, whereas β-tubulin is at the plus end (+). The polarity of tubulin has important ramifications in the subsequent steps of microtubule assembly.

The next step in microtubule assembly occurs when multiple tubulins assemble end-to-end. Like a line of magnets, the plus end of the growing chain attracts the minus end of a free dimer. The chain, or **protofilament**, grows until it reaches a critical length. The protofilaments then line up side by side to form a sheet that eventually rolls into a tube to form the microtubule. Because the angle between adjacent protofilaments is about 28°, 13 protofilaments are required to form a complete circular tube. Once the microtubule is formed, it can continue to grow by incorporating more dimers, or it may shrink by shedding them.

Microtubules show dynamic instability

Microtubule dynamics, such as the rates of growth and shrinkage, regulate many cellular functions. Any chemical that disrupts microtubule dynamics can become a potent poison. Some plants use mi-

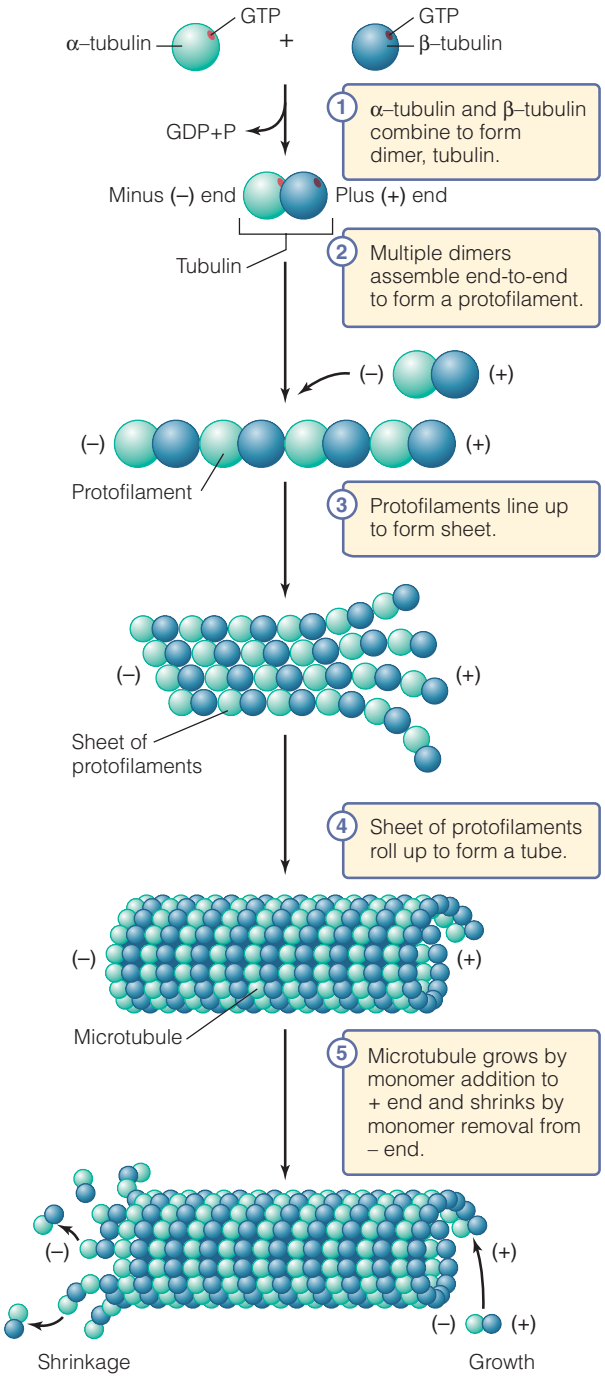


Figure 4 Microtubule assembly Microtubules are composed of repeating units of the protein tubulin, a dimer of two GTP-binding proteins, α-tubulin and β-tubulin. Tubulin dimers connect end-to-end to begin the construction of a protofilament. The protofilaments join side by side to start the formation of a sheet. Once the sheet reaches a critical width, it rolls into a tube to form the microtubules. Microtubules grow by adding tubulin and shrink by losing tubulin.

crotubule poisons as part of their defense against animal grazing; for example, the Pacific yew tree (*Taxus*) produces taxol, the periwinkle plant (*Vinca*) produces vinblastine, and the autumn crocus (*Crocus*) produces colchicine. Animals that graze on these plants are sickened as a result of the effects of these alkaloids on their own microtubule dynamics. Many of these plant defense agents have been developed as anticancer drugs because of their ability to kill rapidly dividing cells. These compounds are also very useful tools in the laboratory, as they allow researchers to dissect the processes that control microtubule dynamics.

The balance between growth and shrinkage determines the length of the microtubule (Figure 5). Many factors influence microtubule dynamics, but the most important is the local concentration of tubulin. If the end of the microtubule is exposed to a high concentration of tubulin, it will tend to grow. At low tubulin concentrations, however, microtubules tend to lose tubulin dimers and shrink. At a specific critical concentration (C_c), growth and shrinkage are in balance and there is no net change in length. However, several factors complicate this simple pattern of concentration-dependent regulation. First, the C_c value at the plus end is lower than at the minus end. This

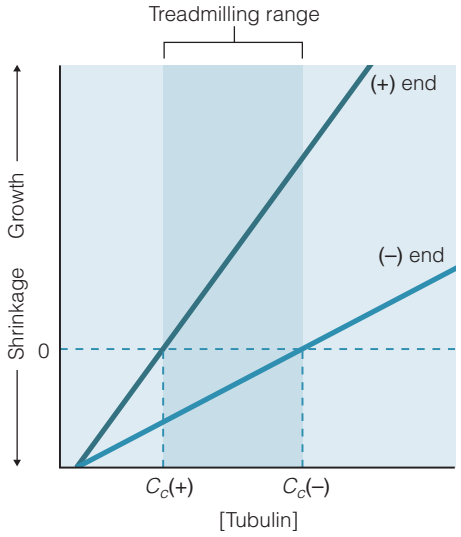


Figure 5 Microtubule dynamics Whether a microtubule grows or shrinks depends on tubulin concentration. Below a critical concentration (C_c) the microtubule is more likely to shrink. Above C_c it will likely grow. While both ends can add or lose tubulin, the plus end has a lower C_c. This means at any particular tubulin concentration, the plus end is more likely to grow than is the minus end.

means that if *both ends* are exposed to the *same tubulin concentration*, the plus end is more likely to grow and the minus end is more likely to shrink. Colchicine and vinblastine are toxic because they prevent microtubule growth. Colchicine binds to free tubulin and prevents it from incorporating into microtubules, while vinblastine prevents microtubule formation by causing free tubulin dimers to aggregate. This reduces the concentration of free tubulin, curtailing microtubule assembly.

The second feature that distinguishes microtubule growth is known as *dynamic instability*. Even when the tubulin concentration exceeds C_c, the microtubule will grow for a few seconds, then spontaneously shrink for a few seconds. This concentration-independent transition is due to a change in the GTP bound by β-tubulin. Once incorporated into a microtubule, the β-tubulin subunit may or may not hydrolyze GTP. As long as the GTP in β-tubulin remains intact, the microtubule tends to grow. Alternatively, if the GTP is hydrolyzed, the microtubule will tend to shrink. Microtubules maintain their constant length by balancing growth and shrinkage, while hydrolyzing a lot of GTP in the process. This may at first seem to be a waste of the cell's energy, but it is a necessary cost. Dynamic instability, despite its energetic costs, enhances the ability of the cell to regulate microtubule growth in space and time. Systems in motion are much easier to alter than static systems.

Microtubule dynamics are also regulated by **microtubule-associated proteins**, or MAPs (Figure 6). These proteins bind to the surface of microtubules, stabilizing or destabilizing the microtubule structure. Some MAPs bind to the plus end of microtubules and prevent the transition from growth to shrinkage. A group of MAPs called *stable-tubule only polypeptides*, or STOPs, are used by many cell types that need long, stable microtubules. For instance, STOPs are abundant in nerves where microtubules are important for the development of long axons and dendrites. Other MAPs act as protein cross-linkers. MAPs can join microtubules together into bundles, or link the microtubules to other cellular structures, such as membrane receptors. Taxol is a potent toxin because it stabilizes microtubules. However, not all MAPs stabilize microtubules. For example, *katanin* (Japanese for "sword") is a MAP that severs microtubules. Normal cell function depends on the regulation of both assembly and disassembly of microtubules. Preventing microtubules from dissociating impairs many cellular processes, including cell division.

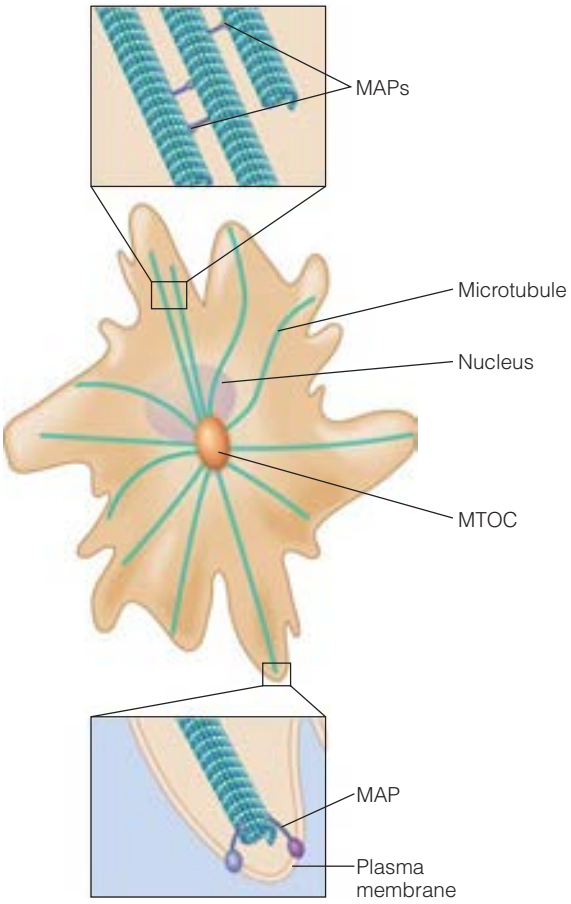
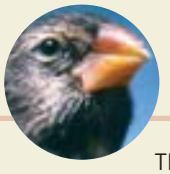


Figure 6 Microtubule-associated proteins
Microtubules are connected to each other and to membrane proteins by microtubule-associated proteins, or MAPs.

The activities of MAPs are regulated by protein kinases and protein phosphatases. Changes in MAP phosphorylation can alter its subcellular location, change its ability to bind a microtubule, or alter its functional properties. Many signaling pathways target MAPs to alter microtubule structure. For example, the hormones that regulate cell division, known as cytokines, induce changes in microtubule structure by regulating the MAP structure and activity. The subsequent changes in the microtubule network ensure that cellular constituents are equally divided between daughter cells.

Temperature is another parameter that affects microtubule dynamics. Early experiments showed that isolated microtubules could assemble and disassemble spontaneously in test tubes. When micro-



BOX 1 **EVOLUTION AND DIVERSITY**

Thermal Adaptation in Microtubules

The thermal instability of microtubules presents a conundrum. If mammalian microtubules spontaneously disassemble at 25°C, what is different about the microtubules of animals that live at even colder temperatures? Many mammalian tissues can stabilize microtubules using a number of microtubule-binding proteins, such as STOPs (stable-tubule only proteins), MAPs, and capping proteins. Do cold-dwelling organisms use these same proteins to prevent thermal instability, or is there something different about tubulin itself? Insight into this question comes from studies using models in which differences arise from both natural selection and genetic engineering approaches.

For many cold-dwelling organisms, microtubule stability can be traced to the structure of tubulin itself. When first discovered, this was a bit surprising because the sequence of tubulin is extraordinarily conserved across animals. Isolated microtubule proteins from cold-water fish spontaneously assemble at lower temperatures than do those proteins from mammals. Antarctic fish have been isolated in Polar Seas for more than 10 million years. Over this time, the sequences of α-tubulins and β-tubulins have accumulated only a few amino acid variations, yet the microtubules from these fish are much more stable than microtubules from warm-water fish. When the genes for β-tubulin from a cold-tolerant cod were transfected into cultured human cells, the microtubules from the transgenic cells were stable in the cold. These studies show that very subtle differences in tubulin structure, even one or two amino acids, can result in profound differences in cold stability. Researchers studied microtubules produced by yeast in which the β-tubulin gene was subtly mutated; a single cysteine was mutated to alanine. This simple mutation made the microtubules cold-stable. Unfortunately for the yeast, the structural changes that increased cold-stability also dramatically impaired processes that depend on microtubule dynamic instability, such as growth and cell replication. These studies illustrate two important aspects of microtubules. First, microtubule function is critically dependent upon maintaining a dynamic balance between assembly and disassembly, or stability and instability. Second, even modest changes in microtubule structure, arising through evolution or genetic engineering, can produce a microtubule with very different properties. Whether these subtle mutations are adaptive or lethal depends on how the specific mutation affects the proteins, and how the structural change influences function in the context of environmental conditions.

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tubules were cooled to 25°C, for example, they disassembled. Although this is a useful laboratory technique to study microtubule dynamics, what does it mean for the animals? Temperature-induced disassembly is not physiologically relevant for most endothermic animals, such as mammals and birds, because they maintain body temperatures well above the threshold temperature. However, many ectothermic animals must endure temperatures low enough to disrupt the microtubules of a mammal. In that case, how do animals that live in the cold avoid spontaneous disassembly of their microtubules? See Box 1, Evolution and Diversity: Thermal Adaptation in Microtubules for an explanation.

Microtubule polarity determines the direction of movement

The extensive microtubule networks within cells provide a complex roadway for the motor proteins. But how do motor proteins identify which road to ride? Once on the road, how do they decide which way to go? Recall that the orientation of the dimers endows a microtubule with a structural polarity, where microtubules have a plus end and a minus end. Since cells organize microtubules by collecting the minus ends at the MTOC, the plus ends are found at the periphery. Motor proteins recognize microtubule polarity, and each motor protein moves in a characteristic direction; kinesin