Microtubule assembly and movement

**Question:** What are microtubules and how are they involved in intracellular movement?

**I. Self-assembly system**

**A. Composition** Like actin, microtubules are formed and function as a self-assembly system. As shown in *V&V p. 1254 Fig. 34-74* and *MBOC p. 803 Fig. 16-21A, C*, an individual microtubule is composed of 13 strands, called protofilaments, arranged parallel to each other around a circle to form a hollow tube. A cross section of the tube has a 25 nm diameter and looks like a ring of 13 globules.

The biggest source of microtubules (MT) is the brain, where actin is also found in abundance. Just as actin filaments are composed of subunits of the protein actin, MT are built from subunits of the protein tubulin, which has a molecular weight of about 56 kD. If total protein from brain cells were fractionated on an SDS gel, we see two discernible bands against a faint smear. One of them is about 41kD, and corresponds to actin. The other 56kD band is tubulin. If purified tubulin is run on an SDS gel, it often shows up as doublet. From this doublet it was learned that a tubulin subunit is itself composed of two distinct polypeptides, called α-tubulin and β-tubulin. Thus, it is the dimer of α- and β-tubulin that forms the stable unit of function and is the building block from which MT are built.

As illustrated in *V&V p. 1254 Fig. 34-74* and *MBOC p. 803 Fig. 16-21D*, each linear protofilament is composed of alternating α- and β-tubulin, consistent with a linear end-to-end alignment of the dimers (α-β-α-β-α-β-). The MT as a whole thus appears as rows of alternating α- and β-molecules. Since the protofilaments are displaced to a small degree, each tubulin molecule contacts six others.

**B. Organizing center**. As with actin, a solution of subunits (dimers in the case of MT) can be induced to polymerize by adding nucleotide (GTP) and salt. If a "MT seed" is included in the solution, MT radiate out from the seed, as shown in *MBOC p. 806 Fig. 16-24*. As with actin, the (-) end is the area of slow growth and the (+) end that of high growth.

Does this pattern of radiating out from an organizing center occur in cells? A look inside a cell in interphase (see *MBOC p.806 Fig. 16-26A*) reveals that it does. The organizing center is a structure called a centrosome, which usually contains two rod-like structures called centrioles positioned at right angles to each other and embedded in a proteinaceous fuzz. All MT originate from the centrosome, although it is not clear what role the various components of the centrosome contribute. The negative ends of the MT are closer to the centrosome, while the faster-growing plus ends radiate outward.
C. Polymerization  As can be expected from the head-to-tail arrangement of dimers, MT are asymmetric, with a fast-growing (+) and a slow-growing (-) end. As shown in the graph in MBOC p. 805 Fig. 16-23, and in keeping with the characteristic features of self-assembly, MT polymerization consists of an initial lag time, during which protofilaments form and come together, followed by a growth phase, and ending with a steady state phase, in which the rate of subunit addition equals the rate of subunit loss. The concentration of MT at the steady state varies with the initial concentration of subunits, but the concentration of subunits left at steady state is the critical concentration.

D. Turnover  Experiments with the plant alkaloids colchicine and taxol demonstrate the constant turnover of MT subunits. Treating cells with colchicine, which binds specifically to free αβ subunits and prevents them from polymerizing, results in the disappearance of MT and the appearance of colchicine-bound free subunits. The fact that simply binding to free subunits can cause the loss of existing MT indicates that MT are continuously losing and gaining subunits, so that colchicine does not actively break down MT but rather capitalizes on the fact that they constantly decompose by themselves and prevents them from rebuilding. Due to its capacity to block polymerization, colchicine is an inhibitor of cell division and has been used as a form of chemotherapy.

In contrast, taxol stabilizes MT by preventing depolymerization, but it too prevents cell division and has been used for chemotherapy. Thus, cell division requires both polymerization and depolymerization.

E. Dynamic instability and GTP hydrolysis  Unlike actin filaments, which maintain a constant length at steady-state, individual microtubules at steady-state appear to shrink and grow dynamically; a period of assembly (growth) may be followed by a catastrophic disassembly, which is just as abruptly reversed by renewed growth. This constant shrinking and growing is known as dynamic instability and can be explained in terms of GTP hydrolysis (see MBOC p. 810, Fig. 16-33 and V&V p. 1254-55). When an αβ dimer is free in solution, both the α and β units are bound to GTP, but when the dimer is incorporated into an MT polymer, the GTP on the β-subunit is hydrolyzed. The rate of subunit dissociation is higher for the GDP-bound state, but the rate of new GTP-bound subunit addition is generally greater than the rate of hydrolysis. As a result, a subunit doesn’t have enough time to hydrolyze its GTP and fall off before the next subunit adds, so by the time it does hydrolyze its GTP its escape route is blocked. Thus a long line of GDP-bound subunits accumulates with GTP-bound subunits only at the growing end. If a GDP-bound subunit does appear at the growing end, it can fall off and doing so clears the way for many more GDP-bound subunits to follow. Each tubulin must exchange GDP for GTP before it can reassociate with the polymer.

II. Movement

A key function of MT is that they serve as a catalyst for movement. This role is observed in nerve cells, in which MT in axons serve as tracks for the transport of...
material between the cell body and the axon terminus. The vesicles in which material is transported travel in both directions, and two MT-associated motor proteins, kinesin and cellular dynein, have been shown to be instrumental in this directed movement.

As illustrated in MBOC p. 814 Fig. 16-37A, kinesin consists of a collection of light chains, two 150 kD heavy chains each with a globular head that has ATPase activity and interacts with the MT. Interaction with MT both stimulates kinesin’s ATPase activity and enables kinesin to walk. In vitro motility assays demonstrate that kinesin walks specifically towards the (+) end of the MT, although other forms of kinesin walk toward the (-) end.

Dynein, as shown in MBOC p. 814 Fig. 16-37A, is composed of 10 - 20 light chains, a 400-500 kD heavy chain, and an amino terminal globular head with ATPase activity. Like kinesin, dynein binds tightly and reversibly to MT, but in contrast to the type of kinesin discussed, it walks towards the (-) end. In both cases, the light chains are probably involved in selecting and transporting the cargo.

Walking mechanism  Walking along a MT is a different concept than actin-myosin movement. The goal is to move a vesicle along the MT in a specified direction, and it appears that the two heads of a kinesin molecule walk along the microtubule so that when one head is attached, the other head is free. The key issue is to guarantee that the kinesin head that is not attached to a tubulin molecule reattaches in the direction of movement. Experiments with light traps like those used in actin-myosin studies indicate that each kinesin head steps 5 - 10 nm per ATP molecule hydrolyzed; there is no lever arm and no power stroke. The mechanism by which kinesin and dynein convert the energy of ATP into vectorial movement through diffusion alone remains unclear.

It turns out that, despite the differences between the two systems, the actin-myosin system can also move vesicles; certain types of myosin (I and V) have short tails that bind to vesicles, and they carry these vesicles with them as they walk up and down actin filaments.

So, in summary, we have seen that cells can move themselves by the force of the polymerization of actin pushing against their plasma membrane, and that, inside cells, vesicles can be transported around on tracks of tubulin or actin by the activity of motor proteins such as kinesin, dynein, different types of myosin. Additionally, microtubules are capable of moving chromosomes.