IV. Microtubules (part 1)

1. **Cellular distribution** (Handout, p.2) In cultured fibroblasts, microtubules are arranged in a dynamic array of long fibers which radiate out from the centrosome to fill the entire cytosol. In mitotic cells, the cytosolic array of MTs reorganizes to form the mitotic spindle apparatus.

2. **Tubulin structure, and structure of microtubules** (figs. 20-3, 20-6, 20-7; handout p.3) Tubulin is the subunit protein of microtubules. Tubulin consists of two polypeptide chains: α-tubulin & β-tubulin (α-tubulin: non-exchangeable GTP; β-tubulin: hydrolyzable, exchangeable GTP). Tubulin polymerizes end to end to form protofilaments; 13 protofilaments align side to side to form a microtubule. Microtubules have a (+) end and a (-) end; the (+) end polymerizes more rapidly than the (-) end.

3. Microtubules (MT’s) form a diverse array of both permanent and transient structures (fig 20-14, p 826; handout p.4). In almost all of these structures, (-) ends are imbedded in the centrosome (or in the case of cilia and flagella, a “basal body”. In the case of axons, the (-) end is oriented towards the cell body, and “plus” ends are oriented away from cell center.) *(Exception: mixed orientation in neuron dendrites.)*

4. **Stable microtubules** (cilia/flagella, axons), versus **labile microtubules** (mitotic spindle, interphase array of cytoplasmic microtubules): carboxy-terminal tyrosine of α-tubulin is removed in stable microtubules (centrioles, cilia) (“detyrosination”); in *Chlamydomonas*, a specific lysine on the α-tubulin in cilia microtubules is also acetylated. [Handout, p.5]

5. Microtubule polymerization is entropy driven: microtubules are cold-sensitive. (Handout, p.6; see also fig 20-5, p.820).

6. Drugs affecting MTs: **colchicine (colcemid)**, **nocodazole** [destabilize] & **taxol** [stabilizes] (Handout, p.7). Labile MT’s are more susceptible to these drugs, and to cold temperature.

7. The **centrosome** (also known as **Microtubule Organizing Center:** “MOC”): (usually, but not always, contains centrioles) (fig. 5-33, p.178; fig 20-13, p. 826) (always contains γ-tubulin, in the form of a γ-tubulin ring complex) (Handout pp.8, 9: Nature 378, 555, 583)


9. Microtubule-associated proteins: **Stabilizing** (MAP-1, MAP-2, MAP-4 & Tau) and **Destabilizing** (Katanin, Stathmin). MAPs bind along side of MT’s, and can govern binding of MT’s to each other or to intermediate filaments. Binding of MAPs affects kinetics of assembly/disassembly; phosphorylation of MAPs inhibits their binding to MTs: MAP Kinase, cdc2 kinase. Phosphorylation of MAP-4 is involved in formation of the mitotic spindle. (Table 20-1, p824; handout, p.12)

   Neurons: MAP-2 in cell body, dendrites; tau in axons.

   *(Inject antisense tau mRNA into neurons: axons collapse; inject tau into fibroblasts: they form “axons”.*
Cellular distribution of microtubules:

Mitotic cell (red: metaphase chromosomes)  

Interphase cell:

Centrosome (Microtubule organizing center) ("MTOC")

In an interphase cell (G2, S, G2) microtubules (MT's) are arranged in a dynamic array of long fibers that radiate out from the centrosome to fill the entire cytosol.

In mitotic cells ("M phase") (upper left insert), the cytosolic array of MTs reorganizes to form the mitotic spindle apparatus.
Structure of tubulin and its organization in microtubules:

Tubulin = $\alpha + \beta$ polypeptides. ($\alpha$ & $\beta$ bind tightly to each other, don’t come apart; act as a single unit.)

Fig 20-3 (5th Ed.) “Tubulin”

Tubulin has a (+) end and a (-) end. Subunits come on and off at both ends, but at concentrations of tubulin exceeding the critical concentration, net addition of tubulin onto the (+) end is more rapid than at the (-) end.

Fig 20-7 (5th Ed.)

In vitro experiment, showing that tubulin adds more rapidly to (+) end than to (-) end

Fig 18-9 (6th Ed.)

This figure shows the seam along the length of the microtubule:

微体 = 13 protofilaments aligned side by side, slightly staggered upward

Fig 18-3 (b) (6th Ed.)
Orientation of cellular microtubules:

**Interphase animal cell**
- Basal body
- Flagellum or cilium
- Nucleus
- Centriole
- MTOC

**Mitotic animal cell**
- Chromosome
- MTOC
- Spindle microtubules
- Centriole

**Nerve cell**
- Dendrite
- Axon
- Nucleus
- Cell body
- MTOC
Enzymes that de-acetylate α-tubulin, and add back tyrosine are located in cytoplasm.

Centrioles, and some stable cytoplasmic MT's are also de-tyrosinated.

Chlamydomonas

Microtubules of cillum or flagellum

Centrioles

Microtubules of cytoskeleton

Pericentriolar regions

α-tubulin de-tyrosinated

tubulin acetyltransferase acetylates a specific lysine on α-tubulin in cilia in Chlamydomonas.
Microtubule polymerization is entropy-driven

\[ \Delta G = \Delta H - T \Delta S \]

(a) Considering only tubulin:

- **tubulin monomers**
- **polymerized tubulin (microtubule)**

(b) Considering the solvent (H₂O)

Hydrophobic interaction between two protein faces results in the release of bound, ice-like water of hydration

(c) Net change in entropy

- **increase temp**
- **decrease temp**

Net change in entropy:

- more ordered (whole system)
  - (low $S$)
- less ordered (whole system)
  - (high $S$)
Consequence of the entropy-driven polymerization of microtubules:

**Microtubules depolymerize at cold temperature**

(Consider ΔG for the polymerization of tubulin:

\[ ΔG = ΔH - TΔS \]

At both cold and warm temperatures, the ΔH term has a small value, and is positive (ie, endothermic). At warm temperatures the [TΔS] term will exceed the value of the ΔH term, so ΔG will be negative; ie, at warm temperatures, polymerization is favored; at cold temperatures, the [TΔS] term will be less than the value of the ΔH term. Therefore, at cold temperatures, the value of ΔG for the polymerization will be positive; ie, polymerization is not favored, and instead, depolymerization is favored.)

Temperature affects whether microtubules (MTs) assemble or disassemble. At low temperatures, microtubules depolymerize, releasing tubulin, which repolymerizes at warm temperatures in the presence of GTP.
Two well-known drugs that affect microtubules are Colchicine and Taxol

At high concentrations, colchicine (and a synthetic derivative, colcemide) causes depolymerization of microtubules; at low concentrations, it "freezes" microtubules. When added at low concentrations to growing cells, cells accumulate in M phase, where they are blocked in metaphase. (After washing away the colchicines, cells resume mitosis.)

Colchicine (also: colcemid, nocodazole) "vinca alkaloids" (vinblastin)

Taxol has the opposite effect: it hyper-stabilizes microtubules, driving the equilibrium in the cell such that all tubulin in polymerized, and dynamic instability is lost. This is just as bad for the events of mitosis as MT depolymerization or "freezing" caused by colchicines. Taxol has proved to be a very effective anti-cancer drug.
Our cells are shaped and supported by a cytoskeleton of interlocking protein filaments. A beautiful star of microtubules, the largest of these filaments, radiates outward from the center of the cell to the cell surface. This “aster” of microtubules is the railway system of the cell. Many types of cargo are carried along these rails. The endoplasmic reticulum is pulled by molecular motor proteins along microtubules, spreading it evenly throughout the cell. Vesicles are delivered to their destinations along microtubules. And, when cells divide, the most valuable cargo of the cell is carried by microtubules. Paired copies of each of the chromosomes are attached to the ends of a doubled microtubule aster and carefully separated into the two daughter cells.

The cytoskeleton, in contrast to our articulated skeleton of bones, is a dynamic structure. It is continually constructed and demolished according to the shifting needs of the cell. A typical microtubule lasts for only 10 minutes before it is disassembled and the parts used to build a new one. Microtubules are nucleated in the “microtubule-organizing center,” at the center of the cell, and then extended one piece at a time into the cytoplasm. Growth proceeds in fits and starts in a process known as “dynamic instability.” Tubules grow slowly and steadily, but are punctuated by periods of rapid disassembly, when large regions peel away from the ends. A small portion of the microtubule may break up, or the fragmentation may extend all the way back to the start, completely destroying the tubule. In special cases, such as the microtubules that support the long axons and dendrites of nerve cells, auxiliary proteins stabilize the microtubule for longer periods of time. But the dynamic structure of microtubules is essential for their everyday function in transport and cell division.

Essential functions make excellent targets for toxins and for cancer chemotherapy. The central role of microtubules in cell division makes them particularly attractive. Many organisms have developed toxins that block the dynamic instability of microtubules, thus blocking the ability of the cell to divide. Two types of natural plant toxins are widely used in medicine. They have the identical result of blocking division, but achieve it in exactly opposite ways. The vinca alkaloids, such as vinblastine, vincristine, and vinorelbine, bind to the end of growing microtubules, blocking the addition of more tubulin dimers. The tubule cannot grow, but it can still disassemble, so the microtubules ultimately break down into nothing. Alternatively, the taxanes, Paclitaxel (in green) binds to beta-tubulin on the inner surface, stabilizing the microtubule and blocking the normal dynamics of assembly and disassembly. Atomic coordinates were taken from entry 1tub at the Protein Data Bank.

Figure 1. Microtubule structure. Microtubules are composed of two similar proteins: alpha-tubulin (in blue) and beta-tubulin (in pink). Heterodimers of one alpha and one beta subunit assemble into a sturdy cylindrical tube.
such as paclitaxel and docetaxel, stabilize microtubules, blocking the disassembly process. When treated with taxanes, cells are choked with large numbers of spurious asters forming throughout the cytoplasm.

Both the vinca alkaloids and the taxanes are large molecules with complex chemistry. The multidrug transporter (discussed in this column in The Oncologist 1999;4:428-429) is designed with these types of molecule in mind, so drug resistance can be a problem. Cancer cells overproduce the transporter and pump all of the drug outside, protecting their delicate flower of cell division from harm.

**ADDITIONAL READING:**


David S. Goodsell: A Macrophage Engulfing a Bacterium
http://mgl.scripps.edu/people/goodsell/

Nucleus

Nuclear pore (Green)

Ribosomes (Pink)

Microtubule (Blue)

Actin filaments (Blue)

The Macrophage

The Bacterium

Blood
The centrosome (microtubule organizing center) contains a pair of orthogonal centrioles, and many $\gamma$-tubulin ring complexes imbedded in an amorphous pericentriolar matrix:

Fig 5-33

Fig 20-13

Centrosome

Centriole pair

Fig 16-23 (Alberts et al, Molec Biol Cell, 4th ed.)

nucleating sites ($\gamma$-tubulin ring complexes)

pair of centrioles

+ microtubules growing from $\gamma$-tubulin ring complexes of the centrosome

(A) (B)

Figure 16-23. Molecular Biology of the Cell, 4th Edition.
tubulin (Fig. 3b) nucleation induced by we have done previously presence of purified We found that tubules at a threshold, which is effective for purified (between Xenopus) suggests that the be similar to that in PCM and is recentrosome, we have nucleating material. It is possible that add γTuRC and connect the centrosome.

Our proposal is that throughout the rings have a diameter, the rings that they disappear are to nucleate the growth, staining lin is located at to be expected if the PCM.

The structure nucleates microtubule (25–28 nm) is similar (25 nm). Second helical turn, allows a three-start helix. It is thought that there are monomers for example that exist. A model for excitement proteins (p195, framework of α/β tubulin

FIG. 6 Seeded nucleation model for microtubule growth. The α/β tubulin dimers bind to the γTuRC and are stabilized by interaction with γ-tubulins. These assembled tubulin molecules act as a seed to nucleate further microtubule assembly, which determines the protofilament number.
Microtubules exhibit “dynamic instability” in cells:

Fig 20-10: Fluorescently labeled MT’s in a living cell; time intervals

Fig 16-11 (Alberts et al, Molec. Biol. of Cell)

Fig 20-9

globulin G), was found to be conformation-specific. It did not recognize denatured tubulin by immunoblotting and seemed not to bind to native nonpolymerized tubulin. However, hMB11 cosedimented specifically with microtubules polymerized in the presence of guanylyl 5′-(β,γ-methylene-diphosphonate) (GMPCPP), a nonhydrolyzable GTP analog, and not with control microtubules assembled in the presence of GTP (Fig. 1A). In this experiment, low concentrations of taxol (0.1 to 1 μM) were used to prevent depolymerization of control microtubules. When a higher concentration of taxol was used, hMB11 bound to both control and GMPCPP microtubules (Fig. 1, B and C), which suggests that it recognized a conformation and not the nucleotide itself.

We then used hMB11 to stain by immunofluorescence a mixture of microtubules polymerized from pure tubulin in the presence of GTP or GMPCPP (Fig. 1D). Under these conditions, hMB11 stained only GMPCPP microtubules [representing 68.6 ± 17.3% (SD) of MB11-positive microtubules] and not control microtubules (1.8 ± 0.9%). The remaining 29.7 ± 16.6% were bundles of both GMPCPP and control microtubules. Despite varying experimental conditions, not all GMPCPP-containing microtubules were stained by MB11, which suggests that only some microtubules possessed conformational defects under these conditions.

**Detection of tubulin in GTP conformation in cellular microtubules.** We next used hMB11 to localize GTP-tubulin in cellular microtubules by immunofluorescence. Because of its conformational binding, hMB11 staining was very sensitive to structural alterations occurring after fixation (10). It was best to use unfixed cells permeabilized in the presence of glycerol and/or low taxol concentration to prevent microtubule depolymerization. In three representative cell lines (HeLa, Ptk2, and MDA-

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**Fig. 4.** A GTP-remnant model for microtubule dynamic instability. (A) Model for microtubule dynamics showing GTP-tubulin (red) in a GTP cap during polymerization (P) and in inner microtubule regions. Upon cap loss, the probability of catastrophe (C) increases and the microtubule depolymerizes (D) until its end reaches a GTP-tubulin remnant. A GTP end is restored and the probability of microtubule polymerization increases, allowing its rescue (R). (B) Ptk2 cells stably expressing GFP-tubulin were imaged at the indicated times. Rescue events (colored arrows) and the tip of a growing microtubule (arrowhead) are indicated. After cytosol extraction, cells were stained with hMB11 (red) and imaged again, often showing GTP-tubulin remnants at rescue locations. Scale bar, 10 μm. The two kymographs show the dynamics of the microtubules highlighted in red and yellow (top) aligned with hMB11 staining (bottom). Note the good coincidence of rescue position and GTP remnants. (C) Quantification of experiments done as in (A), showing the proportion of polymerizing microtubules stained by hMB11 at their plus ends and the proportions of GTP-tubulin remnants that colocalized with rescue locations in Ptk2 cells (means ± SEM). The proportion that would be expected in stochastic conditions is shown for reference at the right (Monte Carlo simulation, table S1) (9). The table shows that the rescue frequency varies with the distribution of GTP-tubulin remnants (means ± SEM, comparison of Ptk2 and RPE1 cells) (9) (table S1).
GTP cap model for dynamic instability:

Figure 6. GTP Cap Model for Dynamic Instability
Microtubules with a cap of GTP-containing subunits, denoted by T, are shown growing slowly by the addition of T-containing subunits. The free T subunits are shown to be in equilibrium with the T-containing end, with a very low $K_{eq}$. At some probability, GTP hydrolysis catches up with assembly, the T cap disappears, and the polymer transits to the "rapid shrinking" phase (at D) shown at the bottom. This polymer rapidly loses subunits from its end (high $K_{eq}$). The GDP-containing subunits that are released, denoted by D, exchange with free GTP in solution and form T subunits. It is not known whether T subunits can add to a depolymerizing end to recap the shrinking polymer.

IIow cells might use dynamic instability to orient the microtubule cytoskeleton:

Figure 11. Morphogenesis by Selective Stabilization
In (A), we depict an unpolarized cell with microtubules that are growing out with no preferred direction and that are spontaneously shortening by dynamic instability. In (B), a local extracellular signal activates some capping structures near the cell periphery. In (C), the selective stabilization of these capping structures gradually leads to a reorientation of the microtubule arrays. In (D), the polarization is complete, with capped microtubules much less dynamic than unpolarized microtubules. Here we also show chemical modification of the less dynamic microtubules (as indicated by the dots).
Microtubule Associated Proteins (MAPs):

Fig 20-12 Sf9 insect cells expressing either MAP2 (left) or Tau (right).

(b) Microtubule

MAP2

Tau

25 nm

Fig 16-31 (Alberts et al, 4th Ed)

stathmin

free tubulin
subunit addition stops
hydrolysis
tubulin subunit
pool shrinks
GTP
hydrolysis
catches up

Microtubule shrinks

Figure 16-31. Molecular Biology of the Cell, 4th Edition.

Fig 16-32 (Alberts et al, 4th Ed.)

MAP2 (orange) in dendrites + cell body

10 μm

Figure 16-32. Molecular Biology of the Cell, 4th Edition.

Tau (green staining) is confined to the axon of a hippocampal neuron (long branched structure on the left), whereas MAP2 staining (orange) is confined to the cell body and to dendrites.

Neurons: block Tau expression, inhibit axon formation

Fibroblasts: express Tau - grow axons!

(Kinesin-13 also promotes disassembly)
Although this review will focus on the mechanism of plus-end tracking and the regulation of plus-end dynamics, it is important to note that there has also been significant recent progress in identifying +TIP-interacting partners and in defining potential mechanisms of plus-end linkage to other cellular structures [15–22,28]. These studies on attachment highlight the important role of +TIPs in coordinating actin and microtubule-dependent processes. They also reveal new interactions important for cell division (such as the interaction between microtubules and the neck separating mother and daughter cells in budding yeast) [23–25]. We refer readers to the cited papers and to several excellent recent reviews for further information [26–29].

Mechanisms for plus-end tracking

Treadmilling

Plus-end tracking was first discovered by live-cell imaging of a fusion between the microtubule-associated protein (MAP) CLIP-170 and the green-fluorescent protein (GFP) [30]. CLIP-170 was originally identified as a nucleotide-sensitive microtubule-binding protein in HeLa cells and was subsequently shown to link microtubules to endocytic vesicles in vitro [34,35]. A technique called fluorescent speckle microscopy (FSM) [31] demonstrated that the plus-end tracking of CLIP-170 is mediated by a treadmilling mechanism (Fig. 2). CLIP-170 molecules appear to be added to the plus-ends of growing microtubules, but shortly thereafter, these molecules dissociate behind the region of new growth. This parade of proteins coming on and then falling off the microtubule end creates an optical illusion: although individual CLIP-170 molecules are stationary, the population of CLIP-170 molecules appears to surf on the growing ends of microtubules as they rocket through the cytoplasm.

Although CLIP-170 was recently found to have a number of binding partners, we suspect that plus-end tracking is an intrinsic property of the protein, based on the following observations [18,32–34]. All CLIP-170 family members (CLIPs) tested so far plus-end track [30,36–38]. The CLIP proteins contain one or more conserved microtubule-binding domains, called the CAP-Gly domain at their N-termini. The structure of the CAP-Gly domain was recently solved and shown to have a unique fold containing three β sheets [39]. The CAP-Gly domain is followed by an α-helical domain and then in some CLIPs by a C-terminal ‘cargo-binding domain’ containing one or more signature zinc-binding motifs (CCHC motifs) [40]. CLIP-170 forms a long extended homodimer arranged in a parallel orientation [41]. Deletion analysis revealed that short CLIP-170 fragments containing little more than the CAP-Gly domain can plus-end track when introduced into cells [30]. Additionally, the CAP-Gly domain is not only found in CLIPs but also in the p150Glued subunit of dynactin (a complex that regulates the motor cytoplasmic dynein), in some tubulin folding factors and in one member of the kinesin motor superfamily [39]. p150Glued was also recently shown to be a genuine +TIP by live-cell imaging, and again a small fragment containing the CAP-Gly domain could plus-end track when introduced into cells [42]. Perhaps the CAP-Gly domain represents the bona fide module for direct plus-end tracking; however, in vitro reconstitution will be needed to definitively rule out an intermediary between the CAP-Gly domain and the polymerizing microtubule end.

Although treadmilling is well described, the underlying mechanisms remain poorly understood. In principle, treadmilling could result from a higher affinity of +TIPs for the microtubule end relative to the microtubule wall, from a faster dissociation (release) from the microtubule wall than from the end, or both (Fig. 3). The most favored mechanism for selective release from the microtubule wall is phosphorylation, which is known to inhibit the binding of CLIP-170 and p150Glued to microtubules [42,43]. Proposed mechanisms for higher-affinity plus-end binding include binding to the GTP cap and recognition of a unique structural feature of the growing plus-end [44]. The fact that all +TIPs can bind along the length of microtubules when overexpressed highlights the delicate balance between binding and release required for selective plus-end accumulation.

Treadmilling as a mechanism for plus-end tracking: evidence for phosphorylation-dependent selective release from the microtubule wall

For CLIP-170, this delicate balance appears to be achieved by the combination of co-assembly of CLIP-170 with...