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CELL BIOLOGY

THE CYTOSKELETON

Topic: Microtubules (MTs)

For

Subject Zoology

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M.Sc. I Sem

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THE CYTOSKELETON

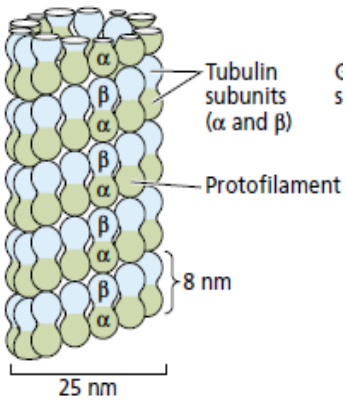
- The cytosol is organized into a three-dimensional network of filamentous proteins called the **cytoskeleton**. This network provides the spatial organization for the organelles and serves as a scaffolding for the **movements of other organelles and cytoskeletal components**.
- It also plays fundamental roles in **mitosis, meiosis, cytokinesis, wall deposition, the maintenance of cell shape, and cell differentiation**.

➢ **Microtubules, Microfilaments, and Intermediate Filaments**

- Three types of cytoskeletal elements have been demonstrated: microtubules, microfilaments, and intermediate filament–like structures.
- Each type is filamentous, having a fixed diameter and a variable length, up to many micrometers.
- Microtubules and microfilaments are macromolecular assemblies of globular proteins.

Microtubules (MTs)

1. Microtubules are **unbranched hollow submicroscopic cylinders** with an outer diameter of **25 nm and** can grow **longer than 20 µm in cells**; they are composed of polymers of the protein **tubulin**. The tubulin monomer of microtubules is **a heterodimer** composed of two similar polypeptide chains (**α- and β-tubulin**), each having an apparent molecular mass of 55,000 daltons. **A single microtubule** consists of **hundreds of thousands of tubulin monomers** arranged **in 13 columns** called ***protofilaments*** (in following figure).



- Microtubules provide support for a variety of **cellular structures** and tracks **for movements** powered by **motor proteins** called **kinesins** and **dyneins**.

- Microtubules help **organize the cytoplasm during interphase** and form the **mitotic spindle**, which separates duplicated chromosomes.
- The head-to-tail arrangement of **dimers of α-tubulin** and **β-tubulin** in the walls of microtubules give the **polymer a molecular polarity**. The **"plus" (β-tubulin) end** grows faster than the **"minus" (α-tubulin) end.**
- A simplifying principle is that **microtubule organizing centers (MTOCs)** containing **γ-tubulin** nucleate and anchor the minus ends of many microtubules.
- The **centrosome** is the **main MTOC** in **many animal cells**, so the microtubules are arranged radially with plus ends in the periphery. Some of these radially arranged microtubules are associated with the nearby **Golgi apparatus**. However, **microtubules are not arranged radially** in **neurons, muscles, and many epithelial cells**.
- Microtubules in polarized epithelial cells originate from a broad zone with **γ-tubulin** near the apical surface and extend the length of the cell.
- Microtubules in **dendrites of nerve cells** have **mixed orientations**.
- **Most plant cells** lack **centrioles** and **centrosomes**.
- During interphase, **γ-tubulin** and **microtubules** are found throughout the cortex, and they form **a bipolar mitotic spindle** during **cell division**.
- The centrosome consists of **a pair of centrioles** and a surrounding matrix containing the **active component in microtubule nucleation**- a complex of proteins including the specialized tubulin isoform **γ-tubulin.**
- The **centrosome duplicates during interphase**, and as cells enter mitosis the two centrosomes separate to form the **poles of the mitotic spindle**.
- **Animal cells and protozoa with cilia and flagella use centrioles** as **basal bodies** to nucleate the **assembly of microtubules for their motile structure, called an axoneme**.
- Microtubules in **fungi** grow from a **spindle pole body**, an organizing center containing **γ-tubulin associated with the nuclear envelope**.
- Drugs that **depolymerize or stabilize microtubules** are useful for exploring their functions in cells and in cancer therapy.

Pharmacologic Tools for Studying Tubulin and Microtubules (Microtubules-specific drugs):

- Tubulin subunits bind several therapeutically active plant alkaloids and synthetic chemicals, including two of the most successful drugs used to treat cancer.
- **1. *Vinblastine*** (from periwinkle) and ***vincristine***, **microtubule-depolymerizing drugs,** interferes with microtubule dynamics by **binding** between **tubulin dimers at the ends of microtubules.** Their bindings prevent **polymerization of microtubules or inhibit microtubule assembly** and ultimately **inhibit mitosis** or **prevent spindle formation during cell division**.
- **2. *Colchicine*** (from the autumn crocus), ***colcemid*** and ***nocodazole*** **(a synthetic chemical), microtubule-depolymerizing chemicals, inhibit microtubule assembly** or **prevent polymerization of microtubules** by binding dissociated tubulin dimers or tubulin subunits. Colchicine binds dimers between the two subunits, **stabilizing a bent conformation** that cannot fit into the microtubule lattice. Colchicine is used **empirically to treat gout**, a painful condition that results from the accumulation of uric acid crystals in joints and other tissues, but no one knows precisely how it works or why it is not more toxic.
- **3. *Taxol*** (from the bark of the Western yew), **a microtubule-polymerizing drug,** binds **β-tubulin** and **stabilizes microtubules**. **It inhibits microtubule disassembly or activates/enhances the assembly of microtubules.**
- At sub-stoichiometric concentrations, ***vinblastine***, ***vincristine*** and ***Taxol*** are effective in **cancer chemotherapy** because they **interfere with the dynamic instability of mitotic spindle microtubules and block cell division**. They preferentially kill dividing cells, since both **microtubule assembly and disassembly are crucial for correct function of the mitotic spindle**.

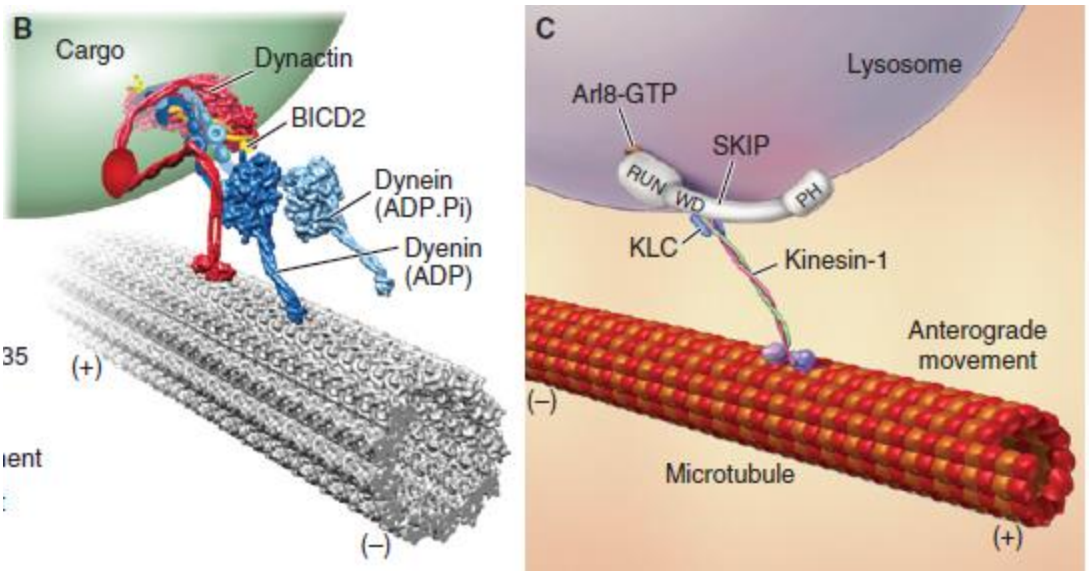
Kinesin and Dynein powered movements:

1. Organelle movements in animals:

- Organelles in most eukaryotic cells can move along linear microtubule tracks at relatively high velocities, on the order of 1 μm/s.
- In cells with a radial arrangement of microtubules (Fig. 37.1) organelles and other cargos associated with **kinesin-1** move toward microtubule **plus ends** located at the periphery of cells. Cargo associated with **dynein** moves in the opposite direction, **toward minus ends** of microtubules.
- Intra-flagellar transport (IFT) of proteins in cilia and flagella (see Fig. 38.18) shares many features with the movements of organelles.

System	Velocity (μm s^{-1})	Mechanism
Microtubule Motors		
Anterograde fast axonal transport, squid	1	Individual kinesin motors
Retrograde fast axonal transport, squid	2	Individual dynein motors
Chromosome movement in anaphase of mitosis	0.003-0.2	Motors plus depolymerization
Endoplasmic reticulum sliding, newt cell	0.1	Individual kinesin motors
Slow axonal transport, rat nerves	0.002-0.1 net 1 (intermittent)	Motors on microtubules
Microtubule Polymerization		
Endoplasmic reticulum tip elongation, Newt cell	0.1	Microtubule polymerization

- **Kinesin-1** favors **acetylated microtubules** and **CENP-E** favors **tyrosinated microtubules** in the center of the **mitotic spindle**.
- In addition, **microtubule-associated proteins**, such as **tau**, can influence the choice of tracks by reducing the run lengths of motors under some circumstances.
- ATTACHMENT OF CYTOPLASMIC MICROTUBULE MOTORS TO MEMBRANES:

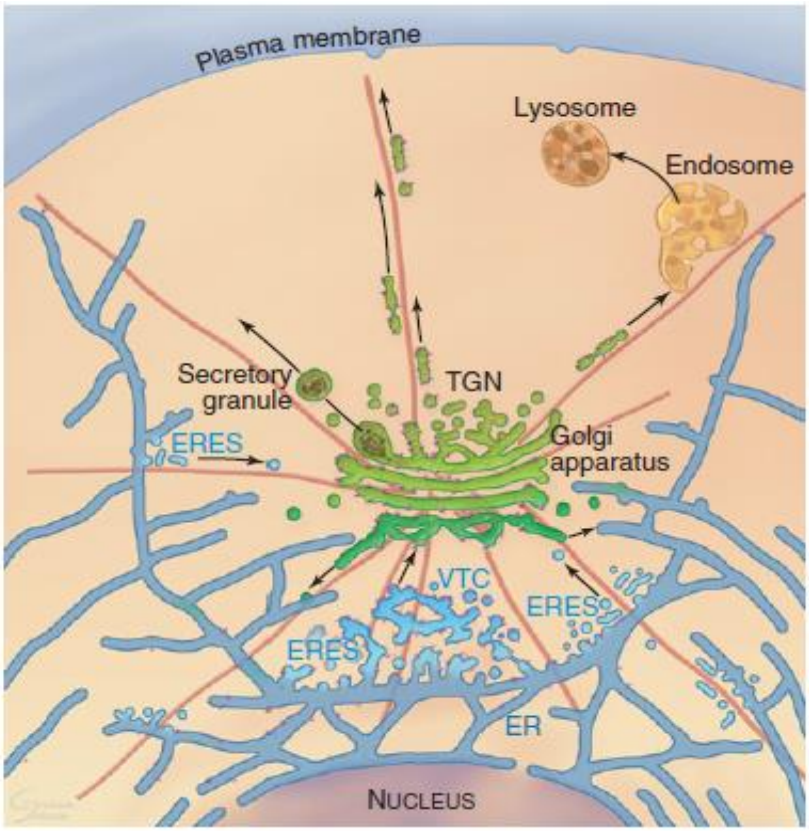


- **B.** Drawing showing **dynein** linked to a **vesicle** by **dynactin complex** and moving toward the minus end of a microtubule.
- **C.** Coupling of kinesin-1 to a **lysosome.** Guanosine triphosphatase (GTPase) Arl8 on the lysosome membrane engages the adapter protein SKIP (SifA-kinesin interacting protein), which binds to the kinesin light chain.
- **Tools for Studying Motor Proteins:**
- A small compound named **monastrol** inhibits **kinesin-5**, resulting in monopolar mitotic spindles that **fail to segregate the chromosomes**. Higher-affinity inhibitors of kinesin-5 are being tested for cancer therapy.
- The small molecule **blebbistatin** inhibits cytoplasmic and skeletal muscle myosin-II (but not smooth muscle myosin-II) and **blocks cytokinesis**.
- **Vanadate and ultraviolet light** can inactivate **dynein**. Vanadate binds to the γ-phosphate site of dynein–adenosine diphosphate (ADP), but it binds similarly to other adenosine tri-phosphatases (ATPase), so it is not specific.
- However, ultraviolet light has a novel effect on the dynein–ADP–vanadate complex: It cleaves and inactivates the dynein heavy chain.
- **Fast Axonal Transport:**
- Living axons reveals that most membrane-bound organelles move either toward (anterograde) or away from (retrograde) the end of the axon (Fig. 37.3) with some pauses and even occasional changes of direction. **Retrograde movements** (2.5 μm/s or 22 cm/day) are faster than **anterograde movements** (0.5 μm/s or 4 cm/day).

- If a 0.1-μm vesicle were the size of a small car, it would move anterogradely at 50 miles per hour and retrogradely at 250 miles per hour.
- In the axons of vertebrate neurons, mitochondria move back and forth in both directions. Their net movement toward the nerve terminal or cell body depends on physiological conditions.
- **Plus-end directed motors of the kinesin family** are responsible for anterograde movements toward the **nerve terminal** and the **minus-end directed motor protein dynein** is responsible for movement in the **retrograde direction**.
- **<u>Other Microtubule-Dependent Movements:</u>**
- Other cells use the same molecular mechanisms as neurons to move organelles in the cytoplasm.

➢ **Transport of vesicles (from Golgi to plasma membrane):**

- **Secretory vesicles** use **plus–end motors (= kinesin**) to move from the **Golgi apparatus** to the **plasma membrane** (**in following figure**).



- **Endosomes** use minus-end motors (=**dynein)** to move from the plasma membrane toward the centrosome.
- The intracellular distributions of the endoplasmic reticulum (ER) depend on microtubules. Strands of the ER align with microtubules in cultured cells. This co-distribution is achieved in two ways:
- (a) Motors transport strands of ER bi-directionally on microtubules
- (b) Other strands of the ER attach to the plus end of microtubules and ride the microtubule tip as it grows and shrinks during dynamic instability. The +TIP EB1 interacts with transmembrane protein STIM1 to couple the ER to growing microtubules. STIM1 has another role in maintaining a supply of Ca^{2+} inside the ER. This is the best example of movement of an organelle driven by forces generated directly by microtubule assembly.
- Microtubules and motors also distribute other organelles inside cells. The concentration of the Golgi apparatus near the centrosome depends on microtubules, because **dynein** motors transport Golgi vesicles **toward the minus ends** of the microtubules nucleated at centrosomes.
- **Mitochondria** move **bi-directionally on microtubules** in animal cells but depend on **actin filaments/microfilaments** in **yeast**. Thus, not only the dynamics of the organelles but also the overall organization of a cell depend on microtubules and associated motors.
- As a result, **cellular architecture** is determined actively, not passively. The intracellular distribution of nucleoprotein complexes also depends on active movements. The most obvious example is the **movement of chromosomes during mitosis**, which depends on **microtubule assembly and microtubule motors** (see Fig. 44.7).
- Microtubules and motors can also produce **asymmetrical distributions of mRNAs** in cells. The adapter protein **La** links kinesins and dynein to specific ribonucleoprotein particles for long distance transport to sites of local protein synthesis (Fig. 37.7). For example, **localization of certain mRNAs in fly oocytes** helps establish the polarity of the embryos.
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- **Transport of mRNAs from cell body to terminal end of axon on microtubules (using Kinesin motor protein) in neurons:**

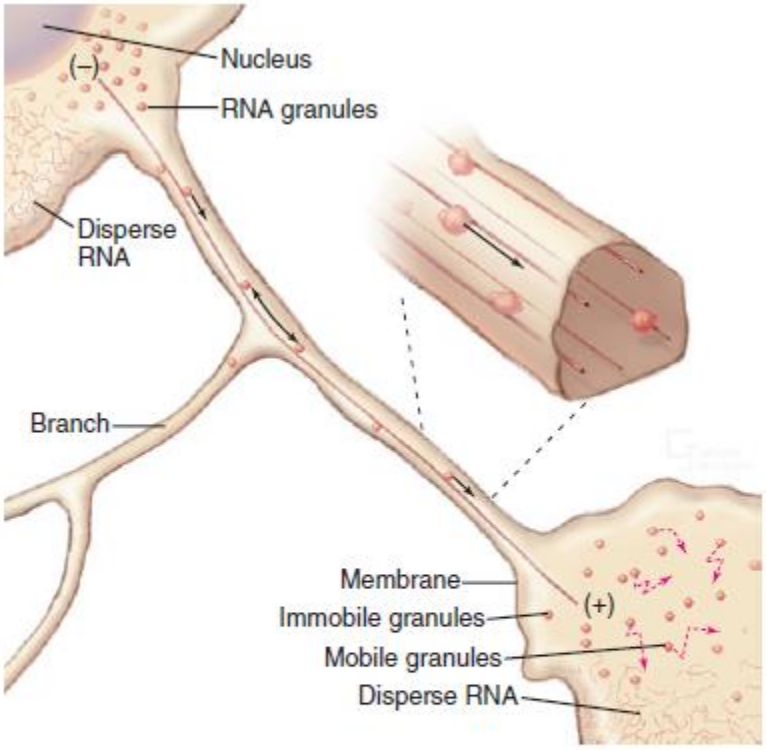

FIGURE 37.7 TRANSPORT OF MESSENGER RNA (mRNA) FOR MYELIN BASIC PROTEIN IN A CULTURED OLIGODENDROCYTE, A GLIAL CELL ISOLATED FROM BRAIN. mRNA synthesized in the cell body (or, in this case, labeled with a fluorescent dye and microinjected into the cell body) is packaged with proteins in a ribonucleoprotein particle, transported from the cell center along microtubules at a steady rate of 0.2 μm s^{-1}, and released at the periphery, where it moves randomly at 1 μm/s. (Modified from Ainger

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<u>Intracellular Movements Driven by Microtubule Polymerization:</u>

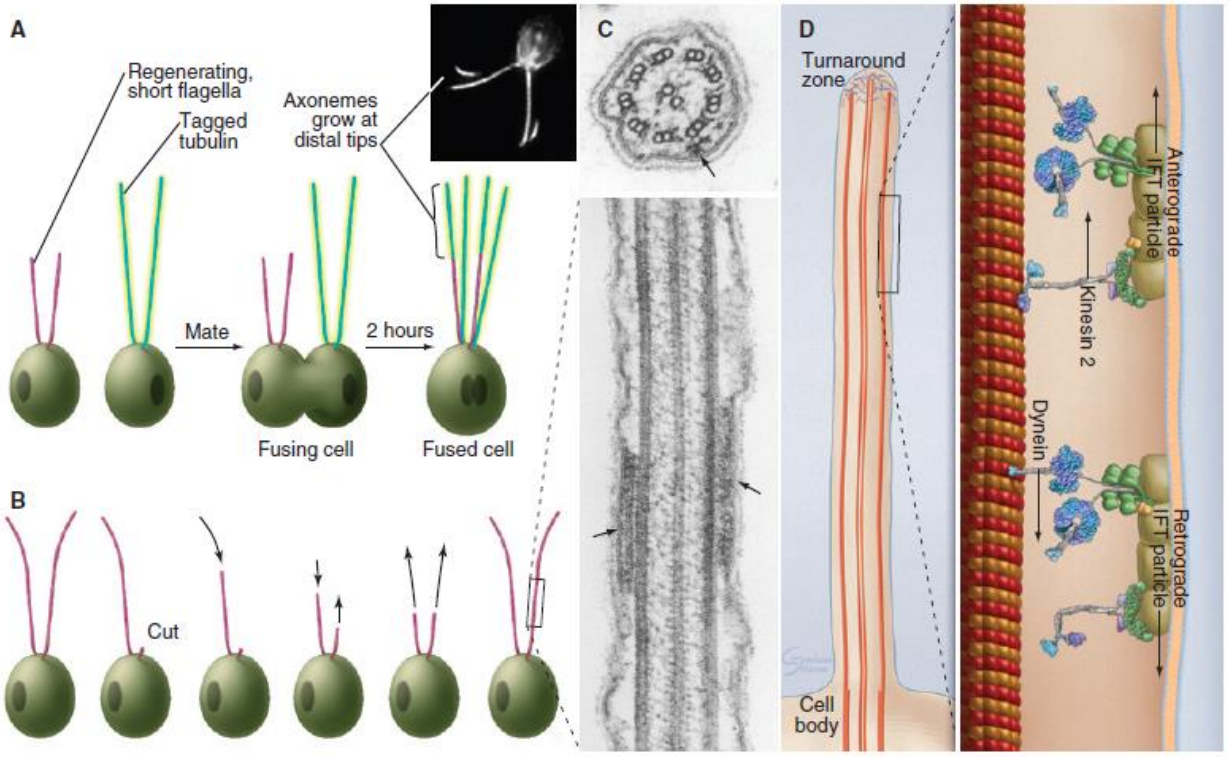
- Microtubule polymerization and de-polymerization have long been known to play a central role in the **assembly of the mitotic apparatus** and the movement of chromosomes (see Fig. 44.7), as well as the establishment of cellular asymmetry (see Fig. 34.2).
- Polymerizing microtubules can exert substantial forces, but strong forces buckle microtubules longer than approximately 10 μm. Consequently, microtubule pushing

mechanisms work best over short distances, such as positioning the ER (Fig. 37.6), the nucleus in fission yeast cells (see Fig. 6.3D) and the mitotic spindle in budding yeast cells (see Fig. 34.2D).

- Remarkably, the end of a ***depolymerizing*** **microtubule can also** ***pull*** on attached cargo. An in vitro proof of-principle experiment (Fig. 37.8) showed that chromosomes could ride on the end of a depolymerizing microtubule, even in the absence of ATP or GTP. Linker proteins associated with the kinetochore region of the chromosome make multiple weak bonds with the walls of the microtubule end (see Figs. 8.21 and 8.22). These interactions rearrange rapidly enough to maintain attachment, even as tubulin subunits dissociate from the end.
- **Transport of Nucleus in neurons**
- **Movement of Melanosomes (in fishes and amphibians) (use of both Kinesin and Dynein)**
- **Transport of Viruses, Vaccinia virus, Herpes virus and Rabies virus (from terminal end of axon to cell body of neuron)**
- **Transport of mRNAs (in Drosophila oocyte)**

2. Movements and cilia and flagella:

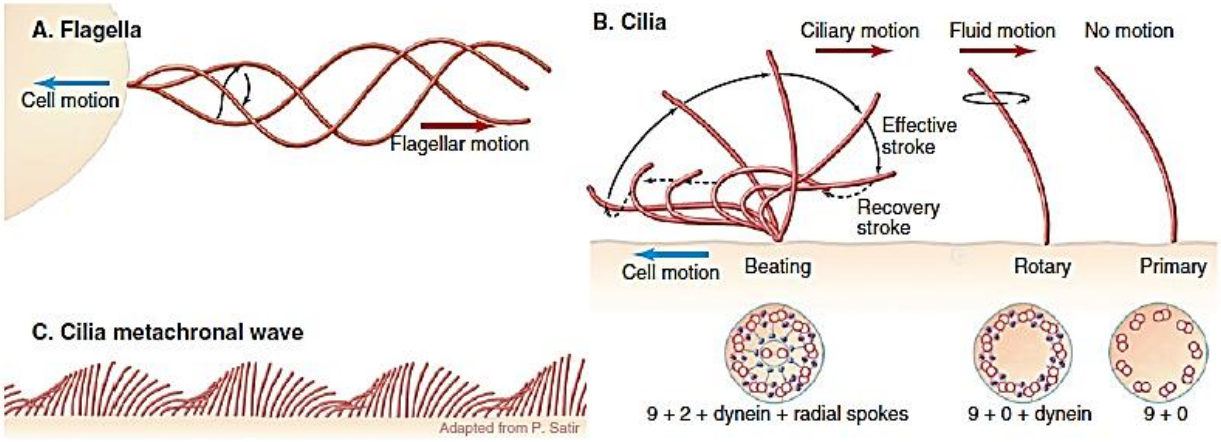
- **FLAGELLAR GROWTH AND INTRAFLAGELLAR TRANSPORT**- *Chlamydomonas* flagella showing intra-flagellar transport particles. D. Model for intra-flagellar transport (IFT).



- Axonemes grow at their tips by incorporation of subunits synthesized in the cytoplasm (Fig. A). A process called **intra-flagellar transport** (IFT) (Fig. C–D) carries individual proteins and subassemblies such as **radial spokes to the growing tip**. These cytoplasmic cargo proteins bind one of two IFT complexes, which associate 1:1 to form larger "trains" visible of electron microscopy (Fig. C).
- **Kinesin-2** moves IFT trains toward the tip of the axoneme, called **anterograde movement of IFT particles**, along the outer doublets just beneath the plasma membrane.
- Cytoplasmic **Dynein 1b** transports **IFT particles back toward the cell body, called retrograde movement**.
- A separate complex, called a **BBSome**, associates with IFT trains and transports transmembrane proteins (including signaling receptors) **bi-directionally** along microtubules of the underlying axoneme. Movements of transmembrane proteins allows

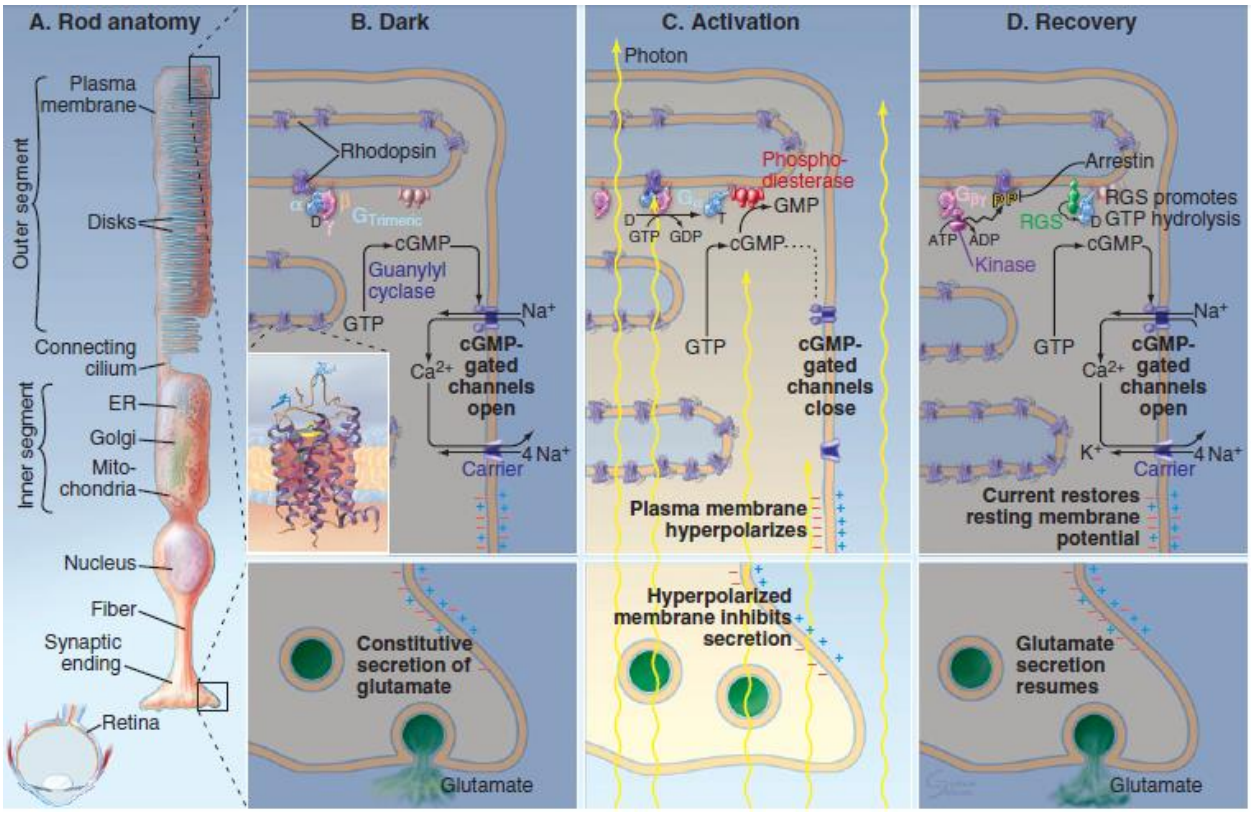
Chlamydomonas to glide on surfaces including the **flagellum of a partner cell during mating.**

- **IFT** is remarkably similar to **fast axonal transport** (see Figs. 37.1 and 37.3) but on a smaller scale.
- **Phosphorylation of kinesin** at the tip of the axoneme may **reverse transport** for the return trip to the cell body. Trains are **full of tubulin** and **other axonemal proteins** in growing cilia; they keep moving **bi-directionally** but are largely empty when cilia are not growing.



- **<u>Rotary Cilia</u>**
- **Single cilia** on the **epithelial cells of the "ventral node" of vertebrate embryos** are required for the asymmetric location of some internal organs, such as the heart and liver, on opposite sides of the body. These nodal cilia lack the central pair microtubules and radial spokes. Rather than beating, the **activity of the dynein arms** causes the **tip of the cilia to rotate clockwise.** This rotary motion propels the extracellular fluid carrying **certain growth factors toward the left side of the embryo**. The absence of this flow explains why **patients with Kartagener syndrome** and mice **missing a single dynein heavy chain** have an equal chance of having their internal organs, such as heart and liver, positioned normally or on the opposite side, a condition called **situs inversus.**
- Rotary cilia may provide clues about **an intermediate stage** in the evolution of axonemes.
- **<u>Primary Cilia</u>**
- Except for blood cells, **differentiated cells in vertebrate tissues** produce a single **primary cilium** by growth of an axoneme from their older mother centriole (Fig. 38.19). The axonemes lack the central pair, and most lack dynein, so they are **immotile** (Fig. 38.12B).

- Primary cilia are **sensory organelles** for both chemicals and mechanical forces.
- For example, **odorant receptors of nematode olfactory neurons** concentrate in the membranes of primary cilia.
- **Rod and cone photoreceptors in the eye** are **modified cilia with a basal body and a vestigial axoneme**.



- Primary cilia on the **epithelial cells of kidney tubules** act as flow sensors, admitting Ca^{2+} into the cilium through mechanosensitive channels in the plasma membrane when bent.
- Many receptors concentrate in primary cilia including those for **developmental morphogens such as sonic hedgehog and Wnt, growth factors including platelet-derived growth factor, and hormones like somatostatin.**
- Some ligands activate local Ca^{2+} release into the cilium.
- **<u>Ciliopathies</u>**
- Genetic deficiencies in the **assembly of cilia or IFT** result in a remarkably wide range of human disease syndromes, known collectively as **ciliopathies**.

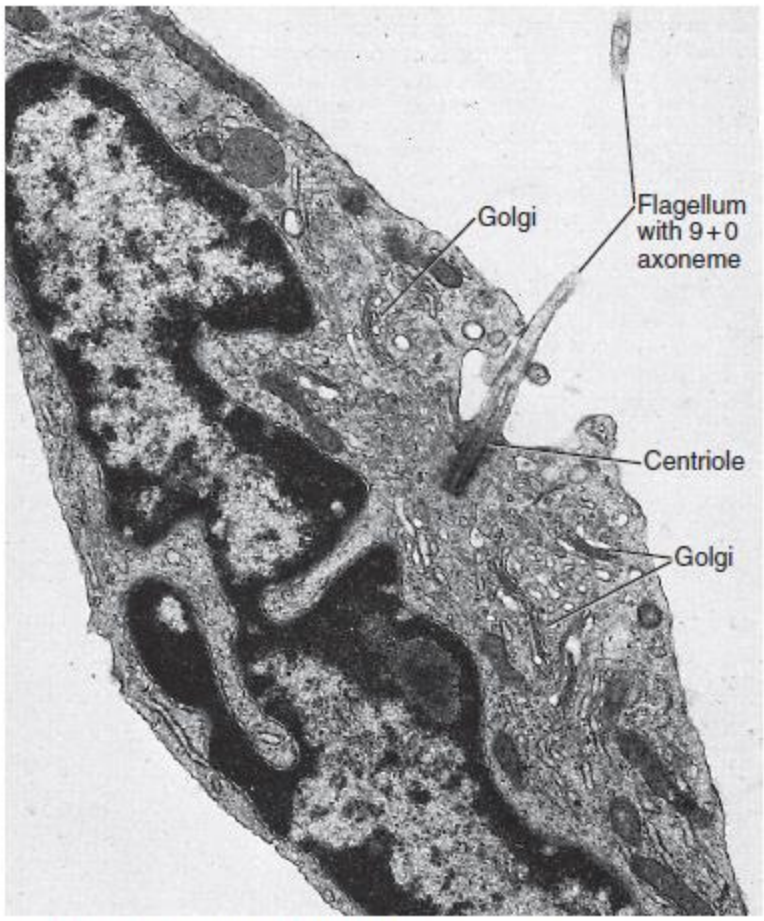


FIGURE 38.19 PRIMARY CILIUM. Electron micrograph of a thin section of a mesenchymal cell with a primary cilium assembled from one of the two centrioles, which serves as the basal body. (From Fawcett DW. *The Cell*. Philadelphia, PA: WB Saunders; 1981.)

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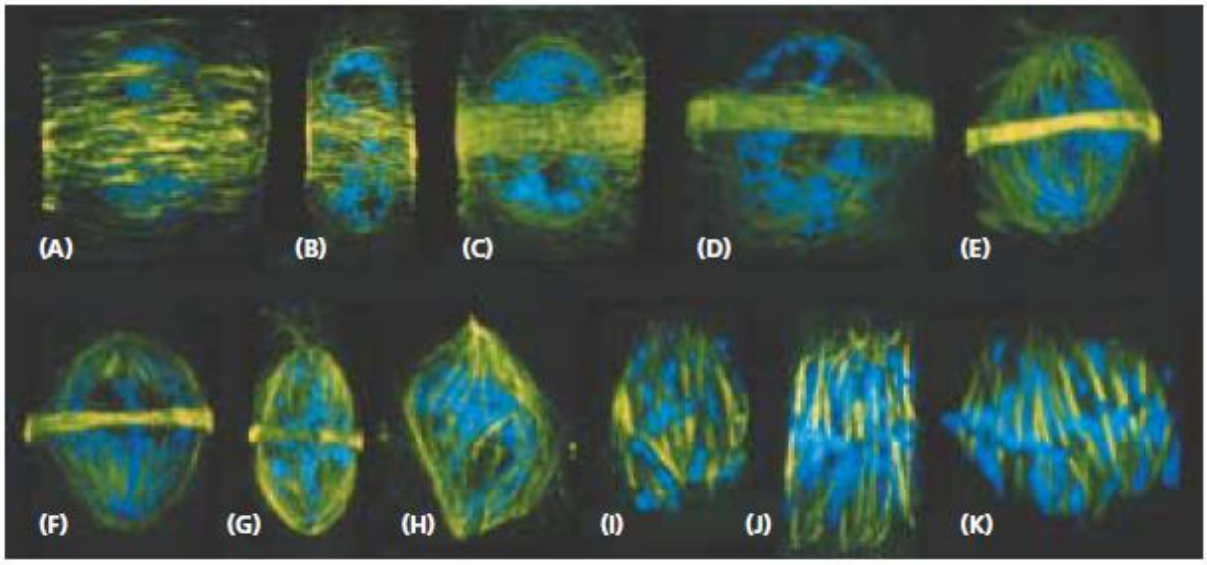
Microtubules Function in Mitosis and Cytokinesis:

Microtubules are an integral part of mitosis. Before mitosis begins, **microtubules** in the cortical (outer) cytoplasm **depolymerize**, breaking down into their constituent subunits. The subunits then **re-polymerize before the start of prophase** to form the **preprophase band** (**PPB**), a ring of microtubules encircling the nucleus. The **PPB** appears in the region where the **future cell wall** will form after the completion of mitosis, and it is thought to be involved in regulating the **plane of cell division.**



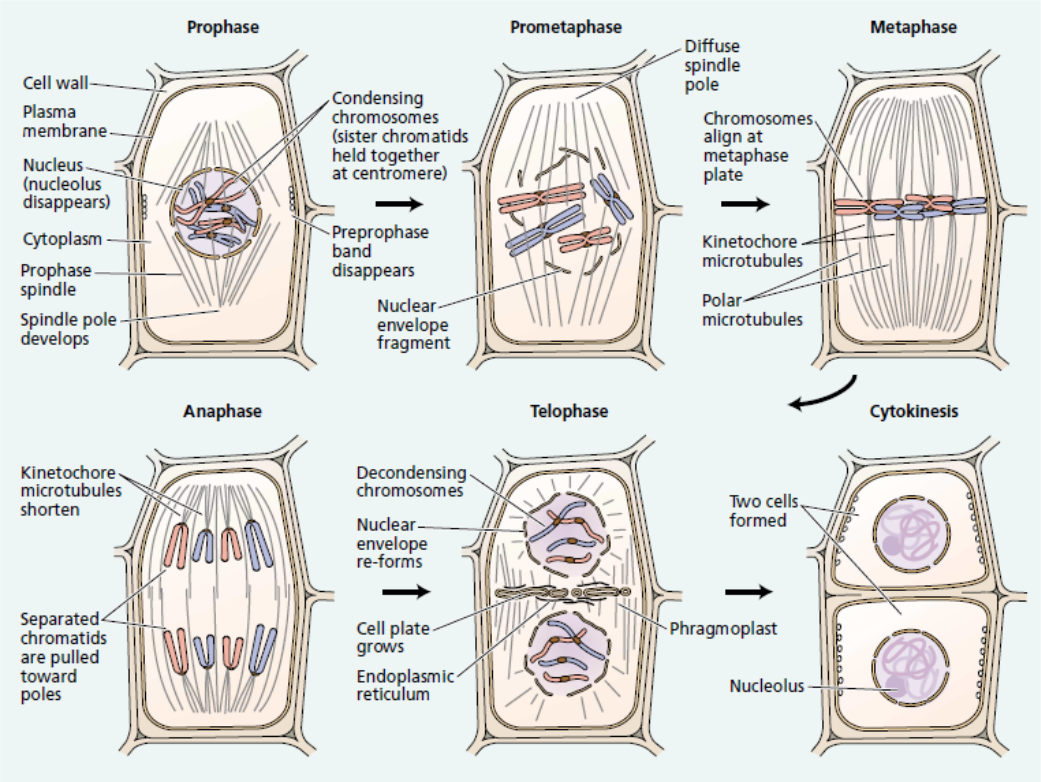


FIGURE 1.24 Diagram of mitosis in plants.

- During prophase, microtubules begin to assemble at two foci on opposite sides of the nucleus, forming the prophase spindle (Figure 1.24). Although not associated with any specific structure, these foci serve the same function as animal cell centrosomes in organizing and assembling microtubules.
- In early metaphase the nuclear envelope breaks down, the PPB disassembles, and new microtubules polymerize to form the mitotic spindle. In animal cells the spindle microtubules radiate toward each other from two discrete foci at the poles (the centrosomes), resulting in an ellipsoidal, or football-shaped, array of microtubules.
- The mitotic spindle of plant cells, which lack centrosomes, is more boxlike in shape because the spindle microtubules arise from a diffuse zone consisting of multiple foci at opposite ends of the cell and extend toward the middle in nearly parallel arrays (see Figure 1.24).
- Some of the microtubules of the spindle apparatus become attached to the chromosomes at their kinetochores, while others remain unattached. The kinetochores are located in the centromeric regions of the chromosomes.

- Some of the unattached microtubules overlap with microtubules from the opposite polar region in the spindle mid-zone.
- Cytokinesis is the process whereby a cell is partitioned into two progeny cells. Cytokinesis usually begins **late in mitosis**. The precursor of the new wall, the **cell plate** that forms between incipient daughter cells, is rich in **pectins**.
- **Cell plate formation** in higher plants is a multistep process. **Vesicle aggregation** in the spindle mid-zone is organized by the **phragmoplast, a complex of microtubules and ER** that forms during **late anaphase or early telophase** from dissociated spindle subunits.

Comparison among microtubules, microfilaments and intermediate filaments:

- In the cell, actin and tubulin monomers exist as pools of free proteins that are in dynamic equilibrium with the polymerized forms.
- Polymerization requires energy: **ATP** is required for **microfilament** polymerization, **GTP** (guanosine triphosphate) for **microtubule** polymerization.
- The attachments between subunits in the polymer are **non-covalent**.
- Both microtubules and microfilaments are polarized; that is, the two ends are different. In **microtubules**, the polarity arises from the **polarity of the α- and β-tubulin heterodimer**; in **microfilaments**, the polarity arises from the **polarity of the actin monomer** itself.
- The opposite ends of microtubules and microfilaments are termed ***plus*** and ***minus***, and **polymerization is more rapid** at the **positive end**.
- Once formed, microtubules and microfilaments can disassemble. The **overall *rate* of assembly and disassembly** of these structures is affected by the **relative concentrations** of **free or assembled subunits**.
- In general, **microtubules are more unstable than microfilaments**.
- **In animal cells**, the half-life of an individual microtubule is about **10 minutes**. Thus microtubules are said to exist in a state of ***dynamic instability***.
- In contrast to microtubules and microfilaments, **intermediate filaments lack polarity** because of the **antiparallel orientation of the dimers** that make up the tetramers.
- Additionally, **intermediate filaments** appear to be **much more stable** than either microtubules or microfilaments.

- Although **very little** is known about intermediate filament–like structures in plant cells, **in animal cells** nearly all of the **intermediate-filament protein** exists in the **polymerized state**.

TABLE 9.1 Properties of Microtubules, Intermediate Filaments, and Actin Filaments

	Microtubules	Intermediate filaments	Actin filaments
Subunits incorporated into polymer	GTP-αβ-tubulin heterodimer	~70 different proteins, likely incorporated as tetramers	ATP-actin monomers
Preferential site of incorporation	+ End (β-tubulin)	Internal	+ End (barbed)
Polarity	Yes	No	Yes
Enzymatic activity	GTPase	None	ATPase
Motor proteins	Kinesins, dyneins	None	Myosins
Major group of associated proteins	MAPs	Plakins	Actin-binding proteins
Structure	Stiff, hollow, inextensible tube	Tough, flexible, extensible filament	Flexible, inextensible helical filament
Dimensions	25 nm outer diam.	10–12 nm diam.	8 nm diam.
Distribution	All eukaroytes	Animals	All eukaryotes
Primary functions	Support, intracellular transport, cell organization	Structural support, mechanical strength	Motility, contractility, intracellular transport
Subcellular distribution	Cytoplasm	Cytoplasm + nucleus	Cytoplasm