Molecular Cell Biology

Prof. D. Karunagaran

Department of Biotechnology

Indian Institute of Technology Madras

Module 3

Microtubule, Actin and Filament Based Motile Systems

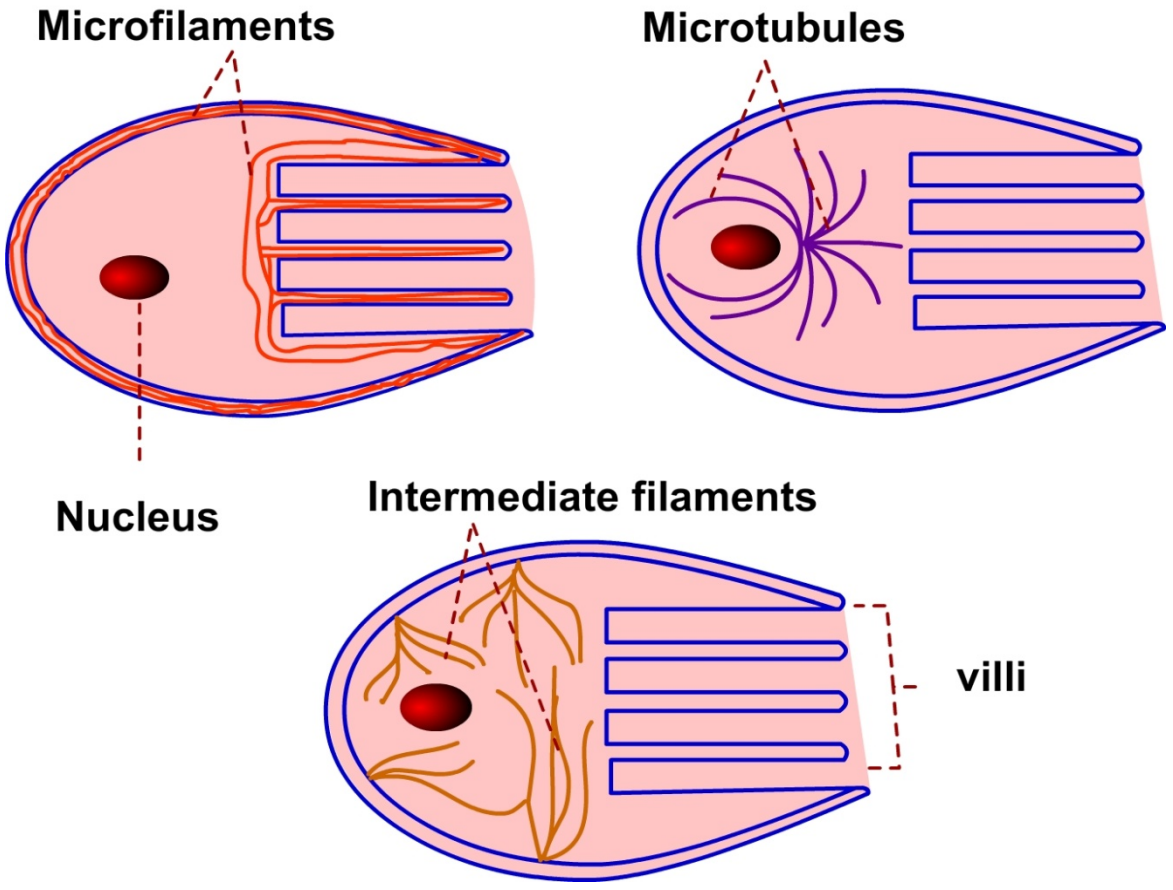
Lecture1

Microtubules

Cytoskeleton

- Eukaryotic cells have an excellent filament system called cytoskeleton to provide shape, movement and stability.
- Animal cells have three types of dynamic filaments filled in their cytoplasm
- Microtubules (20-25 nm in diameter) help in intracellular transport and in maintaining the positions of many organelles. They also help in chromosome separation during cell division
- Intermediate filaments (about 10 nm) that provide mechanical strength
- Microfilaments (3-6 nm) such as actin help in maintaining the shape and in locomotion
- A large no. of accessory proteins are needed for the effective functioning of all these filaments

Cytoskeletal components of intestinal epithelial cells:



- Intestinal epithelial cells contain all the three types of cytoskeleton filaments
- Villi contain the microfilaments giving a shape to the surface of epithelium
- Microtubules originate from centrosomes
- Intermediate filaments connect the desmosomes of adjacent cells

Microtubules

- Microtubules are present in the cytoplasm of eukaryotic cells with the characteristic tubular appearance
- They are about 25 nm in outer diameter
- Their length may be about a few micrometers
- At one end they are attached to centrosomes or microtubule organizing centers
- About 13 subunits are visible in a cross section with a center to center spacing of 4.5 nm
- Cilia, flagella and the cytoplasmic microtubules contain tubulin, a protein existing as a dimer of mol.wt. 110,000-120,000
- Tubulin alpha and beta are present in flagella and it is usually heterodimer in many cells

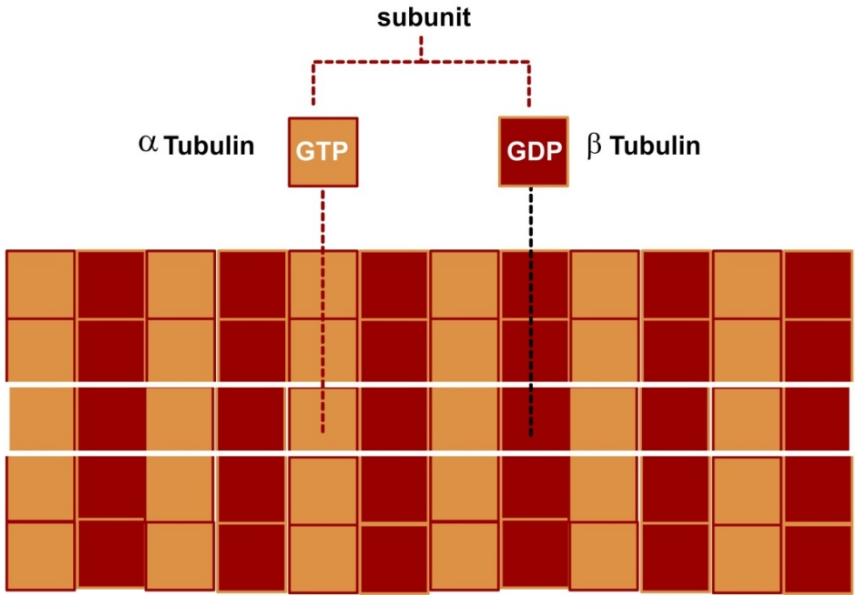
Tubulin

- Tubulin is known to bind colchicine and vinblastine at different sites
- Tubulin exhibits kinase activity
- Tubulin can also exist as free dimers in equilibrium with polymerized tubulin and may sometimes be seen integrated with membranes
- Polymerization originates from centrioles directed by microtubule organization centers
- At interphase and metaphase the polymerized tubulin is high but low at prophase and anaphase
- Phosphorylation of tubulin monomers by a cAMP dependent kinase results in polymerization
- Thus cAMP promotes polymers and enhances fibroblastic shape to cells

Tubulin polymerization

- At one end there may be polymerization while at other end depolymerization may occur often called plus and minus ends
- The shuttling of subunits from one end to the other of a microtubule is called "tread milling"
- Colchicine inhibits the assembly of monomers but the disassembling goes on with a net result of disorganization of microtubules
- GTP hydrolysis favors assembly while lack of GTP stops the assembly
- The in vivo control of assembly and disassembly involves calcium and calcium binding protein calmodulin
- Thus calcium and calmodulin addition inhibits polymerization

Protofilament formation



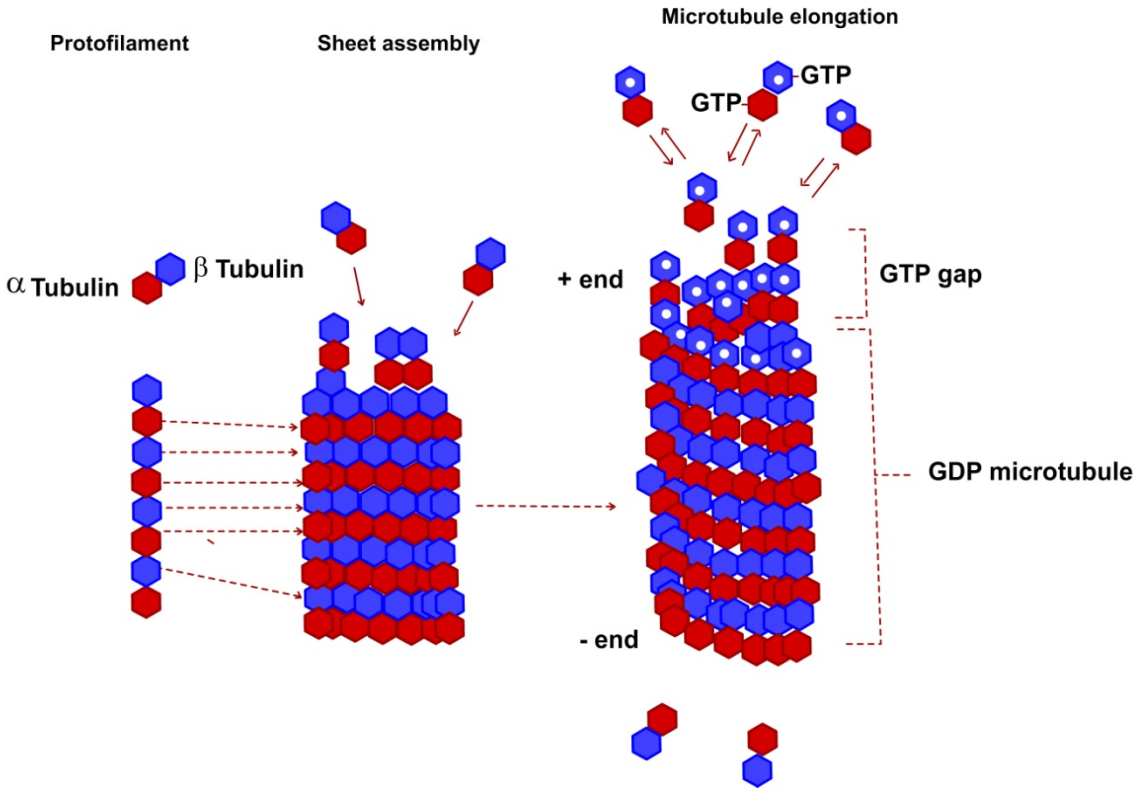
Microtubules contain a non-exchangeable GTP on alpha-tubulin and an exchangeable one on the amino terminal domain of beta-tubulin

In vitro microtubule assembly

- Tubulin heterodimers join end-to-end to form **protofilaments with alternate alpha and beta subunits**.
- Tubulin heterodimers can join laterally to form **sheets**, and eventually **microtubules**.
- Assembly of **13** protofilaments yields a **helical** arrangement of tubulin heterodimers
- A **bacterial** protein **FtsZ (molwt 40,000)** is homologous to tubulin and has GTPase activity
- FtsZ also assembles into protofilaments that can join to form sheets or tubules. Thus it can also polymerize and participate in cell division

Protofilament

- A linear protofilament is formed by linking the subunits head to tail longitudinal contact
- There is a repeat of dimeric subunits after every 8 nm
- The lateral interactions ensure the side by side contact of protofilaments into a sheet or cylinder**—** a microtubule
- The structure is adjusted in such a way that one alpha subunit is in contact with another alpha subunit in an adjacent protofilament
- This is called a staggering arrangement that also confers polarity in the sense that at one end called plus end beta subunits form the ring and in the other end it is done by alpha subunits
- A singlet microtubule is built from 13 protofilaments
- Cilia and flagella contain doublet microtubules
- Centrioles and basal bodies contain triplet microtubules



Critical concentration

- Tubulin polymerization is temperature dependent. Low temperature favours depolymerization whereas at 37^0C polymerization occurs in the presence of GTP
- Polymerization is also dependent on the critical concentration of alpha and beta tubulin dimers
- Above the critical concentration polymerization occurs and below the critical concentration depolymerization occurs
- Polymerization preferentially occurs at the plus end especially when the critical concentration is on the higher side and depolymerization occurs at the minus end where the critical concentration is less for the dimers
- GTP on the beta tubulin (but not on the alpha-tubulin) is hydrolyzed to GDP
- If the rate of polymerization is faster than the rate of GTP hydrolysis, then a cap of GTP-bound subunits is formed at the (+) end

Dynamics of microtubule stability

- Dissociation (**“** off **”** rate) of a GDP-tubulin dimer is four orders of magnitude as fast as that of a GTP-tubulin dimer
- If the (+) end becomes capped with subunits containing GDP**–**beta tubulin rather than GTP**–**beta-tubulin, a microtubule is destabilized and depolymerizes rapidly
- The stability of a microtubule is determined by the growth rate, the shrinkage rate, the **catastrophe** frequency, and the **rescue** frequency

Nucleation

- Centrosomes or microtubule organizing centers (MTOC) are involved in the nucleation of microtubules and the consequent polymerization
- Pericentriolar material of centrosomes contain pericentrin and another tubulin called gamma tubulin that can interact with beta tubulin and alpha tubulin dimers
- The complex set of proteins form gamma tubulin ring complex that helps in nucleation at the minus end of microtubule

Microtubule associated proteins (MAPs)

- A no. of microtubule associated proteins are thought to be involved in the assembly of microtubules
- MAPs are of two types (Stabilizing and Destabilizing)
- **Stabilizing MAPs** contain a basic domain that binds with microtubules and an acidic projection domain that extends from the wall of the microtubule like a filamentous arm. The projection domain binds to membranes, intermediate filaments or to other microtubules.
- For example spacing between microtubules in MAP2-containing cells is larger than in Tau-containing cells
- MAP2 is found only in dendrites, where it forms fibrous cross-bridges between
- microtubules and links microtubules to intermediate filaments
- Tau, which is much smaller than most other MAPs, is present in both axons and dendrites
- Tau is present in both axons and dendrites

- Tau is actually a complex of four proteins and these can act as calmodulin binding proteins influenced by calcium
- MAP4, is found in neuronal and non-neuronal cells
- In mitosis, MAP4 regulates microtubule stability
- CLIP170 cross-links microtubules to chromosomes
- Phosphorylated MAPs cannot bind to microtubules and promote microtubule disassembly
- MAP kinases and CDKs are known to be involved in the phosphorylation
- **Destabilizing MAPs** directly destabilize microtubules
- katanin disrupts intact cytosolic microtubules by an ATP-dependent process
- Internal bonds between tubulin subunits in the microtubule wall are disrupted resulting in the fragmentation of microtubules
- Op18 or stathmin, increases the frequency of rapid disassembly of microtubules in the mitotic spindle
- Stathmin binds tubulin dimers, thereby reducing the poolof dimers available for polymerization
- Phosphorylation inactivates Op18 and inhibits its destabilizing effect

Functions of cytoplasmic microtubules

- In axons and dendrites of neurons microtubules are important for their shape
- In some tissues such as the eye, induction of lens placode is accompanied by the formation of a no. of microtubules
- Similarly they are also important for spermiogenesis
- Microtubules are important for the cell polarity and motility
- Pseudopodia formation in some protozoan involves microtubules
- Microtubules play important roles in the transport of particulate materials in melanocytes

Association of microtubules with organelles and vesicles

- Membrane containing organelles such as ER, golgi, mitochondria and endosomes are associated with microtubules
- ER membranes elongate along with microtubules
- Golgi complex is present very near to MTOC
- Golgi vesicles move along microtubules
- Microtubules are thought to play a role in the intracellular transport of membrane-limited organelles and vesicles

Microtubular organelles – Cilia, flagella and centrioles

- Specially differentiated appendices called cilia and flagella are important for cell motility
- Flagella are generally few in number but are long
- Cilia on the other hand are short but more in number
- Some protozoa contain thousands of cilia
- Flagellata contains many flagella
- Spermatozoa move with the help of flagella
- Ciliated epithelium is common in air passages of the respiratory system and in various parts of the genital tract

Cilia

There are two types of cilia

- Non-motile or primary cilia
- Motile cilia

Non-motile or primary cilia

- They are present in all mammals
- They do not beat
- They are present in sensory organs (eye and nose)

Motile cilia

- Present on the surface of cells.
- They beat in a rhythmic manner
- Present in the lining of the trachea (windpipe)
- sweep mucus and dirt out of the lungs
- cilia in the fallopian tubes move the ovum from the ovary to the uterus

Flagella

There are three types of flagella

- Bacterial flagella
- Archaeal flagella
- Eukaryotic flagella

Bacterial flagella

- They are helical filaments rotating like screws
- Present in E. coli, Salmonella typhimurium
- There may be one, two or many such flagella per cell

Archaeal flagella

- Similar to bacterial flagella
- They lack a central channel

Eukaryotic flagella

- complex cellular projections that move back and forth
- e.g., sperm cell propels itself through the female reproductive tract.

Ciliary apparatus

Ciliary apparatus has several components

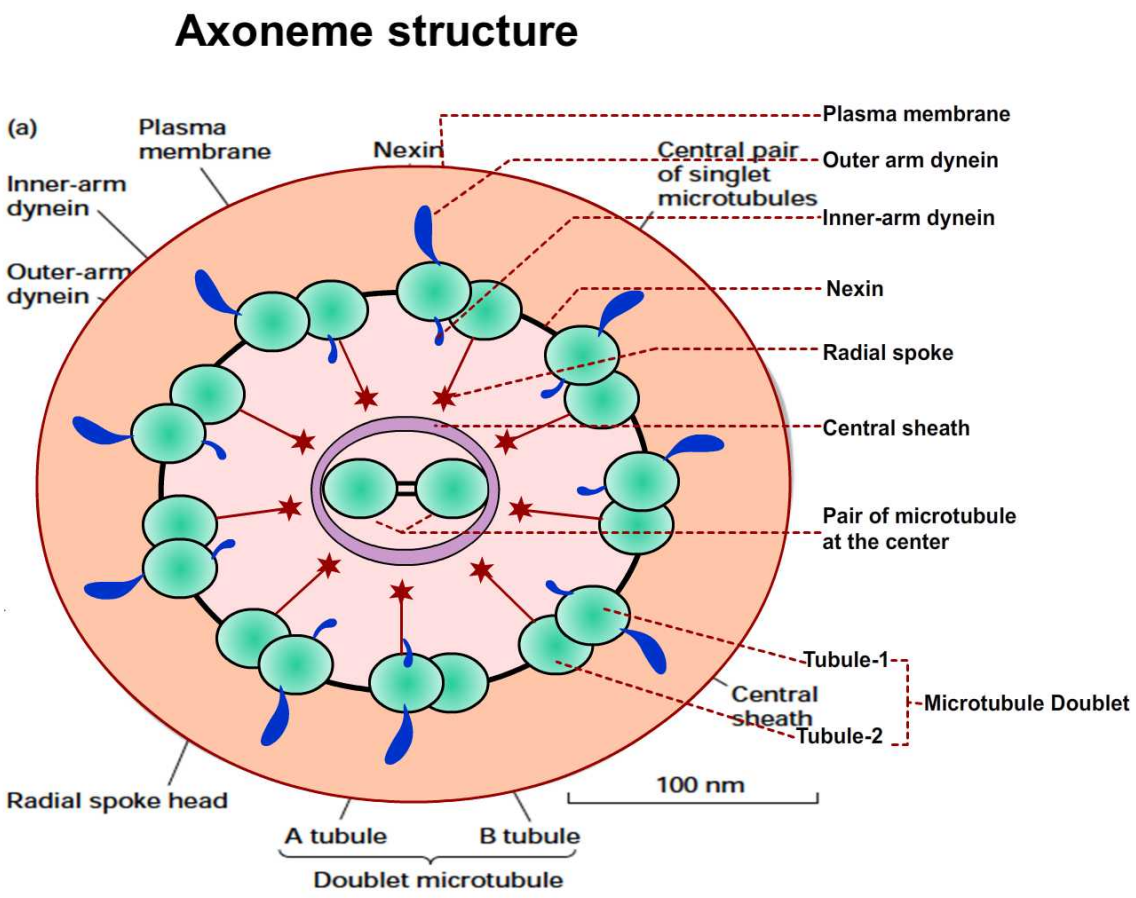
1. Cilium projects from the surface of a cell as a slender cylinderoid process
2. Basal body is an intracellular organelle that originates from centriole and is also called granule
3. Ciliary rootlets are fine fibrils arising from the granule and appears like a conical bundle very near to the nucleus

4. Some epithelial structures are similar to cilia in their shape but are immobile and they are known as stereocilia. An example is the epididymal appendages

Axoneme is the motile element

- The basic microtubular structure of cilia and flagella is the motile element called axoneme
- The length of axoneme may vary from microns to millimeters
- Its outside diameter is however very small (0.2 mm)
- It has an outer membrane that continues with the plasma membrane
- It also has a matrix called ciliary matrix.

Axoneme structure



Structural features of axoneme

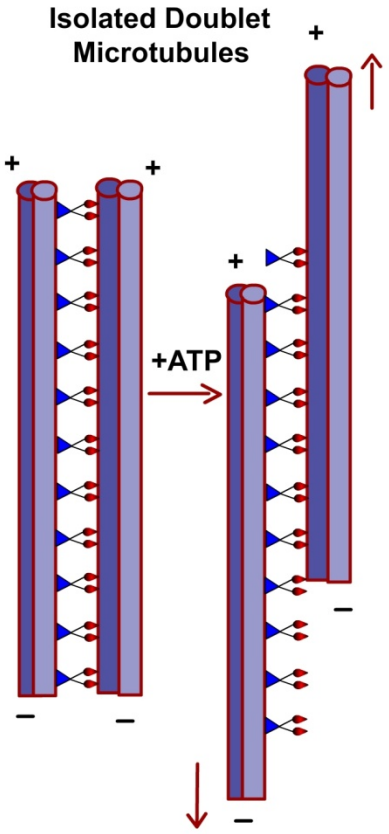
- Axoneme contains a typical 9 + 2 pattern of microtubules
- There are two central tubules and from this one can make out 2 symmetrical halves for the axoneme
- Ciliary beat is usually perpendicular to this plane of symmetry
- The peripheral tubules exist as doublets and are called subfiber A and subfiber B
- Subfiber A is small and complete
- Subfiber B is larger but incomplete as it lacks the wall adjacent to A
- Subunit has 13 microtubules whereas B has only 11 of them

Dynein

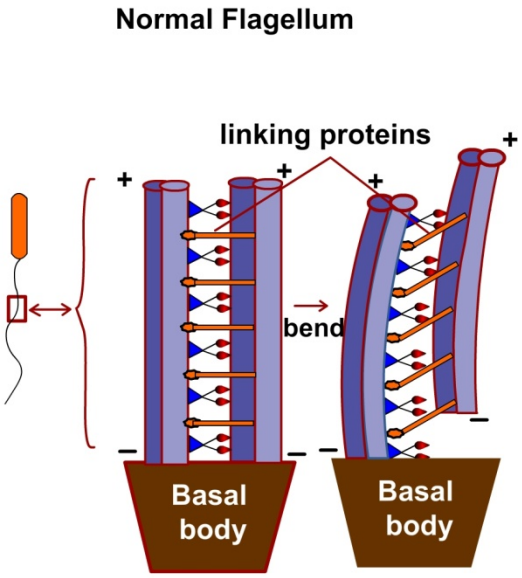
- The processes orienting in the same direction emanate from subunit A are called dynein arms
- Dynein arms contain an elliptical head, two spherical feet that are in contact with subfiber A and a stalk that bridges the head with subfiber B of the adjacent doublet
- There is also a link between dyneins
- Dynein is basically an enzyme called ATPase (300,000-400,000 daltons)
- This enzyme is activated by magnesium and calcium
- Dynein I and II represent the two isozymes for this enzyme

Nexin

- The doublet microtubules are linked by nexin links
- Nexin is a protein (mol. wt. 150,000-160,000)
- Nexins are thought to help in maintaining the integrity of the axoneme when it is sliding
- There is a sheath around the central microtubules and it is linked with the subfiber A by radial spokes having a fork-like structure
- The radial spokes are perpendicular to the ciliary axis and may participate in the sliding between the outer doublets into local axial bending

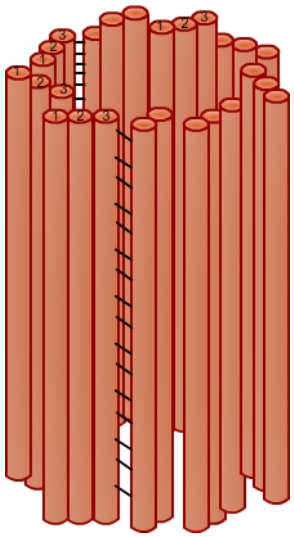


Dynein Produces Microtublet sliding

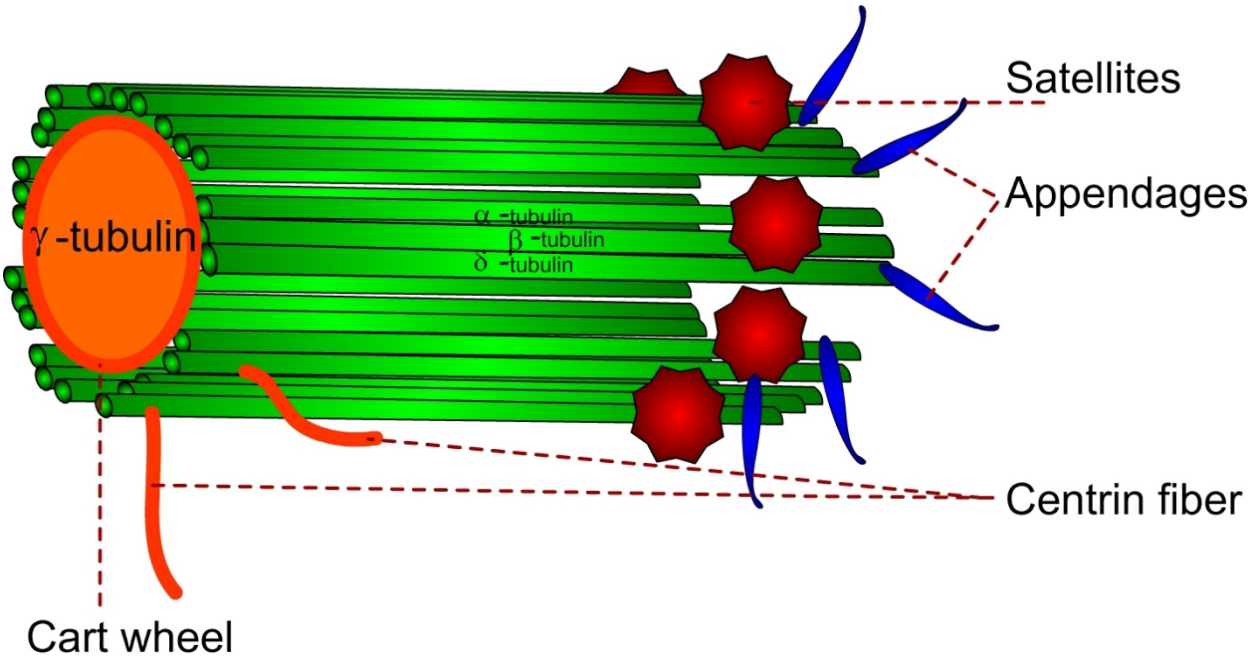


Dynein causes Microtublet bending

Basal bodies



- Basal bodies or kinetosomes of cilia and flagella are similar to centrioles
- Centrioles are cylindrical structures (0.2 X 0.5 mm) open at both ends or may carry a cilium at one end
- The cylinder part is separated from the cilium by a ciliary plate



Centrioles

- Two to six rows of membrane particles are sometimes present below the ciliary plate and they are called ciliary necklace
- Centriole is made up of nine triplets arranged in a circle.
- Each triplet is called a blade and the tubules within this blade form a helical structure
- The triplet tubules are designated A B and C from centre to periphery
- A and B cut across the ciliary plate and continue as tubules in the axoneme
- In this way centrioles can have a triplet while the cilia have a doublet

Ciliary rootlets and satellites

- Ciliary rootlets originate from the basal body and are usually striated
- The striated fibers are parallel microfilaments formed by globular subunits
- It is thought that ciliary rootlets have a role in contraction

- Perpendicular to the basal body there are basal feet and satellites
- Basal feet are dense processes composed of microfilaments
- Satellites are pericentriolar bodies that are electron dense structures
- They are close to the centrioles and serve as nucleating sites for microtubules

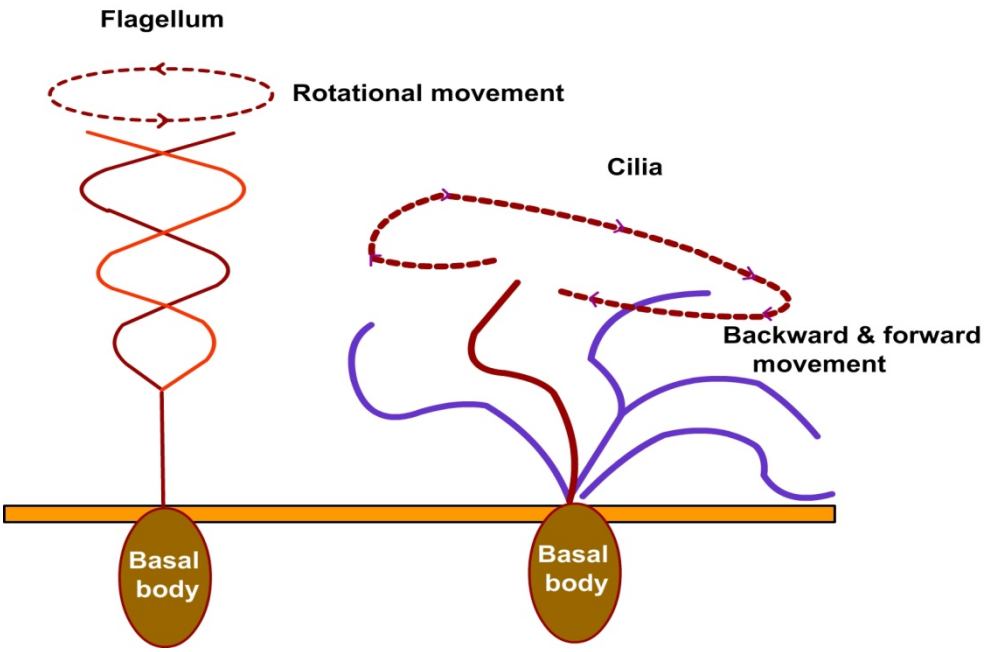
Function of centrioles and basal bodies

- They have important role in the formation of cilia
- They are involved in the ciliary and flagellar beat
- Their role in mitosis will be discussed later
- Higher plant cells that do not migrate lack centrioles
- Lower plant cells that migrate do have centrioles
- It is said that centrioles may have a role in locating the directions of signal sources (optic, acoustic and olfactory)

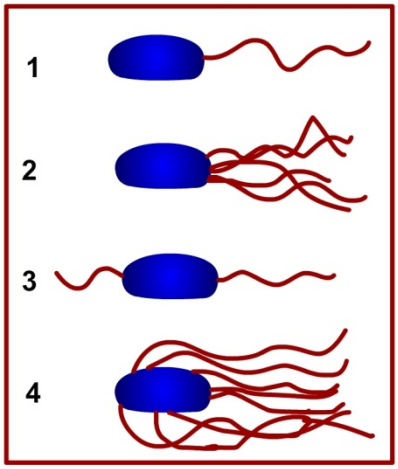
Ciliary movement

- On the surface of epithelial cells the movement of cilia can be observed
- With a row of cilia one can observe a wave of contraction and this is called metachronic contraction
- The contraction starts before or after the contraction of next cilium
- If the contraction of all cilia is in the same phase at a given time it is said to be isochronic
- Intraciliary excitation may end up in interciliary conduction thus constituting a rhythm to the ciliary beat
- Ciliary contraction in a frog pharynx is about 10-17 per second
- In protozoa the cilium is rigid and the by flexing the base a motion is created that is usually called pendulous ciliary movement.
- In metazoa contraction results in doubling of the cilia taking a shape of a hook and this is called unciform movement of the cilia
- Other types of movement known are the indundibular movement in which the cilia or flagella rotate and attain a conical or funnel shape.

- Flagella also display undulant motion in which the contraction waves start from the site of implantation and pass to the free border.



Various types of flagella movement in prokaryotic and eukaryotic cells.



Sliding involves dynein

- Ciliary and flagellar motions are somewhat similar to muscular contraction
- The sliding of microtubule doublet involves the hydrolysis of ATP
- Two large families of motor proteins-the kinesins and the dyneins-are involved in the variety of movements in which microtubules participate
- Kinesin and dynein move along microtubules in opposite directions-most kinesins toward the plus end and dyneins toward the minus end
- The movement of kinesin I transports vesicles and organelles away from the cell body toward the tip of the axon
- MAP-1C is a motor protein that moves along microtubules in the minus end direction.
- MAP-lC is related to the dynein isolated from cilia (axonemal dynein)
- MAP-1C is also called cytoplasmic dynein

Immotile cilia syndrome

- This is a condition in which cilia and flagella are immotile
- Nonmotile sperm for example leads to infertility
- Chronic brochitis and sinusitis also involve immotile cilia
- In these conditions dynenin arm is deficient and there is lack of ATPase activity
- In some cases defective nexin links, spokes may be involved
-

Photoreceptors and cilia

- Retinal rods and cones contain a short and fibrous segment that connects the outer and inner segment that resembles cilia
- The connecting cilium contains nine pairs of filaments very similar to cilia
- However, there are no central microtubules in this structure

Origin of cilia and flagella

- Centrioles programmed to form mitotic spindle or single cilia develop directly from the wall of pre-existing centriole

- The daughter centrioles called procentriolesstretch into cylinders
- Half the way during this development they are released into the cytoplasm and complete their maturation
- Those destined to become kinetosomes as in a ciliated epithelium follow a different mechanism for development
- The daughter centrioles are aligned in rows below the apical plasma membrane
- Each centriole produces satellites from the side, roots from the base and a cilium from the top

Study Questions

1. What are microtubules?
2. What is meant by ‘tread-milling”?
3. A bacterial protein homologous to tubulin is

   a) Nexin b) myosin c) actin d) FtsZ
4. Match the following

Dynein	E. coli
Axoneme	MTOC
Centrosomes	9+2 pattern
Flagella	ATPase

5. If the contraction of all cilia is in the same phase at a given time it is said to be ----------.