

# The Motile Machinery and Cytoskeleton of the Cell

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## Objectives

By the end of this session the student should be able to:

- ☛ Distinguish between microtubules, microfilaments and intermediate filaments
- ☛ State their main locations and functions
- ☛ Explain how they are assembled and disassembled
- ☛ Name the associated molecules interacting with the various filaments and tubules
- ☛ Give examples of clinical disorders involving intracellular filaments

## Recommended reading

The World of the Cell: Becker WM, Kleinsmith LJ, Hardin J. 4<sup>th</sup> Edition

- ☛ Chapter 22 Cytoskeletal Systems
- ☛ Chapter 23 Cellular Movement: Motility and Contractility
  - Intracellular microtubule-based movement
  - Microtubule-based motility
  - Actin-based motility

Note: The other topics on filament-based movements in muscle will be dealt within the sessions on muscle.

## The Motile Machinery and Cytoskeleton of the Cell

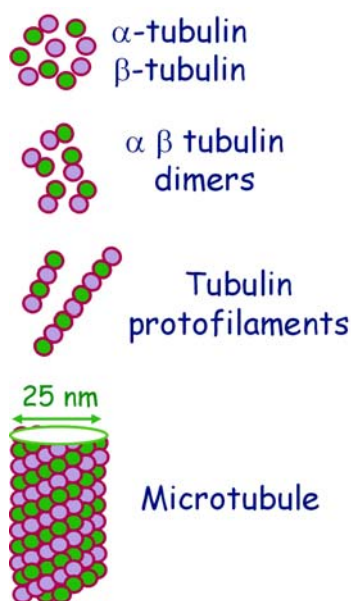
A system of microfilaments and microtubules in the cytoplasm maintains the shape of cells and provides mechanisms of movement of the whole cell or its component parts.

There are three main classes of filaments, classified according to diameter

- Microtubules - 25 nm
- Intermediate filaments - 10 nm
- Microfilaments - 5 to 7 nm

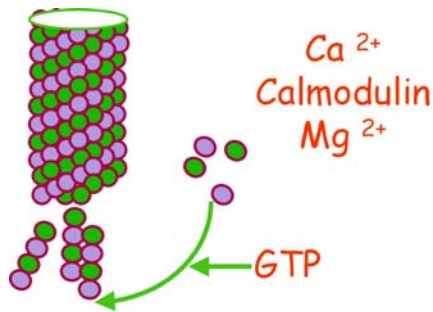
The three types of filaments can be observed in electron micrographs. The individual fibres are beyond the resolution of the optical microscope but their distribution in cells can be localised specifically using **monoclonal antibody techniques**. A fluorescent dye is used to visualise the antibody.

### Microtubules



- Microtubules are assembled by polymerization of  $\alpha$ -tubulin and  $\beta$ -tubulin molecules.
- $\alpha$  and  $\beta$  tubulin unite to form heterodimers.
- $\alpha$  -  $\beta$  tubulin dimers assemble into tubulin protofilaments, each consisting of alternating  $\alpha$ - and  $\beta$ - units.
- Tubulin protofilaments are assembled into cylindrical structures, the microtubules.
- There are 13 protofilaments along the circumference of microtubule. The protofilaments are arranged longitudinally giving the appearance of alternating spirals of  $\alpha$ - and  $\beta$ - units.
- Microtubules have a diameter of 25 nanometers.

Microtubules increase in length by polymerisation.



The process of microtubule assembly requires:

1. Tubulin monomers bound to GTP
2.  $Mg^{2+}$  and  $Ca^{2+}$  ions.

Microtubules may also be dismantled. The disaggregated tubulin monomers are bound to GDP.

Note that GTP binding is necessary for microtubule assembly, but GTP hydrolysis does not provide the energy to drive the process. GTP remains bound to the tubulin in microfilaments. When GTP in the microtubules is hydrolysed to GDP the monomers become less stable and disaggregate.

Polymerisation and disaggregation of microtubules may occur at either end, and may proceed independently.

Microtubules have + and - ends. The + end is where microtubule assembly or disassembly occur faster than in the - end.

(When assembly at the + end equals disaggregation at the - end the process is called tread milling. This is probably unimportant and may not occur frequently.)

Substances that act on microtubule assembly *in vitro*:

1. Colchicine, vinblastine, vincristine and podophyllotoxin inhibit microtubule assembly
2. Taxol promotes and stabilises microtubule formation

Note that all these substances are plant extracts.

Colchicine, and the other substances that inhibit microtubule assembly, prevent mitotic spindle formation and are therefore termed anti-mitotic drugs. Vinblastine and vincristine are used clinically as anticancer drugs that inhibit mitosis. Taxol stabilizes the mitotic spindle and prevents its disaggregation, so that Taxol is also an anti-mitotic drug.

## There are two main classes of Microtubules.

These are responsible for different types of movement in the cell.

### 1. Axonemal microtubules

- Are found in cilia and flagella and responsible for their movement
- Are very stable (do not disaggregate readily)
- Are highly organised in regular patterns

Cilia are found mainly lining the epithelia of the respiratory system and the uterine tubes. Flagella form the tails of spermatozoa.

### 2. Cytoplasmic microtubules

- Occur in the cytosol of most cells.
- Are responsible for movement of structures within cells:
  - Movement of chromosomes in mitosis ( microtubules form the mitotic spindle)
  - Movement of organelles:
    - Vesicle transport e.g. transport of vesicles from RER or SER and to Golgi complex and transport of secretory vesicle to the surface of the cell
    - Movement of vesicles in axoplasmic transport (i.e. along axons)
    - Movement of melanin pigment in pigmented cells

(Note: the microtubules at different sites and in different organisms are similar in structure, but differ slightly in the details of the amino-acid sequence of the  $\alpha$  and  $\beta$  tubulin.)

## Microtubule Associated Proteins (MAPs)

These are proteins that:

1. Bind at intervals to microtubules
2. Allow interaction with other microtubules and organelles
3. Provide energy for movement.
4. Promote and regulate microtubule assembly
5. Stabilise microtubules

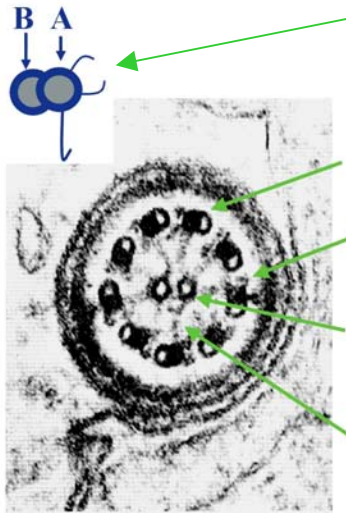
The following are some MAPs:

- **Dynein** - is found in cilia and flagella. It is a  $Mg^{2+}$  activated ATPase that utilises ATP to provide energy for bending of cilia

- **Kinesin** - binds microtubules to organelles and provides energy for their movement by utilisation of ATP
- **Dynamin** - is a group of molecules that bind adjacent microtubules providing interaction between them.

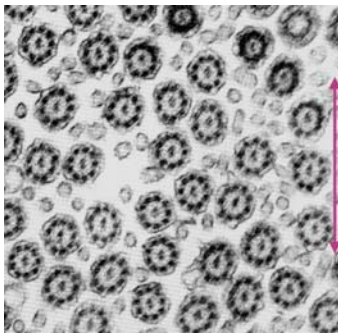
## Cilia and Flagella are composed of Microtubules

Each cilium consists of:



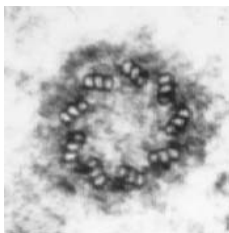
- Doublets formed of two microtubules termed A & B sub-fibres. The A sub-fibre is the one that forms a complete circle.
- 9 peripheral doublets (microtubule pairs)
- Side arms connecting the A sub-fibre of one doublet to the B sub-fibre of the adjacent doublet
- A pair of central microtubules. (These are separate and do not form doublets.)
- Radial spokes connecting the central tubules to the peripheral doublets.

In ciliated cells, the cilia are all aligned in the same direction.



The orientation of the central pair of microtubules determines the direction of ciliary movement. The arrow indicates the direction of ciliary beat in this epithelium.

## Centrioles and Basal Bodies are made of microtubules



- The microtubules are arranged in triplets
- There are 9 peripheral triplets oriented obliquely
- They have no central pair

**Basal bodies** organize the formation of cilia and flagella.

There is a basal body associated with each cilium or flagellum. The basal bodies are aligned below the apical plasma membrane of the cell.

**Centrioles** are found in the center of the cell. They frequently occur in pairs arranged at right angles to one another. They organize the formation of microtubules e.g. the spindle of microtubules in mitotic cells.

## Microtubule Organising Centres (MOCs)

These are sites are sites that:

1. Promote initial nucleation of microtubules
2. Organise microtubule assembly and orientation
3. Anchor microtubule ends

The following are examples of MOCs:

- **Basal bodies** - organize the formation of cilia
- **Centrioles** - form the mitotic spindle
  - form centriole satellites that form other centrioles
- **Kinetochores** on the centromeres of chromosomes
  - are attachment sites for microtubules of mitotic spindle
- **Centrosomes** in non-dividing cells assemble microtubules
- Other MOCs in plant cells. (Plant cells do not have centrioles.)

## Clinical Applications

**Kartagener's syndrome** is a genetic defect caused by a mutation in the gene that codes for ciliary dynein. The defective dynein impairs ciliary and flagellar movement. Therefore, affected individuals suffer from:

- Male sterility due to non-motile spermatozoa
- Chronic chest infections because of accumulation of dust particles and mucus in the bronchi
- Impaired ciliary movement in the uterine tubes may impair movement of fertilized ova towards the uterine tubes but this does not cause sterility.

***Situs inversus*** is a condition in which the positions of the viscera on the left and right sides of the body are reversed. The left or right orientation of the heart, spleen, aortic arch, stomach, liver etc. is reversed. This condition is caused by a mutation of the *inversus viscerum (iv)* gene, which encodes for a dynein molecule similar to that of axonemal dynein. Microtubules appear to

be very important in early embryonic development. Their role is still incompletely understood.

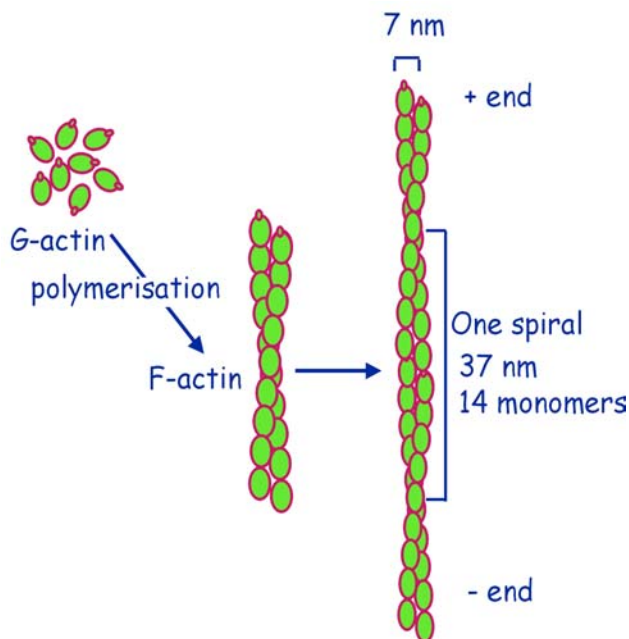
Vinblastine and vincristine are used as anti-cancer drugs because they inhibit mitosis.

## Actin Filaments

Actin filaments are responsible for movements that bring about changes in cell shape and contribute to the cytoskeleton. They are found in the cytosol of most cells but are particularly abundant in the following cells:

1. Muscle (skeletal, cardiac and smooth muscle)
  - $\alpha$ -actin interacts with myosin to produce muscle contraction
2. Macrophages and other cells having amoeboid movement
  - Actin filaments form the pseudopodia and change in cell shape
3. Cells in mitosis or meiosis
  - Actin filaments are responsible for cytokinesis
4. Microvilli and the terminal web
  - $\beta$ -actin is present at these sites

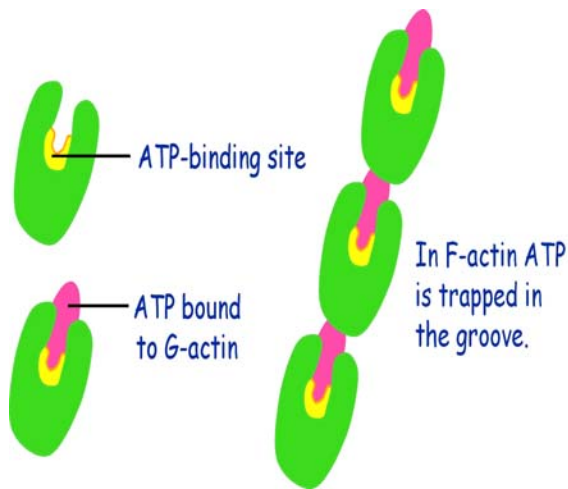
## Molecular structure of actin filaments



- Actin filaments (known as F-actin) are assembled from globular molecules of G-actin.
- Actin filaments consist of double strands of G-actin arranged in a spiral.
- Each spiral turn spans a length of 37 nm and contains about 14 monomers (7 per strand)
- Each filament has a diameter of 7 nm.
- Actin filaments have polarity indicated as + and - ends.



## G-actin binds to ATP



- G-actin is a globular molecule with a central groove.
- The groove has an ATP binding site.
- G-actin-ATP complexes are necessary for binding to occur.
- ATP remains trapped in the molecules and does not provide the energy to drive the binding process.
- ATP hydrolysis to ADP promotes disaggregation of

## Actin filaments are dynamic structures.

They undergo assembly and disassembly, and may be lengthened or shortened. Assembly and disassembly occur at either end, but proceed faster at the + end than at the - end.

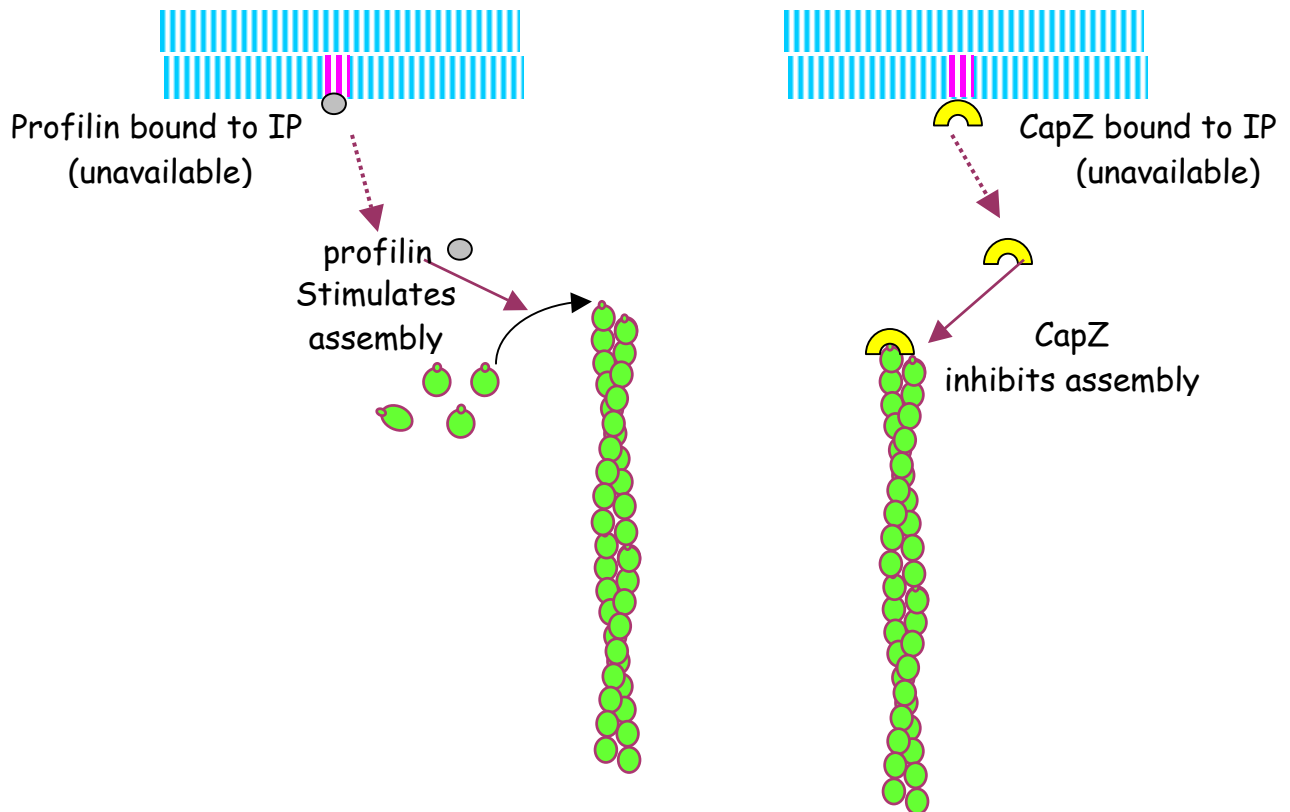
## Assembly and disassembly of actin filaments are controlled by various proteins:

1. Proteins that **promote** the assembly of F-actin:
  - **Profilin** transfers G actin to the growing + end
  - **Cofilin** increases the rate of turnover at the - end
2. Proteins that **inhibit** the assembly of F-actin:
  - **Thymosine** binds to G-actin preventing its availability for assembly
  - **Capping proteins** bind to the end of F-actin preventing assembly. Examples are
    - **Cap Z**
    - **Gelsolin** caps the ends and breaks F-actin
3. Proteins that **modulate** assembly / disassembly
  - The **G-proteins** Rac, Rho and Cdc42 regulate actin assembly and disassembly (G proteins are Guanine-nucleotide binding



proteins that are activated by binding to GTP; this class of proteins regulates many cellular functions.)

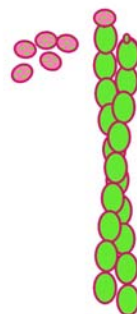
- **Inositol phospholipids** in membranes can bind to regulatory proteins preventing them from functioning
  - Binding to profilin inhibits assembly
  - Binding to CapZ promote assembly



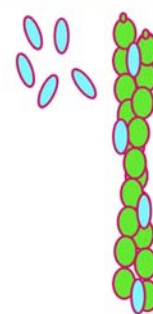
The above diagram shows that inositol phospholipids can both inhibit and stimulate actin filament assembly. When it binds to profilin it inhibits actin filament assembly; when it binds to capZ it stimulates actin filament assembly.

Certain chemicals can be used to control actin filament assembly and disassembly *in vitro*. They have been used extensively for studying the functions of these filaments

Cytochalasin B binds to F-actin and inhibits assembly



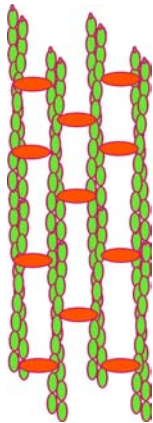
Phalloidin binds to F-actin and inhibits disassembly



These substances also affect all the cell processes that involve assembly or disassembly of F-actin.

- They will inhibit all amoeboid movement.
  - Amoeboid movement is the result of cytoplasmic flow, which is associated with sol-gel transformation.
  - Gelation is associated with assembly of F-actin, while solation is associated with its disaggregation to G-actin.
  - Phagocytosis depends on amoeboid movement; it is inhibited by cytochalasin-B or phalloidin *in vitro*.
  - Cytokinesis following mitosis is brought about by actin filaments and is inhibited by these substances *in vitro*.

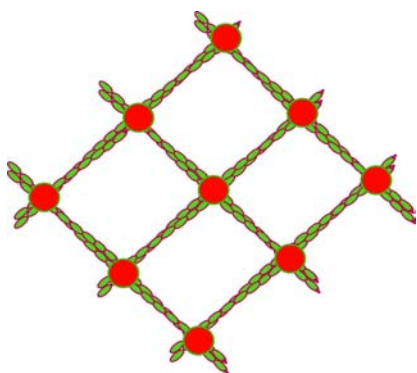
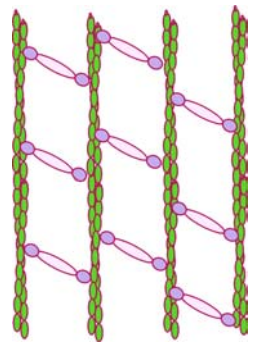
Some actin-binding proteins regulate the organisation of actin filaments by cross-linking:



- Villin and Fimbrin are actin-bundling proteins that cross-link actin filaments to form compact bundles. They are found in microvilli and other surface projections

$\alpha$ -actinin binds F-actin to form a loose meshwork

- It allows interaction with myosin - like molecules to promote contraction.
- It is found in motile cells - pseudopodia, lamellopodia and filopodia



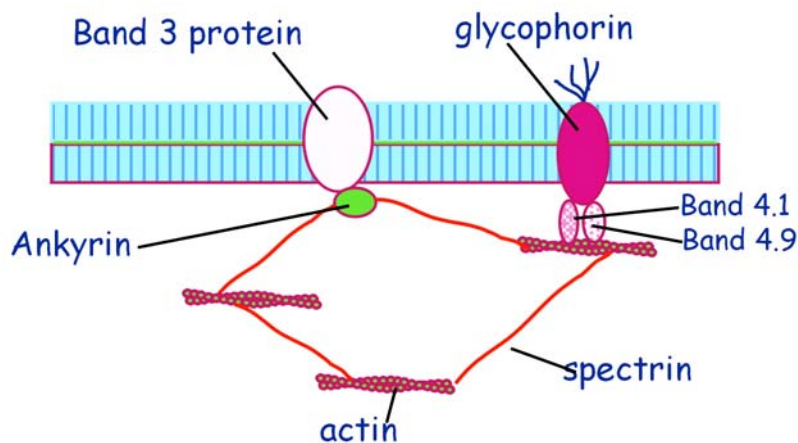
Filamin binds actin filaments where they cross one another to form networks.

Such networks are found beneath the plasma membrane and nuclear envelope and are called "stress fibres".

### Actin filaments become re-organised in motile cells

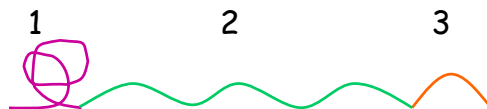
In resting cells, actin filaments form meshworks below the surface and around the nucleus. In motile cells, bundles of actin filaments are oriented mainly in one direction.

Spectrin and ankyrin bind to actin and to membrane proteins forming a 3-D network in erythrocytes.



### Intermediate Filaments (IF) - 10nm Filaments

The IF protein molecules that form intermediate filament are composed of three domains:

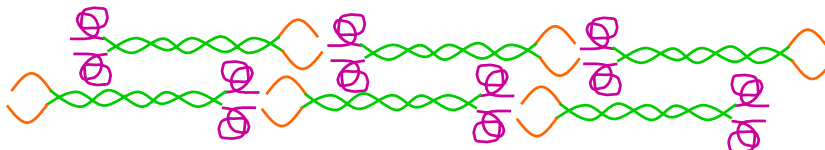


1. N-terminus head (ending in an NH<sub>2</sub> group)
2. Central rod domain consisting of 310 amino acids ( $\alpha$ -helical)
3. C-terminus (ending in a COOH group)

Dimers consist of two intertwined molecules



Protofilaments consist of dimers staggered next to each other



There are various types of IF protein.

The central  $\alpha$ -helical domain is constant, but the N- terminal head and C-terminal tail are very variable.

Intermediate filaments are tissue-specific

The IF proteins vary according to the tissue. The following types of IF are known

1. **Keratins** - they are found in epithelial cells:
  - Skin keratin - soft and hard keratin in horny layer of epidermis, cuticle, hair, and nails.
  - Tonofilaments  
(Tonofilaments are 10 nm filaments that are attached to desmosomes, which bind epithelial cells firmly together.)  
Keratins are also classified as
    - Class I - acidic keratin
    - Class II - neutral or basic keratin
2. **Vimentin**: (Class IIIa) specific for connective tissue cells including fibroblasts, adipocytes, endothelium and mesenchyme
3. **Desmin**: (Class IIIb) specific for muscle cells (provide attachment for actin filaments)
4. **Glial fibrillary acidic (GFA)**: (Class IIIc) specific for neuroglial cells that surround neurons in the central nervous system.
5. **Neurofilaments**: (Class IV) specific for axons and including subtypes NF-L, NF-M and NF-H (light, medium and heavy neurofilament proteins). They also include  $\alpha$ -internexin (in early stages of neuron development).
7. **Nuclear lamins A, B and C**: (Class V) form nuclear scaffolding supporting the chromatin. They give and maintain the shapes of nuclei
8. **Nestin**: (Class VI) specific for neuronal stem cells

Note: The classification into classes I to VI is based on the amino acid sequence similarity. Class III refers to three closely related but distinct intermediate filaments.

Intermediate filaments are all morphologically similar but can be distinguished from one another using monoclonal antibody techniques.

Monoclonal antibodies are highly specific for the different tissue specific types of IF. Monoclonal antibodies can be tagged with a fluorescent dye, and can then be visualized using fluorescence microscopy.

### Intermediate filaments have important clinical applications

Monoclonal antibodies of different types of IF can be used to detect the source of origin of cells e.g. undifferentiated metastatic cells that cannot be identified morphologically can be distinguished as being epithelial, muscle, fibroblasts, glial etc.

### Many diseases involve abnormalities of microtubules or microfilaments

Kartagener syndrome - involves defective microtubule formation

Epidermolysis bullosa - defective keratin

Amyotrophic lateral sclerosis - defect of neurofilaments

Alzheimer's disease - neurofibrillary deposits in neurons

### The Tensegrity Structure or Microtrabecular Lattice of Cells

A meshwork composed of all the microtubules, actin and intermediate filaments in the cell that form the cytoskeletal-supporting framework. Initially it was called a "tangled web" or "microtrabecular lattice", but is now known to form a structure with tensile and compression forces, and is therefore called a **tensegrity structure** rather like a "space frame" in modern architecture.