CANCER 9

THE CYTOSKELETON
Professor Mike Ferenczi

Principal components of the cytoskeleton

Principal components of the cytoskeleton

Review previous outline knowledge of the cytoskeleton and the three filament systems which make it up.
Describe the assembly and organisation of subunits in intermediate filaments.
Define the role of intermediate filaments, their size and localisation.
Describe how the structure of microtubules results from polymerisation of subunits consisting of α,β-tubulin dimers.
Describe the polymerisation dynamics of microtubules in terms of preferred (+) and non-preferred (-) ends and GTP hydrolysis.
Summarise the functions of motor proteins associated with the microtubule cytoskeleton.
Describe the structure of monomeric actin.
Summarise the structure of F-actin filaments and describe their polymerisation from monomeric (G-) actin.
Explain treadmilling in the context of filament polymerisation
Describe what is meant by molecular motors
Describe the role of molecular motors

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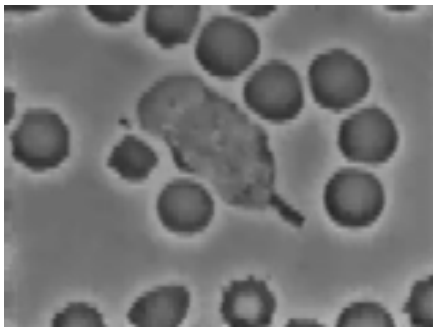
Chemotaxis: Crawling Neutrophil Chasing a Bacterium



Human polymorphonuclear leukocyte (neutrophil) on a blood film "chasing" *Staphylococcus aureus* microorganisms, added to the film.

16mm movie made in the 1950s by David Rogers at Vanderbilt University. Written by Tom Stossel, June 22, 1999. Cell Biology & Cytoskeleton Group Division of Hematology, Brigham & Women's Hospital, Harvard Medical School

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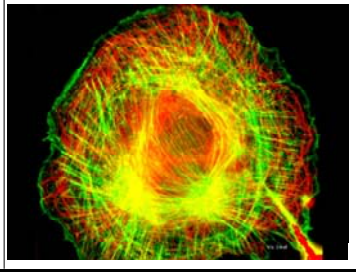
The cytoskeleton consists of three types of filaments

Three types of filaments form the cytoskeletal system (skeleton of the cell):

1. Intermediate filaments. ∅: 8-12 nm
2. Microtubules (MT): ∅: 25 nm
3. Actin filaments – microfilaments and stress fibres: ∅: 7 nm

Each filament type consists of a predominant protein that polymerises to form the filament.



Each filament may also contain additional proteins that give the filaments particular properties: binding specificity, regulation, localisation

LO: Review previous outline knowledge of the cytoskeleton and the three filament systems which make it up.

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Filament systems

Microfilaments. Thin filaments in muscle, made up of *actin, troponin* and *tropomyosin*,

Microtubules (MTs), made up of *tubulin*, and

Intermediate filaments, made up of a number of proteins depending on the cell type.

Each is formed by polymerisation of subunits
There is a wide variety of filament-associated proteins:

- Control polymerisation/depolymerisation
- Link filaments to membranes, organelles, extracellular material via other proteins
- Move organelles, or the cell itself : motor proteins
- Control the movement of motor proteins

Highly dynamic.
Controls cell shape, rigidity and motility.
Cross-linking turns cytoplasm from liquid to gel

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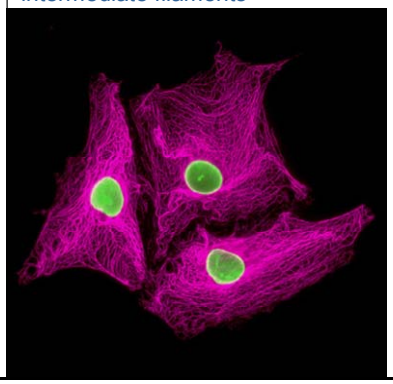
Cytoskeletal filament dynamics

All 3 filament types are made by polymerisation of monomer

Free monomer must be in equilibrium with polymer - but the equilibrium can be altered by other proteins that bind to either free monomers or to filaments near the site of monomer addition

The equilibrium dynamics are similar in principle for actin filaments and microtubules (they differ in detail though), but rather different for intermediate filaments



The intermediate filament cytoskeleton in epithelial cells: keratins (purple) give the cell resilience by making cytoplasmic networks and lamins (green) protect the DNA by reinforcing the nuclear envelope.
Professor Birgit Lane FRSE, University of Dundee

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Intermediate filaments

www.colorado.edu/MCDB/MCDB1150/ohd/overhead.html

Intermediate filaments are stable, durable. Diameter from 8-12 nm (intermediate in size compared with thin filaments and microtubules).
Prominent in cells that withstand mechanical stress
The most insoluble part of the cell. Can be dissociated by urea.
Highly regulated – polymerisation - depolymerisation
Distribution is cell-type specific .
Can be phosphorylated.
Mutations lead to degenerative diseases.

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Intermediate Filaments types

Types I and II: *Acidic Keratin* and *Basic Keratin*, respectively: in epithelial cells (bladder, skin, etc).

Type III. Number of cell types, including:
vimentin in fibroblasts, endothelial cells and leukocytes;
desmins found in desmosomes which link cells together (e.g. cardiac muscle, skeletal muscle);
glial fibrillary acidic protein (GFAP) in astrocytes and other types of glia (binds prion protein), and
peripherin in peripheral nerve fibers.

Type IV: *Neurofilament H* (heavy), *M* (medium) and *L* (low). Modifiers refer to the molecular weight of the NF proteins;
mainly in axonal cells.
internexin and some nonstandard IV's are found in lens fibres of the eye (*filensin* and *phakinin*).

Type V: *lamins* which have a nuclear signal sequence so they can form a filamentous support inside the inner nuclear membrane. *Lamins* are vital to the re-formation of the nuclear envelope after cell division.

Some unclassified protein filaments

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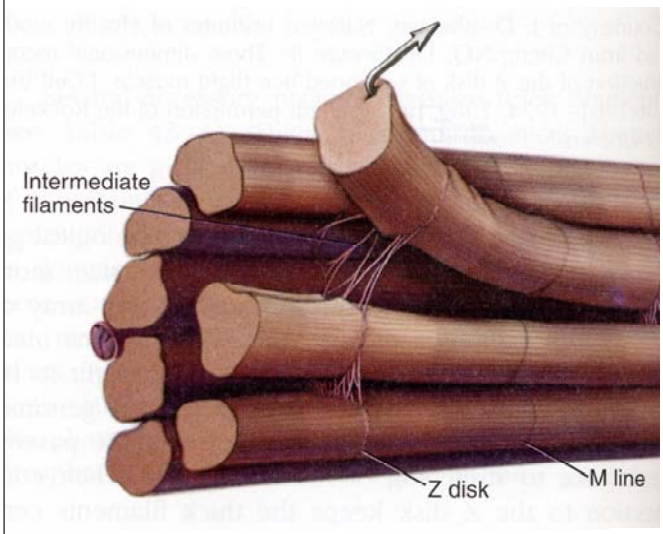
A function of desmin in a muscle cell



In a skeletal muscle cell, desmin filaments join myofibrils, to help maintain sarcomere alignment

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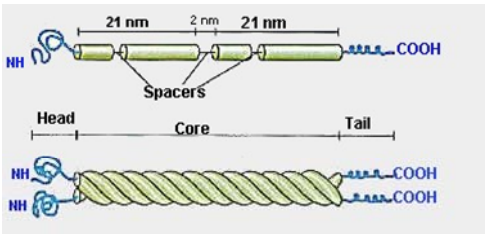
Intermediate filament assembly

The polymerising subunits are tissue specific (though they all have stretches of homologous structure), e.g. cytokeratins in epithelial cells, vimentin in connective tissue cells, neurofilament proteins in neurons, etc.

Usually found as a cytoplasmic network in the central region of the cell
Provide mechanical strength to cells. In epithelial layers links at desmosomes connect intermediate filaments in adjoining cells to create strength of cell sheet



Intermediate filament subunit

Filament start forming by **parallel** assembly of monomers

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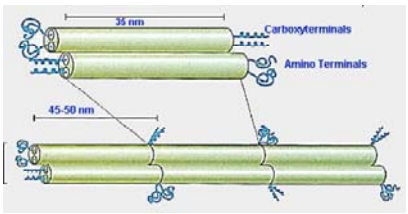
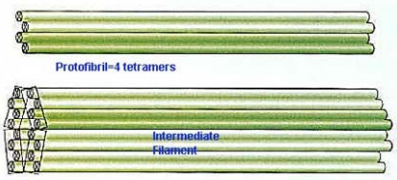
Intermediate filament assembly



Alberts et al, Molecular Biology of the Cell, Garland Publishing, NY, 1996)

Dimers form staggered **anti-parallel** tetramers

Four tetramers (16 filaments) form **protofilaments**
Which then assemble into **intermediate filaments**



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Microtubules (MTs)

•Microtubules are polymers of tubulin.

Microtubules are hollow cylinders. Their circumference is usually made up of 13 tubulin monomers

•The microtubules have polarity (the two ends are not identical)

•One end is called the 'plus' end, the other is the 'minus' end

•Elongation occurs preferentially at the 'plus' end.

•Tubulin dimers bind to MTs. The dimers are made of one molecule of α-tubulin and one of β-tubulin.

•The β-tubulin is at the 'plus end'.

•They are found 'singly' or in more complex structures: cilia, flagella or centrioles.

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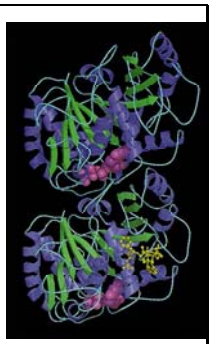
Tubulin

- Tubulin is a highly conserved protein with subdomains which are required for subunit interactions during microtubule assembly
- Tubulin has sites for:
  - GTP binding
  - interaction with MAPs
  - drug binding sites



α-tubulin with GTP

β-tubulin with GDP and taxol

Nogales et al. 1998. Nature 391:199-203

Two type of tubulins:
α-tubulin has a bound GTP that does not hydrolyse.
β-tubulin has a bound GTP or GDP. The bound GTP can be hydrolysed, releasing P_i. GDP can be released and exchanged for GTP
γ-tubulin is found in centrosomes and act as nucleating sites for microtubule assembly

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Microtubule (MT) Assembly

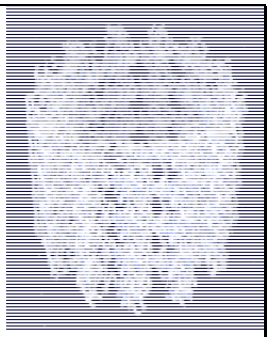
Polymerisation occurs in 2 phases:

1. Nucleation: The process requires tubulin, Mg^{++} and GTP and also proceeds at 37ºC. This stage is relatively slow until the microtubule is initially formed. An α and a β tubulin molecule join to form a heterodimer.

2. Elongation: Made by polymerisation of tubulin (αβ dimer, 2 × 55kDa proteins) to form a hollow filament, 25 nm diameter. Around the periphery are 13 tubulin dimers, forming longitudinal, staggered 13 protofilaments.

GTP bound to β-tubulin is hydrolysed to produce GDP and P_i. GTP bound to α-tubulin is not. Hydrolysis stops at 4 ºC.

MTs have an inherent polarity, determined by polarity of tubulin assembly.



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Microtubule (MT) Assembly

During microtubule assembly GTP binds to tubulin β-subunits and is subsequently hydrolysed to GDP. Tubulin subunits are added to GTP-capped microtubules much more efficiently than to GDP-microtubules

So, if GTP-containing subunits are present at the 'plus' end, then the filament is relatively stable and further polymerisation will occur.

If polymerisation is slow so that hydrolysis occurs faster than new subunit addition, GDP-containing subunits may be present at the end in which case depolymerisation is favoured.

Replacing GTP with a non-hydrolysable analogue of GTP does not prevent polymerisation, but does prevent depolymerisation.

In a healthy cell, the presence of GTP maintains microtubules.

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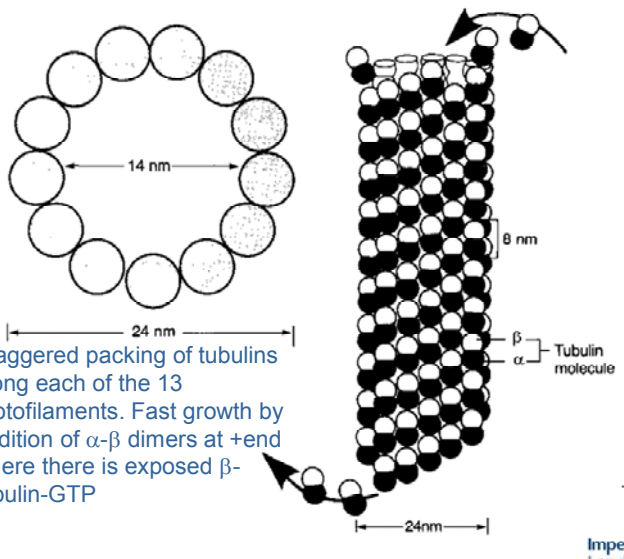
Microtubule structure



Staggered packing of tubulins along each of the 13 protofilaments. Fast growth by addition of α-β dimers at +end where there is exposed β-tubulin-GTP

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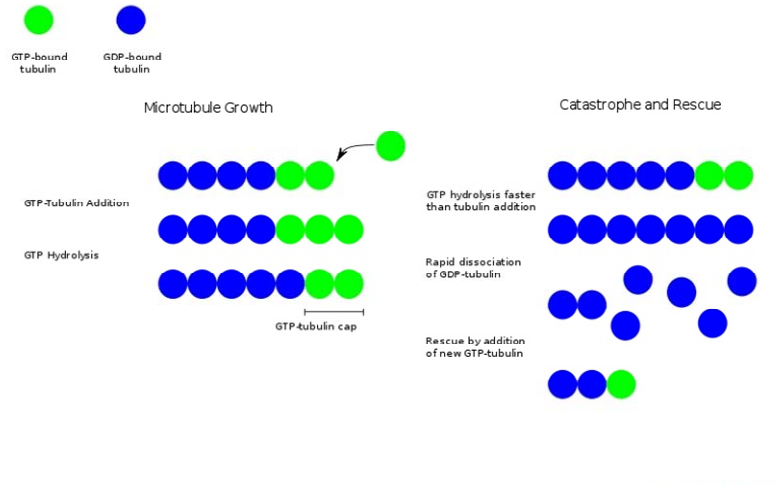
Filament nucleotides



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Filament nucleotides

Microtubule (and actin filament) subunits contain a nucleotide

For tubulin this is GTP

After polymerisation this hydrolyses slowly to GDP

[In the case of actin filaments the nucleotide is ATP/ADP]

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Dynamic instability

The switch between filament growth and depolymerisation is known as dynamic instability.

Polymerisation/depolymerisation of MTs depend on cellular concentrations of MTs, GTP, GDP, tubulin and **microtubule associated proteins** (MAPs) which affect the stability of the plus and minus-ends of MTs.

Free GDP-subunits resulting from depolymerisation are converted to GTP-subunits by nucleotide exchange

Since GTP hydrolysis is energetically favoured, microtubule polymerisation can do work in the cell

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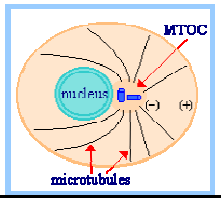
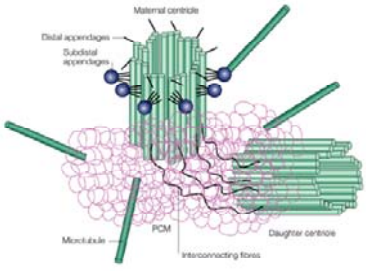
Cellular organisation of microtubules

In interphase cells, MTs extend radially from the microtubule organising centre (MTOC) associated with the centrosome. The minus ends are capped by the **centrosome** (2 centrioles arranged at right angles to each other from which 9 triplet MTs radiate). The plus-end of MTs is at the periphery. Capping of MTs by pericentriolar material (PCM) gives stability to MTs even at low tubulin concentration.

Ability of spindle microtubules to be broken down by cell during mitosis is essential for chromosomal separation





Doxsey (2001) Re-evaluating centrosome function Nature Reviews Molecular Cell Biology 2(9), 688-698.

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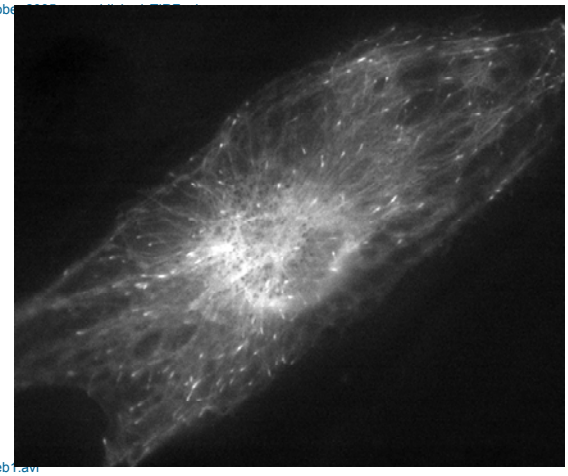
Microtubule elongation

Microtubule elongation

Melanocytes labelled with GFP-EB1 protein. EB1 binds to the tips of microtubules. Alistair Hume, Dmitryi Ushakov, Miguel Seabra, Michael Ferenczi, Imperial College – Octobe



eb1.avi

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Microtubules during mitosis

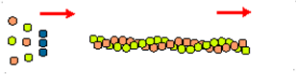
During mitotic spindle assembly, some microtubules are stabilized by the proteins of the kinetochore

During metaphase, subunits are added to the plus end of a microtubule at the kinetochore and are removed from the minus end at the spindle pole (microtubules maintain constant length)

At anaphase the chromatid is released from attachment to its sister at the metaphase plate and the kinetochore moves up the microtubule, removing subunits from its plus end as it goes (chromatid carried to spindle pole).

Part of chromatid movement is due to the simultaneous loss of tubulin subunits from the minus end of the microtubules at the pole.

Treadmilling: growth at one end, and depolymerisation at the other end. Proteins attached to MTs eventually end up at the minus end of the MTs.



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Microtubule associated proteins (MAPs)

Microtubules are essential for many critical cellular processes including cell division. Their polymerisation/depolymerisation is controlled by a number of regulatory systems and accessory proteins.

The microtubule system is a target for anti-cancer drugs:
colchicine, vinblastine, nocodazole, taxol and vincristine, and a target for herbicides and pesticides

MAPs bind to MTs to
•Stabilize them
•Cross-link them
•Attach them to other cellular components: membranes, intermediate filaments, other microtubules

•Microtubule-dependent motor proteins (ATPases): kinesins (anterograde – towards periphery) and dyneins or ncd (retrograde – towards centre)
Tau protein (plaques in Alzheimer)

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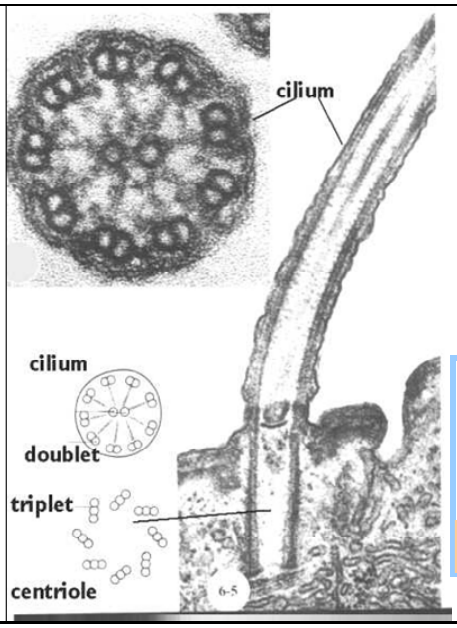
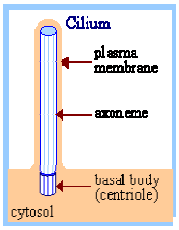
Cilia and flagella



Composed of microtubules

flagella (longer than cilia) - beating provides the force that allows sperm to swim

cilia – hair-like appendages found in respiratory tract (function to clear mucus) and epithelia of oviduct (function to transport ova toward the uterus)



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Microtubule diseases

Inhibition of the axonal transport in the brain accelerates neurodegenerative diseases, such as Alzheimer's disease (AD). Loss of cellular function and cell death might result from an accumulation of microtubule associated proteins (MAP) like tau in brain tissues.

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Actin filament system

EM-view of negatively stained F-actin filaments (Julie Hodgkinson, IC)

Filaments made by polymerisation of G-actin (43 kDa globular protein)

In most cells found at the periphery, underlying the cell surface

Examples include thin filaments of muscle; core of each microvillus in a brush border; involved in cell surface shape changes including those underlying cell migration

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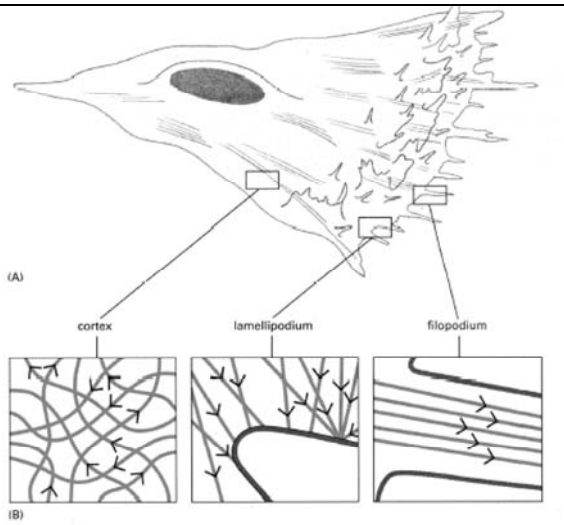
Stress fibres



Stress fibres are cables of actin
Stress fibres are found in fibroblasts and other cells where cell adhesion is important.

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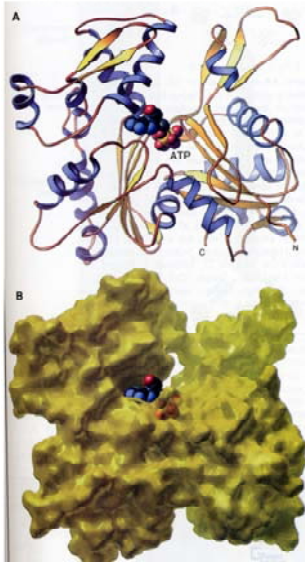
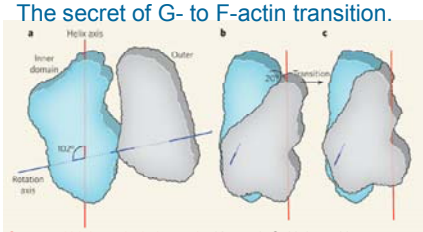
Actin structure



Actin monomers (G-actin for Globular actin) contain ATP or ADP. ATP is slowly hydrolysed upon polymerisation.

The secret of G- to F-actin transition.



Structural biology: Actin in a twist Kenneth C. Holmes Nature 457, 389-390(22 January 2009) doi:10.1038/457389a

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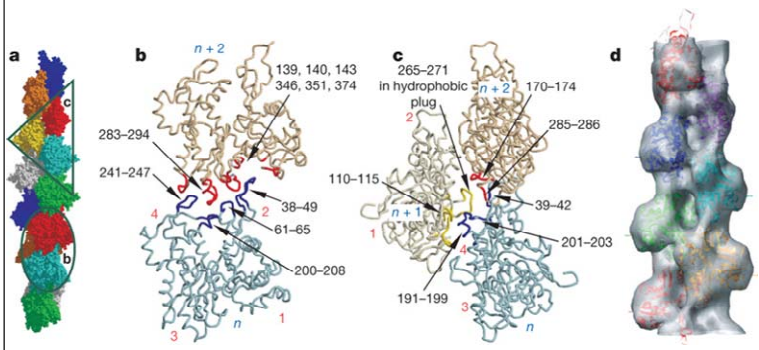
Actin structure



The nature of the globular- to fibrous-actin transition Toshiro Oda, Mitsusada Iwasa, Tomoki Aihara, Yuichiro Maéda & Akihiro Narita Nature 457, 441-445(22 January 2008) doi:10.1038/nature07685

Each repeat contains 13 molecules (35.7 nm).
Rotation/molecule = 167°. Looks like two long-pitch helices slowly winding around each other.

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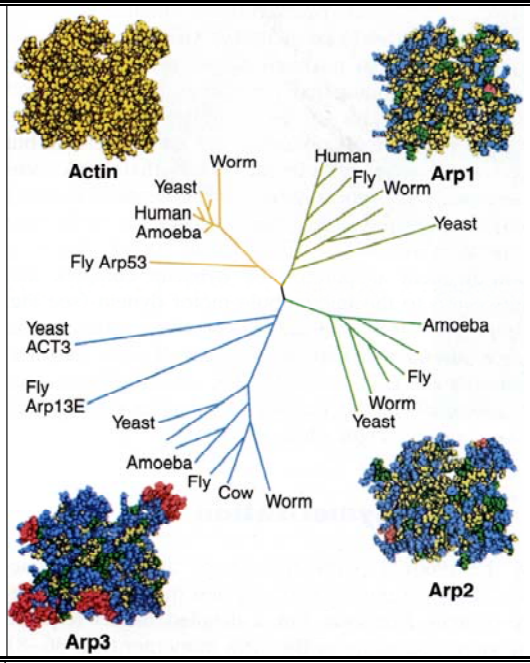
Actin phylogenetic tree



Actin (the amino acid sequence of actin) is highly conserved.

Several actin-like proteins are found. These usually bind actin and co-polymerise with it.

The flat F-actin structure is very much like the bacterial analogue of actin MreB. Actin is a bridge between eukaryotes and prokaryotes.

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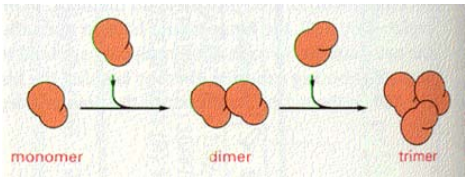
Actin polymerisation

Actin exists in two forms:
G-actin (globular actin) are actin monomers
F-actin (filamentous actin) are actin filaments
Actin monomers bind ATP. The ATP can be hydrolysed to form ADP-actin and P_i.
Two step polymerisation:

1. **Nucleation:** requires ATP-actin monomers at a high concentration to form trimers of ATP-actin. Under some conditions, actin dimers are formed. This allows the formation of branching actin structures. Nucleation rate is proportional to $[actin]^3$.

In motile cells, the leading edge nucleates actin polymerisation by the Arp2/3 complex and WASP family proteins



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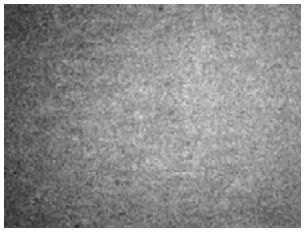
Actin elongation

2. **Elongation:** this is fast in the presence of ATP-actin. ATP-actin monomers are added to actin trimers to form new filaments and at the 'barbed'-end of actin filaments to elongate existing actin filaments. All subunits have identical polarity. Filament is helical. Polymerisation at 'barbed' end is 10x faster than at 'pointed'-end.

Elongation at the leading edge may push the membrane forward



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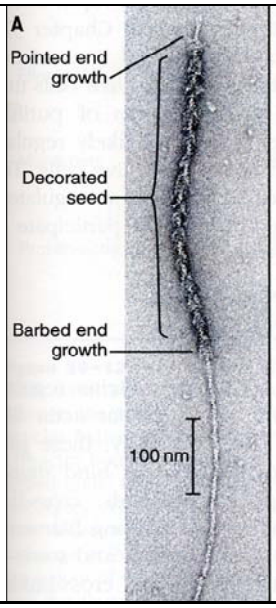
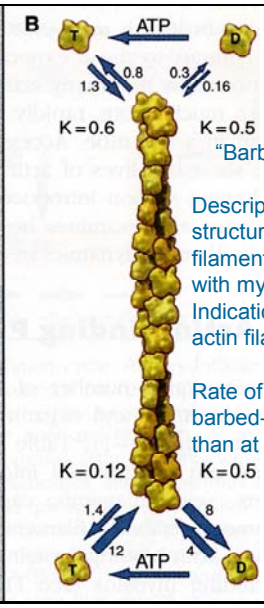
Actin elongation





"Barbed" and "pointed" ends:
Description of herring-bone structure seen when actin filaments are 'decorated' with myosin molecules. Indication of polarity of the actin filaments.

Rate of growth at the barbed-end is ~10x faster than at the pointed end.

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Actin depolymerisation - shortening of filaments

The ATP in F-actin hydrolyses so that the bulk of actin filaments contains ADP-actins.

At low concentrations of ATP-actin monomers, Actin monomers at the 'barbed'-ends of F-actin will end up with ADP-actins. These will dissociate from F-actin, causing shortening, unless stabilised by ligands.

ADP in ADP-actin monomers can be exchanged for ATP.

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Actin-binding proteins (ABPs) in muscle

Examples:
Myosin motors: many classes

Tropomyosin: part of thin filaments in smooth and striated muscle. Involved in regulation

Troponin: confers ca-regulation of contraction to thin filaments in skeletal muscle.

Caldesmon: smooth muscle regulation of contraction

Calponin: smooth muscle regulation

α-actinin: cross-linking protein

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Examples of actin binding proteins in the cytoplasm

α-actinin: cross-linking protein

Gelsolin: capping, nucleating and severing activity. Calcium activated capping and severing. Also bundles actin filaments

Villin: nucleates, severs and caps actin filament. Similar to Profilin: binds actin monomers (ATP-actin) and provides pool for actin elongation at barbed end

Cofilin: regulated by phosphorylation. Binds to G- and F-actin. Increases filament turnover 20-30x

Fimbrin: α-actinin-like domains. Can bundle actin filaments.

Vimentin: found in intermediate filaments. Maintains myofibril alignment in striated muscle

Vinculin: actin cross-linking and bundling

Ezrin, Radixin, Moesin (ERM): regulation by phosphoinositide lipids (membrane components). Active in unfolded tail conformation.

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Actin binding molecules

Cellular poisons active at low concentrations: used as research tools

Cytochalasin: from aspergillus clavatus fungus, inhibit polymerisation/depolymerisation

Latrunculin: from sea sponge: inhibits polymerisation

Phalloidin: toxin from amanita fungus. Binds to and stabilises F-actin. Fluorescent derivative (eg rhodamine-phalloidin) used to stain F-actin in vitro.

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Cell motility examples

Migration of phagocytic cells towards site of infection

•Migration of cancerous cells away from site of primary tumour - invasion

•Migration of cells during embryological development

•Cytoplasmic streaming

•Muscle contraction

•Swimming: waving of cilia/flagellae; movement of liquids

•Transport of organelles, Movement of vesicles

•Phagocytosis

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Cellular motors

Microtubule-dependent motors:

kinesins
dyneins
ncd (nonclaret disjunctional – name of a drosophila gene)

Actin-dependent motors:
myosins

Polymerisation engines: microtubules, actin (eg listeria)

Treadmilling: when filaments shorten at one end and grow at the other.

Rotary motors:
flagellar engine
F1 ATPase

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How do cells move?

•Involves coordinated shape changes due to cytoskeleton (actin mainly)

•Needs appropriate signalling to coordinate parts of cell and control direction

•May depend on extracellular signals and receptor pathways

•Cells become polarised - line up with a thin actin-containing extension (lamellipodium) forming the leading edge

•Microtubule system also is aligned - MTOC (microtubule organising centre) is forward of the nucleus, as is the Golgi

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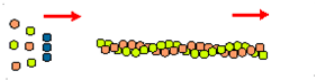
Treadmilling:

Movement of polymers brought about by polymerisation at one end, and depolymerisation at the other.

Treadmilling is observed in the laboratory for microtubules and actin filaments.



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Molecular motors

Molecular Motors:

These are proteins which have evolved to move cells or parts of cells.
The movement requires energy, so molecular motors are usually ATPases (hydrolyse MgATP to MgADP and inorganic phosphate).
Sometimes they harness ion gradients (bacterial flagellae).

ATP hydrolysis results in ADP, P^i, work and heat

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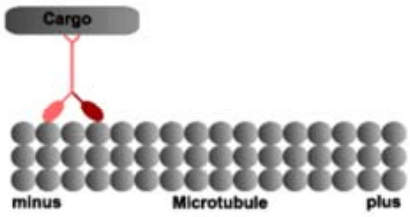
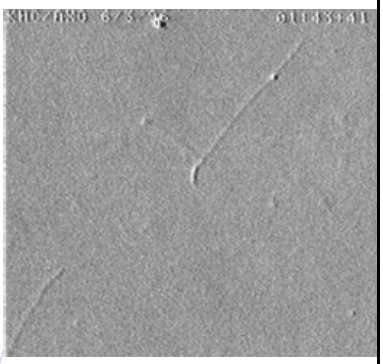
Microtubule based motors

Kinesin moves vesicles along microtubules



Kinesin plated on a slide move microtubules



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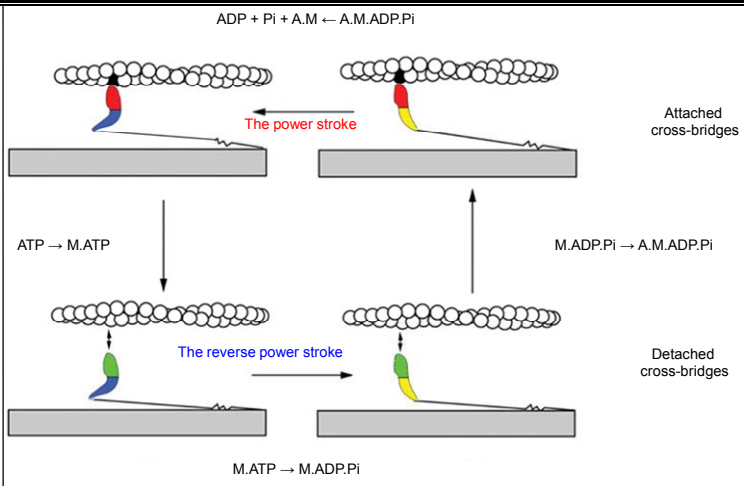
Lymn-Taylor scheme of cross-bridge cycling



What happens in the absence of ATP?

Rigor mortis

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