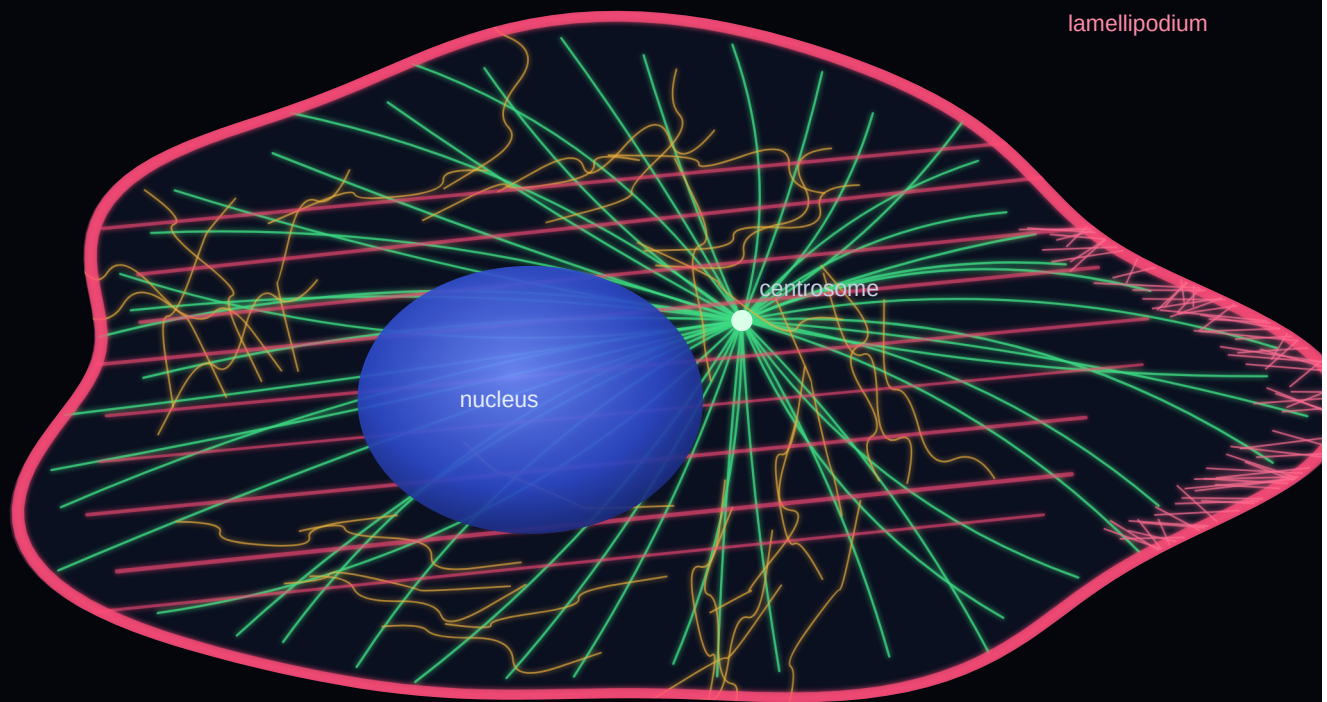




Stylised fluorescence view of a crawling fibroblast, coloured the way it would look under three stains. The same colours mark each filament system throughout these notes.

The cytoskeleton

Actin filaments, microtubules and intermediate filaments: structure, assembly, motor proteins, cilia, and the diseases that follow when they fail.

— Actin (microfilaments) — Microtubules — Intermediate filaments — Nucleus (DNA)

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1. What the cytoskeleton is

The **cytoskeleton** is a dynamic, three-dimensional network of protein filaments running through the cytoplasm of eukaryotic cells. Unlike the bony skeleton it is not a fixed scaffold: most of its filaments are built from small soluble subunits that are added and removed continuously, so the network can be dismantled in one place and rebuilt in another within seconds to minutes.

Three filament systems make it up, each a polymer of a different protein family:

- **Actin filaments** (microfilaments), about 7 nm wide, built from actin.
- **Microtubules**, hollow tubes 25 nm wide, built from α/β -tubulin.
- **Intermediate filaments**, about 10 nm wide, built from a family of fibrous proteins such as keratins, vimentin and lamins.

Around these filaments work hundreds of **accessory proteins**: nucleators that start new filaments, capping and severing proteins that control length, cross-linkers that bundle them into gels or cables, and **motor proteins** (myosin, kinesin, dynein) that convert the chemical energy of ATP into movement along them.

Functions

- **Shape and mechanical strength.** Resists deformation; intermediate filaments give tensile strength, microtubules resist compression, the actin cortex supports the plasma membrane.
- **Internal organisation.** Positions the nucleus, Golgi, ER and mitochondria.
- **Intracellular transport.** Microtubules and actin act as tracks for vesicles, organelles, mRNA and chromosomes.
- **Cell division.** Microtubules form the mitotic spindle; an actin-myosin contractile ring divides the cytoplasm (cytokinesis).
- **Cell movement.** Crawling (lamellipodia, filopodia), muscle contraction, and the beating of cilia and flagella.
- **Signalling and polarity.** Links to cell junctions and the extracellular matrix; transmits mechanical signals (mechanotransduction).

A useful mental model

Think of a tent. **Microtubules** are the poles that resist compression and also serve as the highways; **actin** is the tensioned fabric just under the surface that can be pulled and reshaped; **intermediate filaments** are the guy ropes that stop the whole structure tearing under stress.

2. The three systems compared

Drawn to scale (1 nm $\approx$ 3.5 px)

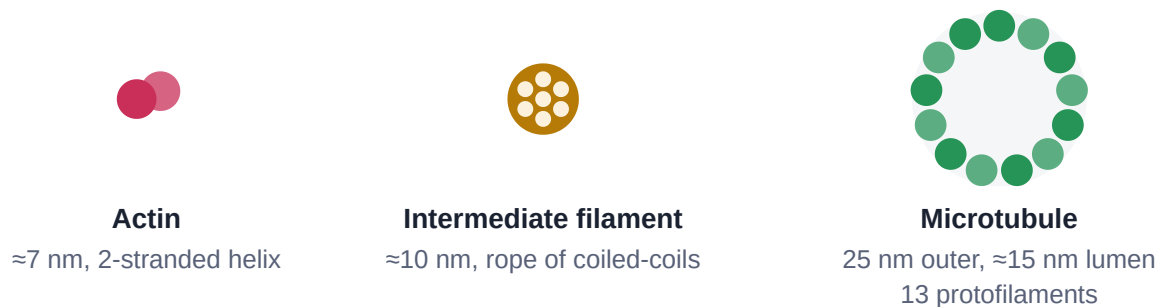


Figure 1. The three filaments in cross-section, to the same scale. Diameter alone is a favourite viva question: 7, 10, 25 nm.

Feature	Actin filament	Microtubule	Intermediate filament
Diameter	≈7 nm	25 nm, hollow	≈10 nm
Subunit	G-actin (42 kDa, globular)	α/β -tubulin heterodimer (~100 kDa)	Fibrous proteins with a central α -helical rod (keratins, vimentin, lamins...)
Nucleotide	ATP (hydrolysed to ADP)	GTP on β -tubulin (hydrolysed to GDP)	None
Structure	Two protofilaments twisted into a helix	13 protofilaments forming a tube	8 tetramers per cross-section, twisted like a rope
Polarity	Yes: barbed (+) and pointed (-) ends	Yes: plus end (β exposed) and minus end (α exposed)	No (antiparallel tetramers cancel out)
Dynamics	Treadmilling; turnover in seconds	Dynamic instability; turnover in minutes	Relatively stable; remodelled by phosphorylation
Nucleation	Arp2/3 complex, formins	γ -tubulin ring complex at the MTOC	Spontaneous self-assembly

Feature	Actin filament	Microtubule	Intermediate filament
Motors	Myosins	Kinesins, dyneins	None
Location	Cortex under the membrane, microvilli, stress fibres, contractile ring	Radiate from centrosome; spindle; axonemes of cilia	Around the nucleus to desmosomes; nuclear lamina
Mechanics	Resist tension, generate contraction	Resist compression; rigid	Very high tensile strength; stretch without breaking
Main roles	Crawling, cytokinesis, muscle contraction, cell shape	Transport, mitosis, cilia/flagella, organelle positioning	Mechanical resilience, nuclear structure
Classic drugs	Cytochalasin, latrunculin, phalloidin	Colchicine, vinblastine, taxol	(no specific drugs in routine use)

3. Actin filaments (microfilaments)

Actin is one of the most abundant proteins in eukaryotic cells, often 5–10% of total protein in non-muscle cells and far more in muscle. It is extraordinarily conserved: yeast and human actin share about 88% sequence identity. Vertebrates have three main isoforms: **α-actin** in muscle, and **β-** and **γ-actin** in non-muscle cells.

From G-actin to F-actin

The monomer, **G-actin** (globular, 42 kDa, 375 amino acids), has a deep cleft that binds one ATP (or ADP) with a Mg^{2+} ion. Monomers polymerise head-to-tail into **F-actin** (filamentous), which looks like two strands of beads twisted around each other with a crossover every ≈ 37 nm.

Because every subunit points the same way, the filament is **polar**. When decorated with myosin heads under the electron microscope it shows arrowheads, which gave the ends their names: the **barbed end (+)** and the **pointed end (–)**. The barbed end grows about 5–10 times faster.

Polymerisation happens in three phases

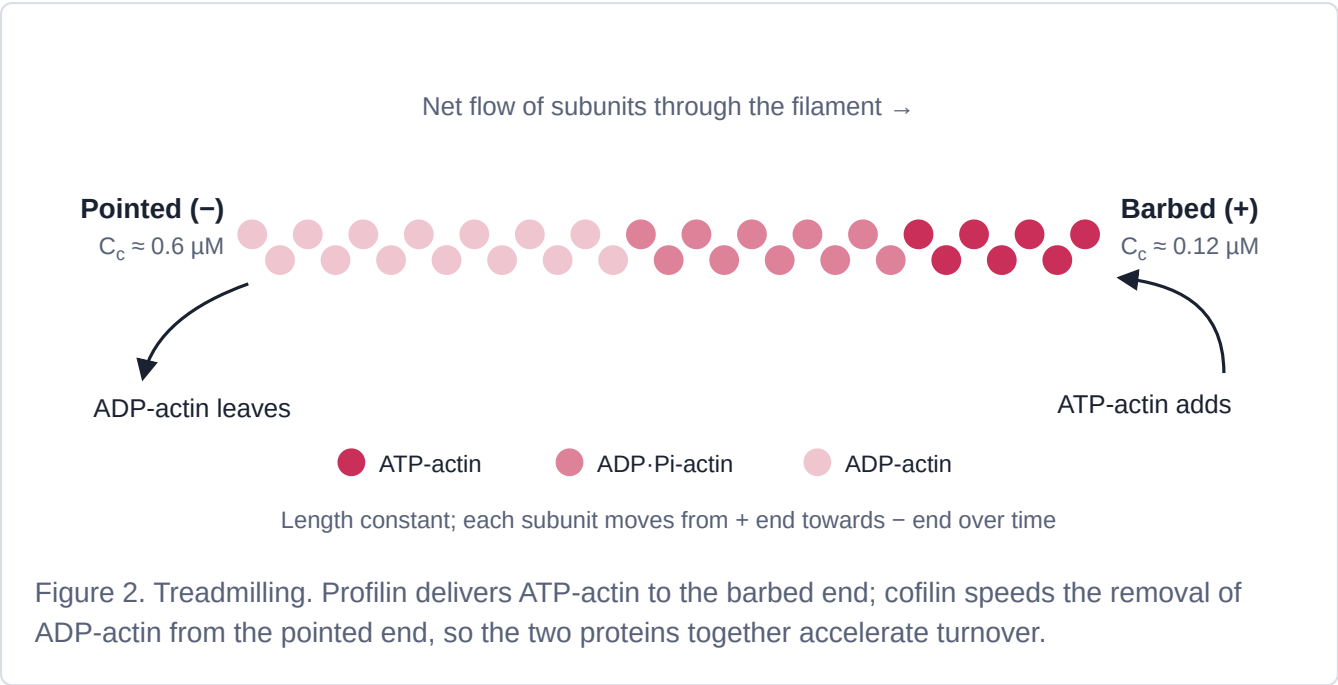
1. **Nucleation (lag phase).** Dimers and trimers are unstable; forming a stable trimer "seed" is the slow, rate-limiting step. Cells bypass it with nucleating proteins.
2. **Elongation.** Monomers add rapidly to both ends, faster at the barbed end.

3. **Steady state.** Assembly and disassembly balance; the free monomer concentration settles at the **critical concentration (C_c)**, below which filaments cannot grow.

ATP hydrolysis and treadmilling

ATP-actin adds to the filament and, shortly afterwards, the bound ATP is hydrolysed to ADP·P_i; the P_i is then slowly released. So a growing filament has an **ATP cap** at its barbed end and older ADP-actin towards its pointed end. ADP-actin binds less tightly and dissociates more readily.

The result is two different critical concentrations: about **0.12 μM at the barbed end** and **0.6 μM at the pointed end**. When the free ATP-actin concentration lies between these values, subunits add at the barbed end and are lost from the pointed end at the same rate. The filament keeps a constant length while individual subunits flow through it from front to back. This is **treadmilling**.

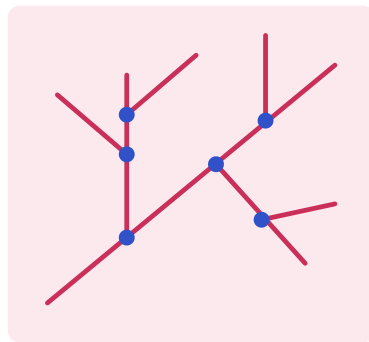


Actin-binding proteins: the control panel

Pure actin behaves predictably in a test tube. Inside a cell, dozens of actin-binding proteins decide where, when and in what shape filaments form. Grouping them by job is the easiest way to remember them.

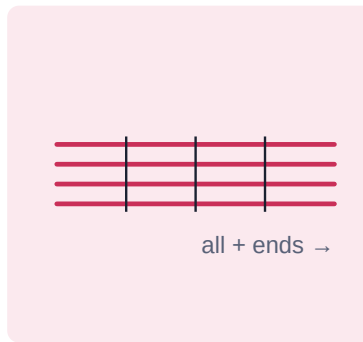
Job	Protein	What it does
Monomer binding	Thymosin β 4	Sequesters ATP-G-actin, keeping a large unpolymerised reserve

Job	Protein	What it does
	Profilin	Catalyses ADP → ATP exchange on G-actin and delivers it to barbed ends (and to formins)
Nucleation	Arp2/3 complex	Binds the side of an existing filament and starts a branch at $\approx 70^\circ$; activated by WASP/WAVE (nucleation-promoting factors)
	Formins (e.g. mDia)	Nucleate unbranched filaments and ride on the growing barbed end, speeding elongation
Capping	CapZ (capping protein)	Caps barbed ends to stop growth; anchors thin filaments at the Z-disc
	Tropomodulin	Caps pointed ends (e.g. in muscle thin filaments)
Severing	Cofilin (ADF)	Binds ADP-actin, twists and severs filaments, speeds pointed-end loss
	Gelsolin	Ca^{2+} -activated; severs and then caps barbed ends (gel → sol transition)
Bundling	Fimbrin, fascin, villin	Tight parallel bundles (microvilli, filopodia)
	α -Actinin	Looser, antiparallel bundles that leave room for myosin II (stress fibres, Z-disc)
Cross-linking into gels	Filamin	V-shaped dimer linking filaments at wide angles into a 3-D gel (cortex)
Membrane links	Spectrin, ankyrin, ERM proteins, dystrophin	Attach actin to membrane proteins
Side binding	Tropomyosin	Stabilises filaments; regulates myosin access in muscle



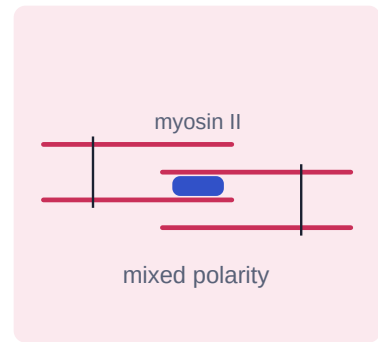
Branched network

Arp2/3 (blue dots) · lamellipodia



Parallel bundle

Fascin, fimbrin · filopodia, microvilli



Contractile bundle

α -actinin + myosin II · stress fibres

Figure 3. The same filament, three architectures. Which cross-linker is present decides the shape, and the shape decides the job.

Where actin structures are found

- **Cell cortex.** A filamin-cross-linked meshwork under the plasma membrane that gives the surface its stiffness. In red blood cells a specialised version, the **spectrin-actin membrane skeleton**, is linked to band 3 by ankyrin and to glycophorin through protein 4.1.
- **Microvilli.** A core of 20–30 parallel filaments bundled by villin and fimbrin, barbed ends at the tip, anchored in the terminal web.
- **Lamellipodia and filopodia.** Sheet-like and finger-like protrusions at the leading edge of a crawling cell.
- **Stress fibres.** Contractile cables that end in **focal adhesions**, where integrins bind the extracellular matrix via talin and vinculin.
- **Contractile ring.** A belt of actin and myosin II that pinches a dividing animal cell in two.
- **Adherens junctions.** An actin belt linked to cadherins through catenins.

How a cell crawls

1. **Protrusion:** Arp2/3-driven branched polymerisation pushes the leading edge forward as a lamellipodium.
2. **Adhesion:** new focal adhesions attach the front to the substratum.
3. **Translocation:** myosin II contraction pulls the cell body forward.
4. **De-adhesion:** adhesions at the rear release and the tail retracts.

Rho-family GTPases act as the switches: **Rac** → lamellipodia, **Cdc42** → filopodia, **Rho** → stress fibres and contraction.

4. Myosins and muscle contraction

Myosins are ATP-driven motors that walk along actin, almost all towards the **barbed (+) end**. Myosin VI is the famous exception and moves towards the pointed end. Each myosin has a **head** (motor domain with the ATP- and actin-binding sites), a **neck** that acts as a lever arm and binds light chains, and a **tail** that decides what the motor carries or binds.

Class	Structure	Function
Myosin I	One head, short tail that binds membrane	Links membrane to actin; microvilli, endocytosis
Myosin II	Two heavy chains + four light chains; long coiled-coil tails assemble into bipolar thick filaments	Muscle contraction, stress fibres, cytokinesis
Myosin V	Two heads, long lever arms; processive, 36 nm steps	Carries vesicles and organelles (e.g. melanosomes)
Myosin VI	Two heads; moves to the pointed (-) end	Endocytic transport; hearing (stereocilia)

The sarcomere

Skeletal muscle is built from repeating **sarcomeres**, running from one **Z-disc** to the next. Thin filaments (actin with tropomyosin and troponin) are anchored at the Z-disc by their barbed ends through α -actinin and CapZ; their pointed ends are capped by tropomodulin. Thick filaments of myosin II sit in the middle (A-band) and are centred by the giant elastic protein **titin**; **nebulin** sets thin filament length.

In the **sliding filament model** (H. E. Huxley & Hanson; A. F. Huxley & Niedergerke, 1954) the filaments themselves do not shorten. Myosin heads pull the thin filaments towards the centre, so the I-band and H-zone shorten while the A-band stays the same length.

The cross-bridge cycle

1. **Attached (rigor)**: a myosin head without nucleotide is bound tightly to actin.
2. **Release**: ATP binds the head and it lets go of actin.
3. **Cocking**: ATP is hydrolysed to ADP + P_i ; the head swings into a "cocked" position.
4. **Binding**: the head attaches weakly to a new actin subunit further along.
5. **Power stroke**: P_i release triggers the lever-arm swing that moves the filament about 5–10 nm; ADP is released and the head returns to rigor.

Regulation. At rest, tropomyosin covers myosin-binding sites on actin. When the muscle is stimulated, Ca^{2+} released from the sarcoplasmic reticulum binds **troponin C**, shifting tropomyosin and exposing the sites. In smooth muscle and non-muscle cells, Ca^{2+} -calmodulin activates **myosin light-chain kinase** instead.

Rigor mortis

After death ATP runs out, so step 2 cannot happen: myosin heads stay locked onto actin and the muscles become stiff.

5. Microtubules

Microtubules were named by Ledbetter and Porter in 1963, once glutaraldehyde fixation made them visible under the electron microscope. They are the stiffest of the three filaments: a microtubule behaves like a hollow rod, which is exactly how engineers make a light but rigid beam.

Structure

- The building block is the **α/β -tubulin heterodimer** (each monomer ≈ 50 kDa; the dimer is 8 nm long). The two are so tightly bound that the dimer never separates under physiological conditions.
- Dimers stack head-to-tail into **protofilaments**. In most cells **13 protofilaments** associate side by side to close a tube 25 nm across with a lumen of about 15 nm.
- Neighbouring protofilaments are slightly offset, so the lattice is a shallow helix with a **seam** where the pattern is interrupted.
- **Polarity:** β -tubulin faces the **plus end** (fast-growing), α -tubulin faces the **minus end**.
- **GTP:** both subunits bind GTP. On α -tubulin it is trapped at the dimer interface and never hydrolysed (the N-site). On β -tubulin (the exchangeable E-site) it is hydrolysed to GDP soon after the dimer joins the lattice.

Dynamic instability

Mitchison and Kirschner (1984) showed that individual microtubules in the same solution behave differently: some grow steadily while others abruptly shrink, and each can switch between the two states. This is **dynamic instability**.

The explanation is the **GTP cap**. GTP-tubulin dimers are straight and pack well into the lattice. Once GTP is hydrolysed, GDP-tubulin prefers a curved shape that the lattice holds under strain. While the growing plus end carries a cap of GTP-tubulin, the tube stays closed and keeps growing. If addition slows so that hydrolysis catches up and the cap is lost, the

strained GDP lattice springs apart: protofilaments curl outward like ram's horns and the microtubule shrinks up to ten times faster than it grew.

- **Catastrophe:** the switch from growth to shrinkage.
- **Rescue:** the switch from shrinkage back to growth.

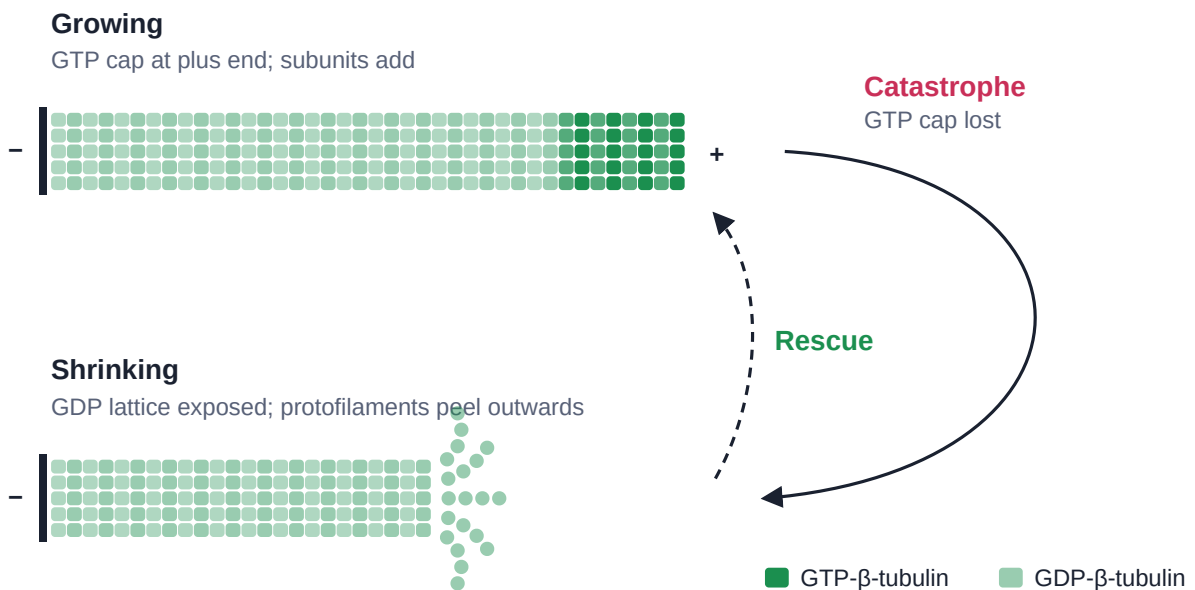


Figure 4. Dynamic instability. The GTP cap (dark dimers) keeps the plus end straight and growing. Without it, the GDP lattice relaxes into curved protofilaments and the polymer collapses. Dynamic instability lets a cell "search" space quickly, as when spindle microtubules probe for kinetochores ("search and capture").

Treadmilling vs dynamic instability

Both depend on nucleotide hydrolysis. Treadmilling is a steady flux of subunits through a filament of constant length and is typical of actin. Dynamic instability is a stochastic switching of the whole filament end between growth and rapid shrinkage and is the hallmark of microtubules (which can also treadmill, for instance in the spindle).

Microtubule-organising centres (MTOC)

Microtubules do not start spontaneously in cells; they are nucleated by the **γ-tubulin ring complex (γ-TuRC)**, a lock-washer of 13 γ-tubulins that acts as a template. The γ-TuRC caps the minus end, so minus ends stay anchored at the MTOC and plus ends extend outward.

In animal cells the main MTOC is the **centrosome**: a pair of **centrioles** set at right angles, embedded in **pericentriolar material** that holds the γ-TuRCs. The centrosome duplicates once per cell cycle, in S phase, and the two centrosomes become the poles of the spindle.

Plant cells and many oocytes have no centrioles yet still build spindles, which shows the pericentriolar material, not the centriole, does the nucleating. Other MTOCs include basal bodies and the spindle pole body of yeast.

Microtubule-associated proteins (MAPs)

Type	Example	Effect
Stabilising	Tau (axons), MAP2 (dendrites)	Bind the lattice, stabilise and space microtubules into bundles. Hyperphosphorylated tau detaches and forms neurofibrillary tangles in Alzheimer disease.
Plus-end tracking (+TIPs)	EB1, CLIP-170	Ride on growing plus ends; promote growth and link ends to the cortex or kinetochores
Polymerase	XMAP215	Adds tubulin dimers to plus ends
Destabilising	Stathmin (op18)	Sequesters tubulin dimers, raises catastrophe frequency
	Kinesin-13 (MCAK)	Motor-like protein that peels protofilaments at the ends
Severing	Katanin, spastin	Cut microtubules along their length (release from centrosome, axon branching)

Post-translational "tubulin code". Long-lived, stable microtubules (axons, cilia) carry marks such as **acetylation** of α -tubulin (on lysine 40, inside the lumen), **detyrosination** and polyglutamylation. These tune which motors and MAPs bind.

Microtubules in mitosis

The mitotic spindle is built from three classes: **kinetochore microtubules** attach to chromosomes; **polar (interpolar) microtubules** overlap at the equator and push the poles apart; **astral microtubules** radiate to the cortex and position the spindle. At anaphase A kinetochore microtubules shorten, pulling chromatids to the poles; in anaphase B, sliding of polar microtubules by kinesin-5 and pulling on astral microtubules by cortical dynein push the poles apart.

6. Kinesin and dynein

Two families of motor walk on microtubules, and they head in opposite directions.

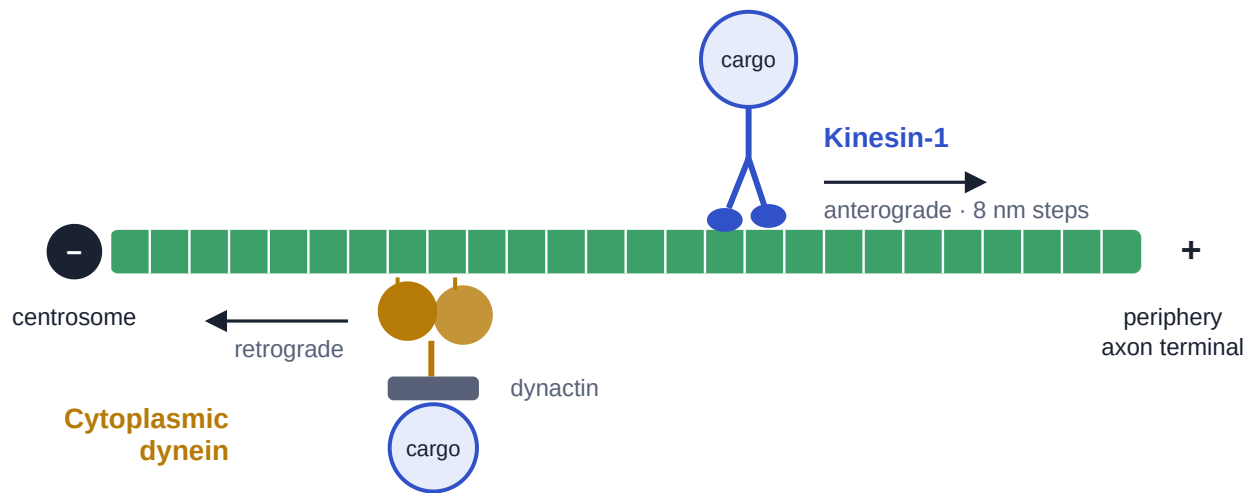


Figure 5. In a neuron, kinesin carries synaptic vesicle precursors and mitochondria out to the axon terminal (anterograde), while dynein returns endosomes and signalling molecules to the cell body (retrograde).

Feature	Kinesin (kinesin-1)	Cytoplasmic dynein
Discovered	1985, squid giant axon (Vale, Reese, Sheetz)	Axonemal dynein 1965 (Gibbons); cytoplasmic 1987
Size	≈380 kDa; 2 heavy + 2 light chains	≈1.4 MDa; 2 heavy chains + many intermediate and light chains
Motor domain	Small, related to myosin and G-proteins	Large ring of six AAA+ ATPase domains with a stalk
Direction	Plus end (most kinesins; kinesin-14 goes to minus end)	Minus end
Stepping	Hand-over-hand, 8 nm per ATP, highly processive	Variable 8–32 nm steps; needs dynactin and an adaptor for long runs
Jobs	Anterograde transport, ER extension, spindle assembly (kinesin-5)	Retrograde transport, Golgi positioning near centrosome, spindle positioning

A common organising principle: in most animal cells microtubule minus ends sit at the centre and plus ends at the periphery, so kinesin moves things outward and dynein moves things inward. The ER is stretched out along microtubules by kinesin; the Golgi is gathered near the centrosome by dynein.

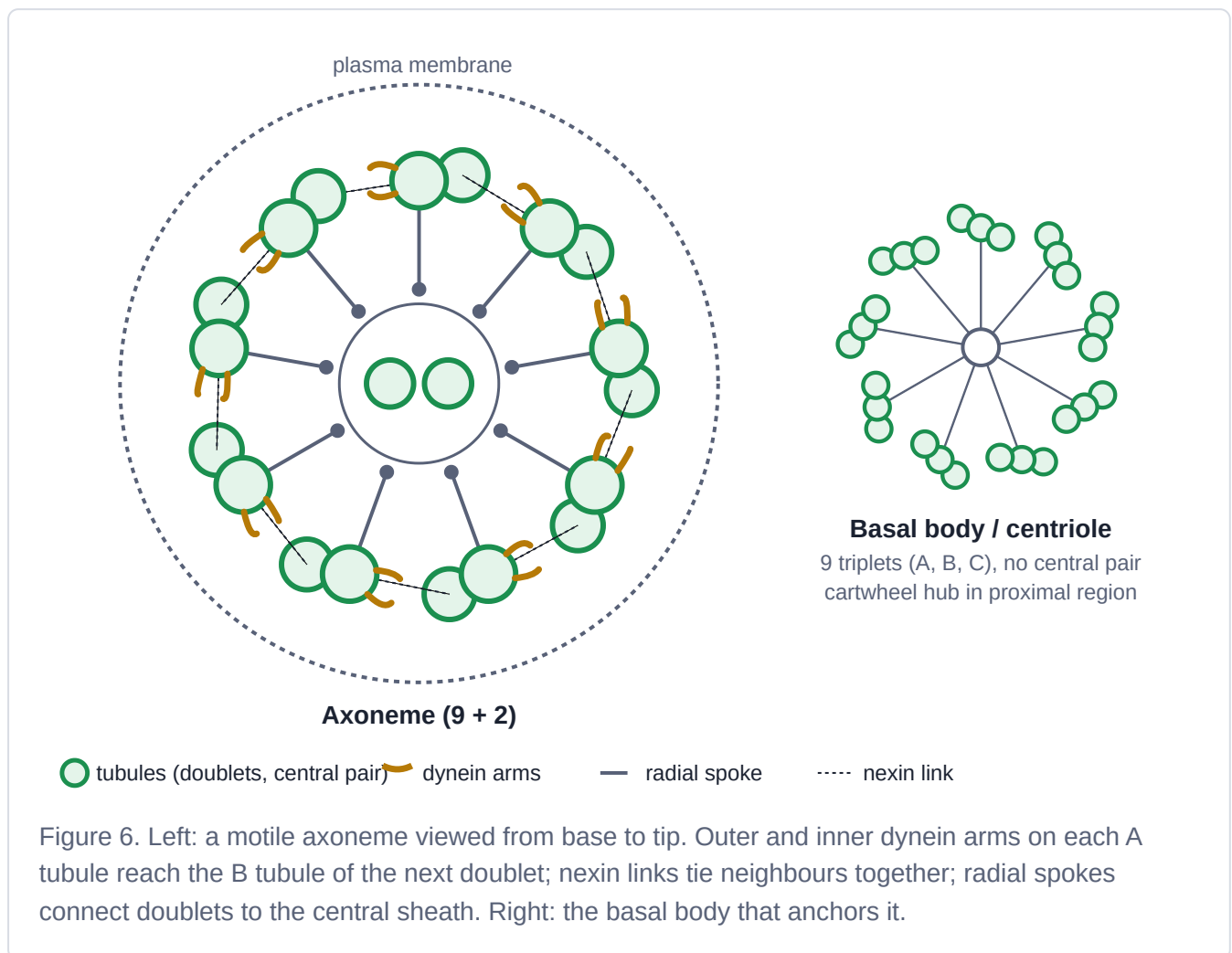
7. Centrioles, cilia and flagella

Centrioles and basal bodies

A centriole is a short cylinder about $0.2\text{ }\mu\text{m}$ wide and $0.4\text{--}0.5\text{ }\mu\text{m}$ long, made of **nine microtubule triplets** (A, B and C tubules) arranged like the blades of a turbine, with no central microtubules: the **$9 \times 3 + 0$** pattern. The proximal end contains a **cartwheel** whose hub and spokes set the nine-fold symmetry. A **basal body** is a centriole that has docked beneath the plasma membrane and templates a cilium; its A and B tubules continue into the axoneme.

The axoneme

Motile cilia and eukaryotic flagella share one core, the **axoneme**, with the **$9 + 2$** pattern: nine outer **doublets** around a central pair of single microtubules. In each doublet the A tubule is complete (13 protofilaments) and the B tubule is incomplete (10–11 protofilaments) and fused to the A tubule.



How a cilium bends: the sliding microtubule mechanism

1. Dynein arms on the A tubule of one doublet bind the B tubule of the neighbour and walk towards its minus end, the ciliary base.
2. If the doublets were free, this would make them slide past one another. Summers and Gibbons (1971) proved it: after gentle trypsin digestion removed nexin links and spokes, adding ATP made axonemes telescope apart to many times their length.
3. In an intact axoneme the **nexin-dynein regulatory complex** and the anchoring at the basal body resist sliding, so sliding is converted into **bending**.
4. Dyneins on opposite sides are activated alternately, controlled through radial spokes and the central pair, producing a rhythmic beat.

Feature	Cilia	Flagella (eukaryotic)
Number	Many per cell (hundreds)	One or a few
Length	Short, $\approx 5\text{--}10\ \mu\text{m}$	Long, up to $\approx 150\ \mu\text{m}$ or more
Beat	Oar-like: power stroke and recovery stroke	Undulating, wave-like (sine wave)
Coordination	Metachronal waves across a sheet	Independent
Moves	Fluid over the cell surface, or the cell	The cell
Examples	Airway epithelium, oviduct, <i>Paramecium</i>	Sperm, <i>Euglena</i> , <i>Chlamydomonas</i>

Do not confuse with bacterial flagella. These are hollow helical filaments of the protein **flagellin**, rotated by a proton-driven motor at the base. They contain no tubulin, no dynein, and are not covered by membrane.

Primary cilia and intraflagellar transport

Most vertebrate cells carry a single, non-motile **primary cilium** with a **9 + 0** axoneme (no central pair, no dynein arms). It works as a sensory antenna, concentrating receptors such as those for Hedgehog signalling, and in kidney tubules it senses urine flow. Cilia have no ribosomes, so their proteins are ferried up and down by **intraflagellar transport (IFT)**: kinesin-2 carries IFT trains to the tip and dynein-2 brings them back. Defects cause **ciliopathies** such as polycystic kidney disease and Bardet-Biedl syndrome. (Nodal cilia in the embryo are an exception: 9 + 0 but motile, and their leftward flow sets up left-right asymmetry.)

8. Intermediate filaments

Intermediate filaments (IFs) are named for their diameter, about 10 nm, between that of actin and the thick myosin filaments of muscle. They differ from the other two systems in almost every way: they are built from **fibrous** rather than globular subunits, bind **no nucleotide**, have **no polarity** and therefore **no motor proteins**. What they offer is toughness. An IF network can be stretched to several times its length without breaking, which is why cells under mechanical stress, such as epidermis, muscle and axons, are packed with them. Not all animal cells have cytoplasmic IFs (many insects lack them), and plants and fungi have none, but the nuclear lamins are found in all metazoans.

Structure and assembly

Every IF protein has the same plan: a non-helical **N-terminal head**, a central **α -helical rod domain** of about 310 amino acids (≈ 350 in lamins) with heptad repeats, and a non-helical **C-terminal tail**. The rod is conserved and drives assembly; the head and tail vary between proteins and give each IF its identity.

Monomer

head · central α -helical rod · tail



Dimer

parallel coiled coil (polar)



Tetramer

antiparallel, staggered (apolar)



Unit-length filament

8 tetramers side by side, ≈ 60 nm long



10-nm filament

ULFs anneal end to end, then compact



width ≈ 10 nm, no polarity

Figure 7. Assembly of intermediate filaments. Because the tetramer is built from two dimers lying in opposite directions, the finished filament has no polarity. That single fact explains why no motor protein can walk along it.

Assembly needs no energy and no nucleator. Its disassembly is controlled by **phosphorylation** of the head domains. The clearest example is the nuclear lamina: at the start of mitosis, CDK1 phosphorylates lamins, the lamina depolymerises and the nuclear envelope breaks down; dephosphorylation at telophase lets it re-form.

Classification

Type	Protein	Where found
I	Acidic keratins (K9–K28)	Epithelia, hair, nails. Always form obligate heteropolymers of one type I + one type II keratin (e.g. K5/K14 in basal epidermis, K1/K10 above it, K8/K18 in simple epithelia).
II	Basic keratins (K1–K8)	
III	Vimentin	Mesenchymal cells: fibroblasts, endothelium, leukocytes
	Desmin	Muscle (around Z-discs)
	Glial fibrillary acidic protein (GFAP)	Astrocytes and other glia
	Peripherin	Peripheral neurons
IV	Neurofilaments NF-L, NF-M, NF-H; α -internexin	Neurons; set axon calibre and so conduction velocity
V	Lamins A/C, B1, B2	Nuclear lamina lining the inner nuclear membrane (all nucleated animal cells)
VI	Nestin	Neural stem/progenitor cells (a stem-cell marker)

Linkers and junctions

IFs form a basket around the nucleus and radiate to the cell surface. There they attach to **desmosomes** (cell-cell, via desmoplakin and plakoglobin) and **hemidesmosomes** (cell-matrix, via plectin and integrin $\alpha 6\beta 4$). Through these junctions the keratin networks of neighbouring cells are joined into a single mechanical web across the whole epithelium. **Plakins** such as plectin also cross-link IFs to actin and microtubules, knitting the three systems together.

IF typing in pathology

Because each tissue expresses its own IF type, and tumours usually keep it, immunostaining for IFs helps identify the origin of a poorly differentiated or metastatic tumour: cytokeratin → carcinoma; vimentin → sarcoma or lymphoma; desmin → muscle tumours (leiomyo-/rhabdomyosarcoma); GFAP → glial tumours; neurofilament → neuronal tumours.

9. Septins and the bacterial cytoskeleton

Septins are GTP-binding proteins that assemble into non-polar filaments and rings, sometimes called the fourth component of the cytoskeleton. First found at the bud neck of budding yeast, they act as scaffolds and diffusion barriers, for example at the cleavage furrow and at the base of cilia.

Bacteria were long thought to lack a cytoskeleton. They in fact carry ancestral versions of all three systems:

Bacterial protein	Eukaryotic relative	Role
FtsZ	Tubulin	Forms the Z-ring at the division site; recruits the division machinery (also used by chloroplasts)
MreB	Actin	Directs cell-wall synthesis to keep rod-shaped bacteria rod-shaped
ParM	Actin	Pushes plasmid copies apart; shows dynamic instability
Crescentin	Intermediate filament	Gives <i>Caulobacter crescentus</i> its curved shape

The sequence similarity between FtsZ and tubulin is low, but their three-dimensional folds and GTP-binding sites are nearly identical, a classic case where structure is conserved longer than sequence.

10. Drugs and toxins

Many natural toxins target the cytoskeleton, and they have been essential research tools as well as medicines.

Agent	Source	Target	Action	Use
Cytochalasin D	Fungi	Actin	Caps barbed ends, blocks elongation	Research
Latrunculin A	Red Sea sponge	Actin	Binds G-actin 1:1 and sequesters it	Research
Phalloidin	<i>Amanita phalloides</i> (death cap)	Actin	Binds and stabilises F-actin; not membrane-permeable	Fluorescent staining of F-actin
Jasplakinolide	Sponge	Actin	Stabilises F-actin; cell-permeable	Research
Colchicine	Autumn crocus	Tubulin	Binds dimers, blocks polymerisation	Gout (blocks neutrophil migration); making polyploid plants; karyotyping (metaphase arrest)
Vinblastine, vincristine	Madagascar periwinkle	Tubulin	Bind dimers at the vinca site, promote depolymerisation	Leukaemia, lymphoma
Nocodazole	Synthetic	Tubulin	Reversible depolymeriser	Cell-cycle synchronisation
Paclitaxel (Taxol)	Pacific yew	Tubulin	Binds β -tubulin inside the lumen, stabilises microtubules	Breast, ovarian, lung cancer

Note the paradox: **colchicine and taxol have opposite effects on polymer mass but the same effect on the cell**. Both freeze the dynamics that the spindle needs, so dividing cells arrest in mitosis. It is the dynamics, not the amount of polymer, that matters.

11. Clinical correlations

Condition	Defect	Key features
Primary ciliary dyskinesia (Kartagener syndrome)	Axonemal dynein arms (often outer arm, <i>DNAH5</i>)	Chronic sinusitis, bronchiectasis, male infertility (immotile sperm), situs inversus in about half
Polycystic kidney disease	Primary cilium proteins (polycystins)	Kidney cysts
Hereditary spherocytosis	Spectrin, ankyrin, band 3, protein 4.2	Spherical, fragile red cells; haemolytic anaemia; splenomegaly
Duchenne muscular dystrophy	Dystrophin (links actin to the sarcolemma)	Progressive muscle degeneration
Epidermolysis bullosa simplex	Keratin K5 or K14	Blistering after minor friction; basal cells split
Epidermolytic hyperkeratosis	Keratin K1 or K10	Blistering and thickened skin, suprabasal split
Hutchinson–Gilford progeria	Lamin A (<i>LMNA</i>), produces "progerin"	Premature ageing, misshapen nuclei
Emery–Dreifuss muscular dystrophy	Lamin A/C or emerin	Muscle weakness, cardiac conduction defects
Alexander disease	GFAP	Leukodystrophy with Rosenthal fibres
Alzheimer disease	Hyperphosphorylated tau	Neurofibrillary tangles; microtubule loss in neurons
Wiskott–Aldrich syndrome	WASP (activator of Arp2/3)	Immunodeficiency, eczema, thrombocytopenia
Chédiak–Higashi syndrome	<i>LYST</i> (lysosomal trafficking)	Giant granules; defective microtubule-dependent lysosome movement in neutrophils

Pathogens exploit actin. *Listeria monocytogenes* and *Shigella* hijack host Arp2/3 to build an actin "comet tail" that pushes them through the cytoplasm and into neighbouring cells. *Listeria*'s surface protein ActA mimics the host WASP family activator.

12. Quick revision

Remember	Value / fact
Diameters	Actin 7 nm, IF 10 nm, microtubule 25 nm
Protofilaments	13 per microtubule; A tubule 13, B tubule 10–11
Nucleotides	Actin: ATP. Tubulin: GTP (hydrolysed on β only). IF: none
Nucleators	Arp2/3 (branched, 70°), formins (linear), γ -TuRC (microtubules)
Motor directions	Myosin $\rightarrow$ barbed (+) except VI. Kinesin $\rightarrow$ plus except kinesin-14. Dynein $\rightarrow$ minus
Step sizes	Kinesin 8 nm, myosin V 36 nm
Axoneme	9 + 2 (motile); 9 + 0 (primary cilium, nodal cilium); basal body/centriole 9 $\times$ 3 + 0
Behaviours	Actin treadmills; microtubules show dynamic instability; IFs are stable, remodelled by phosphorylation
Stains	Phalloidin (F-actin); anti-tubulin antibody; DAPI (DNA)

13. End-semester question bank

Long answer (10–15 marks)

1. Define the cytoskeleton. Give a comparative account of microfilaments, microtubules and intermediate filaments in terms of structure, chemical composition, assembly and function.
2. Describe the ultrastructure of a microtubule. Explain dynamic instability and the role of the GTP cap, and add a note on microtubule-associated proteins.
3. With a labelled diagram, describe the ultrastructure of a eukaryotic cilium, including the basal body. Explain the sliding microtubule mechanism of ciliary movement and the experimental evidence for it.
4. Describe the polymerisation of actin. Explain critical concentration and treadmilling, and discuss how actin-binding proteins regulate the actin cytoskeleton.

5. Give an account of molecular motors. Compare myosin, kinesin and dynein in structure, direction of movement and function.
6. Describe the structure, assembly and classification of intermediate filaments. Discuss their importance in disease and in tumour diagnosis.
7. Explain the sliding filament theory of skeletal muscle contraction, describing the cross-bridge cycle and its regulation by calcium.
8. Describe the role of the cytoskeleton in cell division, covering both the mitotic spindle and cytokinesis.

Short notes (5 marks)

1. Treadmilling
2. Centrosome and the γ -tubulin ring complex
3. Arp2/3 complex and formins
4. Kartagener syndrome
5. Nuclear lamina
6. Colchicine and taxol
7. Spectrin membrane skeleton of the erythrocyte
8. Intraflagellar transport and primary cilia
9. Stress fibres and focal adhesions
10. Prokaryotic cytoskeleton

Differentiate between

1. Microfilaments and microtubules
2. Cilia and flagella
3. Kinesin and dynein
4. Centriole and basal body
5. Treadmilling and dynamic instability
6. Eukaryotic and bacterial flagella

Draw and label

1. T.S. of a 9 + 2 axoneme
2. T.S. of a centriole
3. Stages of intermediate filament assembly

4. Dynamic instability of a microtubule
5. A sarcomere, showing A-band, I-band, H-zone and Z-disc

▼ **Very short answer (2 marks), with answers**

1. Outer diameter of a microtubule? **25 nm**
2. Protofilaments in a typical microtubule? **13**
3. Which tubulin subunit hydrolyses its GTP? **β -tubulin**
4. A toxin that stabilises F-actin and is used to stain it? **Phalloidin**
5. A microtubule motor moving towards the minus end? **Dynein** (also kinesin-14)
6. Microtubule arrangement in a primary cilium? **9 + 0**
7. Intermediate filament protein of astrocytes? **GFAP**
8. Protein that caps actin barbed ends at the Z-disc? **CapZ**
9. Actin monomer-binding protein that promotes ADP→ATP exchange? **Profilin**
10. Bacterial homologue of tubulin? **FtsZ**
11. Protein that nucleates microtubules? **γ -tubulin (γ -TuRC)**
12. Kinase that phosphorylates lamins at mitosis? **CDK1 (cyclin B-CDK1)**

14. Case-based MCQs

Ten hard questions that test application rather than recall. Choose an answer before opening the explanation.

Q1. A 9-year-old boy has had recurrent sinusitis and a chronic wet cough since infancy. Chest imaging shows bronchiectasis and his heart lies on the right side. A nasal brush biopsy is examined by transmission electron microscopy. The finding most likely to be reported is:

- A. Nine outer doublets with no central pair in all cilia
- B. Absence of outer dynein arms on the A tubules
- C. Nine triplets in place of doublets in the axoneme
- D. Absence of the B tubule in each doublet

▼ **Show answer**

Answer: B. This is primary ciliary dyskinesia with situs inversus (Kartagener syndrome). The commonest ultrastructural defect is loss of outer dynein arms, often from *DNAH5* mutations. Without the arms, doublets cannot slide, so airway cilia do not clear mucus and nodal cilia fail to set left-right asymmetry, leaving situs to chance (hence situs inversus in about half). Option A describes a normal primary cilium; C describes a basal body.

Q2. Fibroblasts are treated with a drug that binds actin monomers in a 1:1 complex. Within minutes the lamellipodia stop, stress fibres disappear and phalloidin staining falls sharply, although the drug never contacts filaments directly. The drug is most likely:

- A. Cytochalasin D
- B. Phalloidin
- C. Latrunculin A
- D. Jasplakinolide

▼ **Show answer**

Answer: C. Latrunculin sequesters G-actin. Because filaments constantly lose subunits at their pointed ends, removing the monomer pool starves the barbed ends and the network disassembles. Cytochalasin caps barbed ends (a filament-binding drug); phalloidin and jasplakinolide stabilise filaments and would increase, not decrease, phalloidin staining.

Q3. A woman receiving paclitaxel for metastatic breast cancer develops numbness and tingling in a glove-and-stockings pattern. Which statement best explains both the anti-tumour effect and the neuropathy?

- A. It binds free tubulin dimers and prevents their polymerisation
- B. It binds β -tubulin in the lumen, stabilising microtubules and suppressing their dynamics
- C. It inhibits the ATPase of kinesin-1
- D. It binds the vinca site and promotes depolymerisation

▼ **Show answer**

Answer: B. Taxanes stabilise microtubules. In dividing tumour cells, the frozen spindle cannot capture and segregate chromosomes, so cells arrest in mitosis. In the longest axons, abnormal, over-stabilised microtubule bundles disrupt axonal transport, and the longest nerves (feet and hands) suffer first. A describes colchicine; D describes vinca alkaloids, which also cause neuropathy but by the opposite mechanism.

Q4. Purified tubulin is polymerised in vitro with GMPCPP, a slowly hydrolysable GTP analogue, instead of GTP. The microtubules grow but almost never undergo catastrophe. The best explanation is:

- A. GMPCPP cannot bind β -tubulin, so only α -tubulin polymerises
- B. Hydrolysis of GTP on β -tubulin after incorporation is what destabilises the lattice; without it every subunit behaves like a cap
- C. Hydrolysis of GTP at the α -tubulin N-site triggers catastrophe
- D. GMPCPP recruits MAPs that stabilise the lattice

▼ **Show answer**

Answer: B. Dynamic instability depends on hydrolysis at the exchangeable site on β -tubulin after the dimer joins the lattice. GTP-tubulin is straight; GDP-tubulin wants to curve. If hydrolysis cannot occur, the whole microtubule is effectively one long GTP cap and stays stable. The α -tubulin GTP is never hydrolysed, and there are no MAPs in a purified system.

Q5. In a cultured neuron, fluorescently tagged mitochondria are seen travelling from the cell body towards the axon terminal at about 1 $\mu\text{m/s}$. Axonal microtubules have their plus ends towards the terminal. The motor responsible is:

- A. Cytoplasmic dynein, walking to the plus end
- B. Kinesin-1, walking to the plus end
- C. Kinesin-14, walking to the minus end
- D. Myosin V, walking on axonal actin

▼ **Show answer**

Answer: B. Fast anterograde axonal transport is powered by kinesin (mainly kinesin-1 and kinesin-3) moving to the plus end. Dynein carries retrograde traffic to the minus end in the cell body. Myosin V handles short-range movement in actin-rich regions, and at speeds of μm per second over long distances the microtubule system is responsible.

Q6. A newborn develops blisters at sites of minor friction, such as where the nappy rubs. Skin biopsy shows the split lies within the basal layer of the epidermis, with an intact basement membrane. The gene most likely mutated encodes:

- A. Keratin 1 or keratin 10
- B. Keratin 5 or keratin 14
- C. Desmin
- D. Collagen VII

▼ **Show answer**

Answer: B. Epidermolysis bullosa simplex is caused by mutations in the keratin pair of basal keratinocytes, K5 and K14. The cells' keratin network collapses and the cells rupture under stress, so the split is inside basal cells. K1/K10 are suprabasal keratins (epidermolytic hyperkeratosis). Collagen VII defects cause dystrophic EB, with a split beneath the basement membrane.

Q7. A 10-year-old child has alopecia, thin skin, loss of subcutaneous fat, and coronary atherosclerosis. Her fibroblast nuclei are lobulated and blebbed. The mutant protein belongs to which class of cytoskeletal filament?

- A. Type III intermediate filament
- B. Type V intermediate filament
- C. Actin-binding protein
- D. Microtubule-associated protein

▼ **Show answer**

Answer: B. This is Hutchinson–Gilford progeria. A point mutation in *LMNA* activates a cryptic splice site, producing progerin, a lamin A that stays permanently farnesylated and anchored to the inner nuclear membrane. Lamins are type V intermediate filaments forming the nuclear lamina, so the nuclear shape is distorted.

Q8. A poorly differentiated soft-tissue tumour from a 7-year-old's orbit is stained for intermediate filaments. It is vimentin-positive, desmin-positive, cytokeratin-negative and GFAP-negative. The most likely diagnosis is:

- A. Squamous cell carcinoma
- B. Astrocytoma
- C. Rhabdomyosarcoma
- D. Adenocarcinoma

▼ **Show answer**

Answer: C. Tumours usually keep the IF type of their tissue of origin. Vimentin marks mesenchymal origin and desmin marks muscle, which together point to a myogenic sarcoma; in a child's orbit that is rhabdomyosarcoma. Carcinomas would express cytokeratin; astrocytomas would express GFAP.

Q9. A solution contains actin filaments with free ends. The critical concentration is 0.12 μM at the barbed end and 0.6 μM at the pointed end. The free ATP-actin concentration is held at 0.3 μM . What happens to the filaments?

- A. Both ends grow
- B. Both ends shrink
- C. Barbed ends grow and pointed ends shrink, so subunits flux through filaments of roughly constant length
- D. Pointed ends grow and barbed ends shrink

▼ Show answer

Answer: C. At 0.3 μM the monomer concentration is above C_c at the barbed end (net addition) but below C_c at the pointed end (net loss). At the steady-state concentration between the two, the rates balance and the filament treadmills. The difference in C_c exists only because ATP is hydrolysed; with a non-hydrolysable analogue both ends would share one C_c .

Q10. *Listeria monocytogenes* moves through the host cytoplasm propelled by a comet tail of actin, and spreads into neighbouring cells. A strain lacking the surface protein ActA is taken up but cannot spread. ActA most directly functions by:

- A. Acting like WASP to activate the Arp2/3 complex, nucleating branched filaments behind the bacterium
- B. Acting like formin to build unbranched filaments
- C. Binding profilin and sequestering monomers
- D. Severing host filaments like gelsolin

▼ Show answer

Answer: A. ActA mimics host nucleation-promoting factors of the WASP/WAVE family. It activates Arp2/3 at the bacterial surface, generating a branched network whose polymerisation pushes the bacterium forward, just as it pushes the leading edge of a lamellipodium. Without ActA, no tail forms and cell-to-cell spread fails.

For further reading: Alberts, *Molecular Biology of the Cell*; Lodish, *Molecular Cell Biology*; Karp, *Cell and Molecular Biology*; De Robertis, *Cell and Molecular Biology*.