Cytoskeleton Dynamics (Chap 16)

Cytoskeleton – system of filaments (actin, microtubule, intermediate filaments) that enhance the spatial and mechanical functions like rearranging components while growing, dividing, etc.

Functions and dynamics

Different classes have different functionality.

1. Actin – • determine shape of cell surface
   • whole cell locomotion
   • pinching of one cell into two

2. Microtubules – • determine positions of organelles
   • direct intracellular transport
   • form mitotic spindle

3. Intermediate filaments – • provide mechanical strength

Accessory proteins → e.g., motor proteins (convert ATP hydrolysis energy to mechanical force)

Actin Filaments

- helical polymers of actin — flexible and diameter 8nm
- organise into variety of linear bundles, 2D networks, 3D gels.
- most highly concentrated in cortex, just beneath plasma memb.

Microtubules

- long hollow cylinders made of tubulin protein
- much more rigid than actin filaments
- long and straight and have one end attached to MTOC (centrosome)

Intermediate filaments

- ropelike fibers made of intermediate filament proteins
- forms nuclear lamina or extends across cytoplasm

Cytoskeletons — Dynamic and Stable

- dynamic and adaptable
- regulation and assembly leads to enormous range of structures

Actin filaments

↳ in cell cortex, provides strength and shape to lipid bilayer

→ dynamic cell surface projections → filopodia
→ lamellipodia
→ pseudopodia
} used to move around

→ more stable arrays → allow cell to brace against substratum (stereocilia in inner ear)

Microtubules

↳ frequently found in an array that extends to cell periphery

→ can quickly rearrange to form bipolar mitotic spindle during cell division

→ form cilia as well as tightly aligned bundles for transport down cell

→ in plant cells, direct the pattern of cell wall synthesis

Intermediate filaments

- line the inner face of nuclear envelope, protective cage for DNA
- in cytosol, twisted into strong cables that hold epithelial sheets together

Rapid reorganisation of Cytoskeleton during cell division

Chromosomes replicate

↓

Interphase microtubule array spreads (mitotic spindle)

↓

Two copies of chromosome transfered into different daughter cell

↓

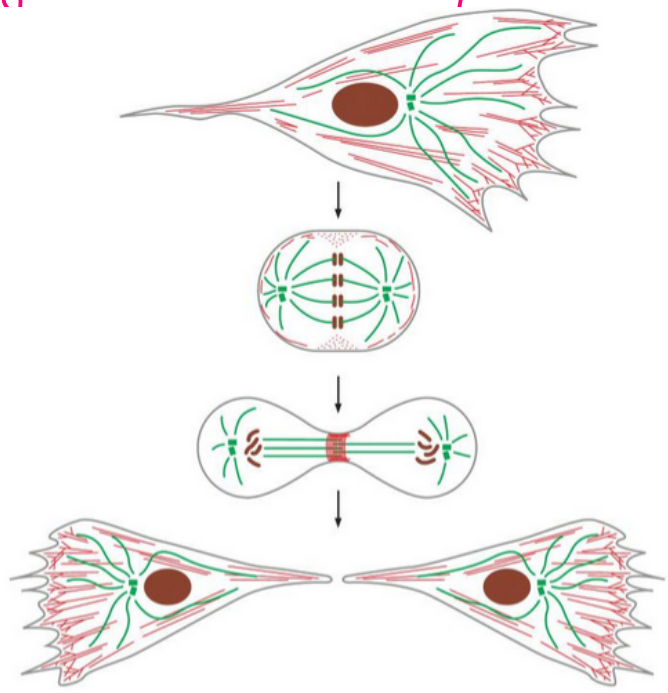
Specialised actin structures stop cell from moving & give circular shape

↓

Actin & myosin form contractile ring around centre of cell (to pinch cell into two)

↓

When division is complete, cytoskeletons of two cells rearrange to form interphase structures



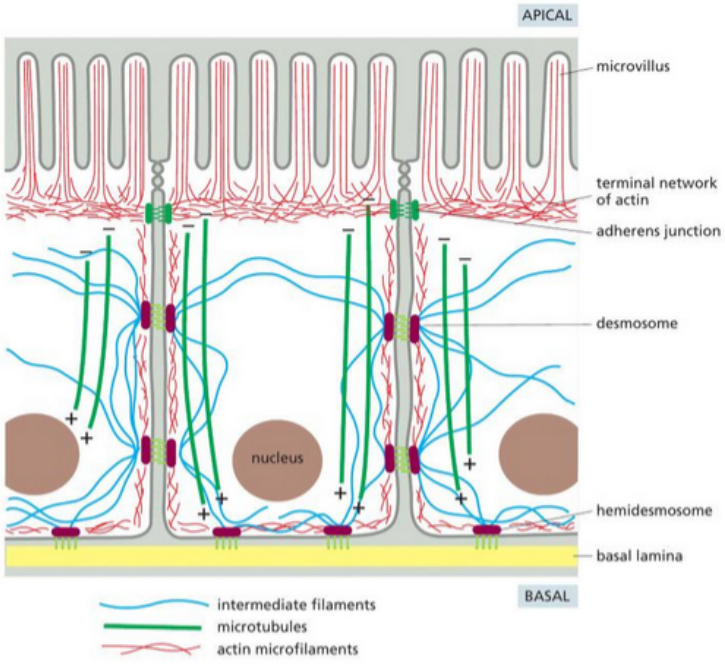
Cellular organisation and Polarity

- In cells with stable differentiated morphology, dynamic elements of cytoskeleton produce stable, large scale structures for cellular organisation.

- at the cores of microvilli on intestinal cells, actin bundles maintain constant location, length and diameter for a few days, but for entire lifetime of cell in case of stereocilia on the hair cells of inner ear.

- also responsible for large-scale cellular — enable cells to Tell difference between Two ends of cell
  ↳ conveyed by cytoskeletal organisation

e.g., polarised epithelial cells use organised array of microtubules, actin & intermediate filaments to maintain critical difference b/w apical and basolateral surface



Protein Subunits of filaments

- subunits are small → can diffuse rapidly in cytosol; long filaments undergo rapid rearrangements by assembling at one site after disassembling at another site

- actin filaments – actin subunits } small, compact,
  microtubule – tubulin subunits } globular
  intermediate filaments – elongated, fibrous subunits

- subunits self-associate, using end to end or side-to-side contacts (weak, non-covalent interaction)
  ↳ no bond to be broken, hence assembly & disassembly fast

- Polarity of filaments – because actin & tubulin subunits are asymmetrical, bind head-to-tail
  subunit polarity → gives filaments structural polarity along length & makes two ends of each polymer behave differently

- Subunits can hydrolyse ATP (actin) & GTP (tubulin) – helps filaments to remodel rapidly

In order to introduce thermal stability, and adaptability microtubules are made of 13 protofilaments – forming a hollow cylinder.

Breaking this filament into two would require breaking bonds of all protofilaments — energetically unfavourable → thermal stability

↳ less bonds broken when removing subunits from ends → rapid dynamics at filament ends.

Accessory proteins and motors act on cytoskeletal filaments

- cell has to maintain a dynamic but stable internal str.,
  - → regulate length & stability of cytoskeleton
  - → number and geometry
  - → attach to each other & to organelles

Some of this is by direct covalent modification of filament

→ Most of regulation done by groups of accessory proteins

↳ bind to filaments or subunits to determine assembly, partitioning & change in rate of assembly & disassembly of filaments

Thus, bringing cytoskeletal str. under control of extracellular & intracellular signals (e.g., cell cycle)

e.g., motor proteins — move along filaments through repeated cycles of ATP hydrolysis

Reynolds no. → depends on ratio of internal to viscous forces acting on an object.

Brownian motion in the cell due to random thermal fluctuations

↙ critical for rate of biochemical rxns

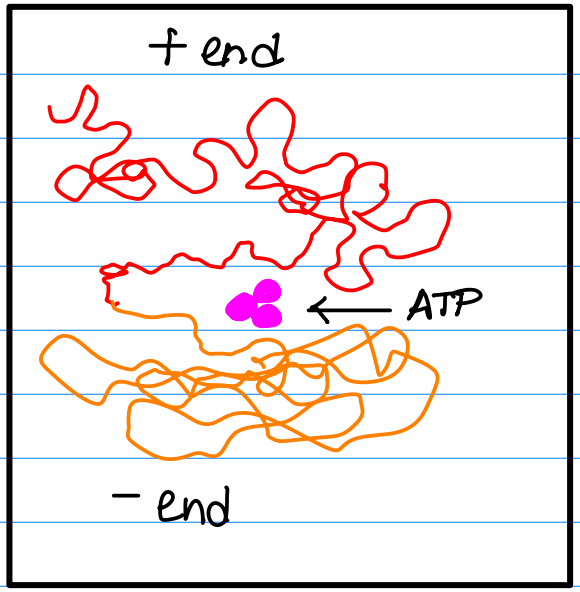
↘ generation of viscous drag forces at small scale → impedes motion of mol. motor → motor activity required

ACTIN

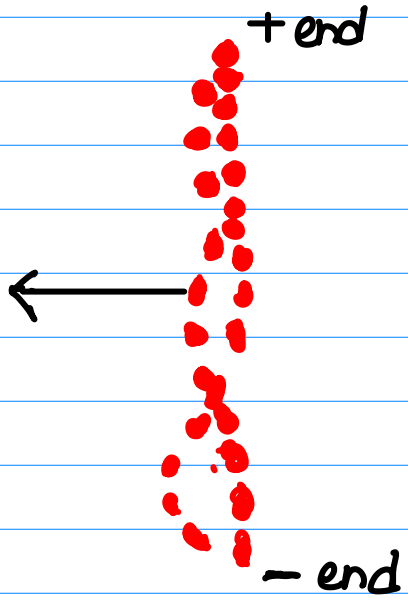
- subunit — globular G-actin (375 amino acid, carrying tightly associated ATP/ADP)
- small variations → isotypes in vertebrates → diff. func.
  - α (muscle cells)
  - β & γ (non-muscle cells)

Assembly

- assemble head to tail to form tight right-handed helix (Filamentous/F-actin)



Monomer



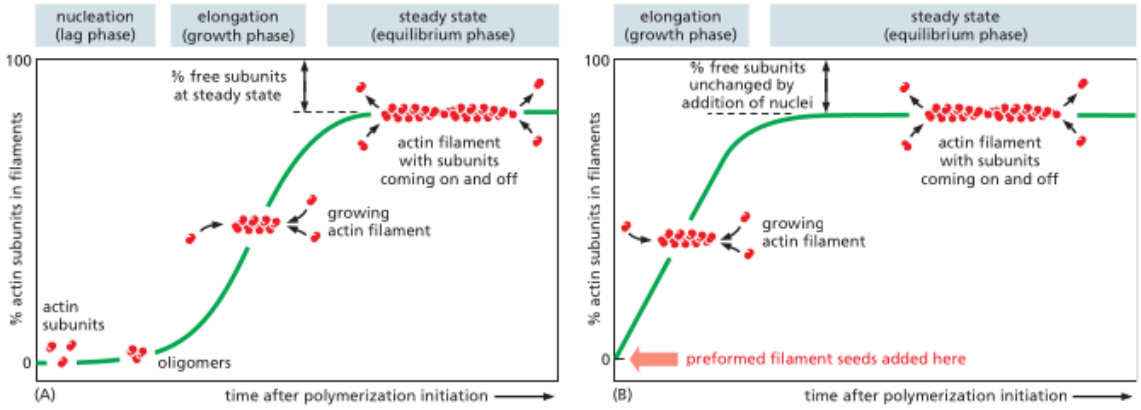
- filaments polar → str. diff. ends → slower growing ⊖ end
  ↳ faster growing ⊕ end
  ↳ monomer ATP binding cleft directed towards ⊖ end

- individually, quite flexible. → crosslink and form large-scale actin bundles (rigid)

Stiffness	characterised by persistence length (length at which random thermal fluctuations can cause it to bend

Nucleation

- actin subunits can spontaneously bind one another — but association unstable until oligomer (nucleus) formation stabilised by multiple subunit - subunit contact & then can elongate rapidly (filament nucleation



Instability of smaller actin filaments → inefficient nucleation

↓

Initiation of polymerisation

↓

Lag phase — no filaments observed

↓

During lag phase, small unstable oligomers → actin filament

↓

Phase of rapid elongation - quick addition to nucleated ends

↓

Conc. of actin monomer ↓

↓

Steady state where rate of addition = rate of subunit dissociation

↓

Conc. of free subunits in soln. → Critical conc. (C_c)

$$k_{on} C = k_{off} \quad \& \quad C_c = \frac{k_{off}}{k_{on}} = k_d \text{ (dissociation const.)}$$