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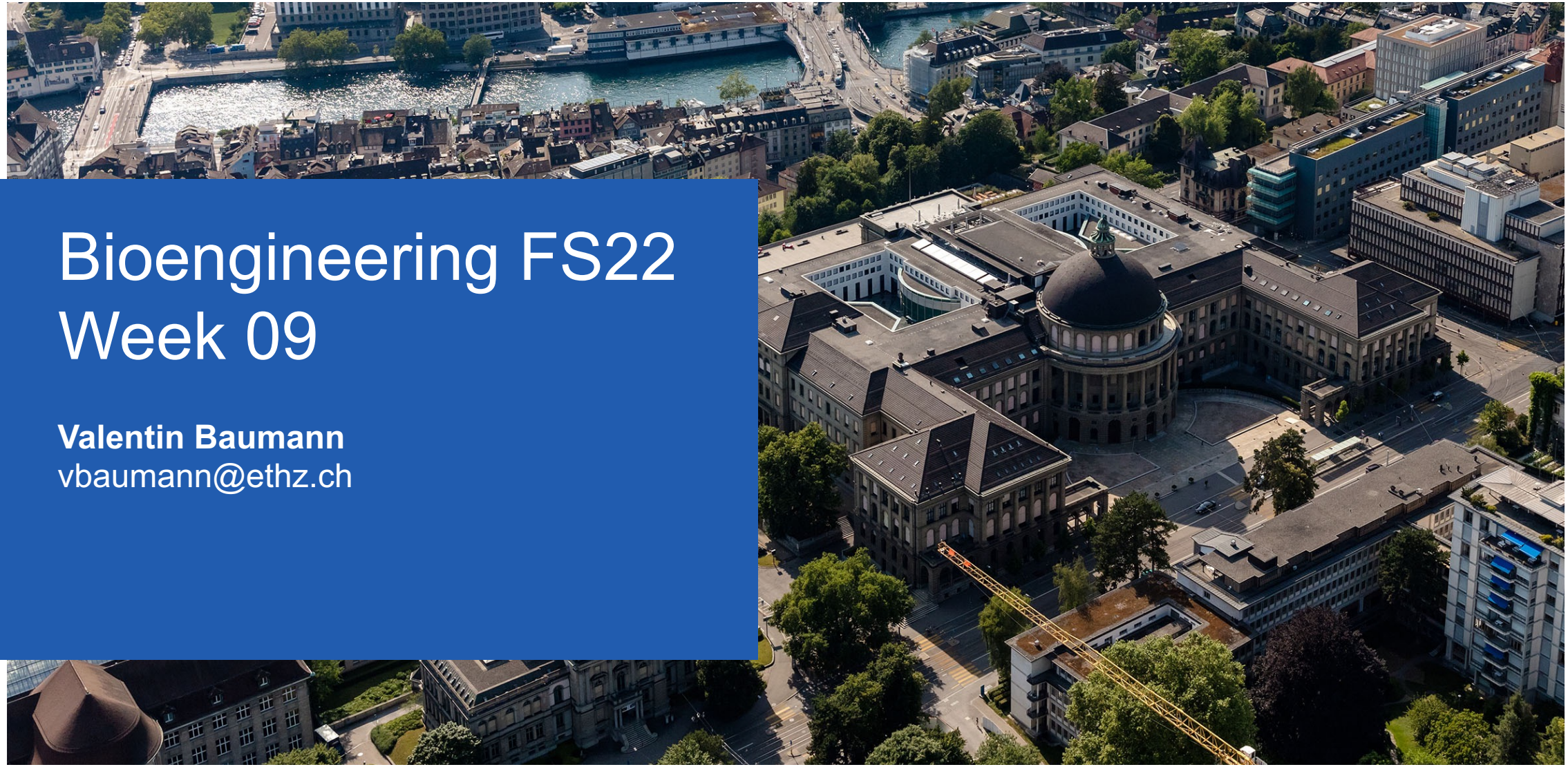

Bioengineering FS22
Week 09

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Agenda heute

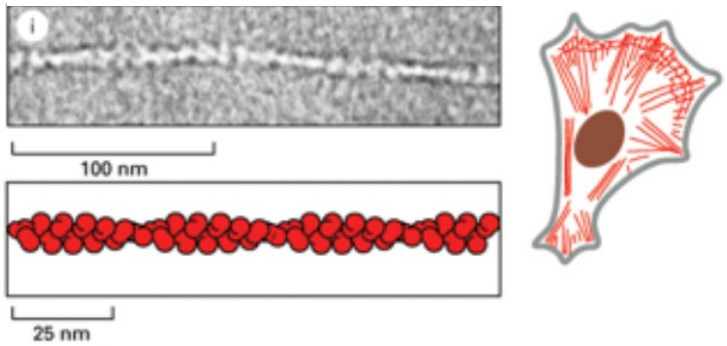
1. Cytoskeleton
    1. Components
    2. Polymerization
    3. Motors

2. Key Cell Behaviours
    1. Migration
    2. Mitosis
    3. Differentiation
    4. Apoptosis

Cytoskeleton

Cytoskeleton - Components

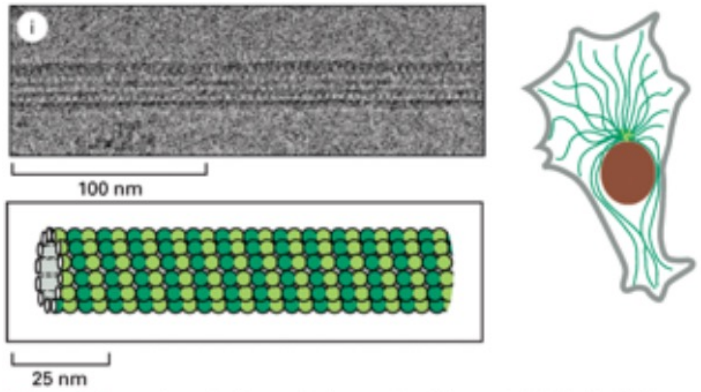
Actin filaments



Actin filaments (also known as *microfilaments*) are two-stranded helical polymers of the protein actin. They appear as flexible structures, with a diameter of 5–9 nm, and they are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane.

- Smallest type (6nm diameter)
- Actin protein monomers, srranged in long spiral chains
- Plus and minus ends
- More ATP powered growth at plus end
- Found beneath cell cortex
- Cytokinesis and cell movement

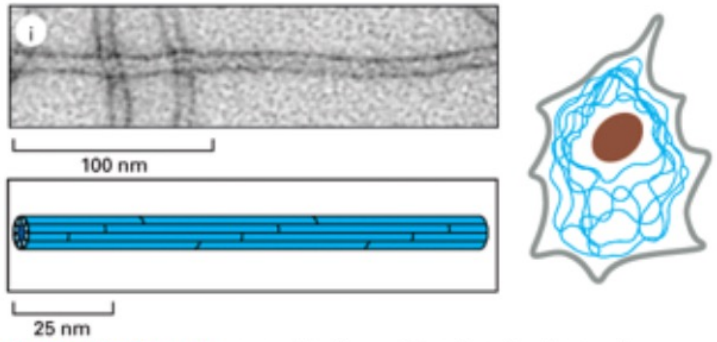
Microtubules



Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and typically have one end attached to a single microtubule-organizing center (MTOC) called a *centrosome*, as shown here.

- Largest type (25nm diameter)
- Tubulin protein subunits – form dimers/protofilaments
- 13 protofilaments form hollow , straw-shaped filaments of microtubules
- Everchanging: constant addition and substraction of tubulin dimers at both ends -> plus/minus ends
- Radiate out from MTOCs (minus end)
- Cell transport & cell division

Intermediate filaments



Intermediate filaments are ropelike fibers with a diameter of around 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength. In an epithelial tissue, they span the cytoplasm from one cell-cell junction to another, thereby strengthening the entire epithelium.

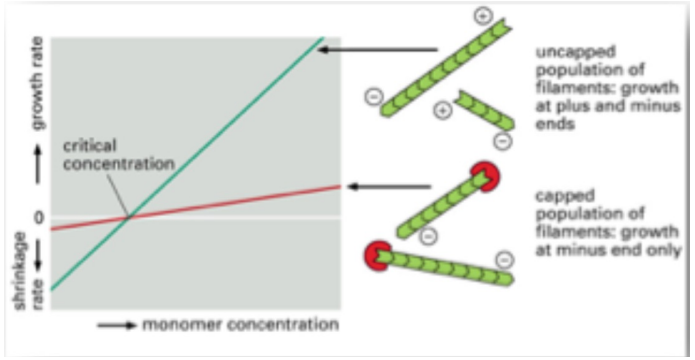
- Mid-sized (10nm diameter)
- Rods form rope-like structures
- Severa types & number of different subunits
- Funcitons mechanical
- Less dynamic than the other filaments
- Strength and support to tubulin structures
- Not polar like other filaments
- Ex.: Lamin, Keratin, vimentin

Cytoskeleton – Polymerization (Assembly and disassembly)

- All Cytoskeleton protein family: composes of small subunits, can assemble and disassemble quickly and is modulated by „accessory proteins“
- Crucial for polymerization kinetcis
  - Concentration of sub-units
  - Temperature
  - Binding affinity of sub-units (major regulator)
- Polymerization allows cytoskeletal structures to be erected and

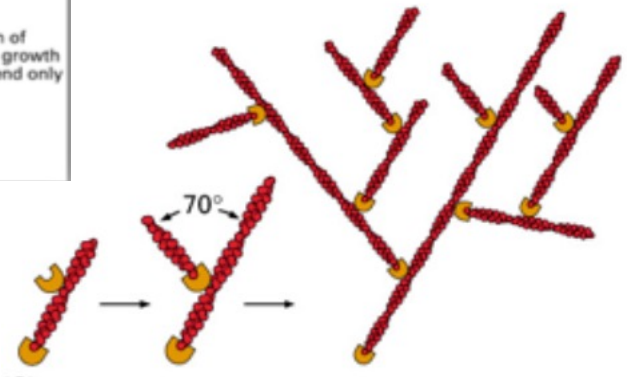
  thus create internal and external forces

- Regulation of Cytoskeletal Polymerization
  - Capping of filament ends
  - Branching (using protein sub-variants)
  - Cutting

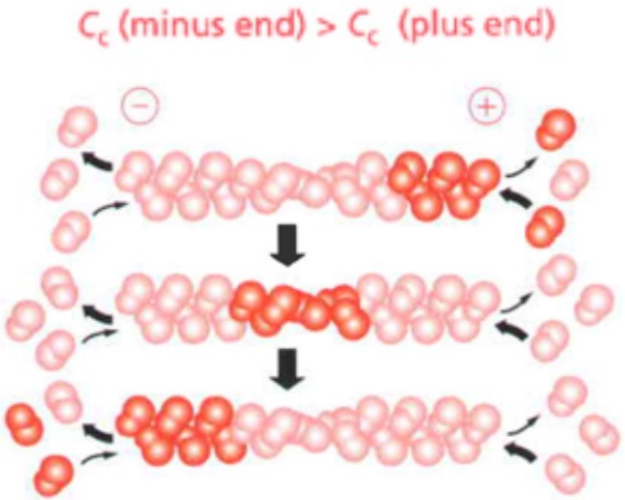


Capping

Branching



Cytoskeleton – Polymerization



- Actin polymerization – **Treadmilling**
  - Sub-unit form favour binding at plus end
  - Polymer maintains constant length but net assembly at plus end and net disassembly at minus end
  - Crucial for propulsion and protrusive forces

- Microtubule polymerization – **dynamic instability**
  - Alternating periods of slow Growth and activ disassembly (if microtubule loses its GTP)

  - Crucial for organisation of cytoplasm in non-dividing cells and chromosomes in dividing cells

- Polymerization motors driven by hydrolysis of ATP/GTP to ADP/GDP
  - Actin polymerization uses ATP and is used for propulsion of the cell parts
  - Microtubule polymerization uses GTP

Cytoskeleton – Motors

- Microtubules used as structure for motors to move on
- Myonsins protein
  - Muscle contraction
  - Intracellular cargo transport
  - Creating cellular tension

- Kinesin
  - Moving cargo inside cells away from nucleus along microtubules (anterograde)

- Dynein
  - Beating of cilia and flagella in single cell organisms
  - Cargo transport along mictrobiulues towards the nucleus (retrograde transport)

Key Cell Behaviours

Wound healing – Combinaiton of all these behaviours

The classic model of wound healing comprises three sequential, yet overlapping phases:

(1) **Inflammation** (cell recruitment to an injury site, involves cell signaling (discussed next lecture)),
   A. Migration (movement of cells to the injured site)

(2) **Proliferation**
   A. Cell division and growth
   B. Cell differentiation (cells that are needed for the repair are "created" from "stem" cells)

(3) **Remodeling** (formation and optimization of the tissue "matrix", will be discussed in coming weeks)

Includes apoptosis of cells, not loner needed

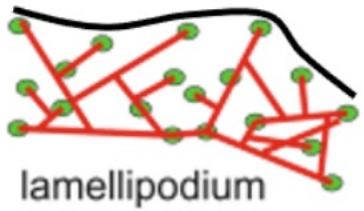
Migration

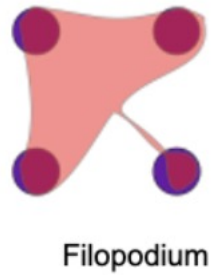
- Type of Migration
  - Chemoattraction (ex. By cytokine signalling)
  - Rolling adhesion (ex. Bidning to selecting molecuels on vessel walls)
  - Tight adhesion (ex. Binding to integrins)
  - Transmigration (ex. By extending Pseudopodia to pass through gaps between cells)

- Motility (Mechanism enabling cell migration)
  - spontaneous movement of cells by swimming, crawling, gliding and swarming
  - Using protrusive structures containing actin filaments
    - Lamellipodia (thin protrusions)
    - Filopodia (plate-like extensions)
  - Forces needed to create these protrusive structures is

  Generated by ATP-facilitated Actin polimerization

  → Pushes membrane in particular direction





Motility

$$F_{\text{stall}} = \frac{\Delta G}{\delta/N} = \frac{N k_B T}{\delta} \ln\left(\frac{k_{\text{on}}}{k_{\text{off}}}\right)$$

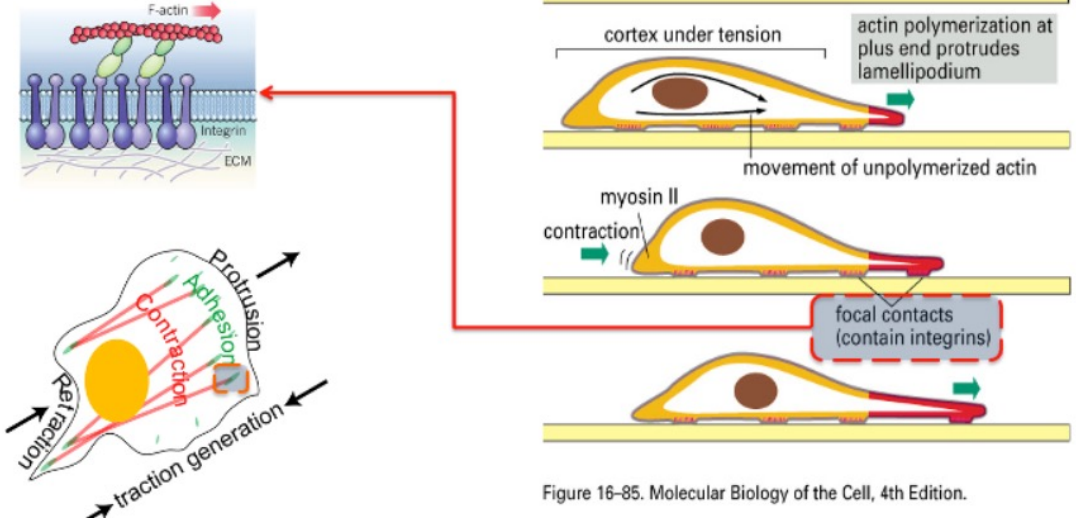
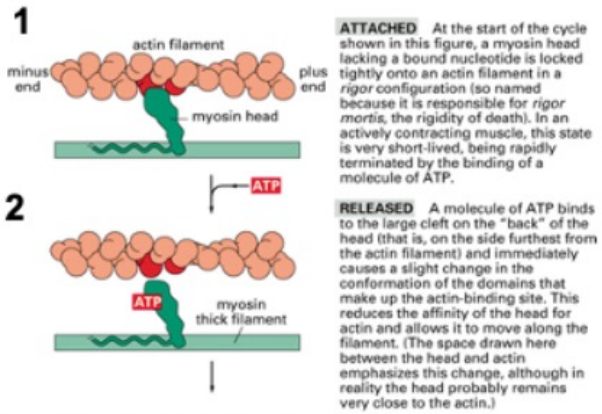


Figure 16–85. Molecular Biology of the Cell, 4th Edition.

The cell front is a site of rapid actin polymerisation: soluble actin monomers polymerize there to form filaments. These actin filaments push the leading front forward and are the main motile force for advancing the cell front. (Lamellipodial and filopodial extension).

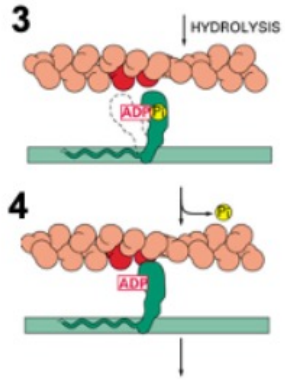
Max protrusion force determined by polymer binding affinity (and temperature)



ATTACHED At the start of the cycle shown in this figure, a myosin head lacking a bound nucleotide is locked tightly onto an actin filament in a *rigor* configuration (so named because it is responsible for *rigor mortis*, the rigidity of death). In an actively contracting muscle, this state is very short-lived, being rapidly terminated by the binding of a molecule of ATP.

RELEASED A molecule of ATP binds to the large cleft on the "back" of the head (that is, on the side furthest from the actin filament) and immediately causes a slight change in the conformation of the domains that make up the actin-binding site. This reduces the affinity of the head for actin and allows it to move along the filament. (The space drawn here between the head and actin emphasizes this change, although in reality the head probably remains very close to the actin.)

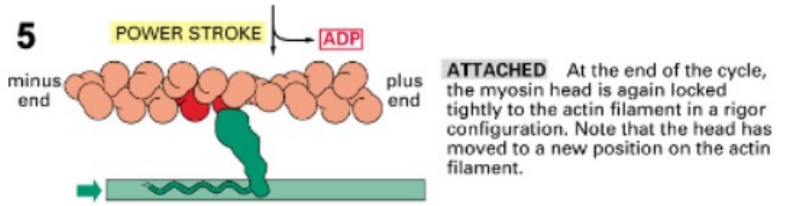
Figure 16–58 part 1 of 3. Molecular Biology of the Cell, 4th Edition.



COCKED The cleft closes like a clam shell around the ATP molecule, triggering a large shape change that causes the head to be displaced along the filament by a distance of about 5 nm. Hydrolysis of ATP occurs, but the ADP and inorganic phosphate (Pi) produced remain tightly bound to the protein.

FORCE-GENERATING A weak binding of the myosin head to a new site on the actin filament causes release of the inorganic phosphate produced by ATP hydrolysis, concomitantly with the tight binding of the head to actin. This release triggers the power stroke—the force-generating change in shape during which the head regains its original conformation. In the course of the power stroke, the head loses its bound ADP, thereby returning to the start of a new cycle.

Figure 16–58 part 2 of 3. Molecular Biology of the Cell, 4th Edition.



ATTACHED At the end of the cycle, the myosin head is again locked tightly to the actin filament in a rigor configuration. Note that the head has moved to a new position on the actin filament.

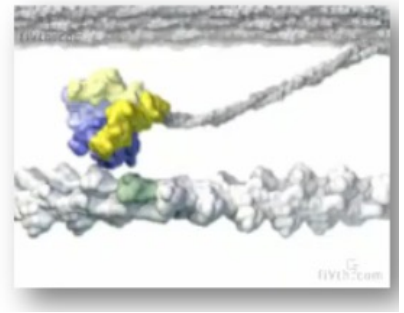


Figure 16–58 part 3 of 3. Molecular Biology of the Cell, 4th Edition.

The rear of the cell is then brought forward by contractile elements of the cytoskeleton – namely acto-myosin motors that are anchored at the cell attachment to its substrate.

Mitosis – Cell division (last stage of “cell cycle“)

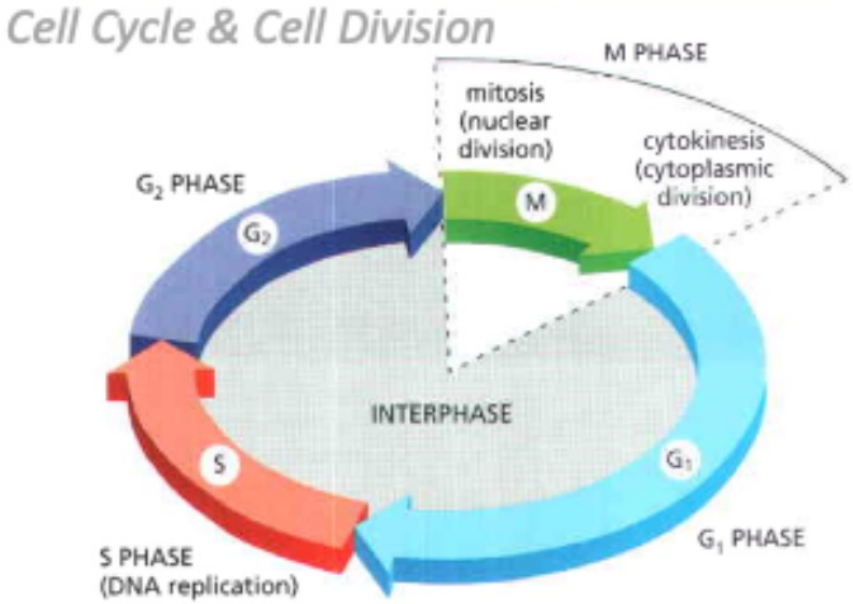
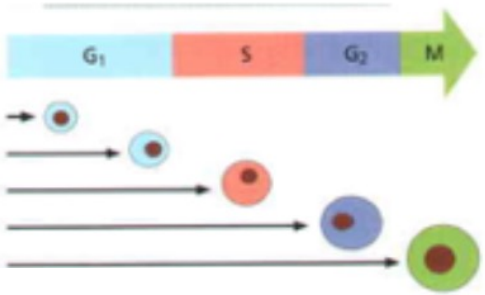


Figure 17–4 The four phases of the cell cycle. In most cells, gap phases separate the major events of S phase and M phase. G_1 is the gap between M phase and S phase, while G_2 is the gap between S phase and M phase.



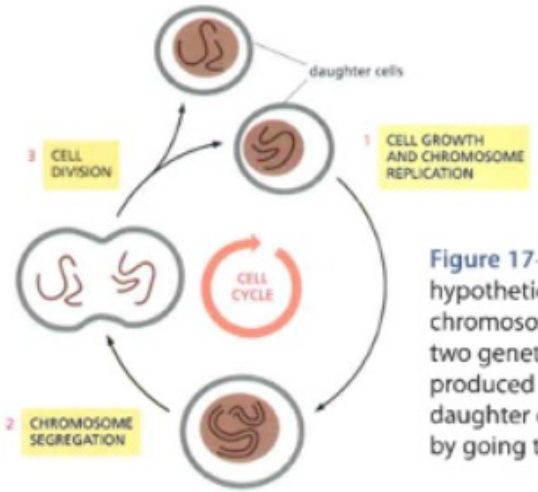
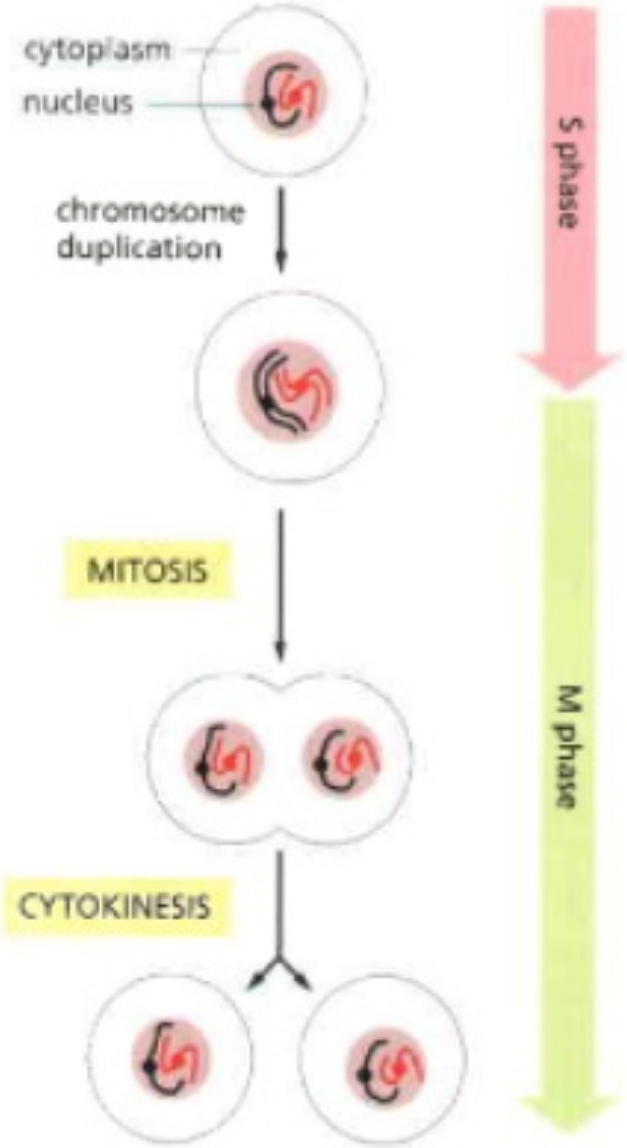
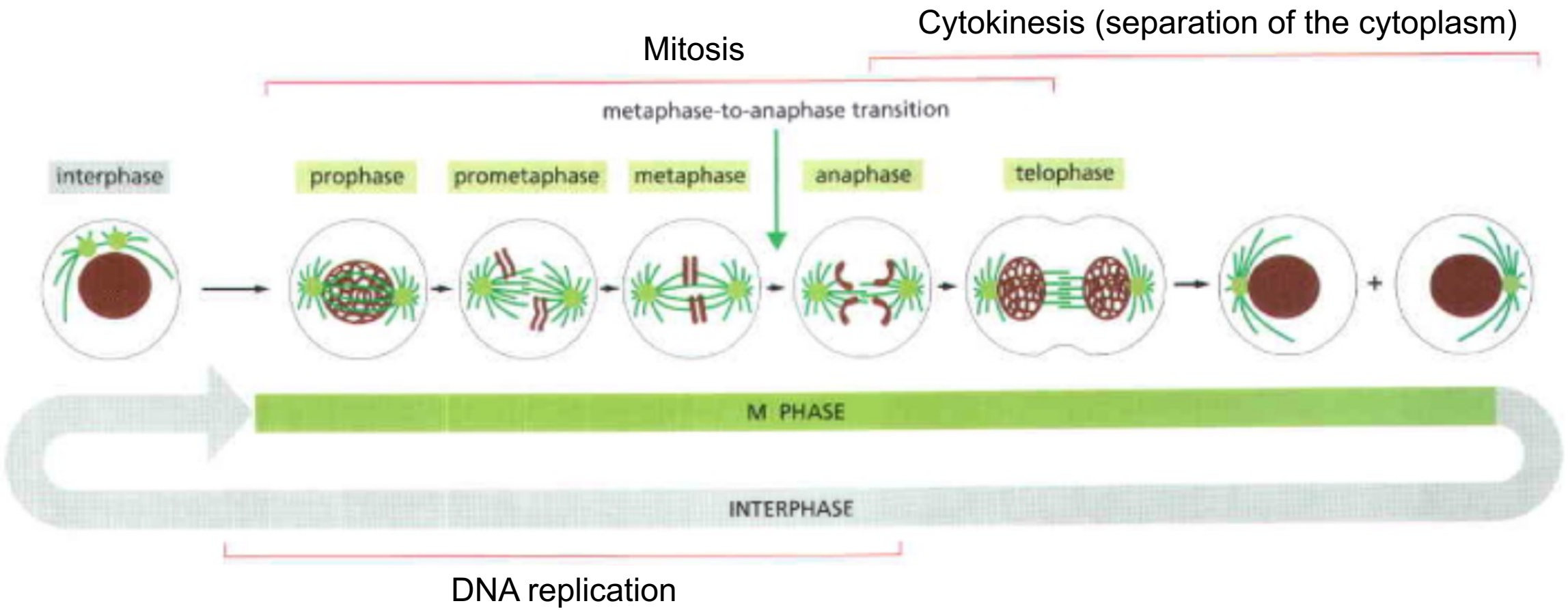


Figure 17–1 The cell cycle. The division of a hypothetical eucaryotic cell with two chromosomes is shown to illustrate how two genetically identical daughter cells are produced in each cycle. Each of the daughter cells will often continue to divide by going through additional cell cycles.



Mitosis – Cell division (last stage of “cell cycle“)

- Primary concern of cell division is the maintenance of the original cell’s genome



Differentiation

Differentiation

Proliferation

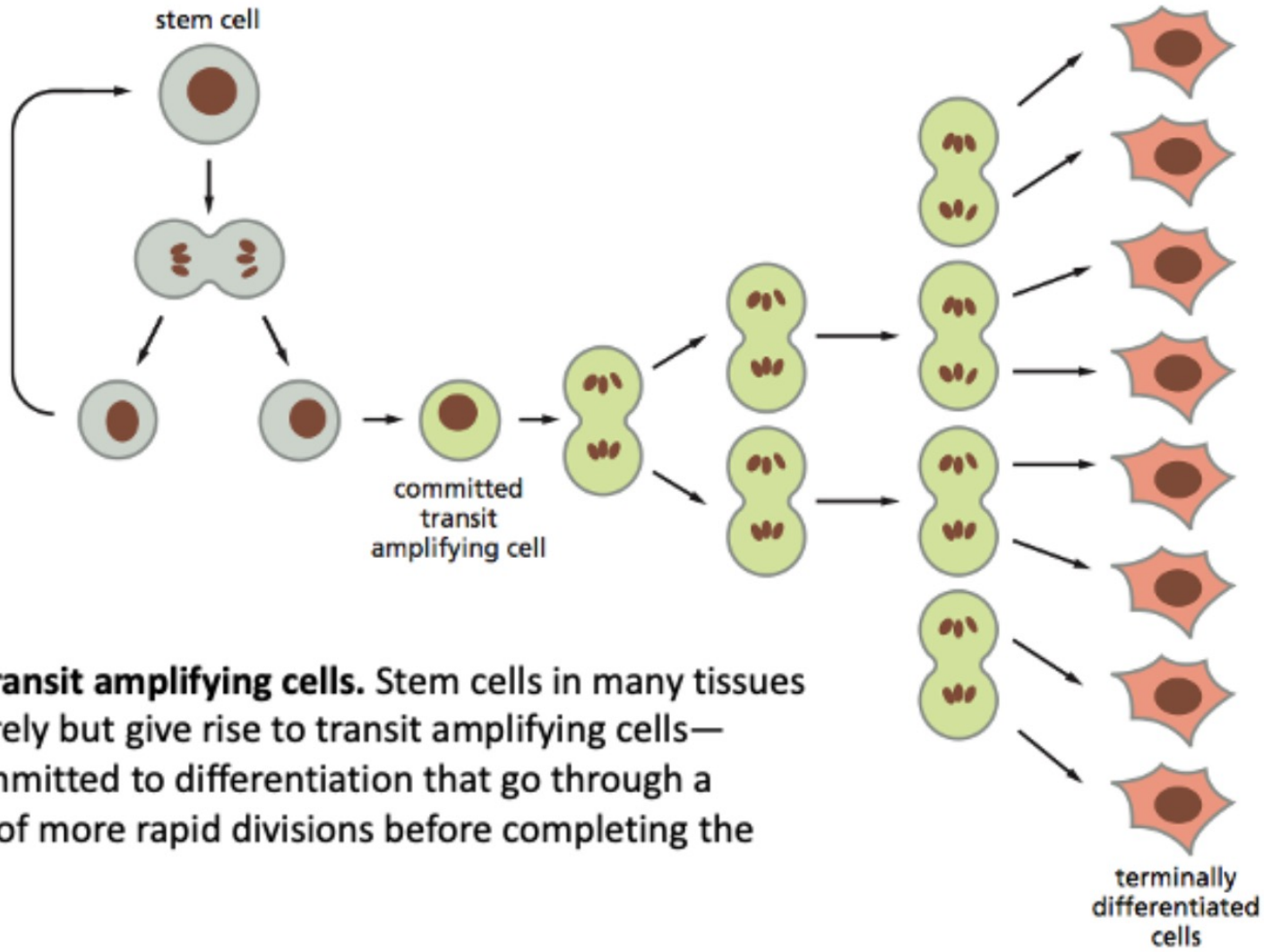
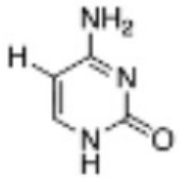
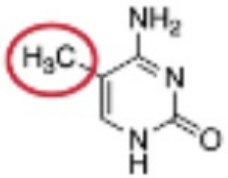


Figure 23–8 Transit amplifying cells. Stem cells in many tissues divide only rarely but give rise to transit amplifying cells—daughters committed to differentiation that go through a limited series of more rapid divisions before completing the process.

Differentiation – Epigenetic changes (ex. Methylation)

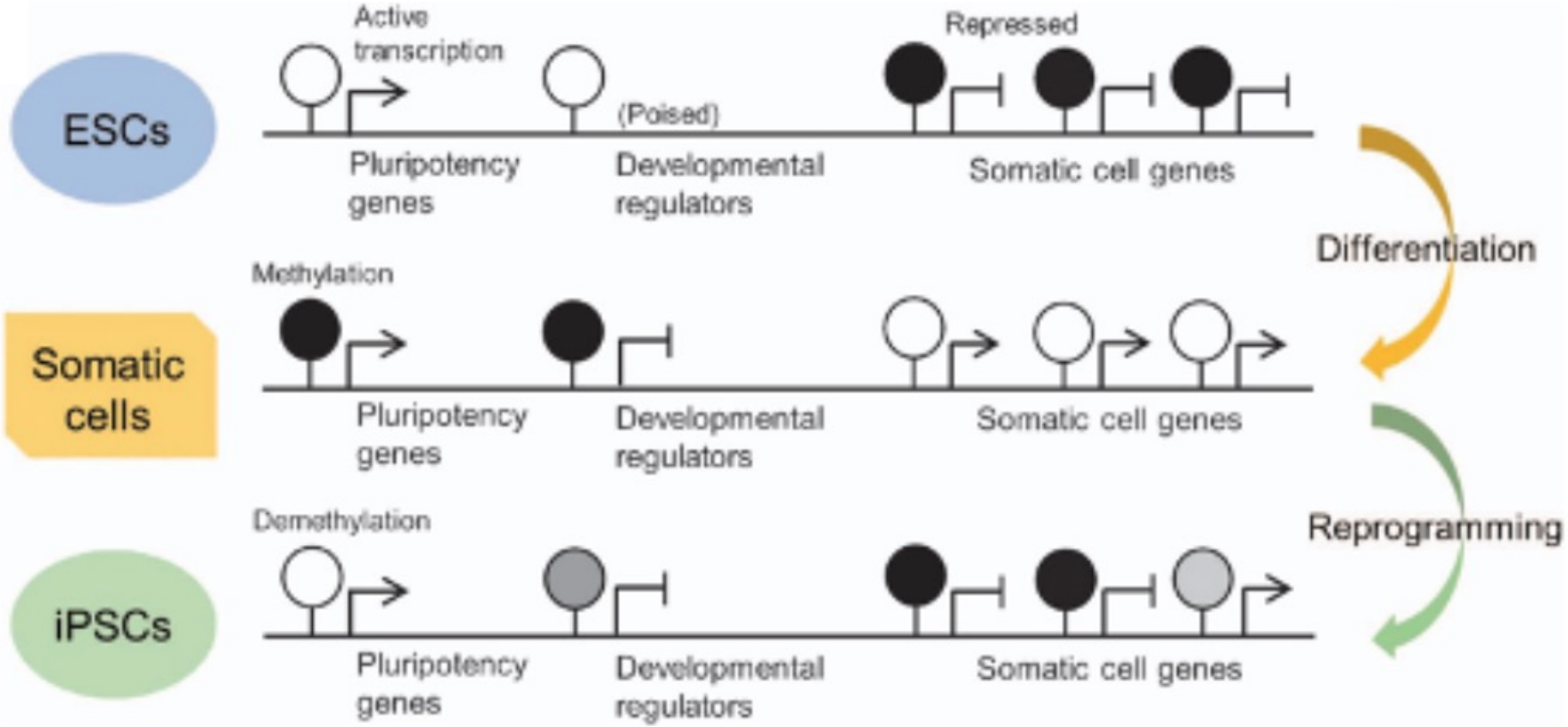


Cytosine



methylated Cytosine

DNA methylation is a process by which methyl groups are added to the DNA molecule. Methylation can change the activity of a DNA segment without changing the sequence. When located in a gene promoter, DNA methylation typically acts to repress gene transcription.

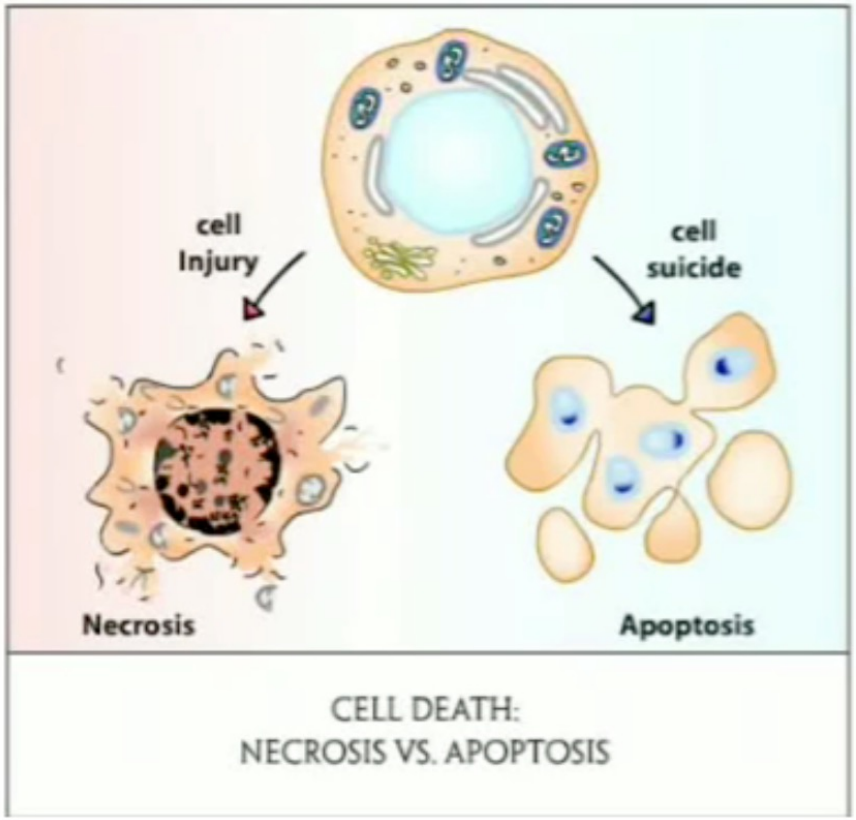


Apoptosis (vs. Necrosis)

- Apoptosis is a programmed cell death (PCD) that is a crucial process in the maintenance of a multicellular organism
- In contrast necrosis is a traumati cell death due to acute celluar injury

- → Apoptosis is programmed, carefully executed and induces no inflammation
- → Necrosis is due to lack of nutrition or an external trauma (infetion, toxicity or injury), typically uncontrolled and induces inflammation

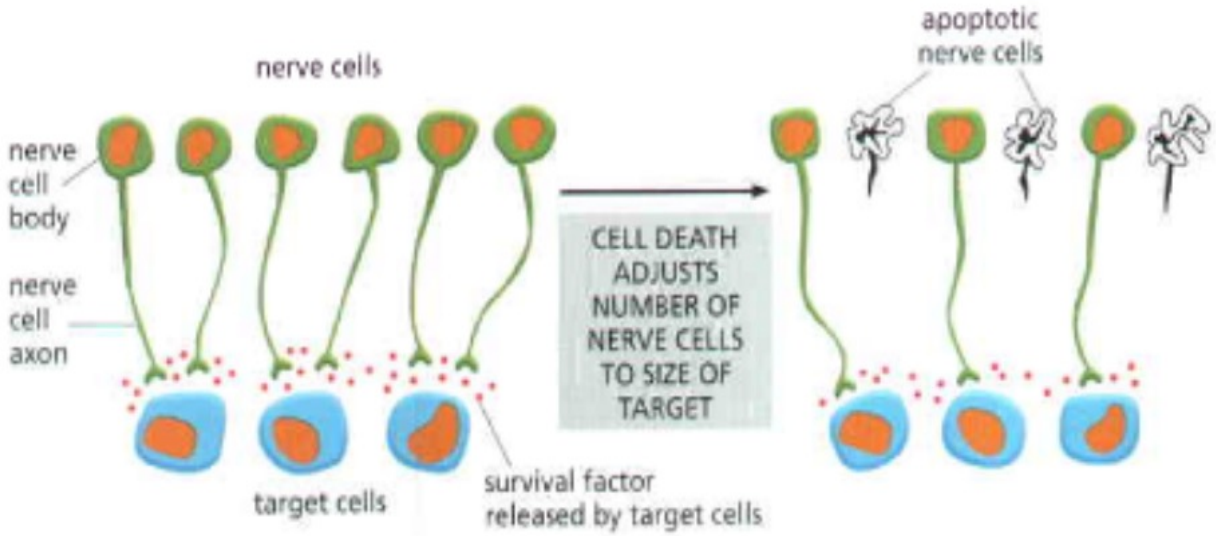
- At the same time defective apoptotic processes are implicated in a wide variety of diseases
  - Excessie apoptosis → Tissue atrophy
  - Insufficient apoptosis → uncontrolled cell proliferation → cancer



Apoptosis

- Apoptosis triggered by many signalling pathways
- A cell requires a specific amount of signalling input in order to just survive

→ Too little, too much, or the wrong signaling can lead to apoptosis



A cell undergoing apoptosis shows a characteristic morphology:

- Cell shrinkage and rounding are shown because of the breakdown of the cytoskeleton. The cytoplasm appears dense, and the organelles appear tightly packed.
- Chromatin undergoes condensation into compact patches against the nuclear envelope
- The nuclear envelope becomes discontinuous and the DNA inside it is fragmented. The nucleus breaks into several discrete chromatin bodies or nucleosomal units due to the degradation of DNA.
- The cell membrane shows irregular buds known as blebs.
- The cell tears apart, triggering elimination by macrophages.