Apoptosis

- the cells of a multicellular organism are tightly regulated.
- if cells are no longer needed → suicide → apoptosis
- different from necrosis in that the cell does not burst and spill toxic contents, damaging neighbours
- while undergoing apoptosis, cells undergo characteristic morphological changes:
  1. shrink & condense
  2. cytoskeleton collapses
  3. nuclear envelope disassembles
  4. nuclear chromatin condenses & fragments
  5. surface blebbing

All these changes trigger phagocytosis by a neighbouring cell / macrophage.

What makes apoptotic cells biochemically recognisable?

1. endonuclease leaves chromosomal DNA into fragments of distinctive sizes → cleavage occurs in linker regions & fragments separate into characteristic ladder pattern on gel electrophoresis.
2. phosphatidylserine migrates from inner to outer leaflet of plasma membrane
3. cells often lose electric potential that exists across the inner leaflet of the membrane.
   This can be visualised by the accumulation of positively charged fluorescent dye in mitochondria driven by ⊖ ve charge on inside of membrane → ↓ in labelling
   Also, release of cytochrome C from inter-memb. space of mitochondria → cytosol

Membrane Blebbing

Caspase 3 → important for membrane blebbing
↳ targets ROCK-1 → need for movement of DNA fragments to blebs
- activation of ROCK-1 by caspase-3 mediated cleavage is Rho-independent
- functions to regulate actin-myosin filament assembly, cell contractility, membrane blebbing through phosphorylation of Myosin Light chain (MLC)

Phosphorylation by ROCK-1 of MLC promotes actomyosin contraction with consequential delamination of Plasma membrane from the cortical cytoskeleton memb.

Phosphatidylserine

- PtdSer & PtdEth are confined to the cytoplasmic leaflet of the memb. & PtdCho & SM on the outer leaflet
- flippase keeps PtdSer inside the cell & opposed by scramblase
- flippase inactivation & scramblase activation are controlled by caspase 3 & 7

Caspases

- family of proteases with cysteine at their active site
- cleaves their target at specific aspartic acids

- synthesised as inactive procaspases → cleavage caspase
  ↳ cleavage occurs at one/two sp. asp acids ⊥ is catalysed by other already active caspases
- procaspase → large + small subunit (heterodimer) ^2 → active tetramer
- amplifying proteolytic cascade
- not all caspases are mediating apoptosis → initiator caspases → activate executioner caspases → cleave target prote
- executioner caspases target nuclear lamins which holds a DNA degrading enzymes → cuts up DNA
- other target proteins include components of the cytoskeleton and cell-cell adhesion proteins
- the apoptotic cell now rounds up and detaches from its neighbours making it easier to engulf it.
- How is the first procaspase activated?

Initiator procaspases have a long prodomain (containing a caspase recruitment domain CARD) that enables them to assemble with adaptor proteins into activator complexes when they receive signal for apoptosis

↓

Once incorporated in such a complex, the initiator procaspases are brought into close proximity so they can cleave each other

↓

activated initiator caspase now activates executioner caspases

Intrinsic and Extrinsic pathways

In the extrinsic pathway,

- extracellular signals bind to cell surface death receptors
- death receptors are transmemb. proteins containing an extracellular domain, a single transmembrane domain and an intracellular doma (death domain)

- death receptors are homotrimers & so are ligands (TNF family)
- e.g., activation of Fas on the target cell surface by FasL on surface of a NK cell → DISC formation
- many cells produce inhibitory proteins that act extracellularly or intracellularly to retain the extrinsic pathway, e.g., decoy receptors (that do not have a death domain) and intracellular blocking proteins (that look like procaspases and competes for binding site in the DISC.

Extrinsic pathway activated through Fas death receptors

FasLig and on the surface of a killer lymphocytes activates Fas death receptors on the surface of a target cell.

↓

Cytosdic tail of Fas then recruits the adaptor protein FADD via the death domain on each protein (Fas-associated death domain)

↓

Each FADD protein then recruits an initiator procaspase forming DISC

↓

within the DISC, initiator procaspase molecules are brought into close proximity, which activates them and they cleave each other.

↓

Activated initiator caspases 8 & 10 then activate executioner caspases, leading to a caspase cascade

↓

Apoptosis

Intrinsic pathway of apoptosis depends on mitochondria

- intrinsic pathway is activated from inside the cell, in response to injury and other stresses

In the intrinsic pathway,

- cytochrome C is released from the mitochondrial intermembrane space into the cytosol

↓

cytochrome C binds to a procaspase activating adaptor protein called Apaf1

↓

they oligomerise into a wheel-like heptamer called apoptosome

↓

the Apaf1 in the apoptosome then recruits initiator procaspase proteins which are activated by proximity in the apoptosome

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The intrinsic pathway of apoptosis is tightly regulated to ensure that cells kill themselves only when it is necessary.

Intracellular regulators of apoptosis

- Bcl2 proteins regulate intrinsic pathway by controlling the release of cytochrome c and other mitochondrial intermembrane proteins
- some Bcl2 proteins are pro-apoptotic and others are anti- and they can form heterodimers and inhibit each other
- In mammals, 6 anti-apoptotic & 9 pro-apoptotic Bcl2
- Pro-apoptotic → BH123 — e.g. Bax, Bak
  → BH3-only
- anti-apoptotic — Bcl2, Bcl-xl, located on cytosolic side of outer mitochondrial membrane, ER & nuclear membrane. (4 Bcl-2 homology domains — BH1-4)
- when an apoptotic stimulus triggers intrinsic pathway, the pro-apoptotic BH123 proteins become activated and aggregate to form oligomers in the mit. outer memb. & release cytochrome c in cytosol
- Bax and Bak are the two BH123 proteins reqd.
  ↳ located in cytosol & migrates to memb. on stimulus → bound to mit. memb.
- anti-apoptotic Bcl2 proteins also located on cytosolic surface of outer mit. memb, ER, nuclear envelope - prevent Bak from oligomerising and getting activated.

In the presence of an apoptotic stimulus, BH3-only proteins are activated and bind - the anti-apoptotic Bcl2 proteins so that they can no longer inhibit the BH123 proteins, which now become activated and aggregate in the outer mit. memb. and promote the release of intermembrane mit. memb proteins into cytosol.

IAPs inhibit caspases

- IAP (inhibitor of apoptosis) — supress apoptosis
  - prevent accidental apoptosis by spontaneous activation of procaspases
  - located in cytosol and bind to & inhibit caspases

- when an apoptotic stimulus activates intrinsic pathway, anti-IAP proteins released from mit. intermemb. space
  ↳ at the same time, released cytochrome c triggers the assembly of apoptosomes → apoptosis
- other extracellular signals that stimulate apoptosis — surge of thyroid hormone in bloodstream, for example, signals cells in tadpole tail → undergo apoptosis at metamorphosis

Extracellular survival factors

- extracellular signal molecules that inhibit apoptosis — survival factors
- most animal cells require continuous signalling from other cells i.e. to avoid apoptosis
- survival factors usually bind to cell surface receptors → intracellular programs that regulate Bcl2 & suppress apoptosis.

Disease from uncontrolled apoptosis

- human diseases where excessive no. of cell undergo apoptosis & contribute to tissue damage — heart attacks & strokes
- inactivation of Fas or Fasl genes → autoimmune disease
- ↓ apoptosis — tumors & cancers
- Bcl2 was identified from a lymphocyte cancer in humans