Mitochondrial Genome, Role of Mitochondria in Cell Metabolism, Signaling and Apoptosis

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Mitochondria:

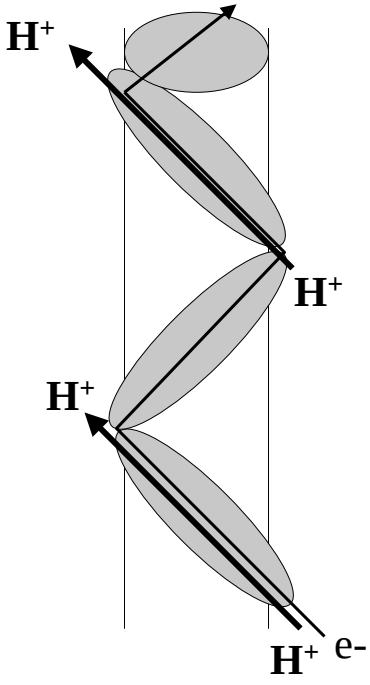
- Originally phagocyted/parasitic bacteria
- Four compartments:
  - outer membrane
  - intermembrane space
  - inner membrane
  - matrix
- In living cell form dynamic network (balance between ‘fission’ and ‘fusion’)

Mitochondria in cell metabolism

- Respiratory chain: final oxidation of substrates and synthesis of ATP (oxidative phosphorylation)
- Decarboxylation of pyruvate
- Citric acid (Krebs) cycle
- Beta-oxidation of fatty acids
- Production of ketone bodies
- Some reactions of urea synthesis
- Some reactions of porphyrin synthesis

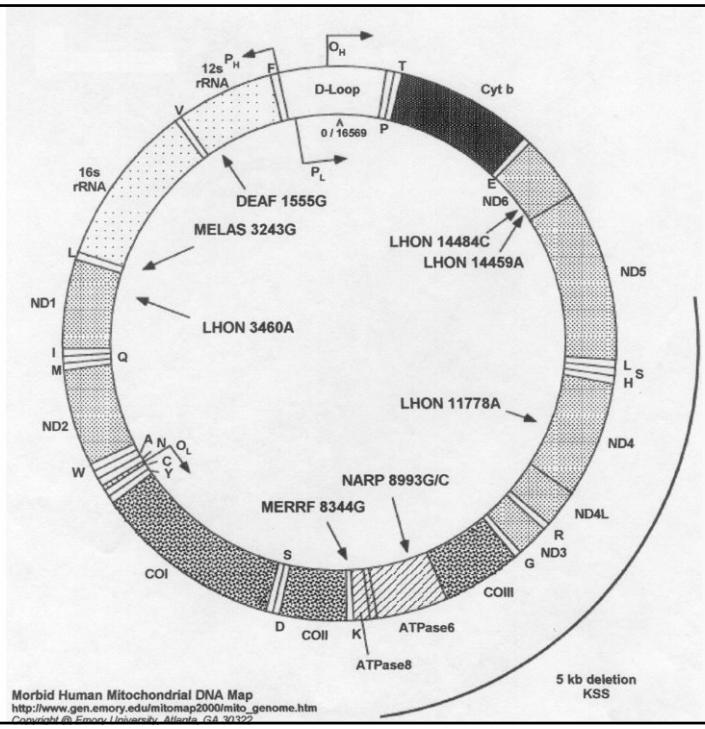
Redox carriers in respiratory chain:

- transfer hydrogen (protons+electrons):
  - NAD+
  - FAD (FMN)
  - Ubiquinone (Coenzyme Q)
- transfer only electrons:
  - Cytochromes
  - Fe-S clusters



Mitochondrial genome

- Circular molecule of DNA, 16569 bp (human)
- Typically 1000-10000 copies in one cell (2-10 in one mitochondria)
- One regulatory region (D-loop); no other non-coding sequences
- 37 genes:
  - 2 ribosomal RNAs
  - 22 tRNAs
  - 13 polypeptides (subunits of respiratory complexes I, III, IV a V)



Mitochondrial genome: **Transcription**

- Promoters for light and heavy strand in D-loop region
- Initiation: binding of transcription factor mtTFA and mtRNA polymerase
- Whole mtDNA strands are transcribed
- Locus of frequent termination at the 16S rRNA/Leu tRNA boundary
- Polycistronic transcripts are cleaved by RNase P to yield tRNAs, rRNAs and mRNAs ("cloverleaves" of tRNAs serve as punctuation...)

Mitochondrial genome: **Replication**

1 Initiation of transcription at light strand promoter

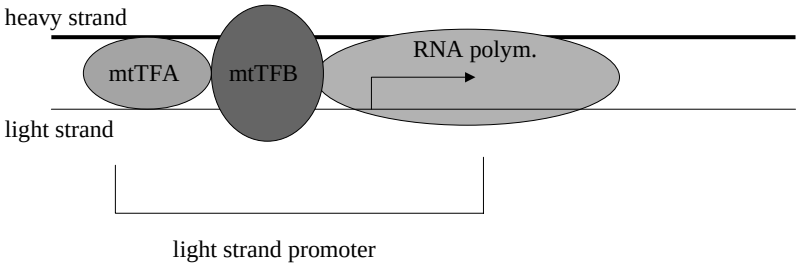
2 RNA/DNA hybrid (R-loop)

3 Processing by RNase MRP: RNA primer results

4 Polymerase γ: initiation of heavy strand replication (+helicase, SSB)

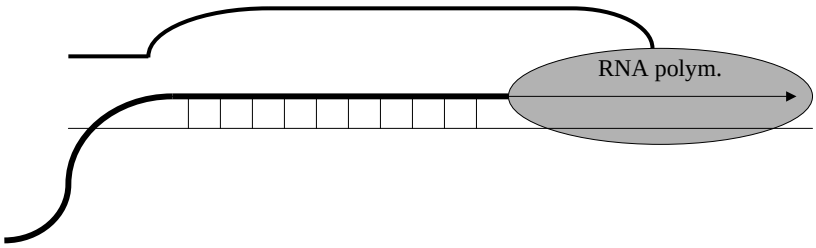
5 Early arrest: D-loop remains; or replication continues

1. Initiation of transcription at light strand promoter:

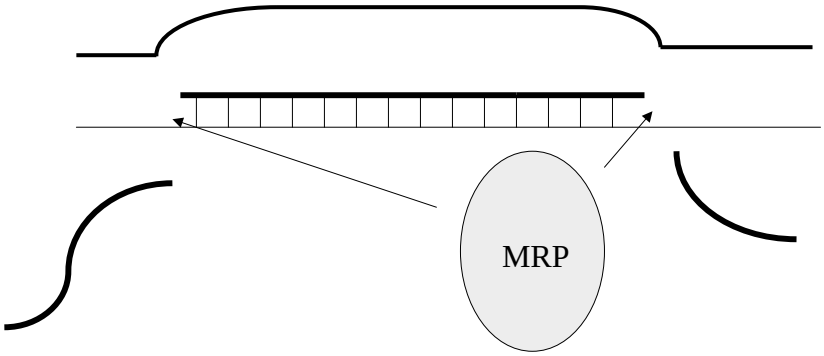


light strand promoter

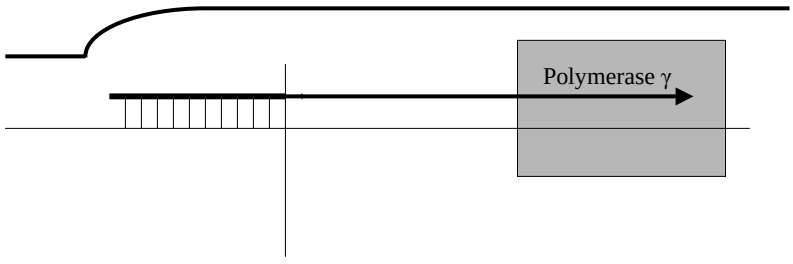
2. RNA/DNA hybrid (R-loop):



3. Processing by RNase MRP: RNA primer results

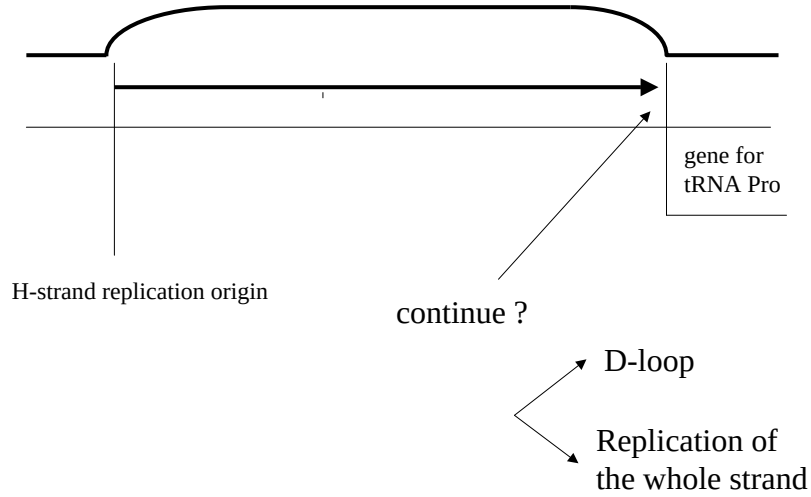


4. DNA Polymerase γ: initiation of heavy strand replication



H-strand replication origin

5. Early arrest: D-loop remains; or replication continues



Mitochondrial proteosynthesis

- Ribosomes in matrix: small, 55S
  - 16S and 12S rRNAs (no 5S rRNA)
- Sensitive to chloramphenicol, but resistant to cycloheximide
- mRNAs lack 5'-cap
  - ... low translation efficiency
- Differences in genetic code (!):
  - UGA: Trp (in cytosol Stop)
  - AGA, AGG: Stop (in cytosol Arg)
  - AUA, AUU: Met (in cytosol Ile)

Protein import into mitochondria

- Mitochondria: cca 1000 polypeptides (respiratory complexes cca 100 polypeptides)
- MtDNA encodes 13 polypeptides
  - ... vast majority of mito proteins is nuclear-coded, synthesised in cytosol and targeted to mitochondria

(Evolution: transfer of mitochondrial genes into nucleus)

Protein import into mitochondria

- N-terminal matrix-targeting signal sequence
- Chaperons in cytosol + ATP: prevent protein folding
- Translocation through mito membranes:
  - receptors & protein channel at sites of inner & outer membranes contact
  - requires mitochondrial potential (proton gradient)
  - Chaperons & chaperonins in matrix + ATP provide correct protein folding
- Proteins for other destinations than matrix: second targeting sequence

Mitochondrial genetics

- Mitochondria are inherited almost exclusively from mother
- Mitosis: random distribution of mitochondria
- Possibility of heteroplasmy (different mtDNA)
  - in tissue
  - in one cell
  - in one mitochondria
- Mitochondria in the cell can exchange mtDNA
- But mtDNA cannot recombinate

Mitochondrial DNA mutates 10times faster than nuclear

- Exposition to oxygen radicals (mito the main source)
- Mito DNA not covered by histones
- Relatively insufficient mito DNA repair

Mitochondrial medicine

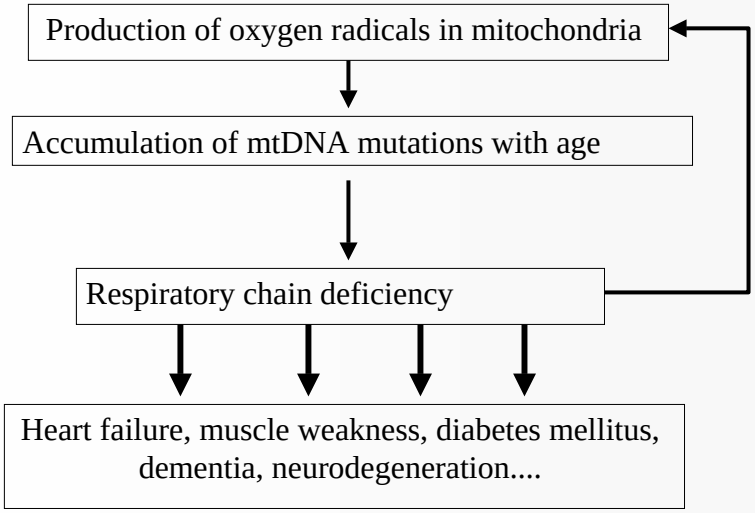
- Defects of oxidative phosphorylation caused by mutation in mitochondrial or nuclear DNA
- Prevalence at least 1 : 8,500
- Mutation in mtDNA:
  - point
    - genes for respiratory subunits
    - genes for tRNA
  - large deletions

- Over 100 mtDNA mutations linked to human disease described
- Usually heteroplasmy
- Symptoms depend on distribution of mutated mtDNA and energy requirements of particular tissues - clinical phenotype pleiomorphic
- Disadvantage of post-mitotic tissues with high energy demand:
  - brain
  - heart
  - muscle

Examples of mitochondrial diseases:

- Luft's disease:
  - hypermetabolism due to loose oxidation-phosphorylation coupling
- LHON (Leber's Hereditary Optical Neuropathy):
  - blindness of young men, cause: point mutations of mito encoded complex I subunits
- MELAS (Myopathy, Encephalopathy, Lactic Acidosis, Stroke-like episodes):
  - cause: point mutations of tRNA genes

Mitochondrial theory of ageing



Mice engineered to express a proofreading-deficient mitochondrial polymerase:

....3-5-times more point mutations in mtDNA, more mtDNA deletions

.... much shorter life span, accelerated ageing

(Nature 429, 2004, 417-423)

Mitochondria and Calcium Signalling

Calcium in the cell:

- In cytosol only 0.1-0.2 µM, about 1 µM is a signal
- Source of the signal is:
  - outside:
    - ligand-operated Ca^{2+} channels
    - voltage-operated Ca^{2+} channels
  - ER stores:
    - PI3 receptor/channel
    - ryanodine receptor/channel
      - cell membrane potential-dependent (skeletal muscle)
      - Ca^{2+} -dependent (heart, CNS)

- **Information in Ca^{2+} signal is encoded by its**
  - **LOCALISATION**
  - **FREQUENCY**
  - **AMPLITUDE**

Ca^{2+} uptake into mitochondria

- Metabolic regulation: Dehydrogenases sensitive to Ca^{2+}:
  - pyruvate dehydrogenase
  - isocitrate dehydrogenase
  - 2-oxoglutarate dehydrogenase
- Sequestration/buffering of cytosolic calcium under certain condition

Mitochondrial calcium transporters:

- Ca^{2+} uniporter: facilitated diffusion down the electrochem. gradient (Vmax cca 1000 nmol mg^{-1} min^{-1})
- Ca^{2+}/2 Na^{+} exchanger: prominent in brain, heart (Vmax up to 18 nmol mg^{-1} min^{-1})
- Ca^{2+} efflux independent on Na^{+}: prominent in liver, kidney (Vmax 1-2 nmol mg^{-1} min^{-1})

(Gunter TE & Pfeiffer DR; Am.J. Physiol. 258, 1990, C755-C785)

Mitochondrial Permeability Transition Pore (MPT)

- Opening of a "megachannel" in the inner mitochondrial membrane
- Permeable for any molecule < 1500 Da
- Collaps of the inner membrane potential, dissipation of proton gradient, uncoupling of respiration
- Swelling of mitochondria

"Megachannel" (MPT) opening is

- Triggered by: matrix Ca^{2+}
- Stimulated by:
  - oxidants
  - depolarisation
  - phosphates
- Inhibited by:
  - protons (low matrix pH)
  - magnesium ions
  - ATP and ADP
  - Cyclosporin A

Function of MPT:

- Physiologic (reversible) MPT opening:
  - energetically “cheap” efflux of Ca^{2+} from mitochondria
  - Calcium signalling:
    - Ca^{2+}-induced calcium release
    - ....mitochondria as a “Ca^{2+} signalling storing memory device”
- Pathologic (irreversible): cell death (apoptosis and necrosis)

Structure of the megachannel:

- Hypothetic (various authors - different views)
- ANT (adenylate transporter, ADP/ATP exchanger) considered necessary (according to some authors sufficient) component of the MPT channel

  ...But: mice with genetic knock-out for ANT still have mitochondria capable of MPT (Nature 427, 2004, 461-465)
- Other associated proteins:
  - Mitochondrial porin (in outer membrane)
  - Cyclophilin D
  - Creatine kinase
  - Peripheral benzodiazepine receptor

Mitochondria and Cell
DEATH

Programmed Cell Death (Apoptosis)

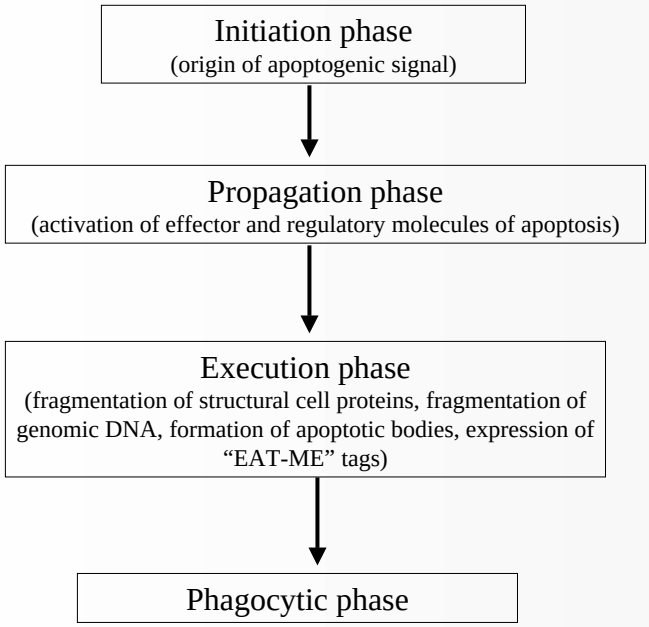
- Essential part of life
  - Regulation of cell number during development
  - Elimination of self-reacting lymphocytes, infected cells, tumour cells, etc.
- Program requiring gene expression, proteosynthesis and ATP.

Apoptotic Signalling Pathways

Constitutively expressed in every cell

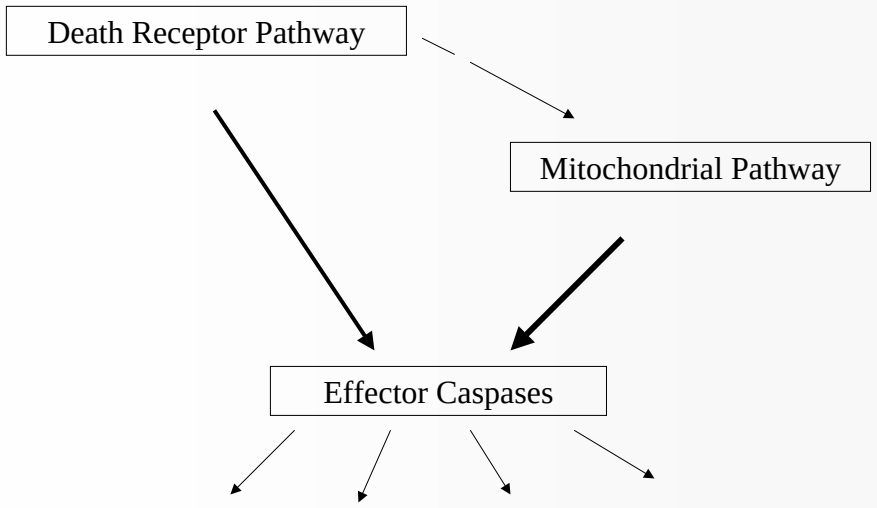
Activation → Lack of survival signals (...”suicide”)

Activation → Due to external pro-apoptotic stimulus (..”murder”)



Caspases (Cysteine ASpartate ProteASES)

- Family of >10 proteases, present in every cell as zymogens (procaspases)
- Executioners of apoptotic cell death
- Limited proteolysis >100 substrates in the cell (...change function)
- Activation of caspases:
  - Proteolysis (effector caspases, short prodomain, e.g. caspase-3, -6, -7)
  - Regulated protein-protein interactions (initiation caspases, long prodomain, e.g. caspase-8, -9)

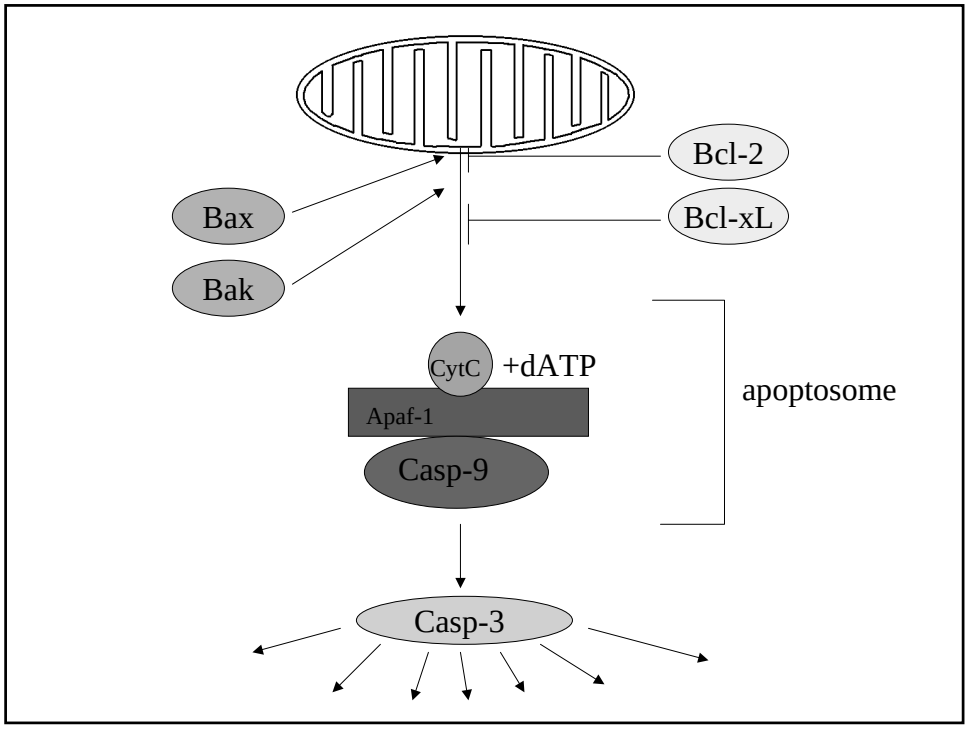


How mitochondria kill

- Proapoptotic factors in intermembrane space:
  - cytochrome c
  - AIF (apoptosis inducing factor)
  - endonuclease G
  - Smac/Diablo (inhibitor IAPs)
  - Htra2/Omi (serine protease, cleaves IAPs)
  - procaspases
- Disruption of cell respiration and ATP production (cytochrome c release, depolarisation)
- Overproduction of oxygen radicals

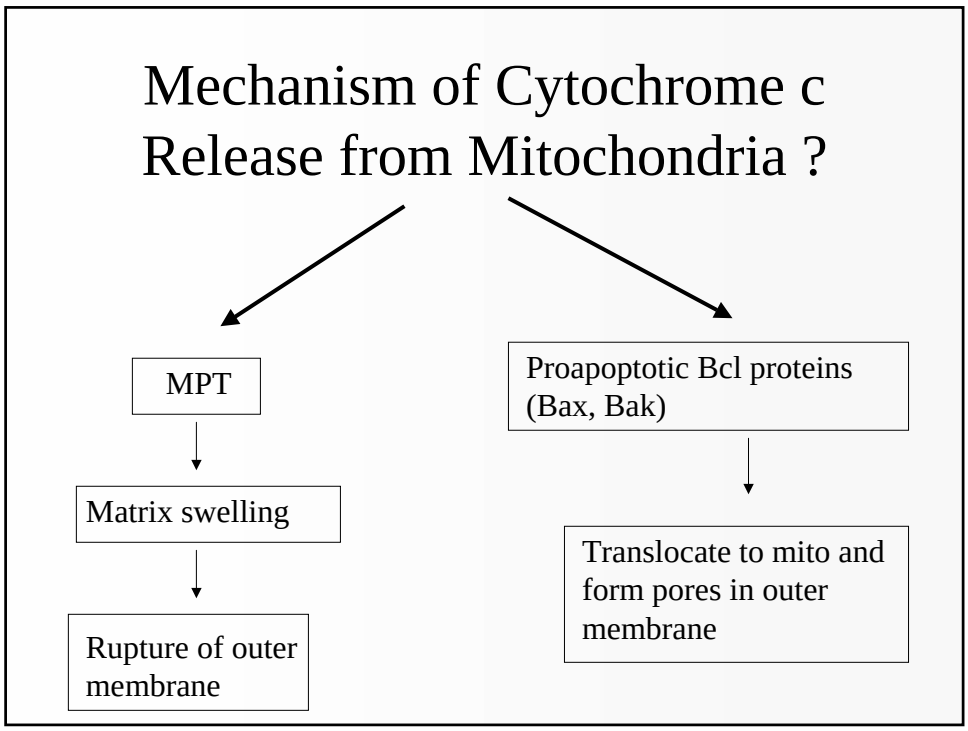
Bcl proteins:

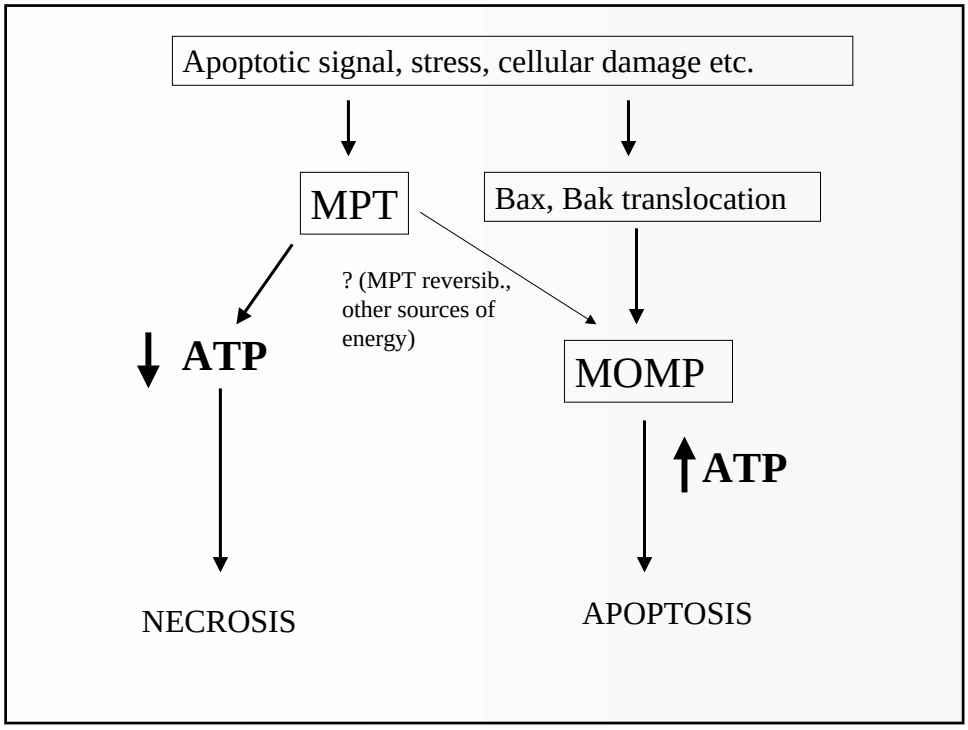
- Family >10 proteins, prototypic member: Bcl-2 (B-cell lymphoma... oncogene)
- Proteins with antiapoptotic activity (Bcl-2, Bcl-xL), or proapoptotic (Bax, Bak, Bid etc.)
- 1-4 BH domains... homo/hetero-oligomerisation
- C-terminal hydrophobic region ... localises to membranes (outer mito, nuclear m., ER)
- Ability to aggregate and form channels in membranes - similarity to bacterial toxins *kolicins*



Release of cytochrome c
from mitochondria ?

MOMP
(Mitochondrial Outer Membrane Permeabilisation)


Mechanism of Cytochrome c Release from Mitochondria ?
MPT
Matrix swelling
Rupture of outer membrane
Proapoptotic Bcl proteins (Bax, Bak)
Translocate to mito and form pores in outer membrane


Apoptotic signal, stress, cellular damage etc.
MPT
Bax, Bak translocation
? (MPT reversib., other sources of energy)
ATP
MOMP
ATP
NECROSIS
APOPTOSIS

Disease pathogenesis as dysregulation of apoptosis ?

- Neurodegeneration, ischemia, AIDS: too much apoptosis...
- Autoimmunity, tumors: too little apoptosis...

- Tumor cells tend to live on glycolysis and "switch off" mitochondria ….
- Dichloroacetate:

  inhibits PDH kinase → activation of PDH
  → activation of mito respiration and production of oxidants
  → activation of apoptotic program
  → and tumor cells die …

(Bonnet S et al.Cancer Cell. 2007 Jan;11(1):37-51).