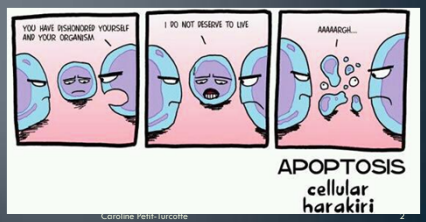
Lecture 11 Mitochondria pt. 2 Apoptosis / Cell communication

Readings: document on blackboard

Objectives

- Describe and differentiate the two cell-death mechanisms and explain the role of mitochondria and calcium in each
- What is mitophagy and how/why does it occur?



Why do cells die?

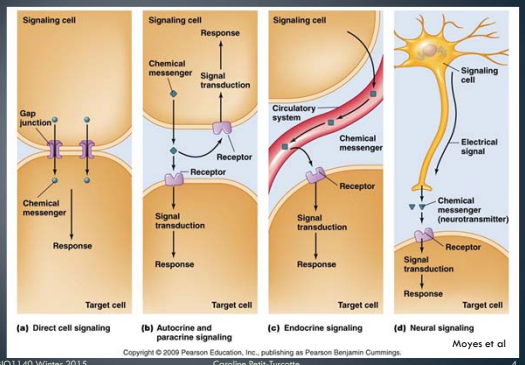
- The size of an organ or organism depends on the Total Cell Mass; the cell number must be controlled

Cells can grow

Cells can divide or die

Total cell number

Cell communication contributes to control



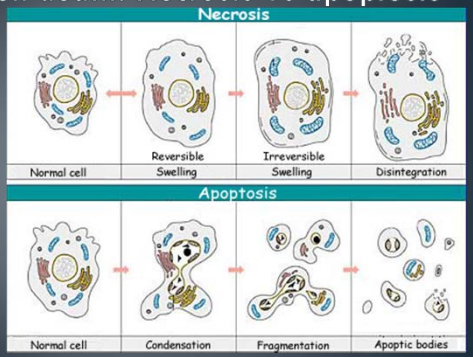
Moyes et al

What are the triggers of cell death?

- Chemical messengers
  - Growth factors
  - Mitogens (cell division)
  - Survival factors
- Toxicity
- Damage
- Cell cycle checkpoints

All 'reasons' for cell death lead to changes in the cell which lead to its demise. The mechanisms 'how' are different.

Cell death: Necrosis vs apoptosis



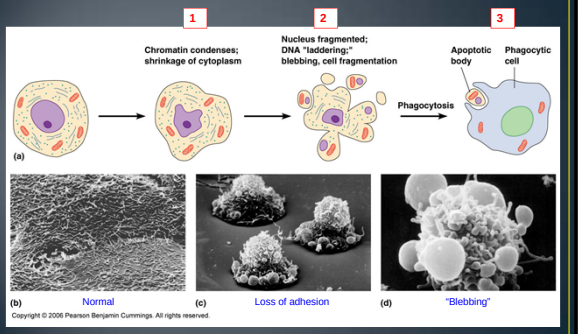
thebrain.mcgill.ca

Cell death

- **NECROSIS**
- Damage
- Ischemia, Excitotoxicity
- Ca2+ surplus
- Loss of ATPase activity, Loss of electrochemical gradient, decreased ATP
- Membrane swells, inflammation, diffusion
- Loss of membrane integrity
- Cell death, lysis, and consequences to neighbours…

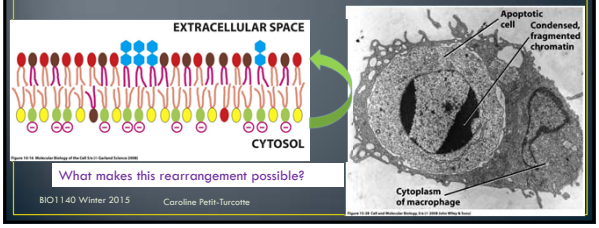
- **APOPTOSIS**
- Stress signal (**Intrinsic** or extrinsic)
- Increase in Ca2+ (cell and mitochondria)
- Pro-apoptotic protein : mitochondria
- Opening of PTP (Permeability transition pore)
- Cytochrome c release (changes to cristae)
- Activation of apoptosomes
- Signalling cascade (Caspases)
- Programmed cell death (autophagy)

Structural Changes During Apoptosis

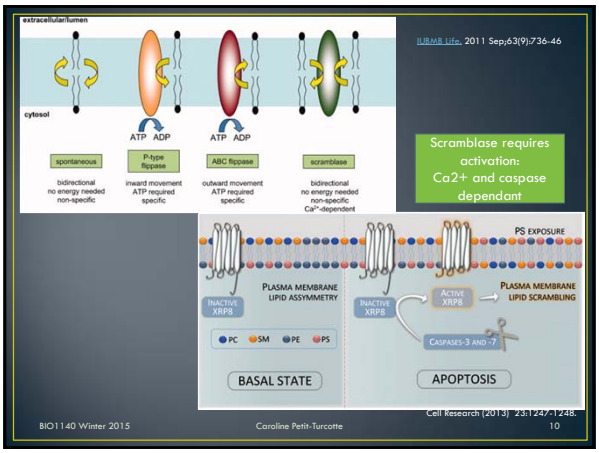


Phagocytosis

- Asymmetric distribution of plasma membrane is lost.
- Negatively charged *phosphatidylserine* then becomes exposed on the outside of cell.
- The cell is then marked for *phagocytosis* by a *macrophage*.



What makes this rearrangement possible?



Apoptosis – Signalling pathways

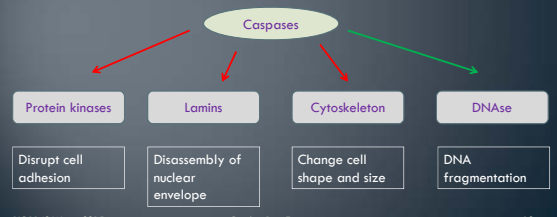
- There are 2 main apoptotic signalling pathways:
  - **Intrinsic pathway** (We will focus on this pathway)
  - Extrinsic pathway

The intrinsic pathway of apoptosis

- Possible triggers: loss of survival factor, DNA damage – Internal stressor
- Leads to dephosphorylation and activation of Bad (pro-apoptotic)
- Bad inhibits Bcl2 (anti-apoptotic)
- Bad also activates Bax and Bak (changes to calcium regulation in ER and mitochondria)
- Caspase cascade

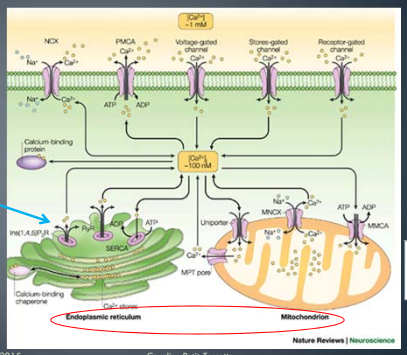
Caspases

- Caspases are a family of *proteases* (enzymes that cleave protein)
- They are split in 2 groups: Initiator (CASP2,8,9,10) and Executioner (CASP3,6,7) Caspases



Importance of calcium…

IP3 gated channel

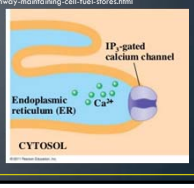


Approx 100 µM Ca2+

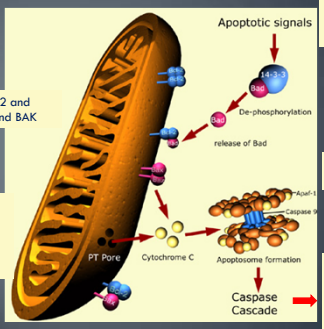
Don't forget about calcium…

- Mitochondria and ER interact via Ca2+
- During stress, ER releases Ca2+ via IP3-dependant channel
- Mitochondria absorb Ca2+ to protect cell
- Excess Ca2+ in mitochondria leads to PTP
- Bcl2 can inhibit IP3 (stops release of Ca2+) while BAX/BAK activate IP3 (promotes Ca2+ release)

http://www.sciencecentric.com/news/10072833-calcium-connections-basic-pathway-maintaining-cell-fuel-stores.html



Mitochondria in apoptosis



1. NO, Toxins, Hormones, Cytokines, etc

2. Inhibition of BCL-2 and Activation of BAX and BAK

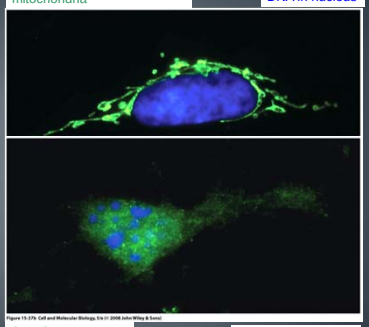
3. Increase in Ca2+ Opening of PTP (permeability transition pore) Release of CytoC

4. Apoptosomes are formed

5. Nuclear condensation
DNA fragmentation
Cytoplasmic shrinkage
Apoptotic bodies
Phagocytosis

Cytochrome *c* in mitochondria

DNA in nucleus

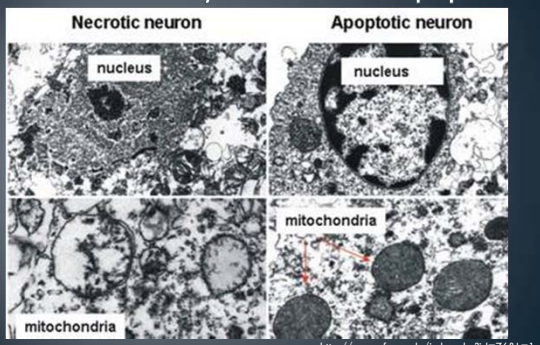


Cytochrome *c* released

DNA fragmented

(Karp, 2008)

Mitochondria stay to the end in apoptosis



http://www.nf.mpg.de/index.php?id=76&L=1

Necrosis and Apoptosis

- http://www.youtube.com/watch?v=Xqw2jihHBm0
- http://www.youtube.com/watch?v=witLM--V2v8

- Apoptosis does not occur only in response to negative stressors – it can also be an essential part of development

Hormone (Thyroxine)

↓

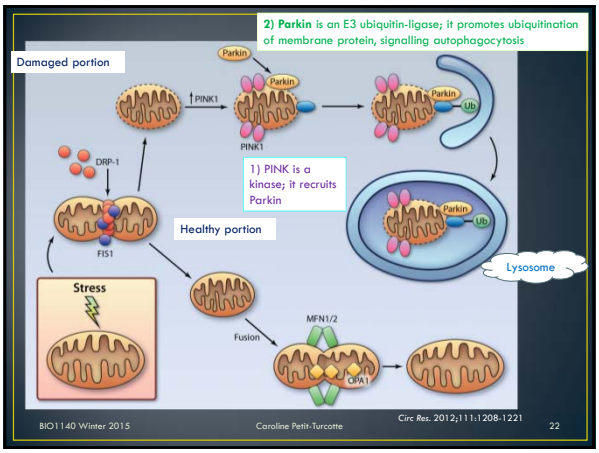
Apoptosis

↓

Tadpole loses tail in favor of legs as a frog

Mitophagy

- Controlled regulation of the number of mitochondria according to the metabolic requirements
- Process that involves recruiting various signalling protein and lysosomes
- The process how mitochondria are 'chosen' remains unclear
- Important for aging, development, and certain pathologies (AD, Parkinson's, etc).


2) Parkin is an E3 ubiquitin-ligase; it promotes ubiquitination of membrane protein, signalling autophagocytosis
Damaged portion
Parkin
PINK1
Parkin
PINK1
Parkin
Ub
DRP-1
1) PINK is a kinase; it recruits Parkin
Parkin
Ub
FIS1
Healthy portion
Lysosome
Stress
MFN1/2
Fusion
OPA1
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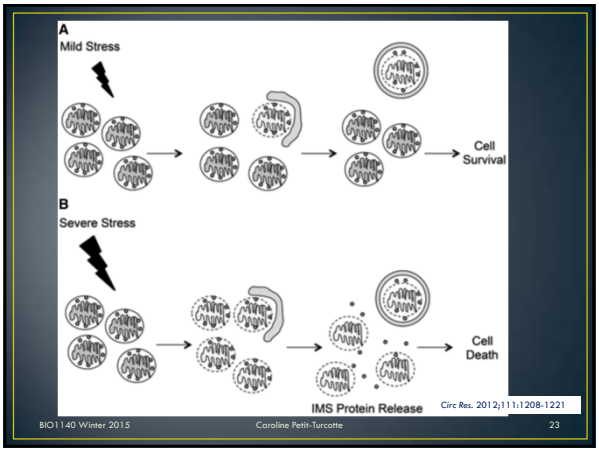
Circ Res. 2012;111:1208-1221
22


A
Mild Stress
Cell Survival
B
Severe Stress
Cell Death
IMS Protein Release
Circ Res. 2012;111:1208-1221
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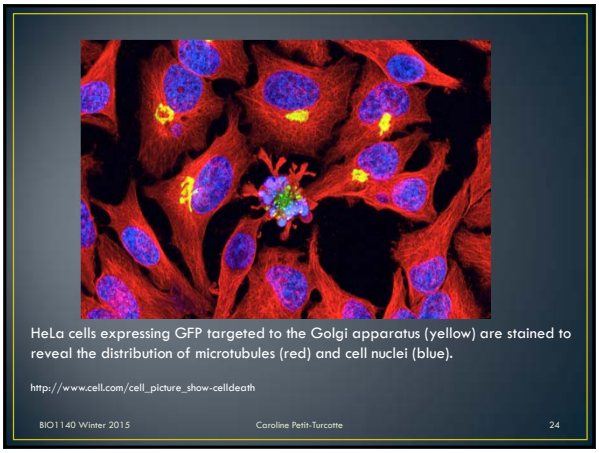
HeLa cells expressing GFP targeted to the Golgi apparatus (yellow) are stained to reveal the distribution of microtubules (red) and cell nuclei (blue).
http://www.cell.com/cell_picture_show-celldeath
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24