MOLECULAR BASIS OF CELL DEATH

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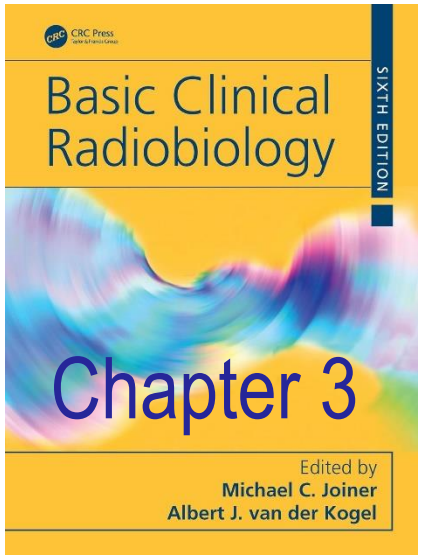
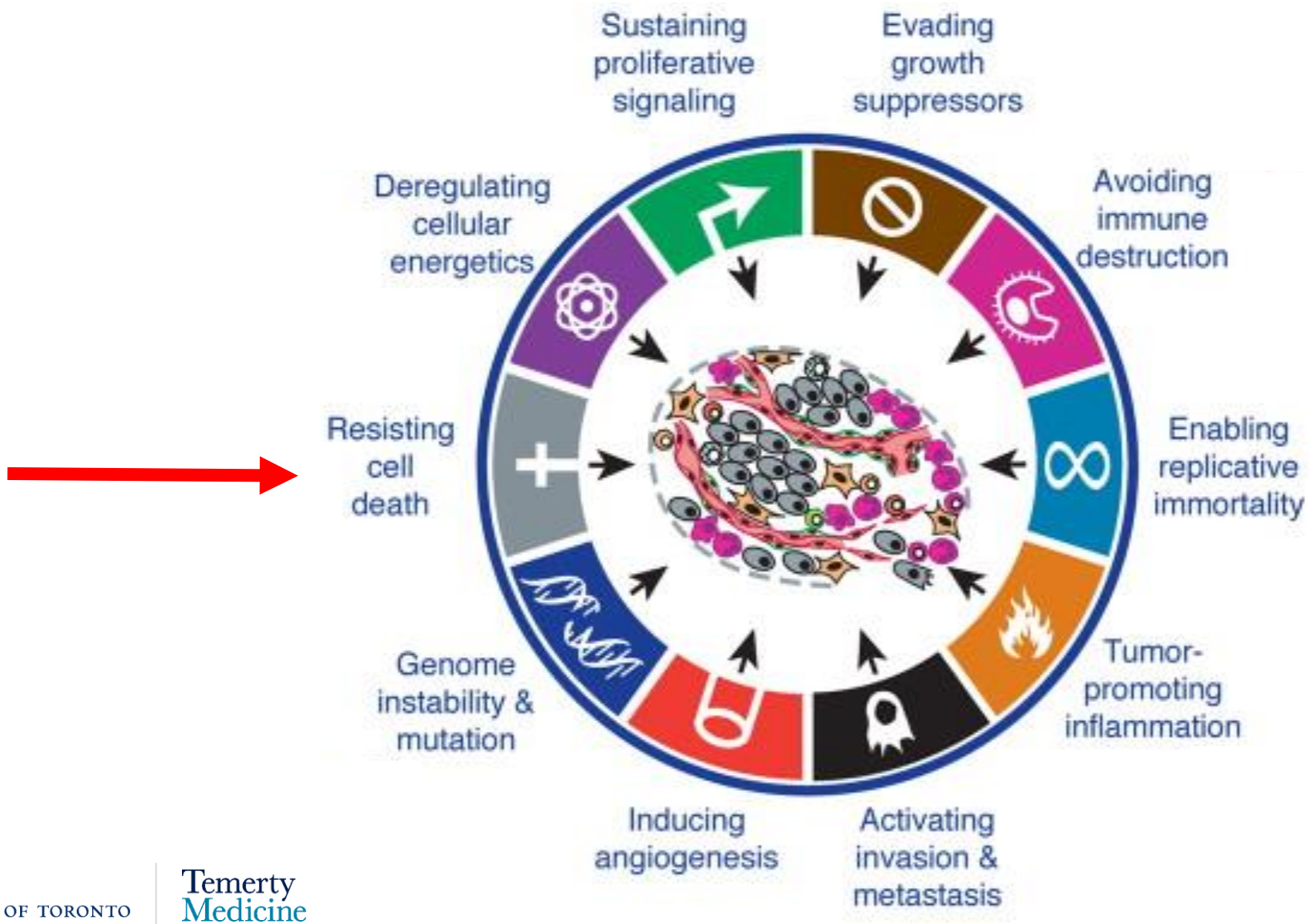


Disclosures: None

Learning objectives

- Define clonogenic cell death / survival
- Identify common cell death pathways
- Describe the relative importance of pathways for cell death caused by ionizing radiation
- Distinguish between early cell death and mitotically linked cell death

The Hallmarks of Cancer



Hanahan & Weinberg, Cell 2011

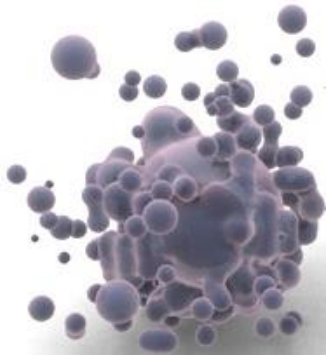
What do we mean by cell death?

- Cell death
  - Loss of reproductive (clonogenic) capacity
  - Cell may or may not appear dead
  - Cells are unable to contribute to tumor growth or metastasis – goal of treatment

- For normal cells, this definition may not be relevant
  - Has no meaning for non-dividing cells
  - Different definitions may be better

How do cells die?

APOPTOSIS



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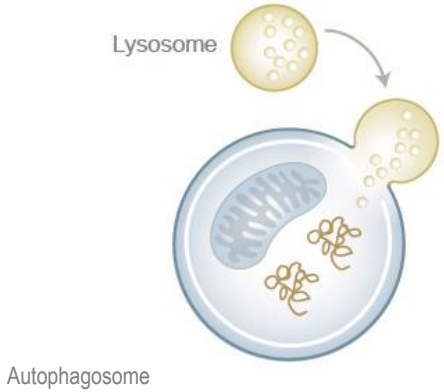

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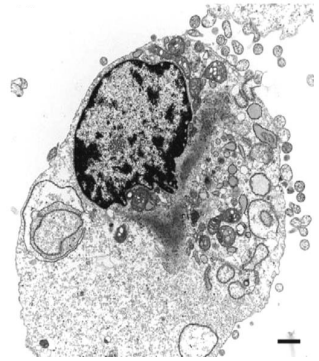

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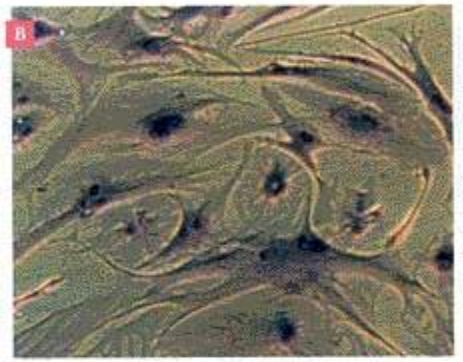
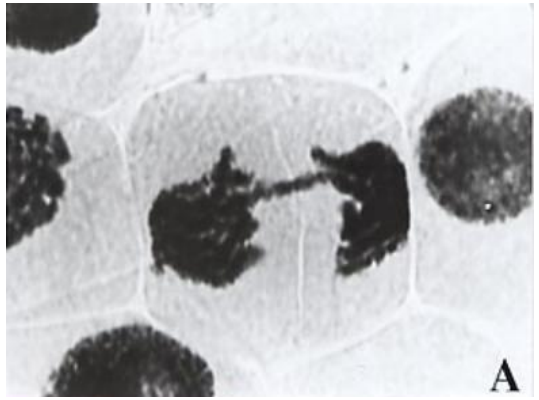
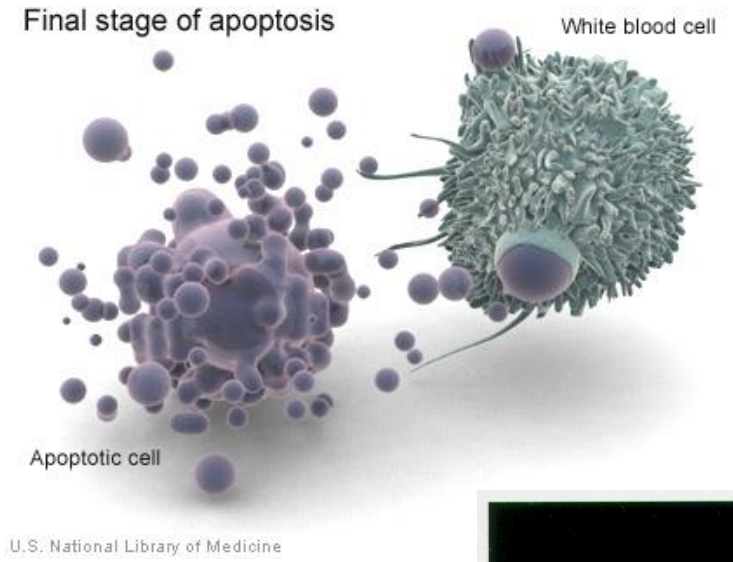


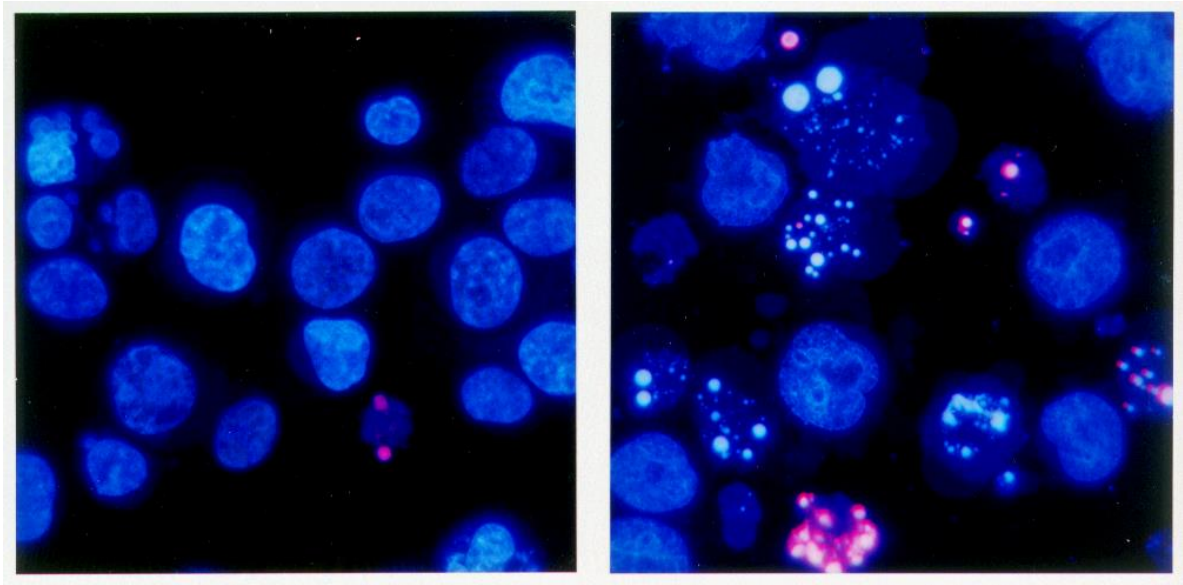
MITOTIC CATASTROPHE



Apoptosis



- Active (programmed) form of cell death
- A decision to die is made

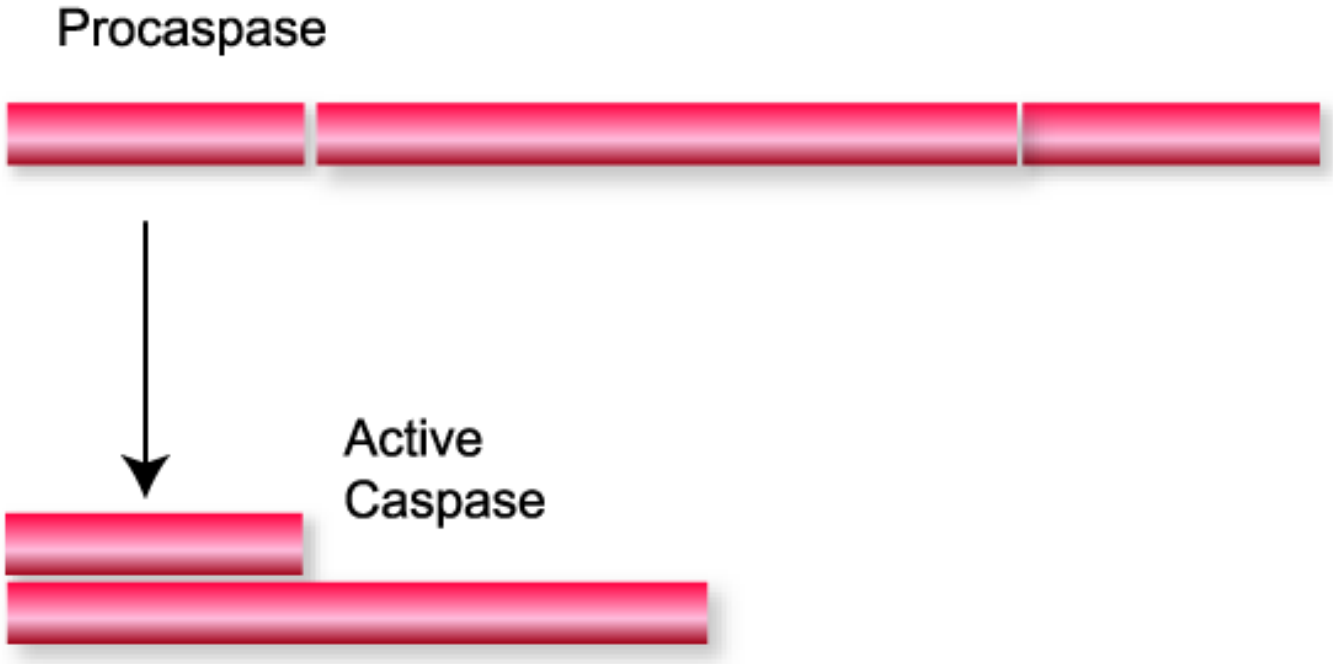


Apoptotic machinery

- Sensors
  - Monitor extracellular (extrinsic pathway) and intracellular (intrinsic pathway) environment for conditions of normality and abnormality e.g. hypoxia, growth factors, damage

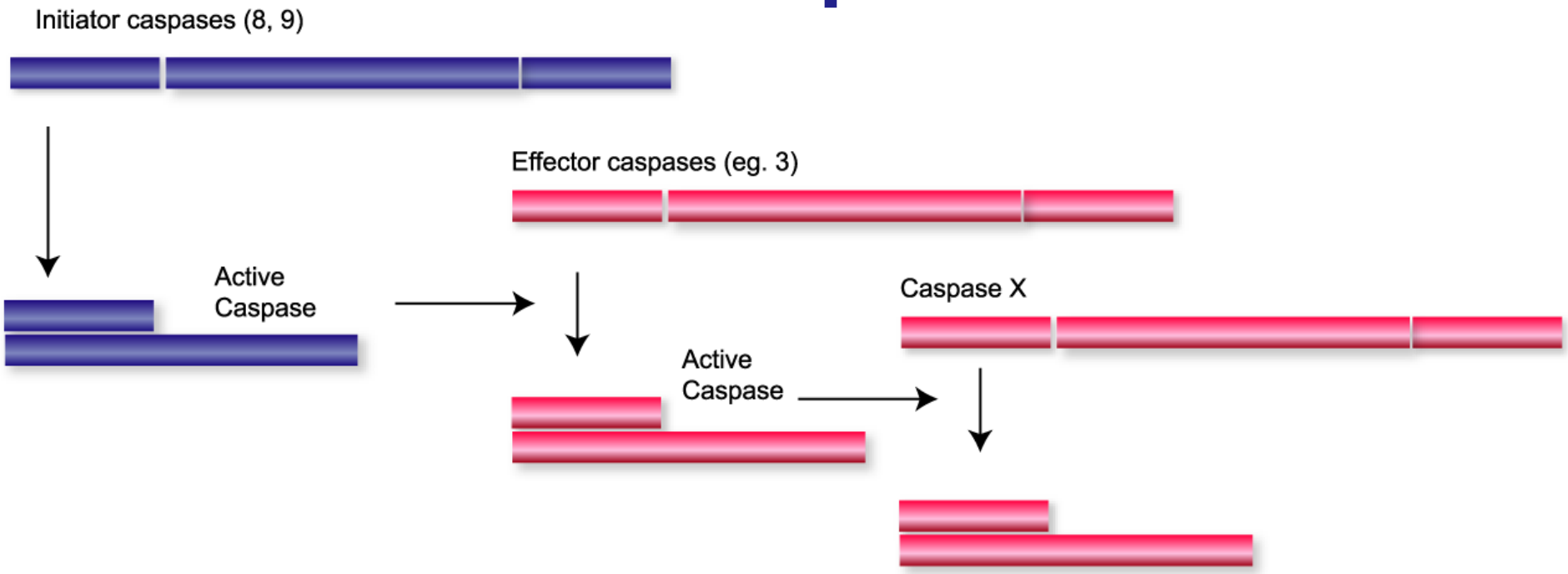
- Effectors
  - Intracellular proteases called caspases

Effectors: caspases



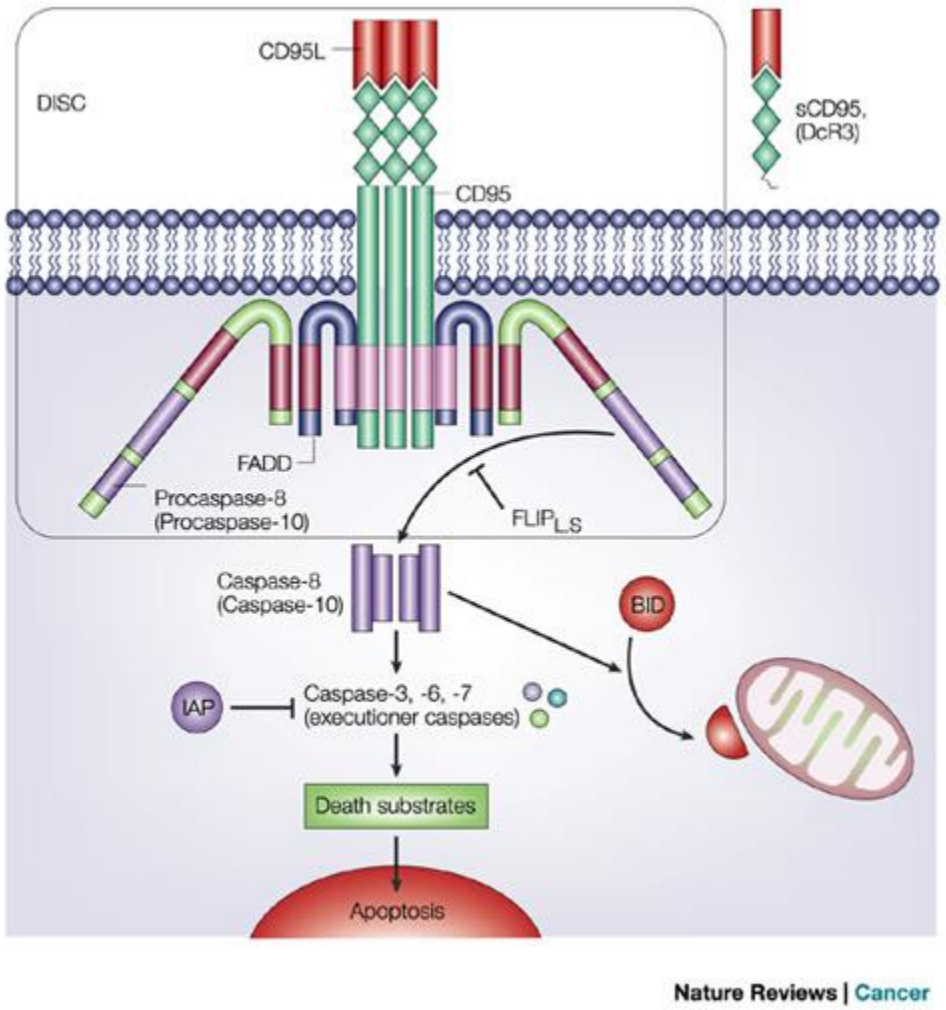
- Executioners of apoptosis
- Cleave proteins at certain sites
- Disassemble the cell
- Present in a pro-form (inactive)

Caspase cascade



Irreversible “switch” for cell death

Extrinsic pathway – death receptors

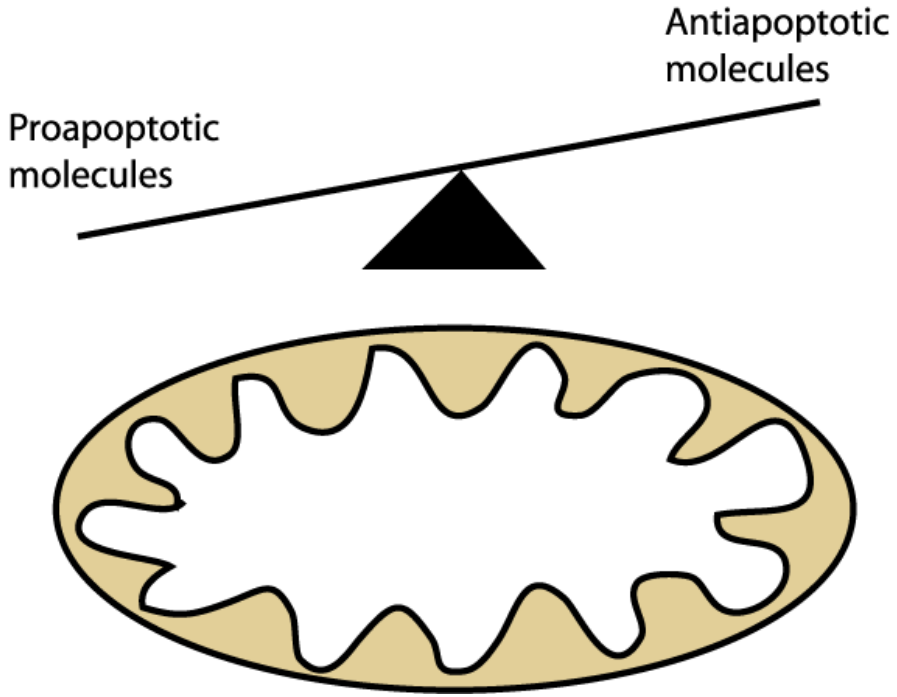


Extrinsic – caspase 8
– signal given to the cell

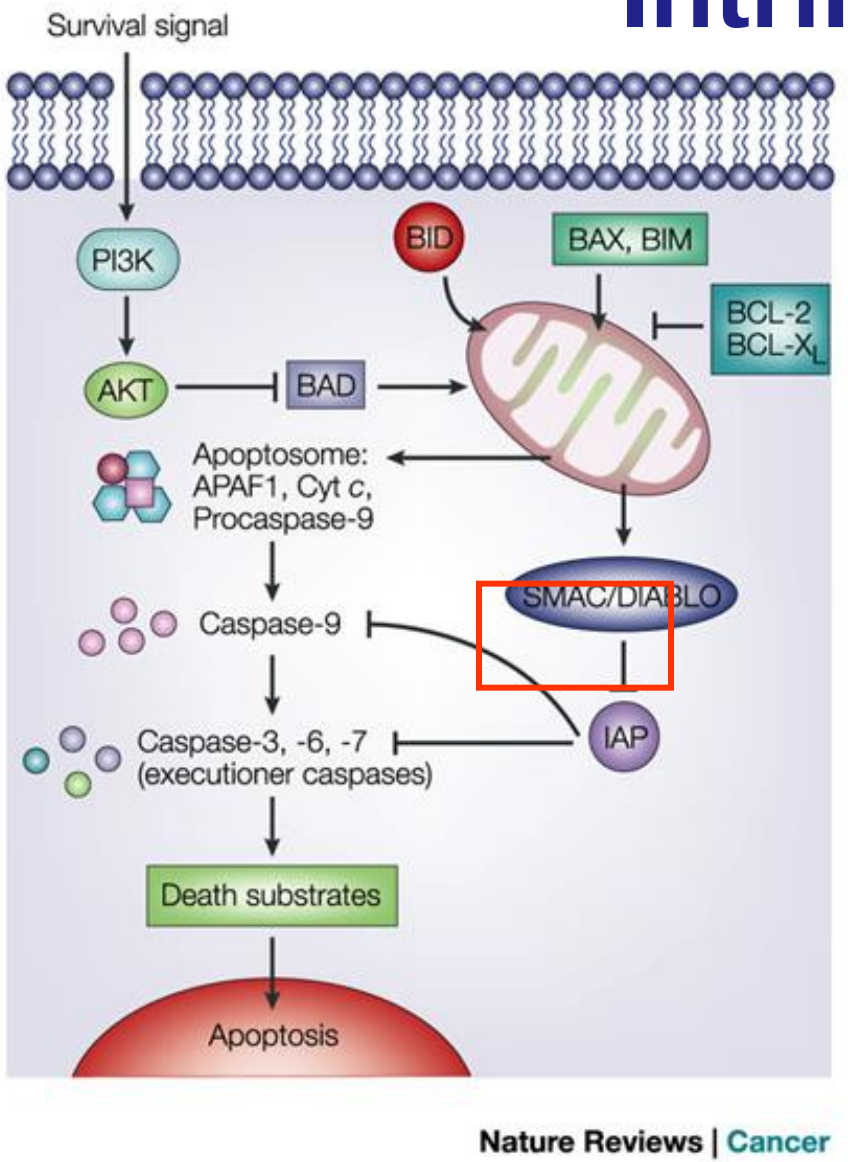
Receptors	Ligands
TRAILR1, TRAILR2	TRAIL
TNFR1	TNF
FAS	FASL

Intrinsic pathway – mitochondria dependent

- Mitochondria induce apoptosis when pro-apoptotic factors outnumber anti-apoptotic factors



Intrinsic pathway



Mitochondria :

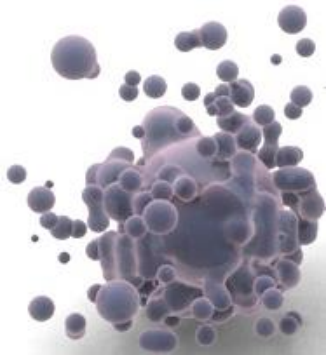
Storage site for apoptosis regulating molecules

Step 2) Release of cytochome C, formation of apoptosome

Step 3) Activation of caspase 9

How do cells die?

APOPTOSIS



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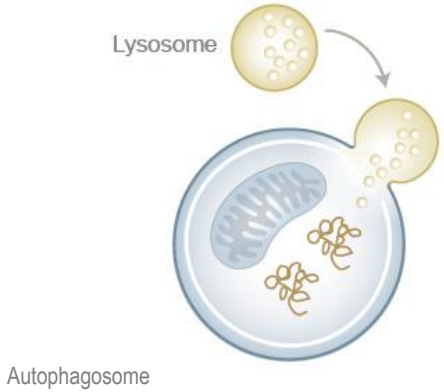

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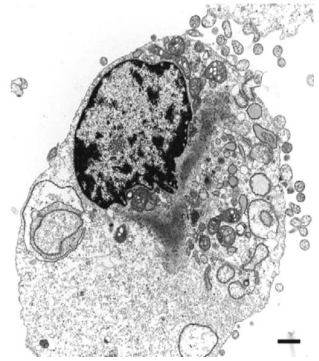

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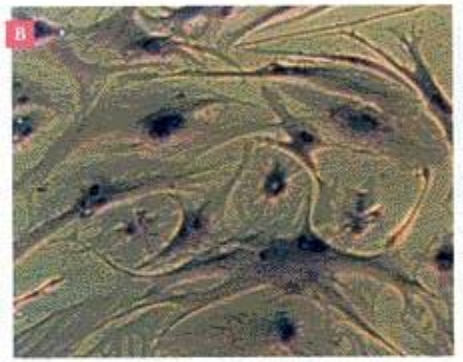

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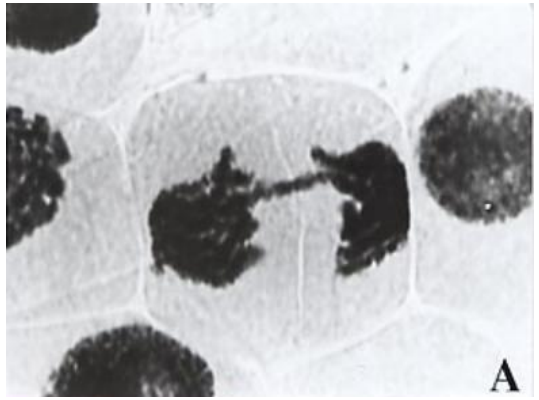
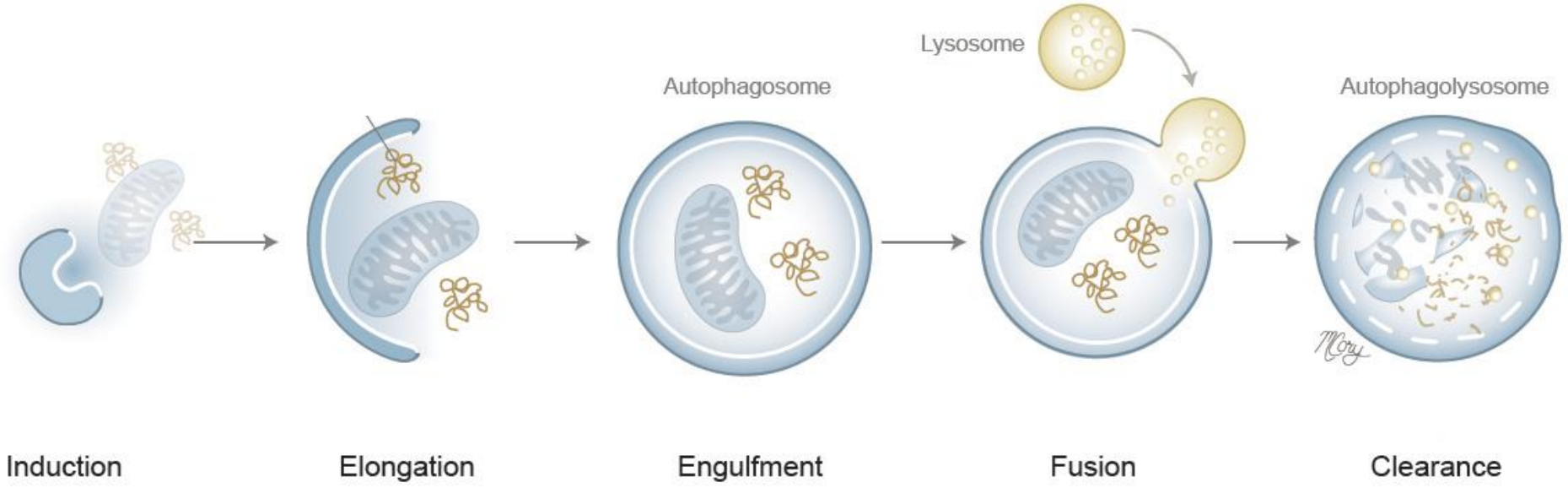


Autophagy –to eat oneself

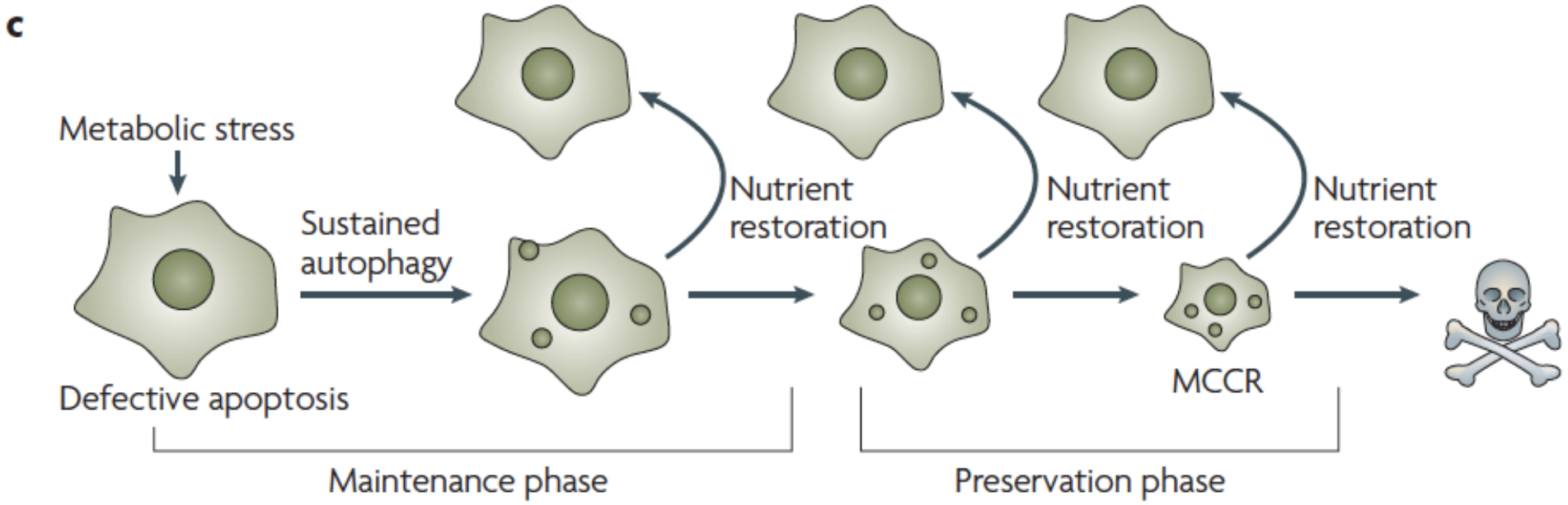


Autophagy

- Important survival mechanism during short-term starvation
  - Degradation of non-essential cell components by lysosomal hydrolases
  - Degradation products are transported back to cytoplasm for reuse in metabolism

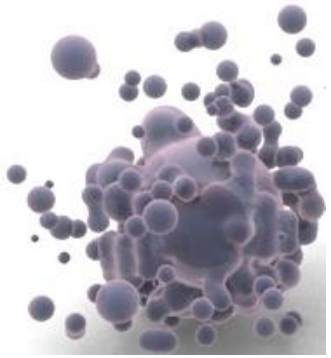
- Important mechanism for quality control
  - Removal of defective organelles, proteins

Autophagy – survival or death?



How do cells die?

APOPTOSIS



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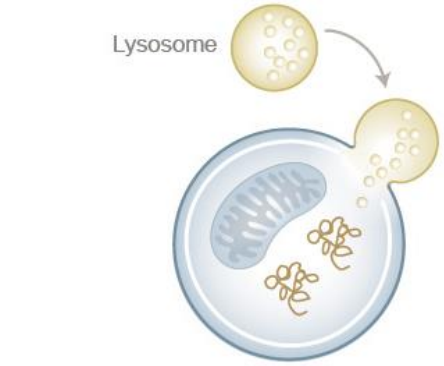

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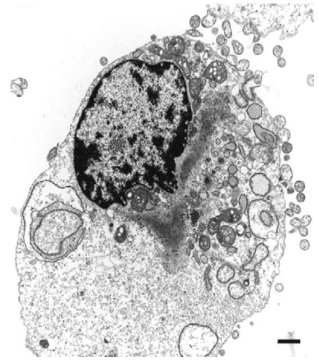

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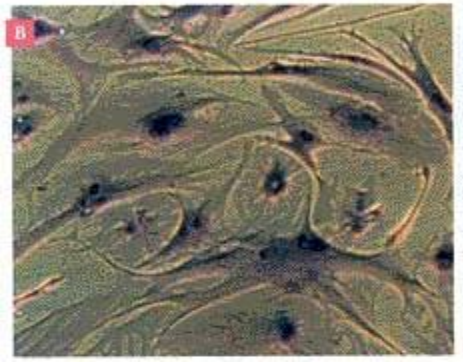

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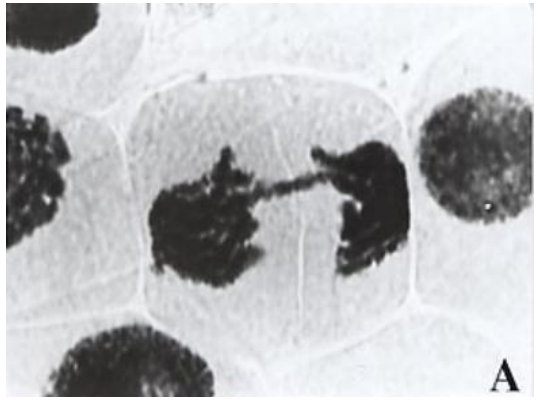
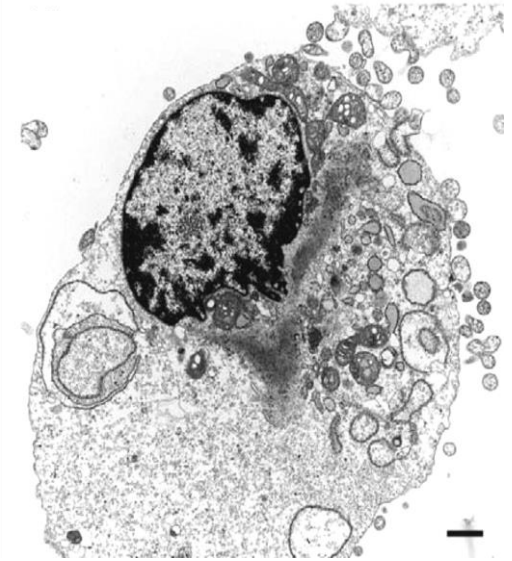
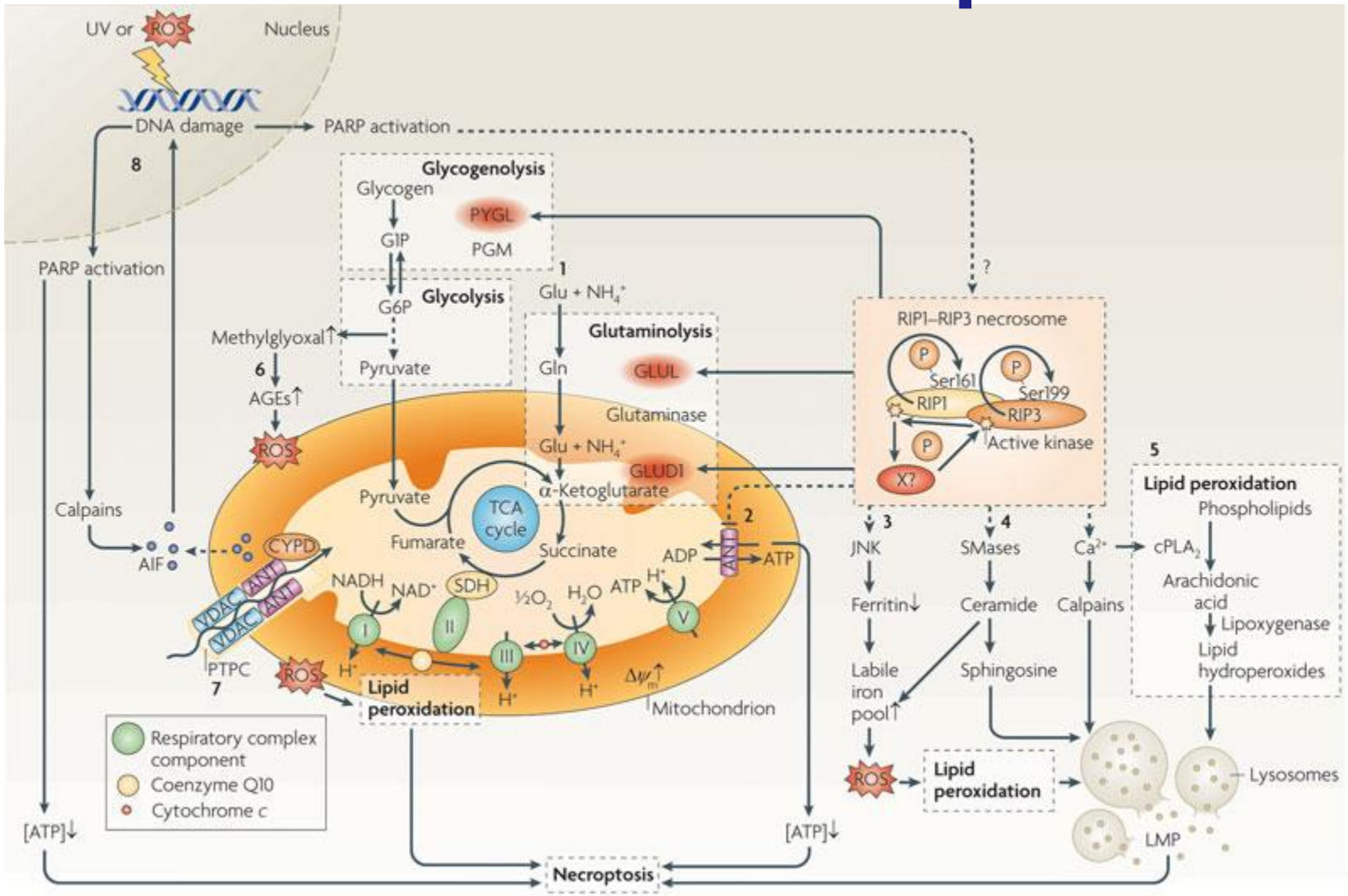


Necrosis

- Insults inducing necrosis
  - Defective membrane potential
  - Cellular energy depletion
  - Nutrient starvation
  - Damage to membrane lipids
  - Loss of function of ion channels/pumps



Execution of necroptosis



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How do cells die?

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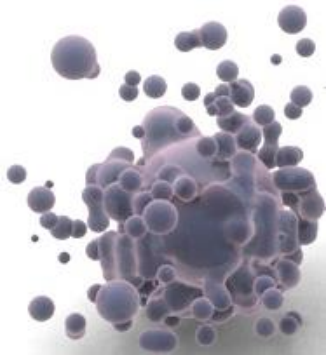

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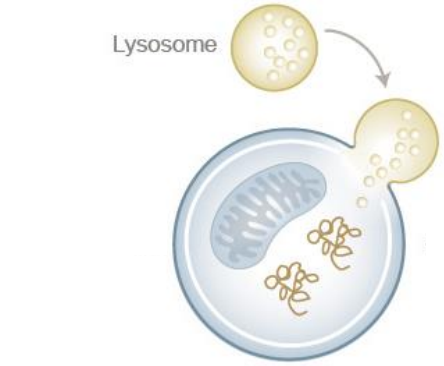

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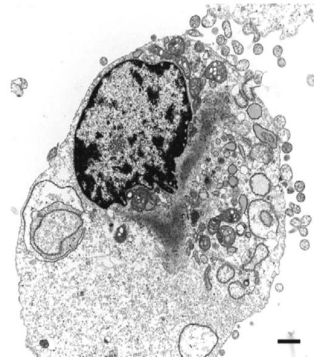

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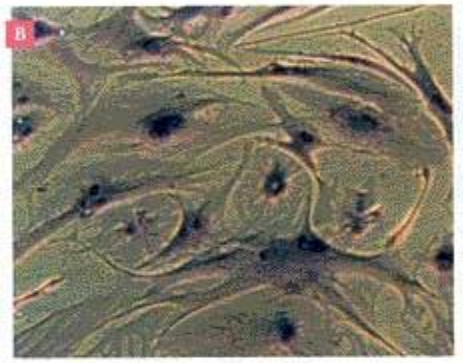

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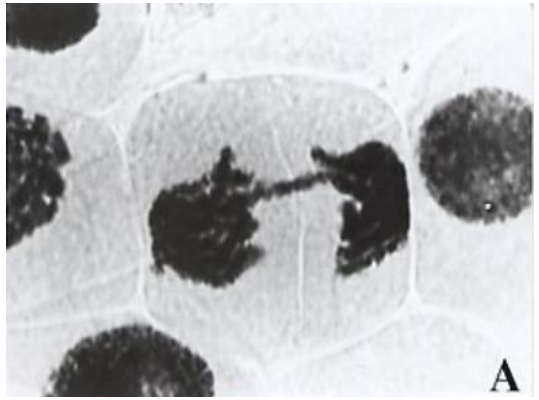
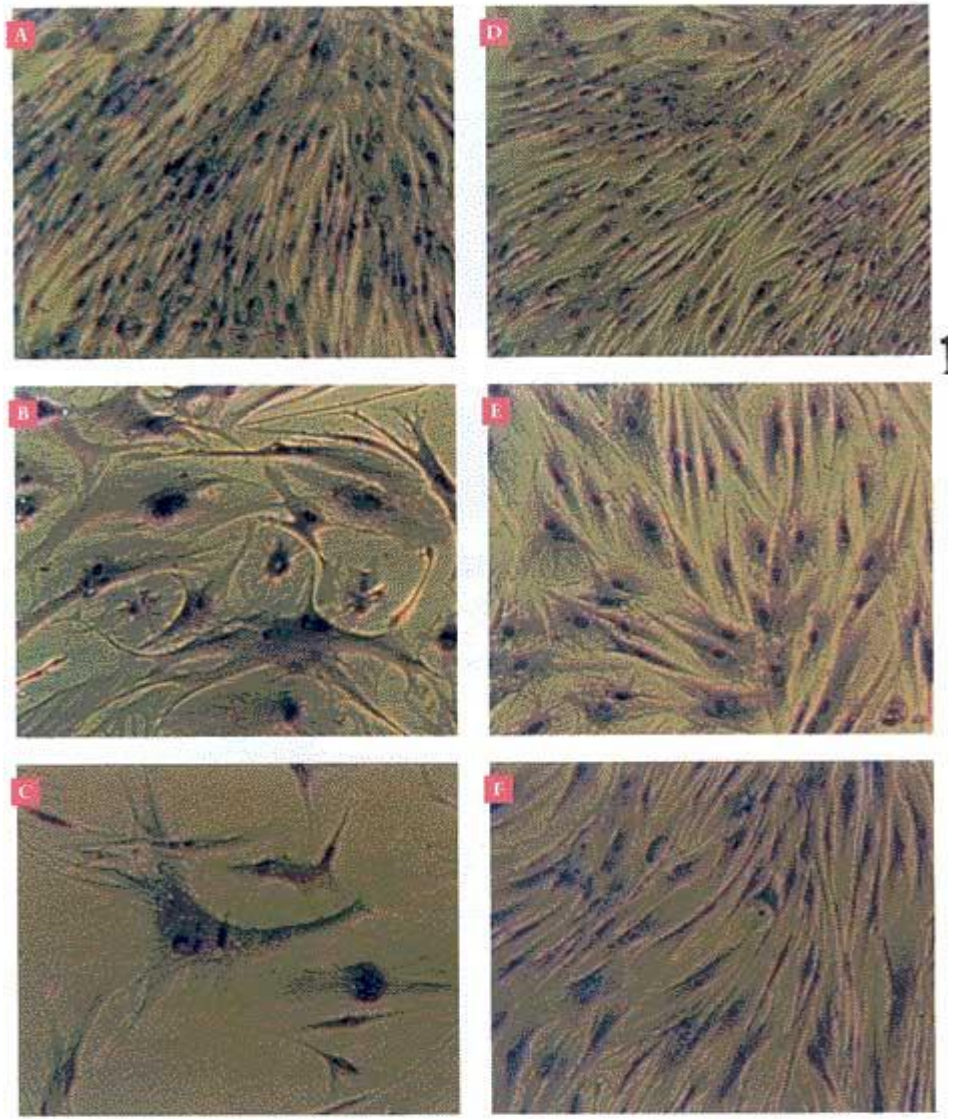
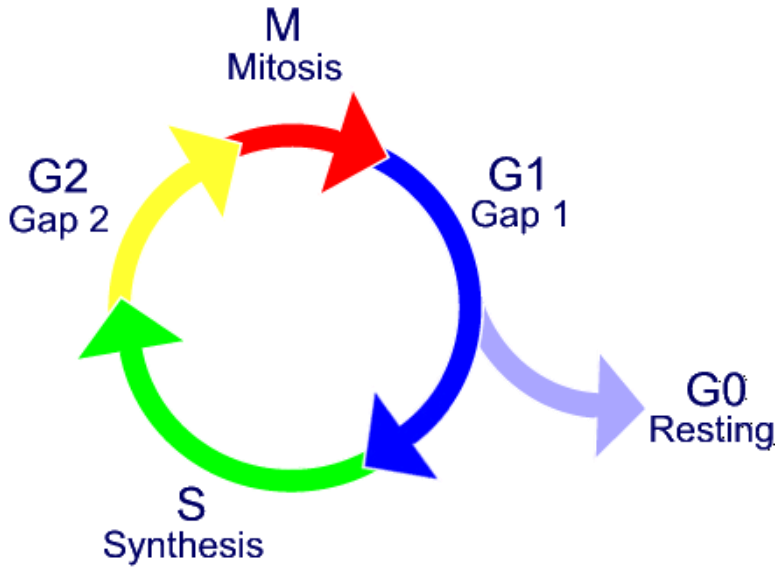


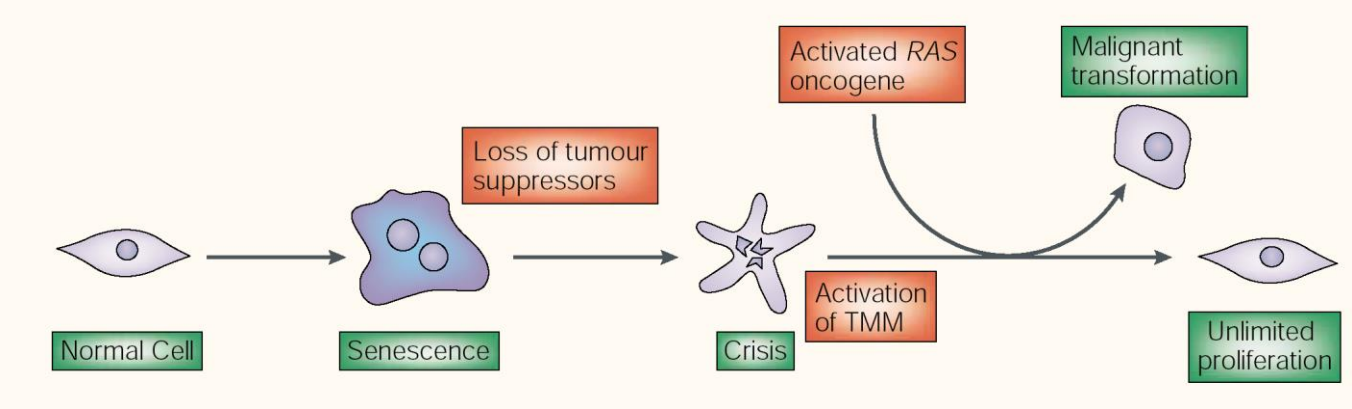
Senescence - permanent loss of proliferative capacity





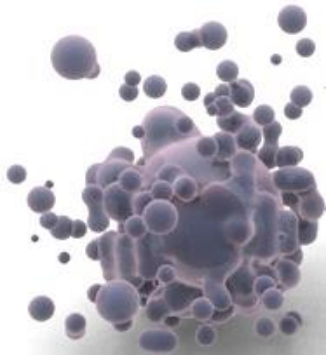
Senescence

- Associated with aging
  - Telomere shortening can induce senescence
  - Limits proliferation in normal cells
- Accelerated senescence
  - Induced by oncogenes, DNA damage
  - Permanent checkpoint activation



How do cells die?

APOPTOSIS



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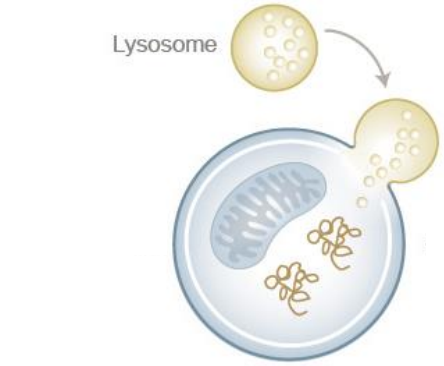

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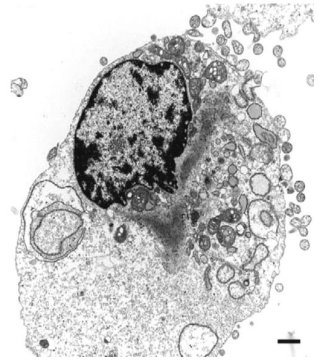

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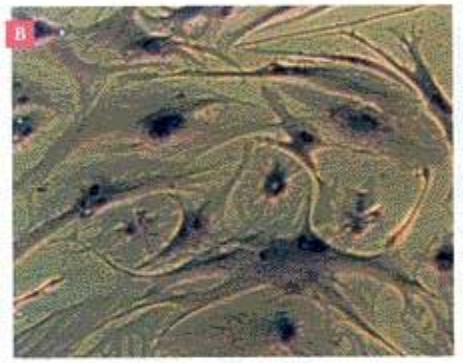

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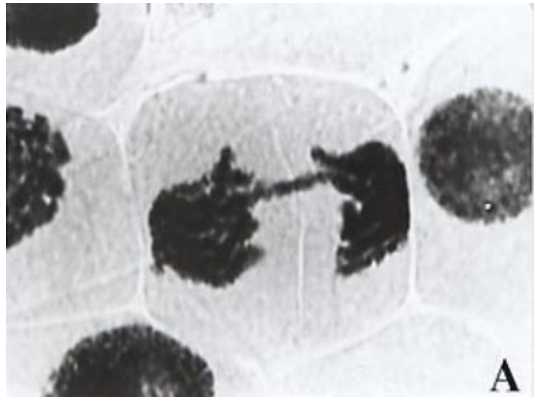
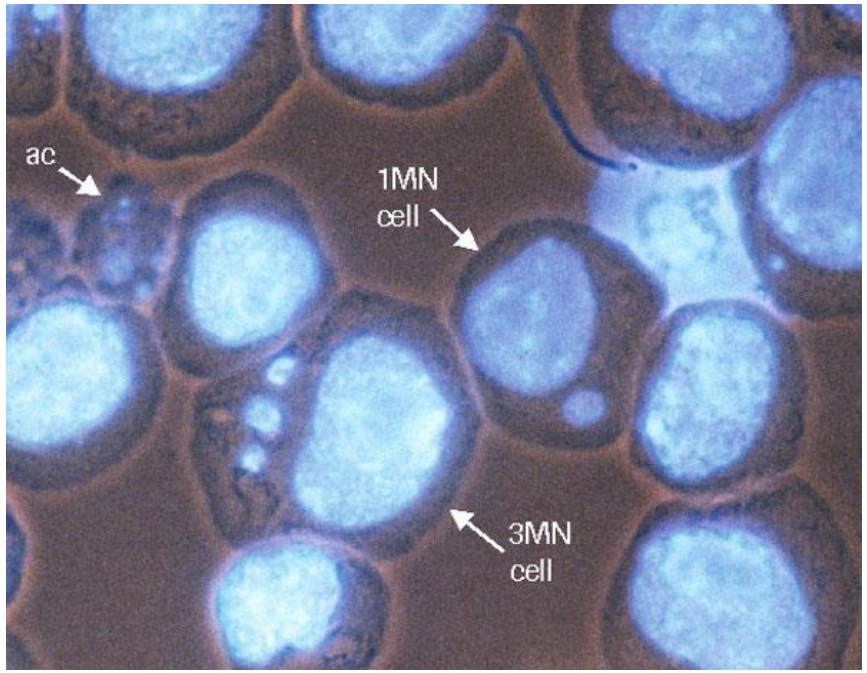
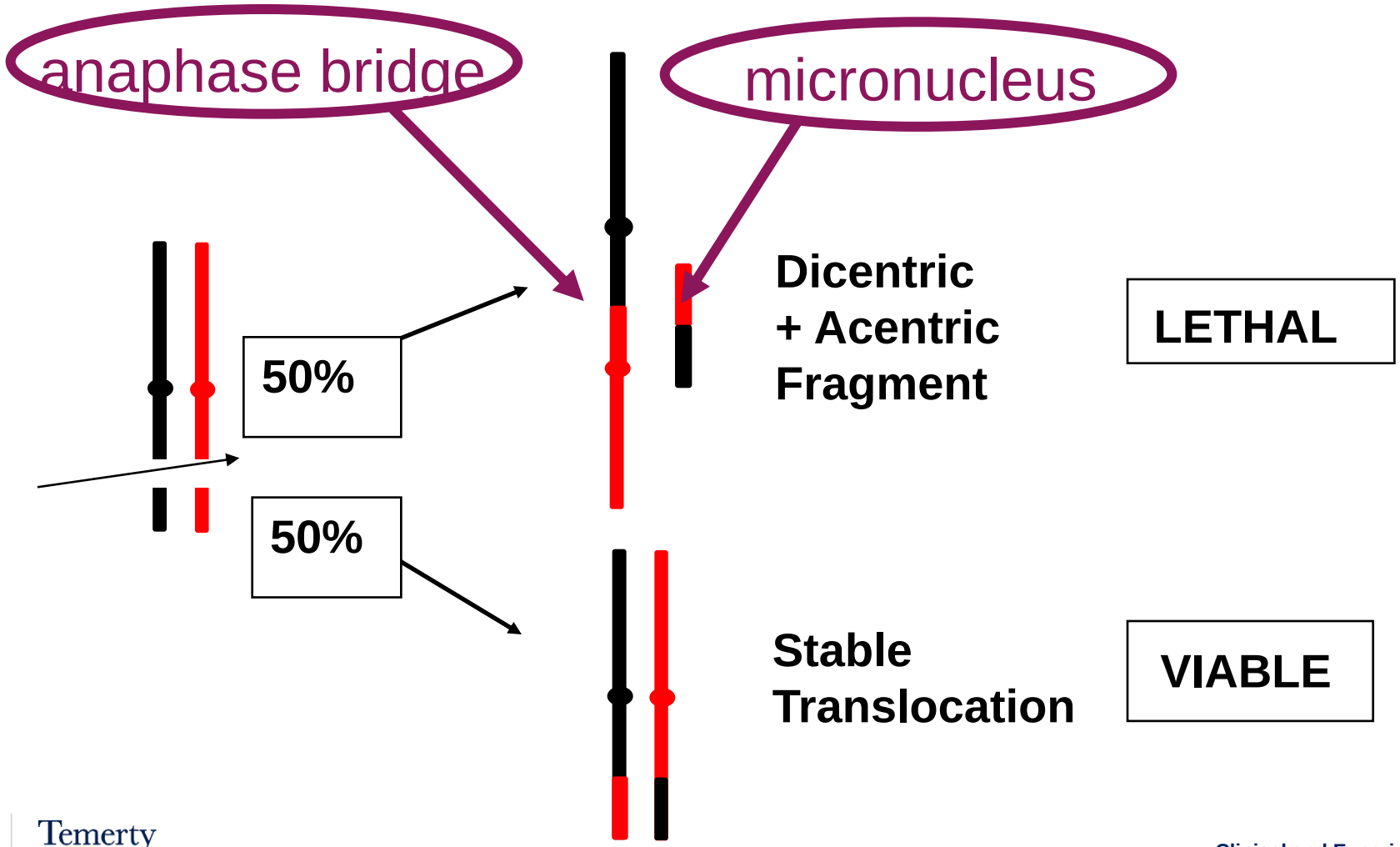


Mitotic catastrophe



- Mitotic catastrophe
  - Cells attempt to divide without proper repair of DNA damage
- May lead to secondary death by apoptosis, necrosis, autophagy, or senescence

Mitotic catastrophe is caused by chromosome aberrations



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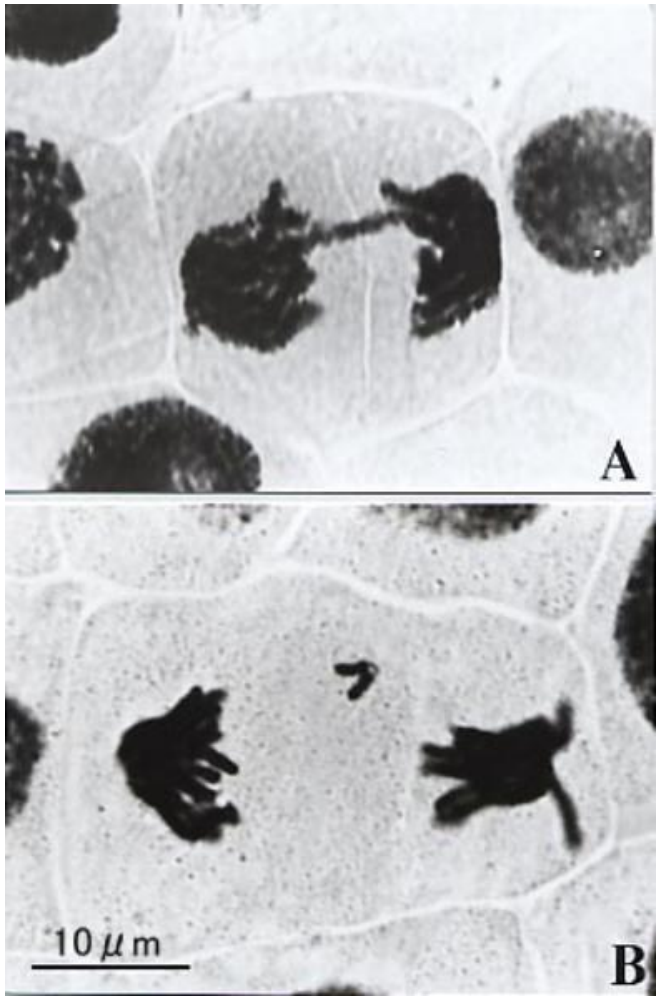


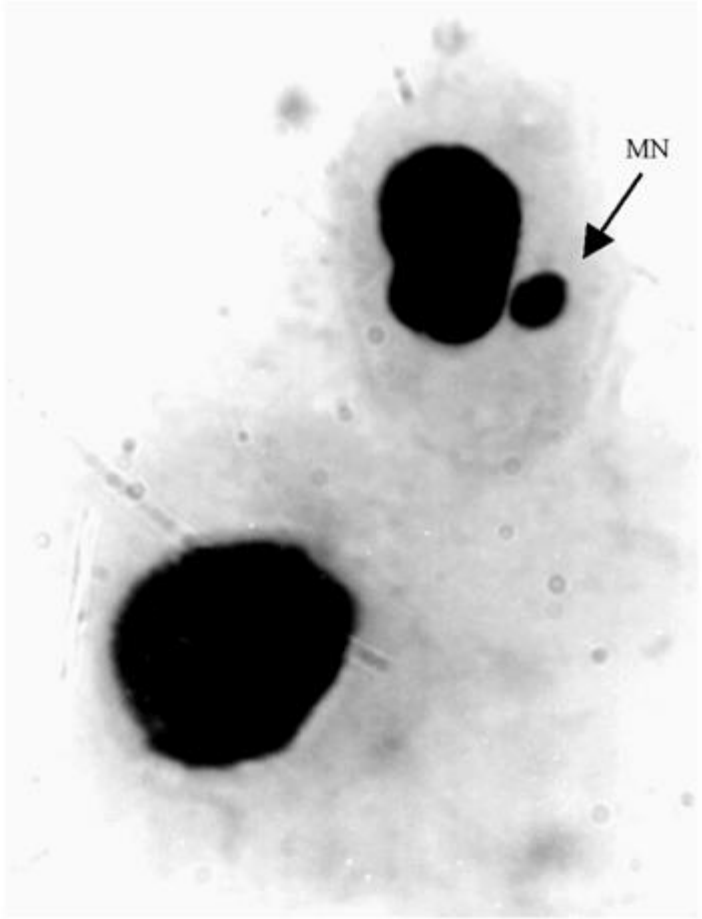


Figure 3 - Micronucleated erythrocyte (arrow) in *R. Catesbeiana* tadpole exposed to lambda-cyhalothrin. Giemsa-stained blood smear 1,000 x.

Mitotic catastrophe

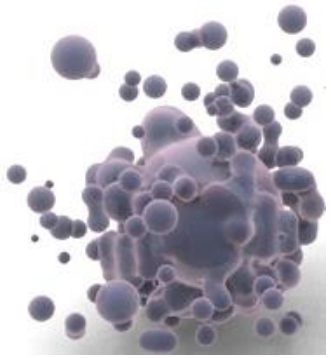
- Mitotic catastrophe takes place at long times after initial damage (irradiation)
  - Depends on proliferation rate
- Influenced by DNA repair capacity
- Cells may attempt several divisions
  - Genome becomes so unstable as to no longer support normal cell function

What about radiation?

- What is the contribution of these death pathways to radiation sensitivity ?
  - The genes controlling these pathways are frequently mutated in cancer
  - The propensity to initiate programmed cell death varies widely

How do cells die?

APOPTOSIS



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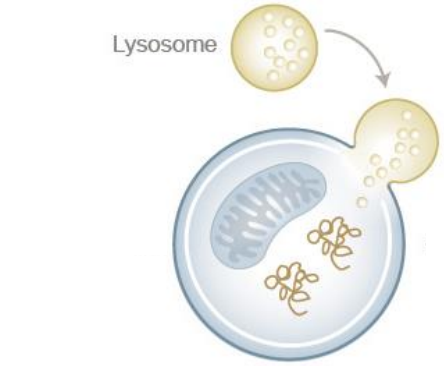
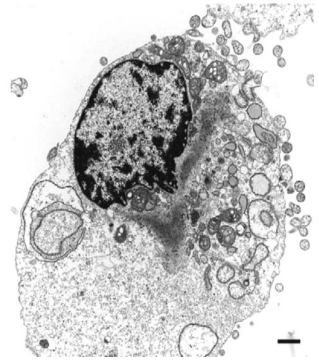
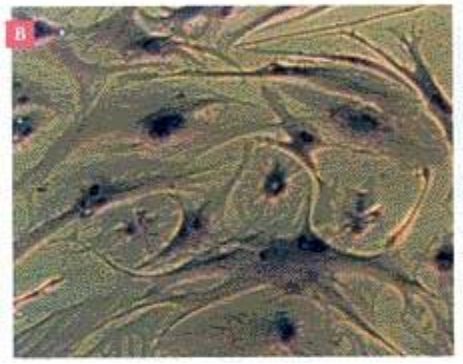


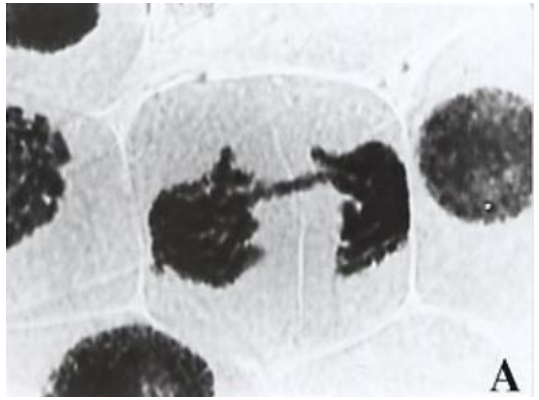
NECROSIS



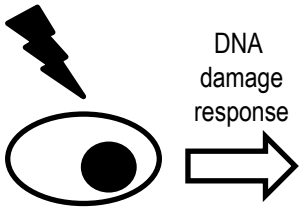
SENESCENCE



MITOTIC CATASTROPHE



What is the *cause* of cell death?



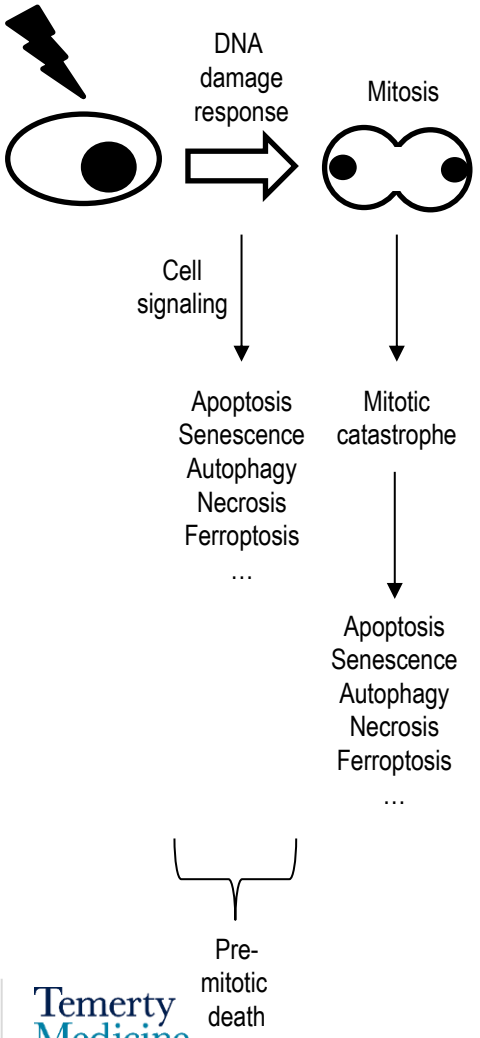
DNA
damage
response

Cell
signaling

Apoptosis
Senescence
Autophagy
Necrosis
Ferroptosis
…

Pre-
mitotic
death

What is the *cause* of cell death?



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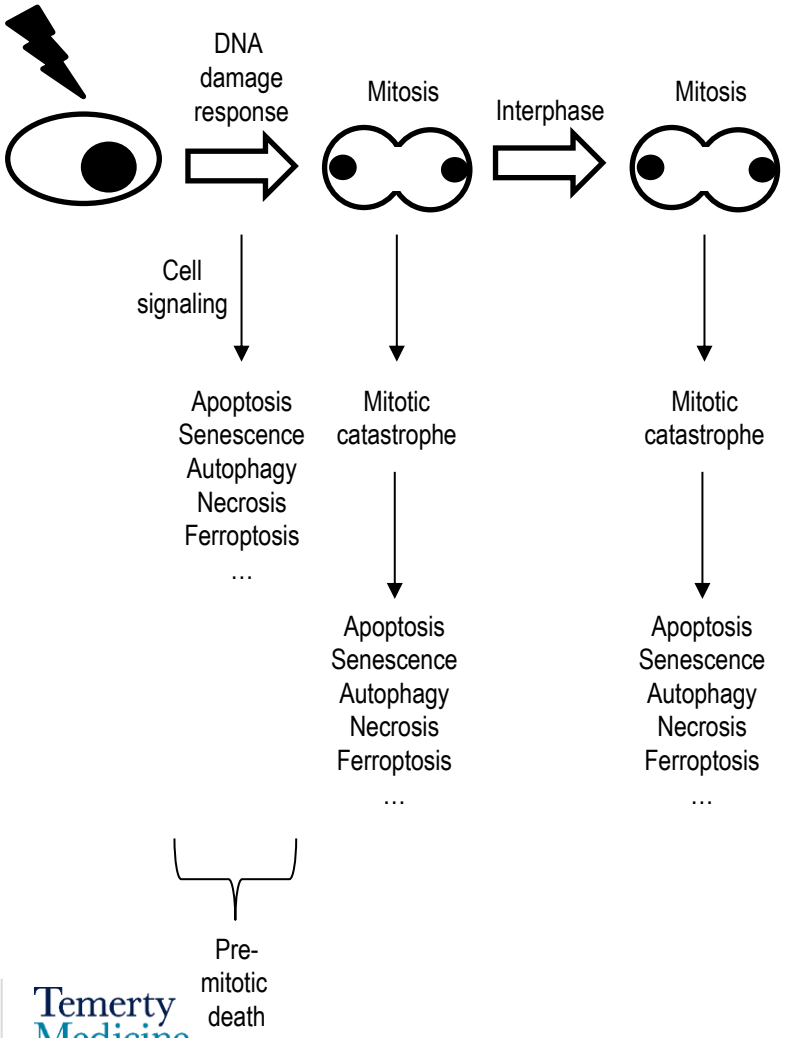

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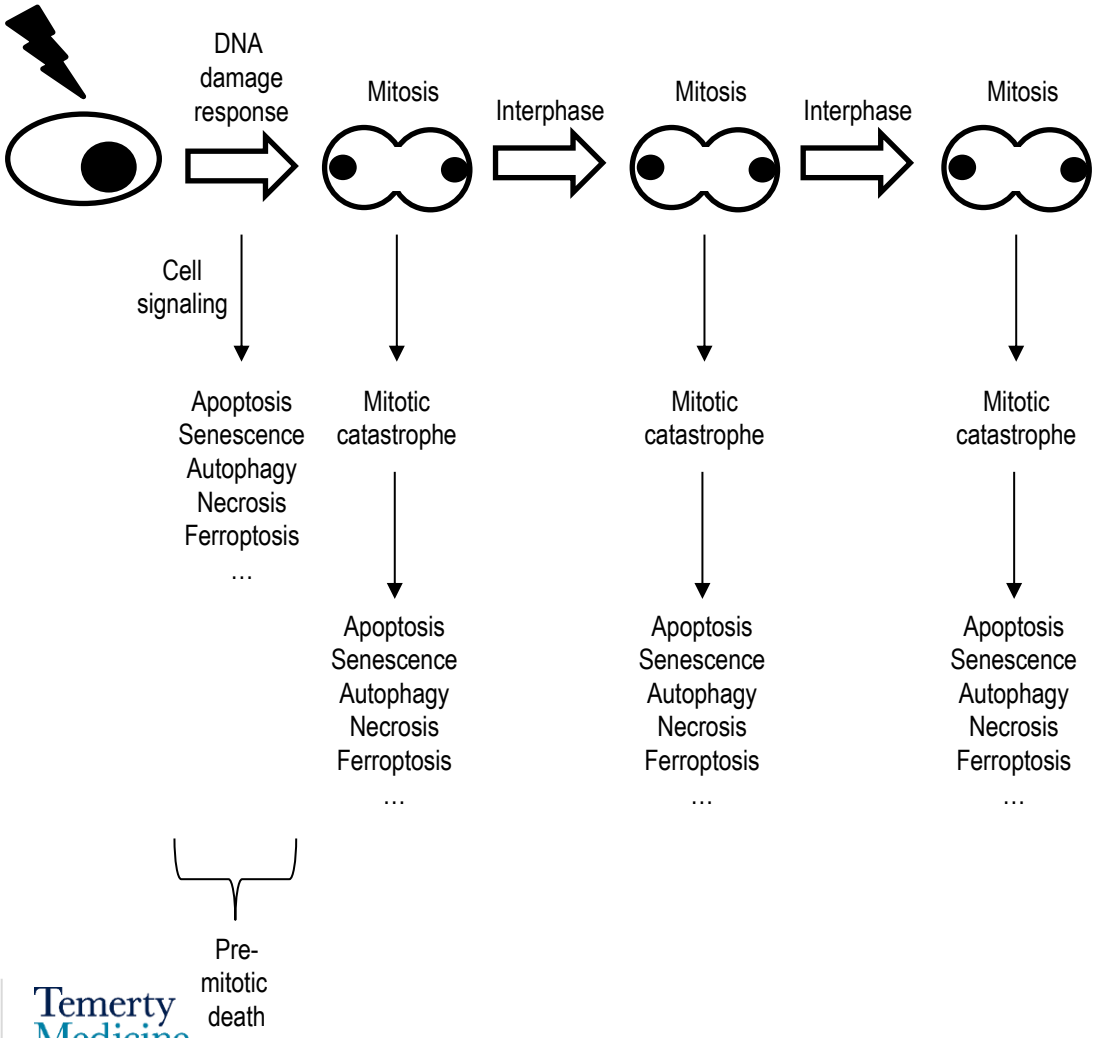

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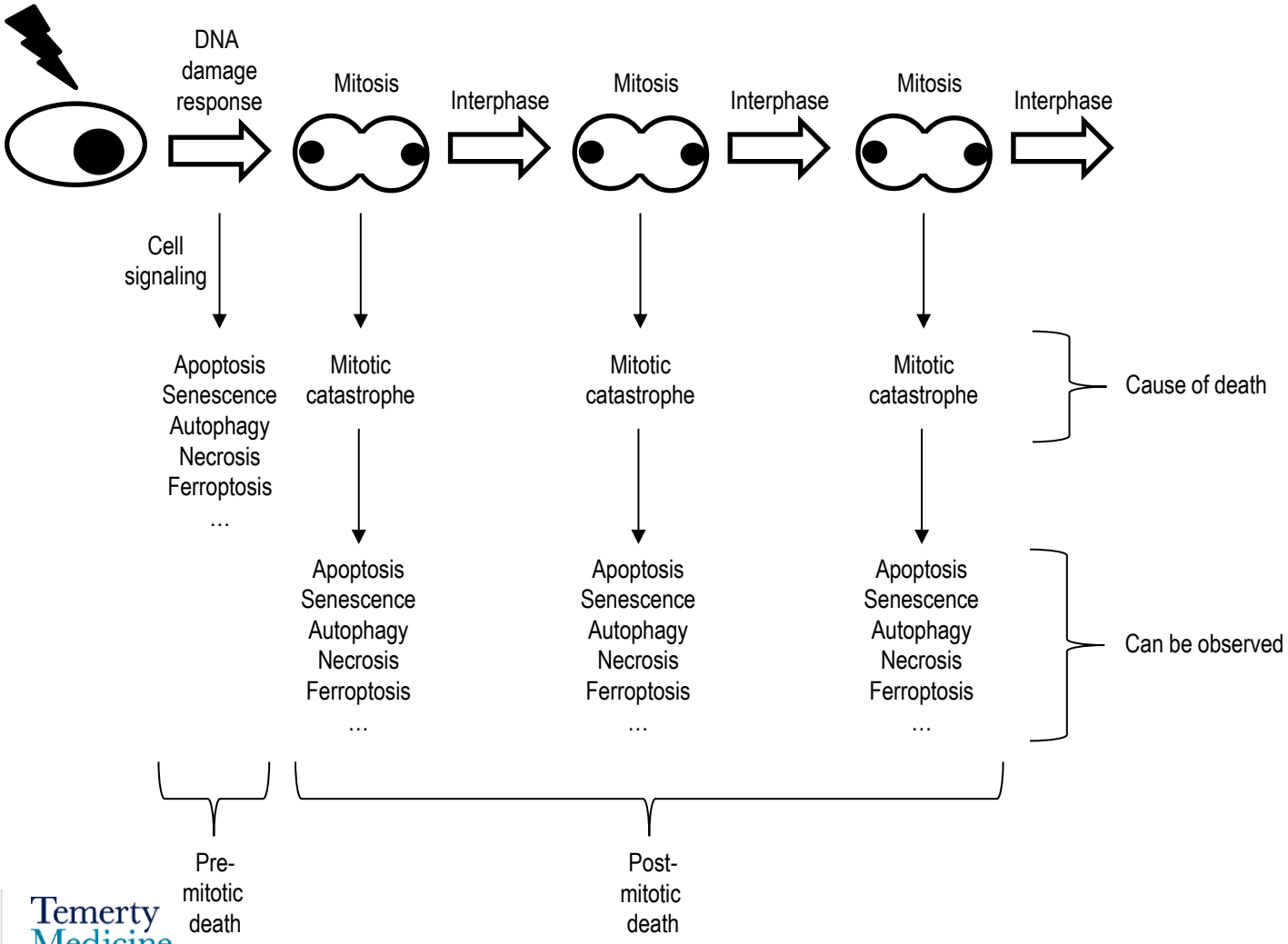
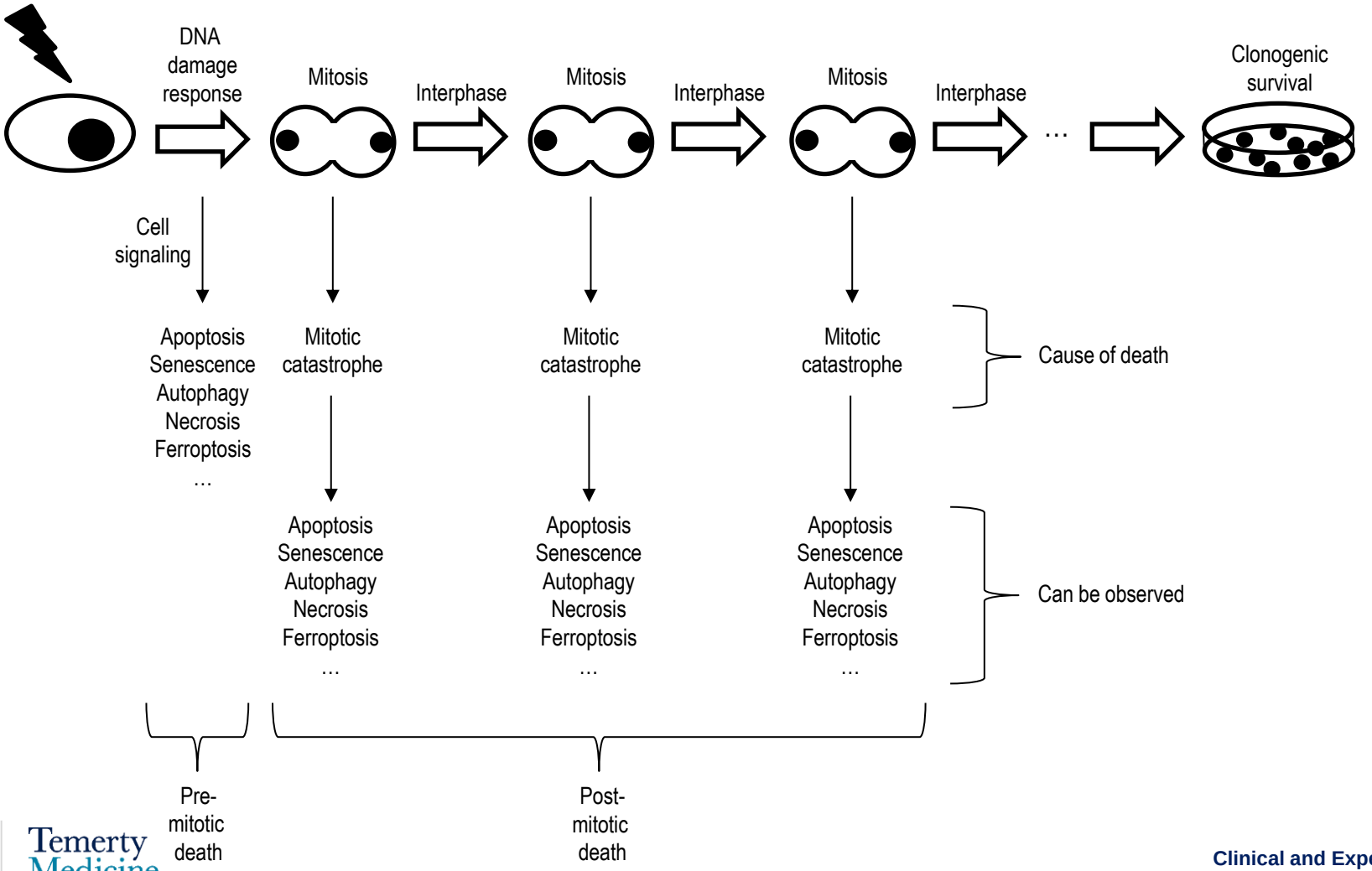
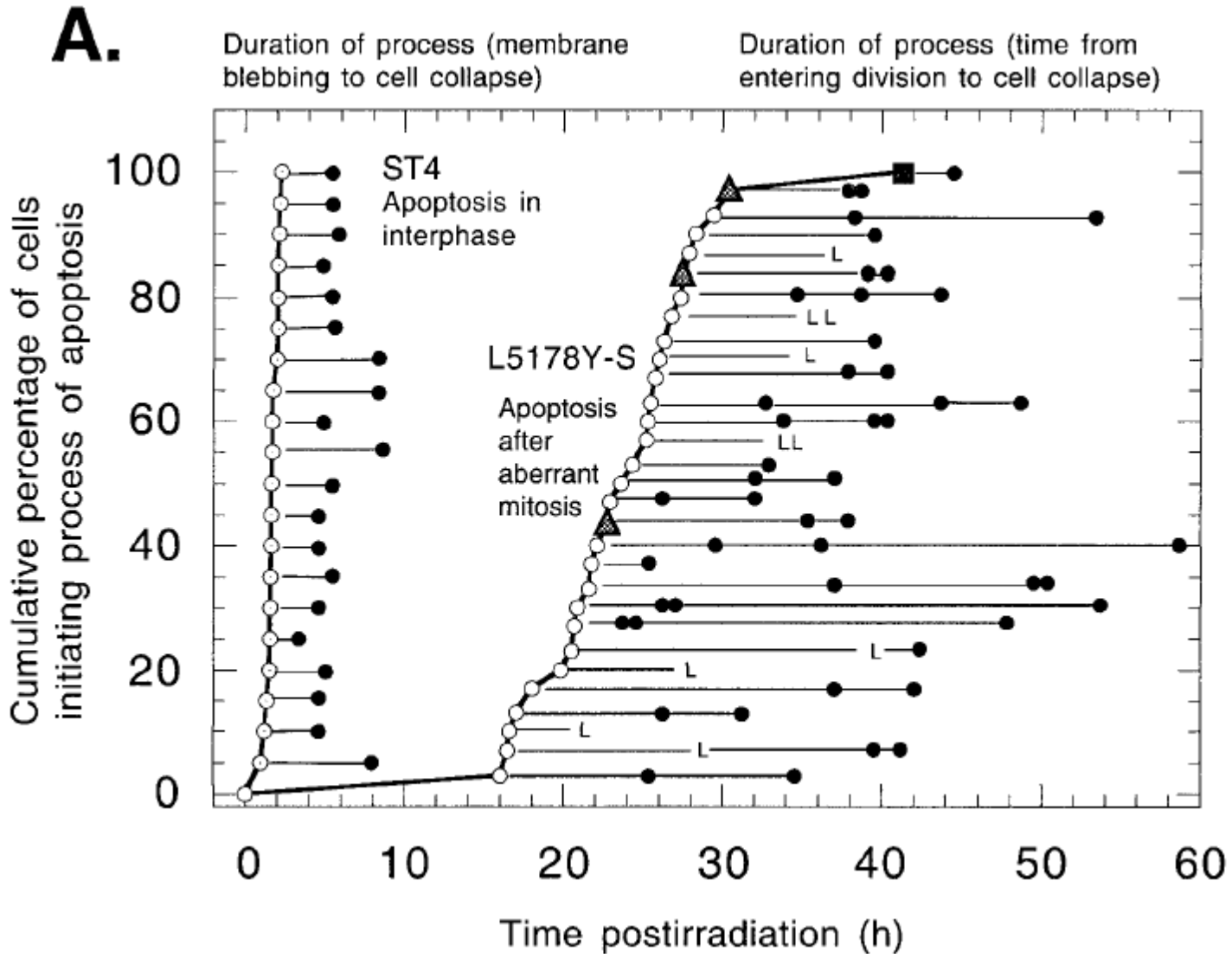


What is the *cause* of cell death?



Two types of apoptosis - pre and post mitotic



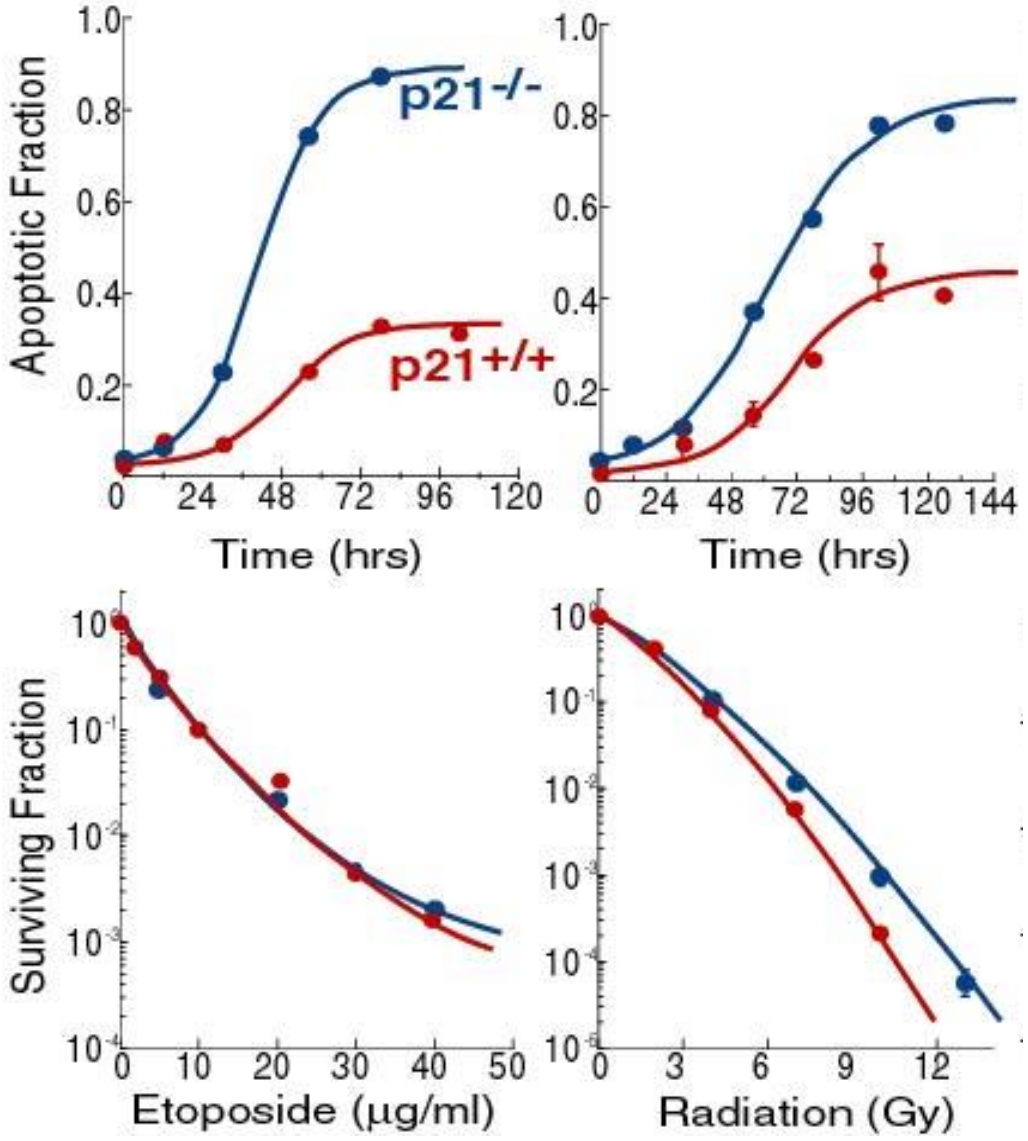
Endlich et al (2000)

Apoptosis is both a reason for cell death and a type of funeral

- Early apoptosis: Apoptosis is the reason the cell dies - it is the most sensitive mode of cell death and genes that affect apoptosis also affect cell death - e.g. some lymphomas and leukemias.

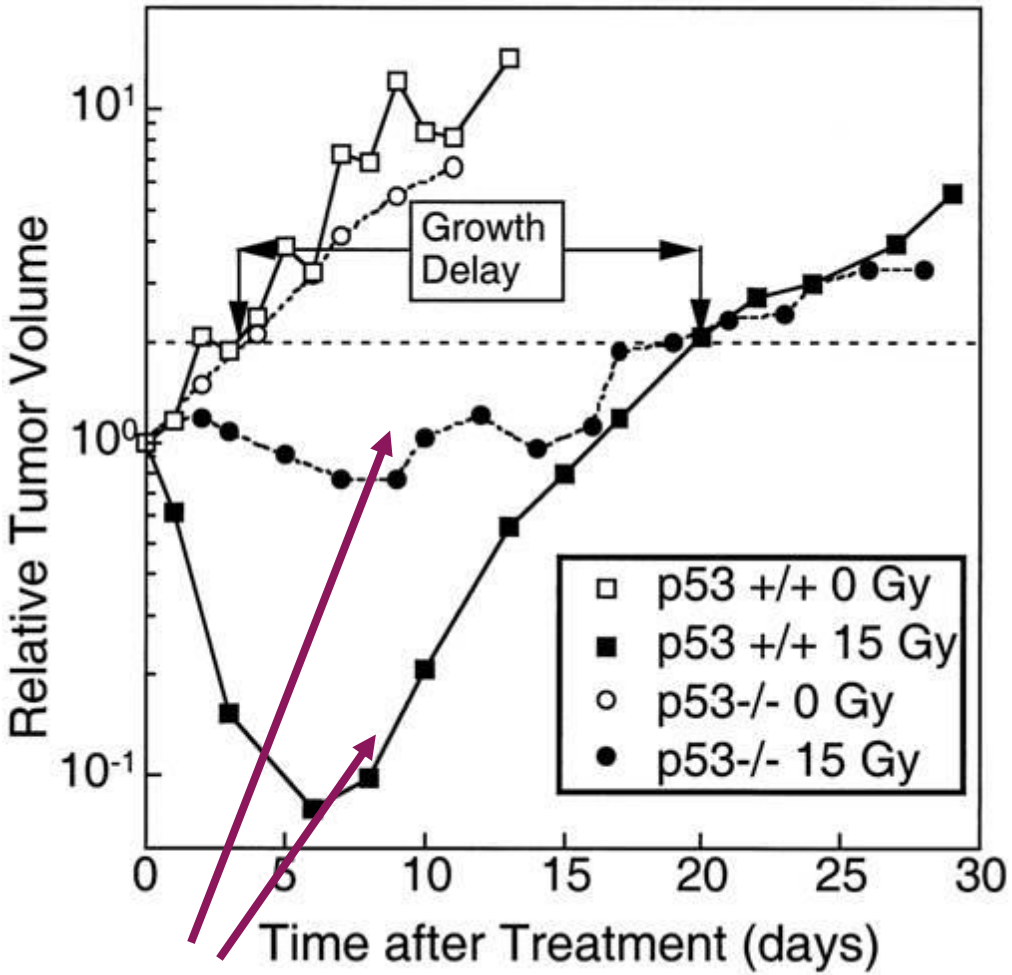
- Delayed apoptosis: The reason the cell dies is usually by mitotic catastrophe. However, the cell may, or may not, have an apoptotic “funeral”. Changing apoptotic sensitivity does not change overall cell killing - e.g. most epithelial cancers.

Apoptosis can change without affecting clonogenic survival



Wouters et al., Cancer Research (1997)

Affecting how cells die can dramatically influence the rate at which cells die



apoptosis difference

Early apoptosis explains:

- The sensitivity of lymphocytes at low radiation dose.

- The efficacy of low dose radiation dose in non-hodgkin lymphomas: 2x2 Gy results in a high proportion of responses in Low grade non-Hodgkin Lymphoma

Summary of many clinical-preclinical studies

- The mechanism of killing of the cells of solid tumors is not by early apoptosis.
- Solid tumor cells may die of apoptosis, but it is by post-mitotic (delayed) apoptosis.
- Modification of post-mitotic apoptosis does not usually change overall cell kill.

(Brown and Attardi, Nat Rev Cancer, 5: 232, 2005)

Conclusions

- Most cell death is controlled or programmed in some way
  - Include apoptosis, senescence, autophagy and necrosis
- Most cell death after radiation occurs in response to mitotic catastrophe and not from the initial damage done by the radiation
  - Cells that proliferate slowly may die at long times after irradiation
- Measuring cell death (e.g. apoptosis) will not necessarily correlate with how many cells die
  - Cells die by other mechanisms
- Genetic changes may dramatically alter how cells die without changing probability of survival

Thank you!

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