# The Cellular Basis of Disease
## Cell Injury 3

## Apoptosis and Necrosis
## Cellular Aging

APPROVED

Christine Hulette MD

# Objectives

This lecture is pretty straightforward. You should be able to address these objectives by the end.

**Discriminate** cell adaptation, reversible cell injury and irreversible cell injury (cell death) based on etiology, pathogenesis and histological and ultrastructural appearance.

**Compare and contrast** pathologic features and the clinical settings in which necrotic and apoptotic cell death occurs.

**List in temporal order** the genetic and biochemical steps in apoptosis.

**Contrast and compare** physiologic and pathologic apoptosis.

**Describe** the mechanisms and implications of cellular aging.

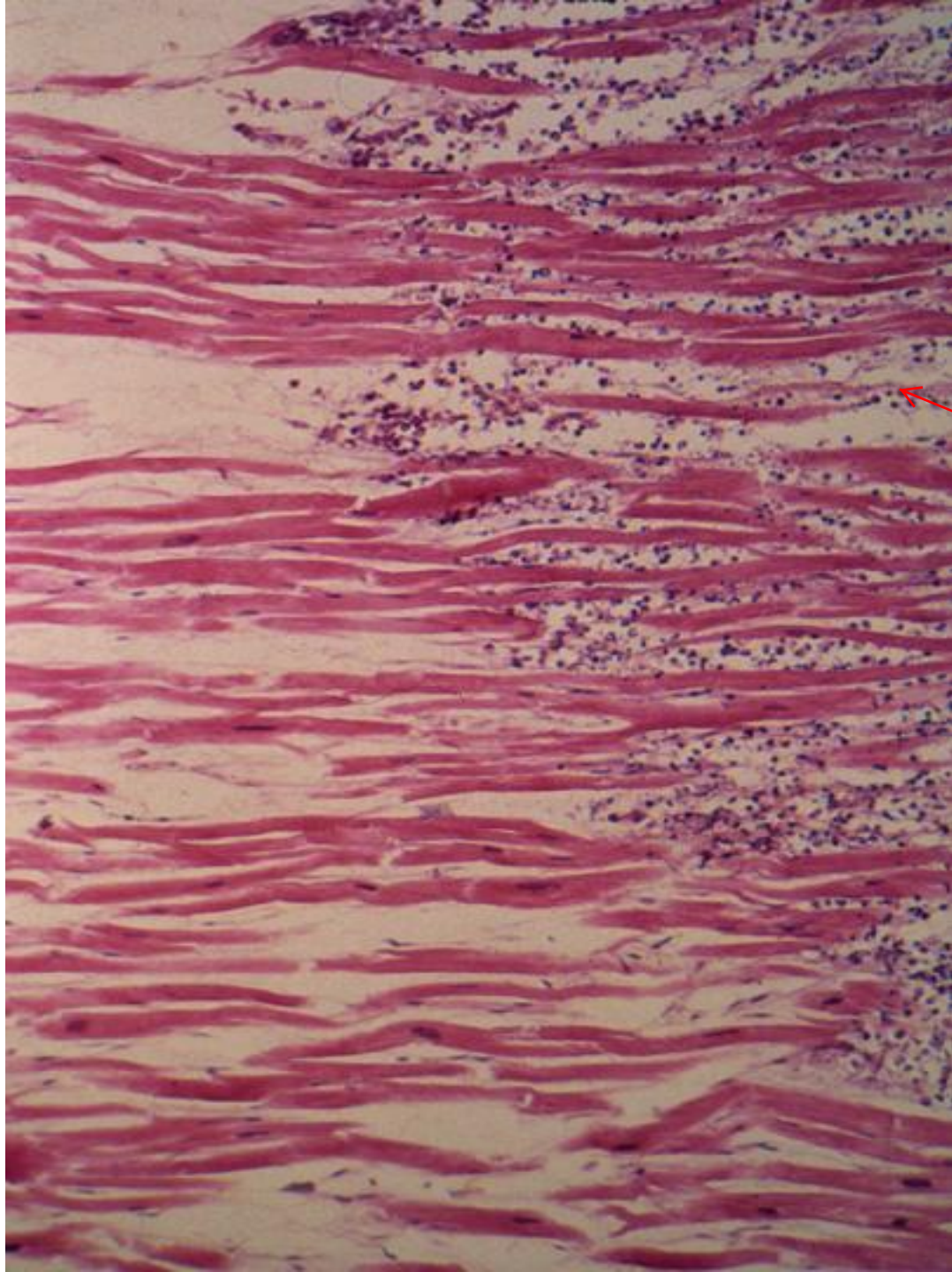
# Necrosis

- **Morphologic expression of cell death**
- **Progressive disintegration of cell structure**
- **Initiated by overwhelming stress**
- **Usually elicits an acute inflammatory cell response (neutrophils may be present).**

Just because Dr. H mentioned it, inflammatory response doesn't occur in immunocompromised patients.

# Apoptosis

- **Pathway of cell death induced by a tightly regulated suicide program.**
- **Controlled by specific genes.**
- **Fragmentation of DNA.** In a regular pattern.
- **Fragmentation of nucleus.**
- **Blebs form and apoptotic bodies are released.**
- **Apoptotic bodies are phagocytized.**
- **No neutrophils.** No inflammation in apoptosis.



# Necrosis or Apoptosis?

Necrosis. Why? Lots of Leukocytes. Does Apoptosis involve an inflammatory response? No!

# Consequences of Cell Death

**Necrosis**

**Loss of functional tissue**

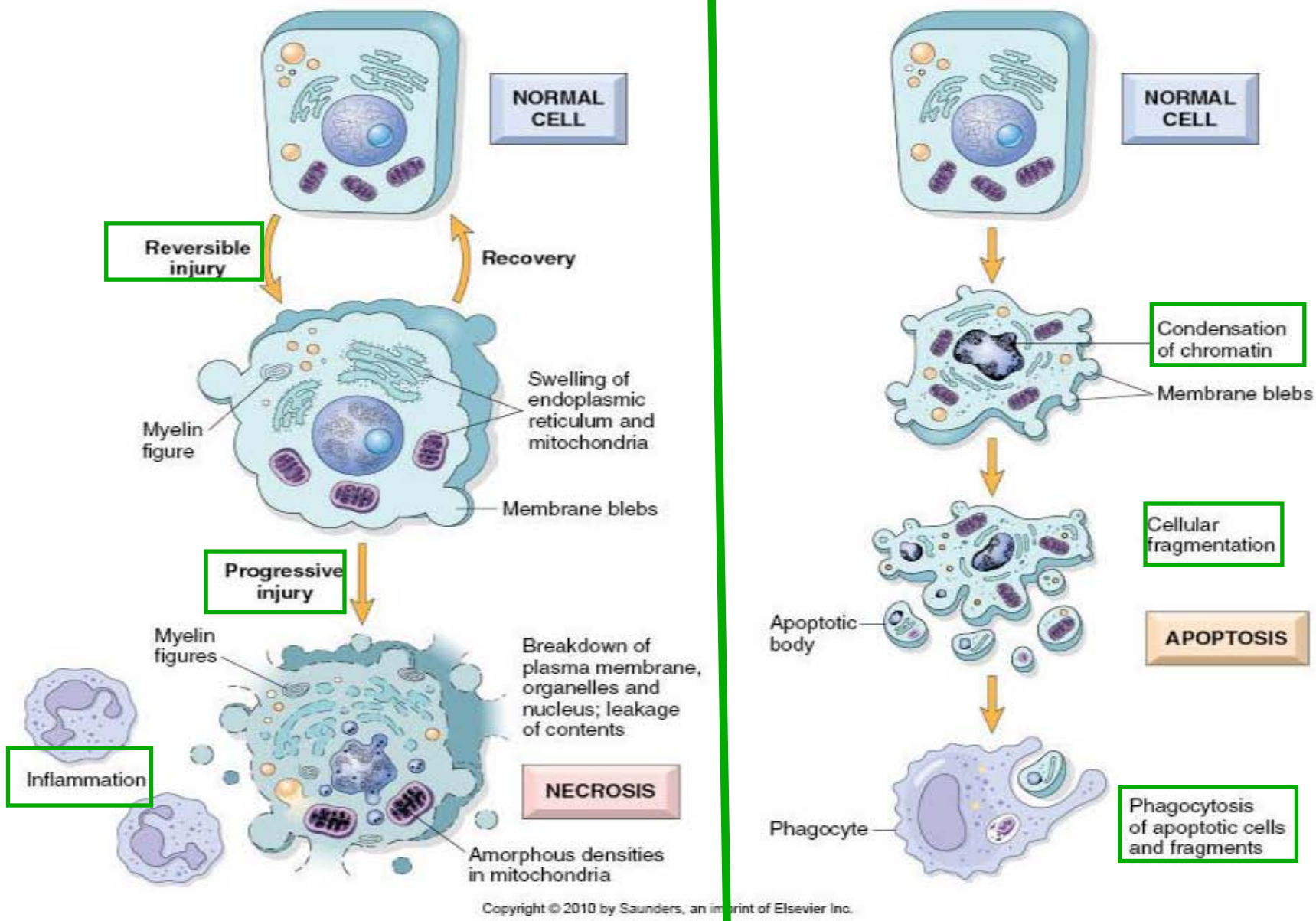
**Impaired organ function, transient or permanent**

**Apoptosis**

**Removal of damaged or unnecessary cells**

Necrosis

Apoptosis



# Causes of Apoptosis

This slide is incredibly hi-yield.

**Physiologic**

**Pathologic**

# Physiologic Apoptosis

**Embryogenesis and fetal development.**

Think development of hands and feet.

**Hormone dependent involution.**

- Prostate glandular epithelium after castration
- Regression of lactating breast after weaning

**Cell loss in proliferating cell populations.**

- Immature lymphocytes
- Epithelial cells in the GI tract

**Elimination of self-reactive lymphocytes.**

Lymphocytes that act against host. If not eliminated, you get autoimmune diseases.

**Death of cells that have served their function.**

- Neutrophils, Lymphocytes

# Apoptosis in Pathologic Conditions

**DNA damage** due to radiation, chemotherapy.

**Accumulation of misfolded proteins** leads to ER stress which ends with apoptosis.

Cell death in **viral infections** that induce apoptosis such as **HIV** and **Adenovirus** or by the host immune response such as **hepatitis**.

**Organ atrophy after duct obstruction**.

# General Characteristics

Compare and contrast Necrosis and Apoptosis.. Look at the chart.

| NECROSIS | APOPTOSIS |
| --- | --- |
| Usually affects large areas of contiguous cells | Usually affects scattered individual cells |
| Control of intracellular environment is lost early | Control of intracellular environment maintained in early stages |
| Cells swell and organelles swell | Cells contract |

# General Characteristics

| NECROSIS | APOPTOSIS |
| --- | --- |
| Nuclear chromatin marginates early, while injury is still reversible | Nuclear chromatin marginates and chromatin condenses, becoming very compact |
| When DNA is cleaved, which is usually a late event, fragments are random in size (smear pattern in gels) | Chromatin condensation and DNA fragmentation occur together; DNA cleaved into multiples of 200 base pair units (ladder pattern in gels) |

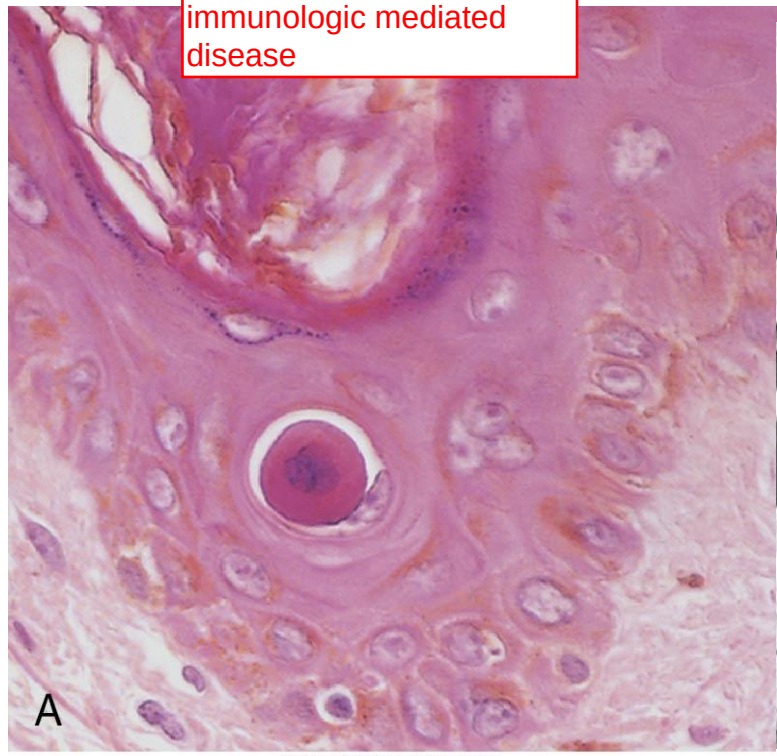
We will see this soon.

# General Characteristics

| NECROSIS | APOPTOSIS |
| --- | --- |
| Cell membrane ruptures as terminal event and cell contents are released, which are chemotactic | Blebs form and apoptotic bodies containing nuclear fragments are shed |
| Chemotactic factors lead to neutrophil infiltration to degrade dead cells | Phagocytosis of intact apoptotic bodies, no chemotactic factors are generated |

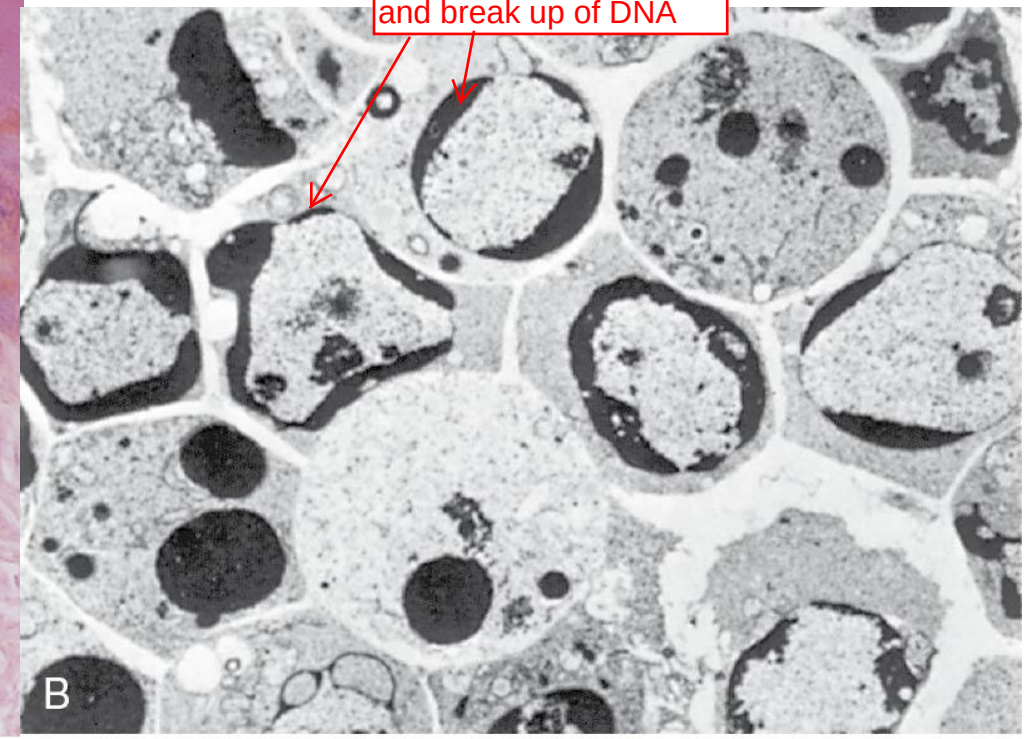
# Apoptosis

Section of skin with immunologic mediated disease

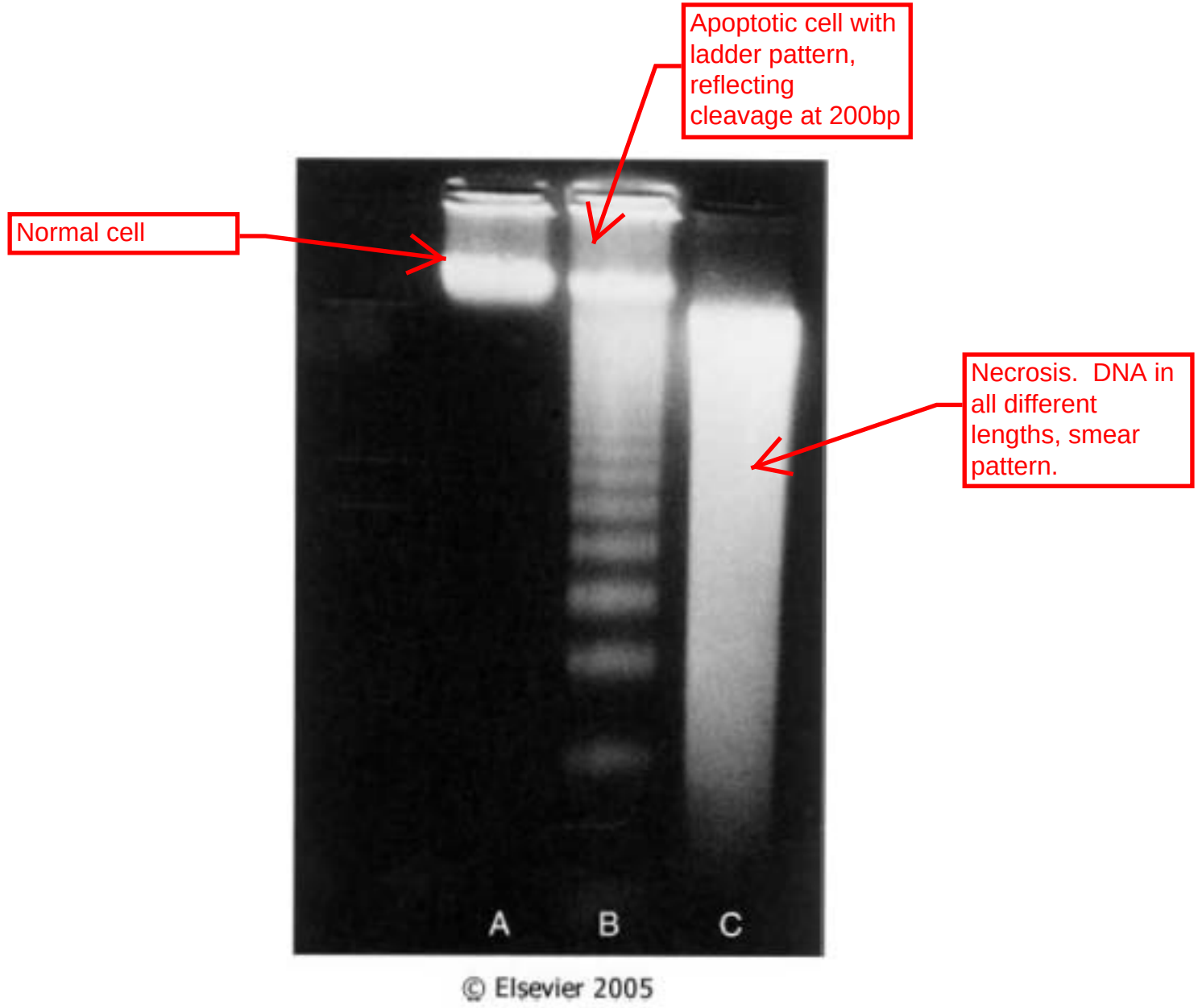


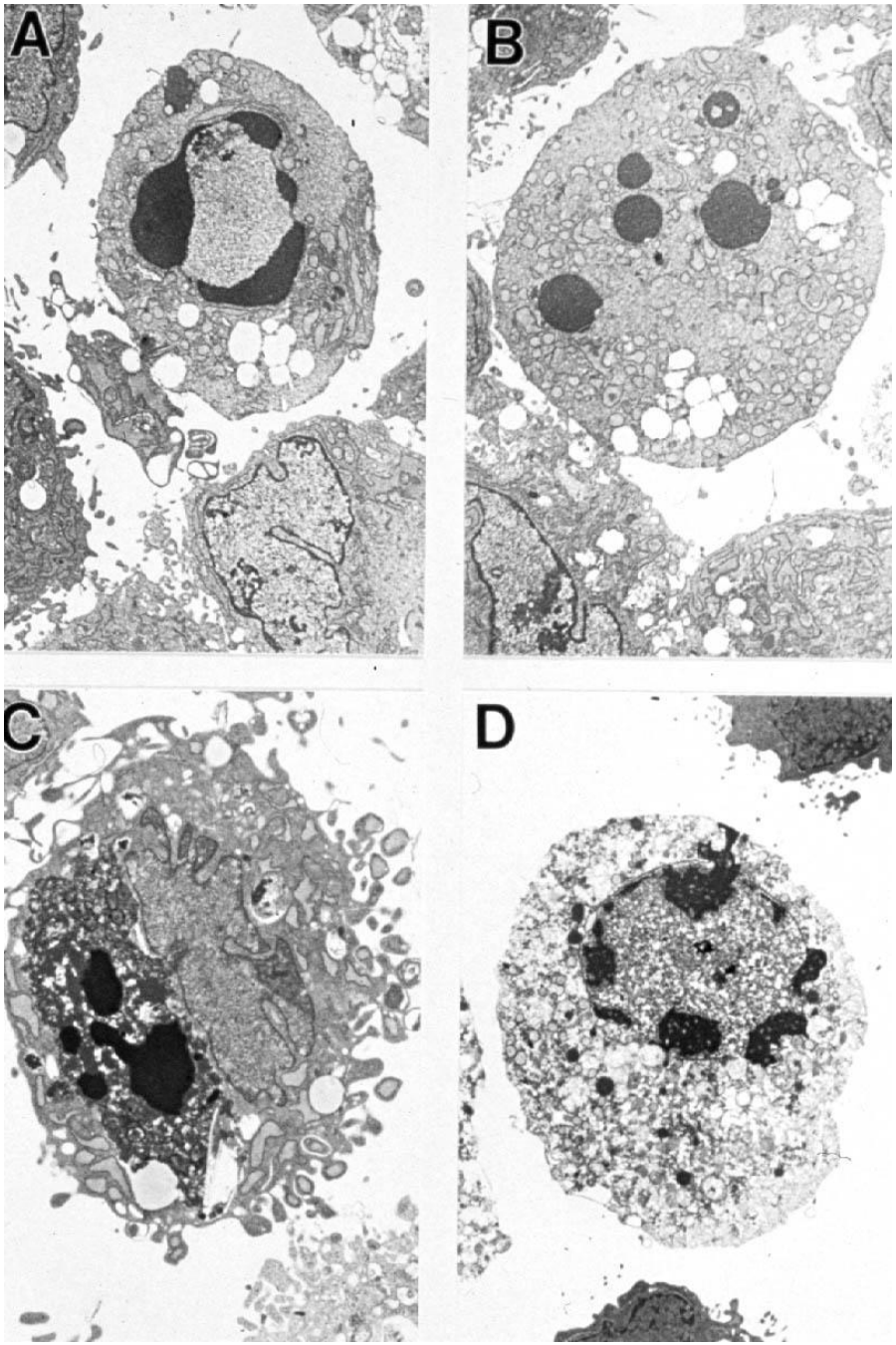



Regular margination and break up of DNA




(From Kerr JFR, Harmon BV: Definition and incidence of apoptosis: a historical perspective. In Tomei LD, Cope FO (eds): Apoptosis: The Molecular Basis of Cell Death. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, 1991, pp 5–29.)






A - early apoptosis; chromatin margination & condensation

B - later in apoptosis; nucleus is fragmented

C - phagocytosis of apoptotic cellular remnants by adjacent cell

D - swollen, necrotic cell for comparison

disruption and dissolution of cytoplasmic components

# Apoptosis

What do you think?

A. Caused by ischemia and inflammation.

B. Responsible for changing cell type in response to stress

C. Beneficial process to eliminate damaged cells

D. Reduces the size of an organ

E. Induced by retinoic acid

# Apoptosis

A. Caused by ischemia and inflammation. Necrosis

B. Responsible for changing cell type in response to stress Metaplasia

**C. Beneficial process to eliminate damaged cells**

D. Reduces the size of an organ Atrophy

E. Induced by retinoic acid Patterning during embryogenesis

# Mechanisms of Apoptosis

**Death receptor (Extrinsic) pathway**

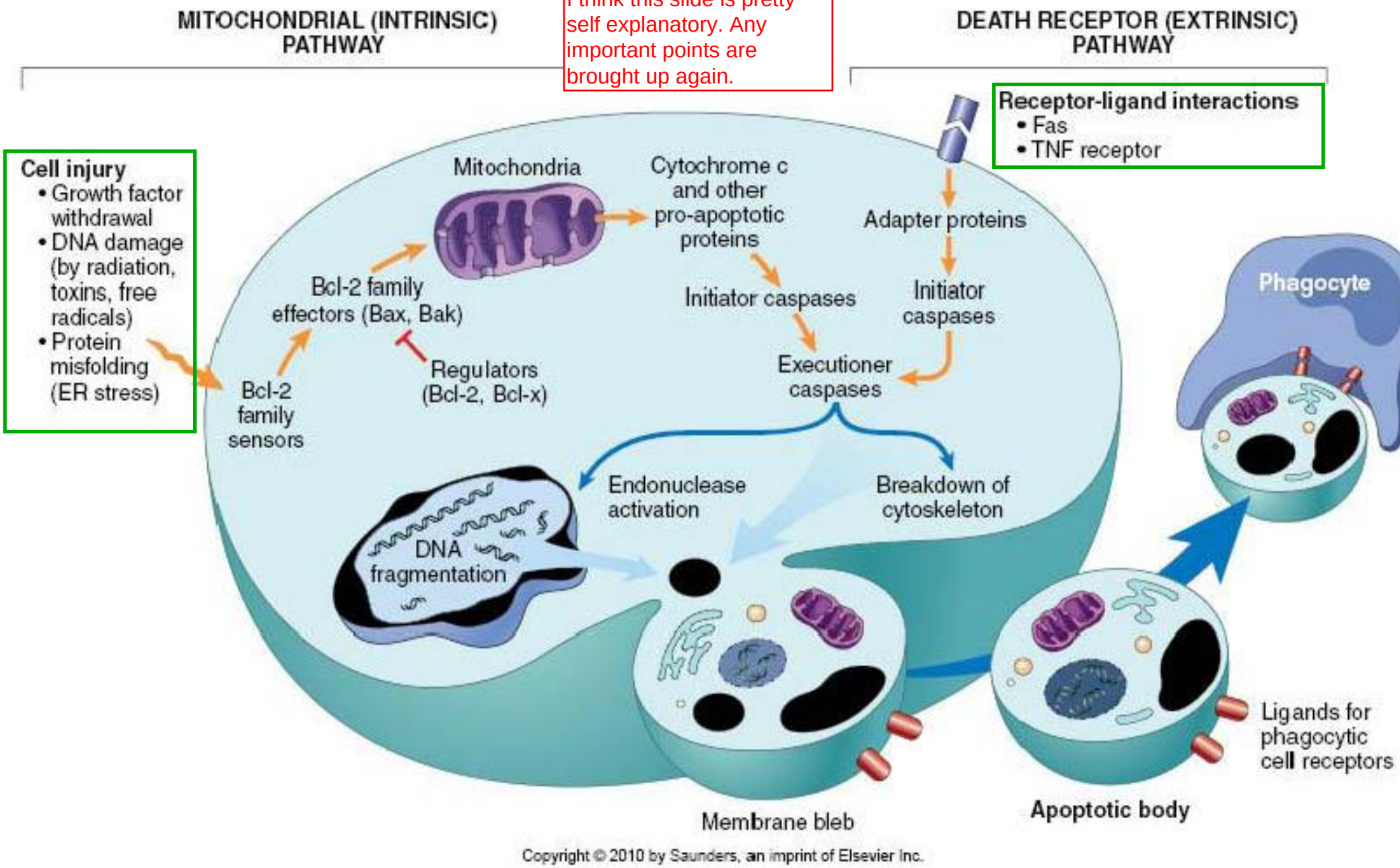
**Mitochrondrial (Intrinsic) pathway**

**Execution Phase**

**Removal of dead cells**

This slide indicates that there are 2 pathways (Intrinsic/Extrinsic) that lead to the execution phase and removal of cells.

I think this slide is pretty self explanatory. Any important points are brought up again.



# Mechanisms of Apoptosis

**Cells contain intrinsic death and survival signals that are genetically regulated.**

**Genes are highly conserved across species and are homologous to ced (cell death abnormal) genes in nematodes that initiate or inhibit apoptosis.**

For more detailed information, check out the Molecule and Cells Apoptosis lecture.

# Intrinsic -Mitochrondrial pathway

**Increased mitochondrial permeability with release of pro-apoptotic molecules into the cytoplasm (cytochrome c).**

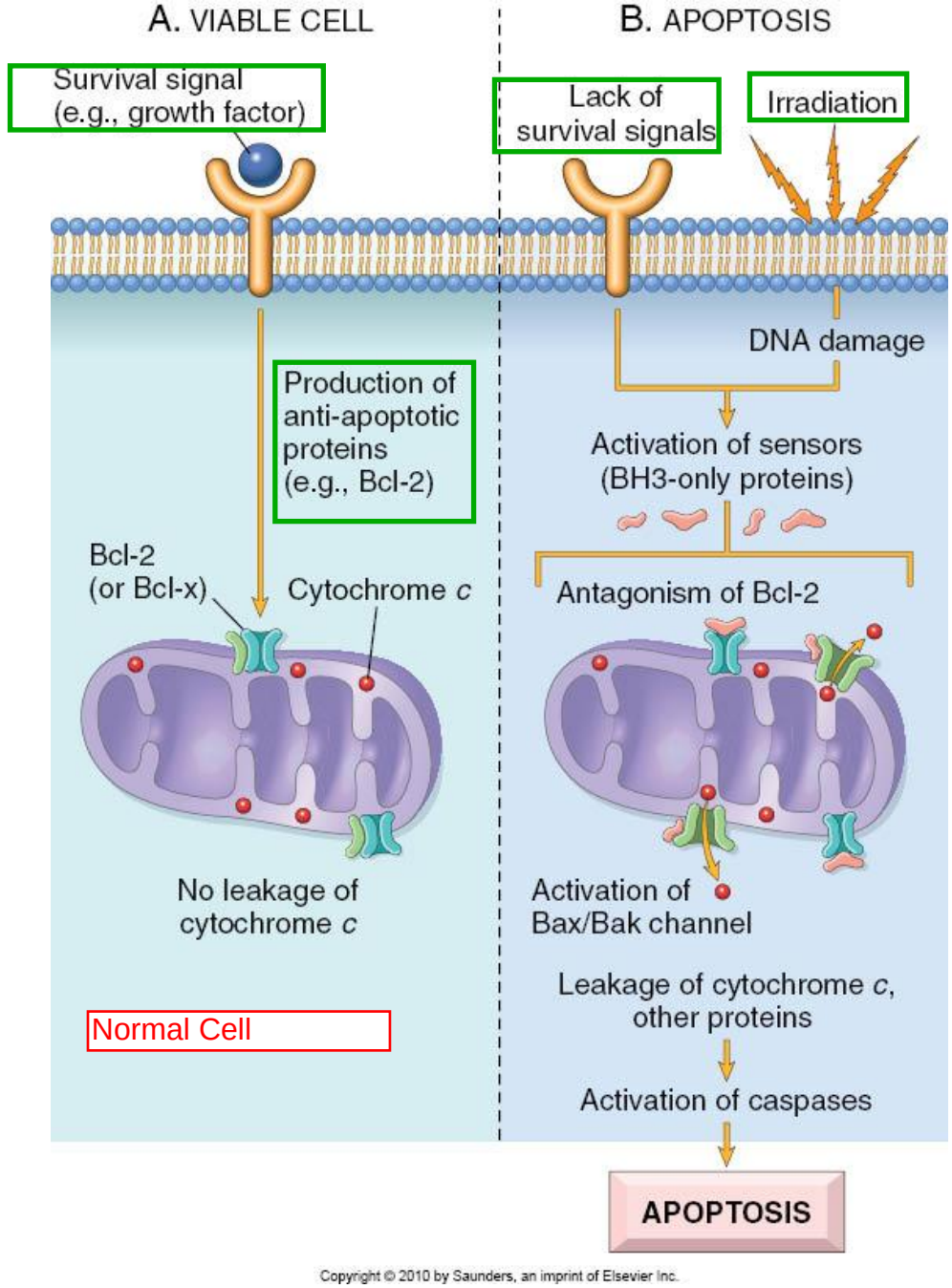
**Synthesis of anti-apoptotic molecules (Bcl-2) promoted by Growth factors.**

**When cells are deprived of growth factors or subjected to stress anti-apoptotic molecules (Bcl-2) are lost.**

***Bcl-2 is over expressed in most follicular B-cell lymphomas – allowing abnormal cells to proliferate.*** Clinical significance.

**Mitochondrial membrane becomes permeable and proteins that activate caspase leak out.**

# Intrinsic (Mitochondrial) Pathway of Apoptosis




Don't memorize details unless you plan on getting a PhD.

Hi MSTPs!!

# Essence of intrinsic (mitochondrial) pathway

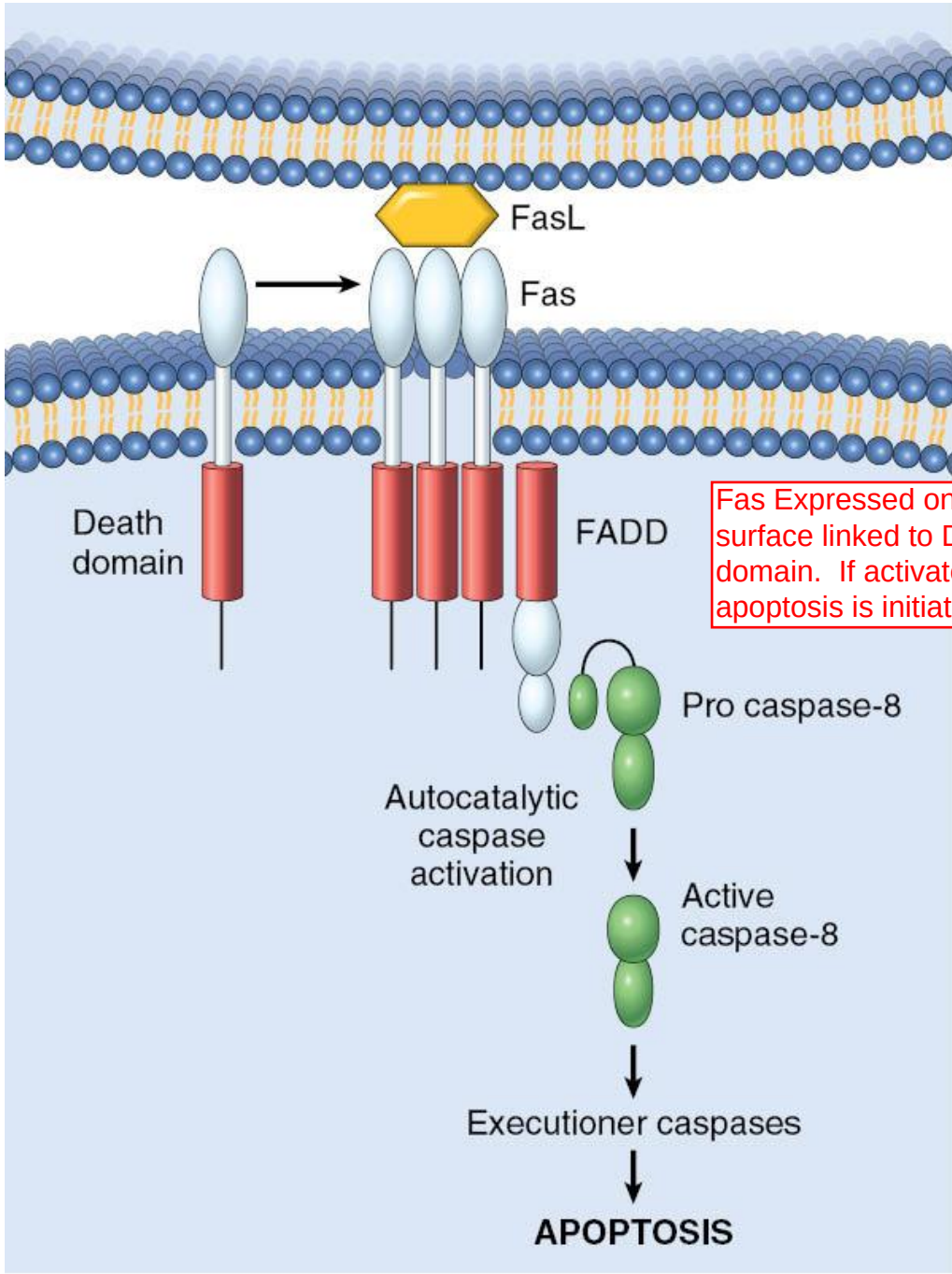
**Pro-apoptotic and protective molecules that regulate mitochondrial permeability and the release of death molecules sequestered in the mitochrondria are maintained in balance normally.**

**Imbalance initiates the death pathway.**

# Extrinsic (Death receptor initiated) pathway

Death receptors are members of the tumor necrosis factor receptor family and a related protein called Fas (CD95).

These molecules contain a death domain.






# Extrinsic (Death Receptor-initiated) Pathway of Apoptosis

# Execution Phase

**The intrinsic and extrinsic pathways converge to a caspase activation cascade.**

**Caspases (*cysteine-aspartic-acid-proteases*) are conserved across species.**

**Synthesized as inactive precursors; activated by proteolytic cleavage.**

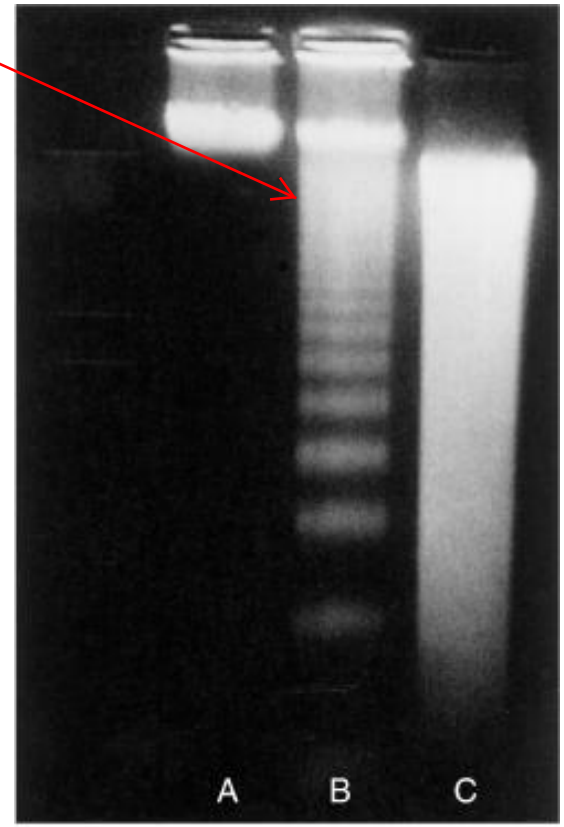
**Family of at least 12 proteases, a few of which are involved in inflammation, and many of which are involved in apoptosis**

# How Caspases Disassemble a Cell

**Cleave structural proteins leading to nuclear breakdown.**

**Converts cytoplasmic DNase to active form.**

**DNase causes characteristic internucleosomal cleavage of DNA.**



# Removal of Dead Cells

**Dying cells secrete factors the recruit phagocytes.**

**This facilitates prompt clearance before they undergo secondary necrosis.**

**Dead cells disappear without a trace and do not produce inflammation.**

# Which of the following features is seen in apoptosis but not in necrosis?

Thoughts?

A. Internucleosome cleavage of DNA

B. Inflammation

C. Pyknosis

D. Cytoplasmic hypereosinoplilia

E. Karyolysis

# Which of the following features is seen in apoptosis but not in necrosis?

**A. Internucleosome cleavage of DNA**

B. Inflammation

C. Pyknosis

D. Cytoplasmic hypereosinoplilia

E. Karyolysis

Features of Necrosis

Reflection of cell injury and not death.

# Examples of Apoptosis

## Lack of growth factor or hormone

**Hormone sensitive cells deprived of hormone.**

**Lymphocytes that are not stimulated.**

**Neurons deprived of growth factor.**

Remember Brain and Behavior?

# Examples of Apoptosis

## Specific activation of death receptors

**DNA damage - Tumor suppressor gene p53 accumulates in damaged cells and arrests the cell cycle. p53 is mutated or absent in some cancers and can not initiate apoptosis in malignant cells.**

**Protein misfolding – unfolded protein response and ER stress – Alzheimer, Parkinson and Huntington diseases.**

**TNF Receptor family.**

**Cytotoxic T lymphocyte**

# Apoptosis in Pathologic Conditions

- Ionizing radiation
- Cytotoxic chemotherapeutic drugs
- Mild thermal injury
- Cell injury in some viral diseases
- Pathologic atrophy after duct obstruction
- Cell death in tumors
- Glucocorticoids induce apoptosis in lymphocytes

You've got this..

# Apotosis Summary

**“Programmed cell death” can be activated by moderate stress which has damaged the cell beyond its ability to recover fully or by viral infection.**

**This has the desirable effect of removing damaged or infected cells.**

**Selective manipulation of apoptotic pathways may be an important approach for treating cancer in the future.**

**We recognize that cell death has occurred by morphologic manifestations which are often influenced by the environment.**

**Is the distinction between necrosis and apoptosis absolute?** NO!!!

# Cellular Aging

We're done with Apoptosis now. Here are the main points to focus on for cellular aging:

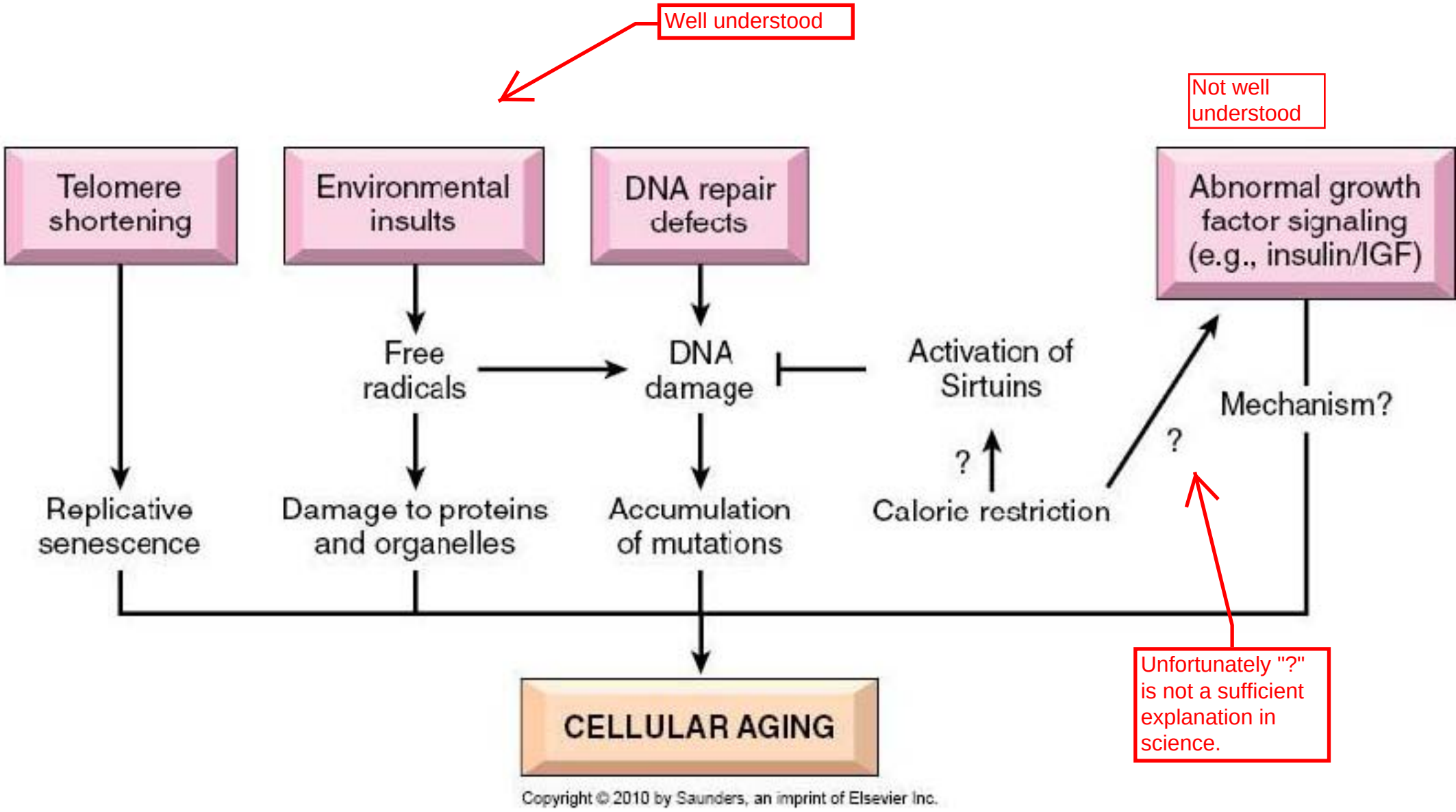
**Structural and Biochemical Changes with Aging**

**Decreased cellular replication**

**Telomere shortening causes cell cycle arrest**

**Accumulation of Metabolic and Genetic Damage**

**Calorie restriction delays aging**


Well understood
Not well understood
Telomere shortening
Environmental insults
DNA repair defects
Abnormal growth factor signaling (e.g., insulin/IGF)
Free radicals
DNA damage
Activation of Sirtuins
Mechanism?
?
?
Replicative senescence
Damage to proteins and organelles
Accumulation of mutations
Calorie restriction
CELLULAR AGING
Unfortunately "?" is not a sufficient explanation in science.
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# Structural and Biochemical Changes with Aging

Oxidative phosphorylation is reduced

Synthesis of nucleic acids, structural proteins, enzymes, cell receptors and transcription factors are reduced

Decreased capacity for nutrient uptake and repair of DNA damage

Cytologic changes

Accumulation of abnormally folded proteins

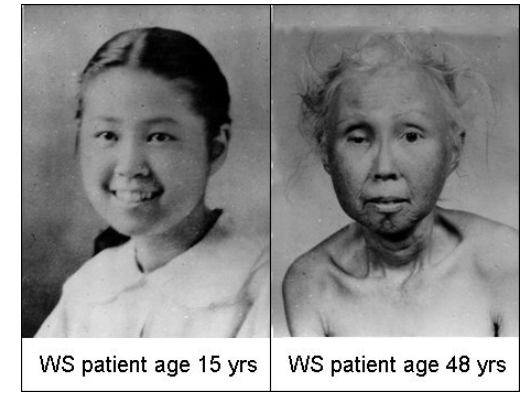
# Replicative Senescence

**Cells have a limited capacity for replication.**

**Cultured human fibroblasts have limited division potential.**

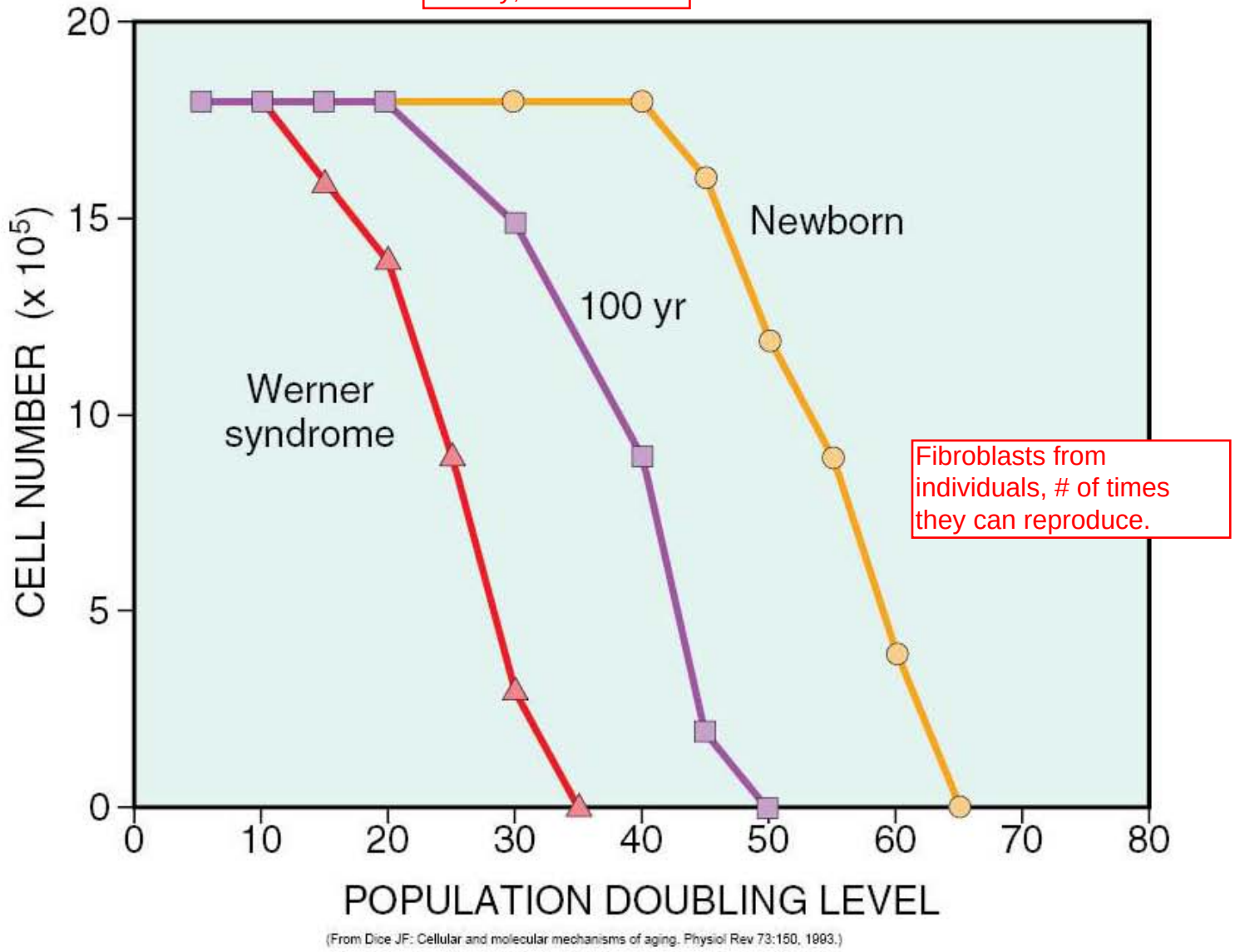
**Werner's syndrome is a rare disease characterized by premature senescence.**

Werner's syndome patients often die as early as age 20, but may look 100.



WS patient age 15 yrs | WS patient age 48 yrs

Oh hey, Robbins.




(From Dice JF: Cellular and molecular mechanisms of aging. Physiol Rev 73:150, 1993.)

Fibroblasts from individuals, # of times they can reproduce.

# Replicative Senescence

With each cell division there is incomplete replication of telomeres.

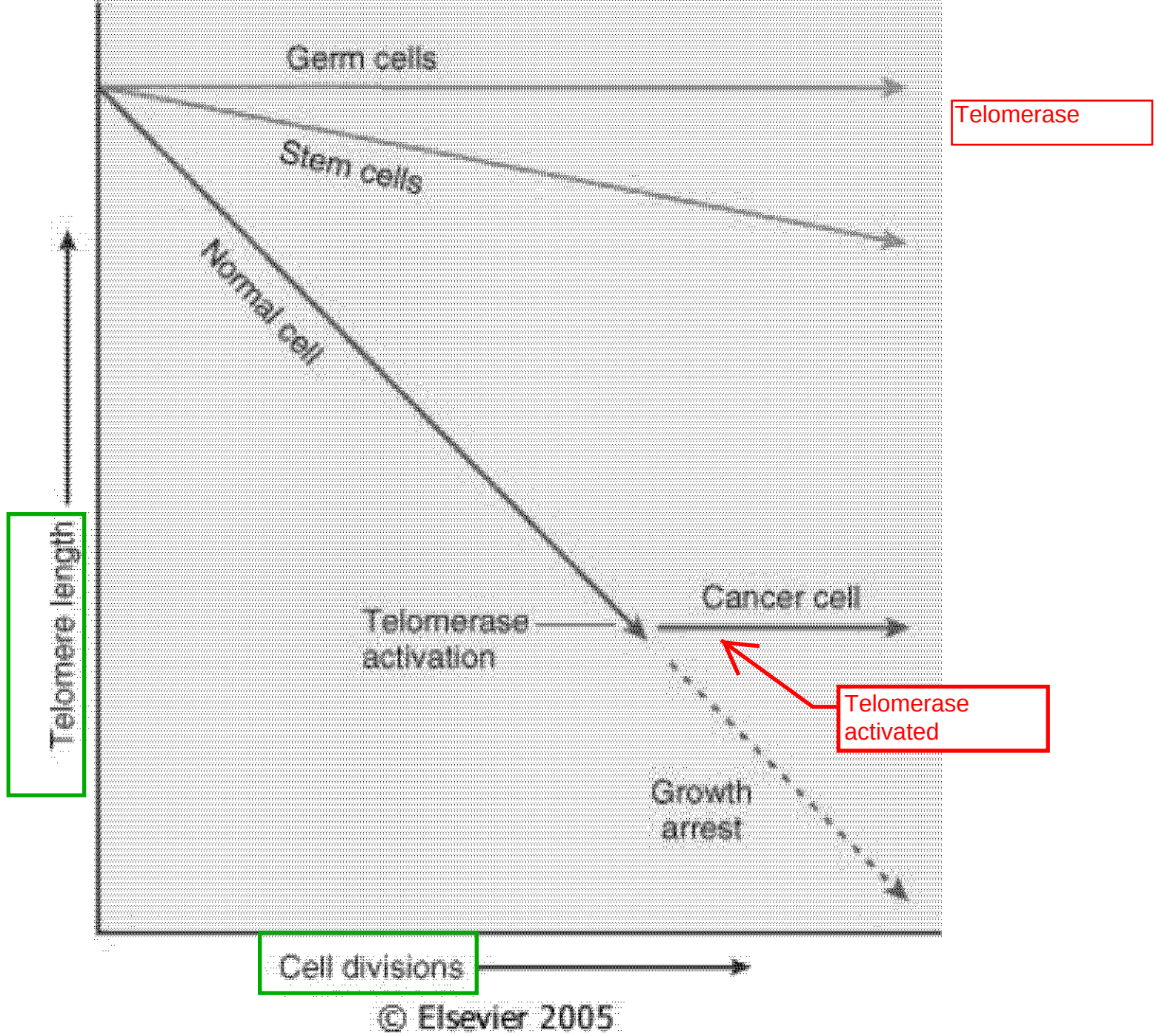
Broken telomeres signal cell cycle arrest.

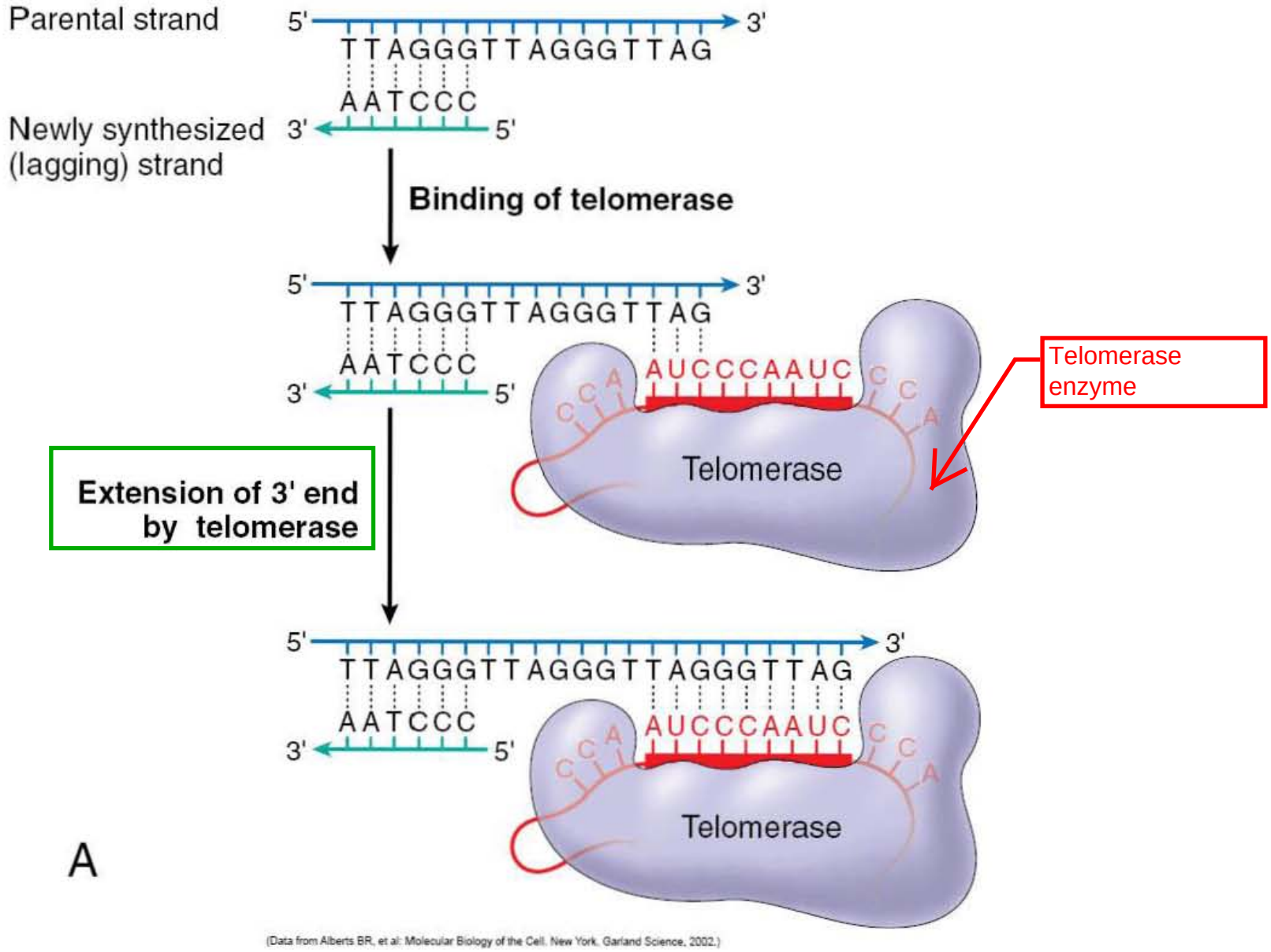
As cells age, the telomere becomes shorter.

Telomerase normally adds nucleotides.

Telomerase is active in germ cells and stem cells but absent in somatic tissue.

Telomerase may be reactivated in cancers


Germ cells
Stem cells
Normal cell
Telomere length
Telomerase activation
Cancer cell
Growth arrest
Cell divisions
© Elsevier 2005
Telomerase
Telomerase activated




(Data from Alberts BR, et al: Molecular Biology of the Cell. New York. Garland Science, 2002.)

# Genes that influence Aging

**Insulin growth factor receptor.**

Active area of research.

**Decreased signaling of IGF -1 receptor is the result of decreased caloric intake or mutations and results in longer lifespan in *C. elegans.***

**Evidence suggests that aging is genetically determined.**

Some families have more individuals that seem to live longer.

# Accumulation of Metabolic and Genetic Damage

Occurs with aging.

**Reactive oxygen species** (lipofuscin).

In older person, more likely to have lipofuscin in cytoplasm

**Over expression of SOD extends life span in *Drosophila.***

**Werner's syndrome – defective helicase.**

**Ataxia telangiectasia – ineffective repair of dsDNA breaks.**

**Damaged organelles accumulate.**

**Which of the following cells has the highest telomerase activity?**

A. Endothelial cells
B. Germ cells
C. Neurons
D. Neutrophils
E. Erythrocytes

# Which of the following cells has the highest telomerase activity?

A. Endothelial cells

**B. Germ cells**

C. Neurons Don't replicate, remember?

D. Neutrophils

E. Erythrocytes Don't even have chromatin!

# Summary

Don't memorize molecular mechanisms.

**Necrosis and Apoptosis**

**Molecular mechanisms**

**Role in health and disease**

**Cellular aging**