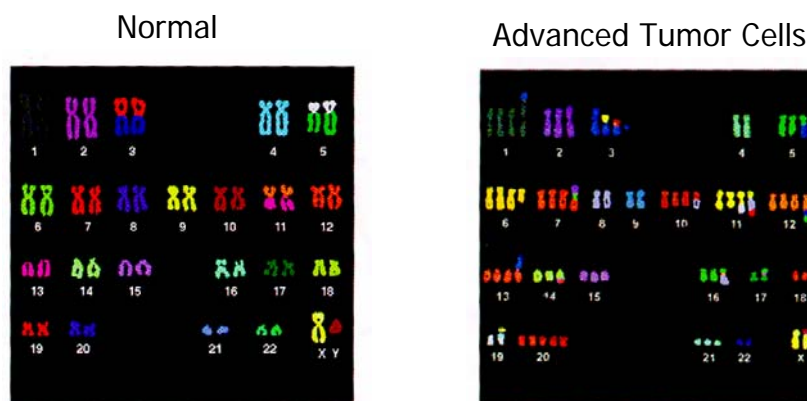
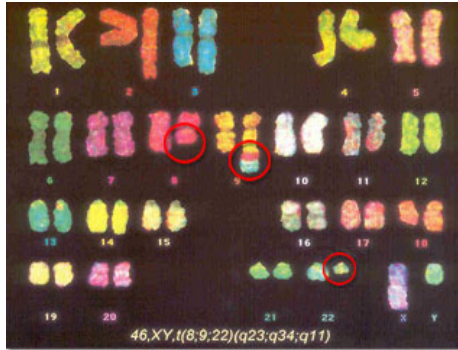


Detection of DNA Damage: DNA repair and mutation

Karyotypic Abnormalities Accumulate During Carcinogenic Progression



Philadelphia Chromosome

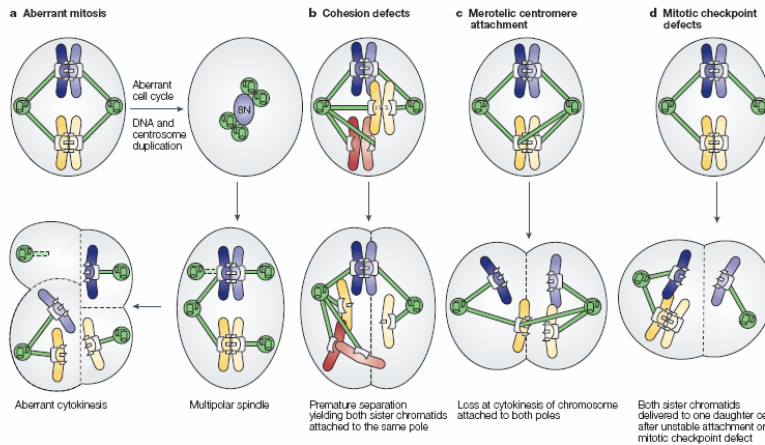


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Note: This karyotype was prepared using a FISH technique known as "chromosome painting". As well as having a translocation from chromosome 22, chromosome 9 also has translocated material from chromosome 8.

- Associated with chronic myelogenous leukemia (90%!!)
- Reciprocal exchange between chromosome 9 and 22.
- Named "Philadelphia" chromosome because that is where it was first identified.

Aneuploidy

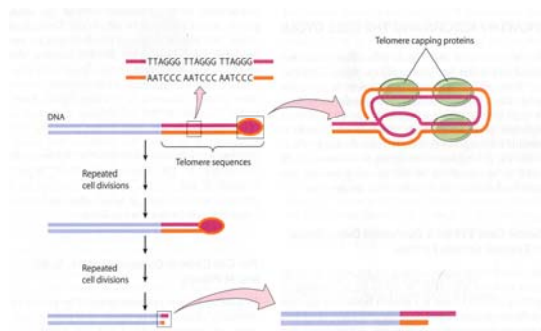


Kops et al.(2005) Nature Reviews (Cancer) 5:773-785.

What are telomeres?

- Telomeres are specialized nucleoprotein complexes at the ends of linear chromosomes.
- Consist of repeats of TTAGGG, a G-rich 3' overhang, and associated proteins.
- Telomere structure prevents the DNA ends from being recognized as "damaged" and targeted for repair.

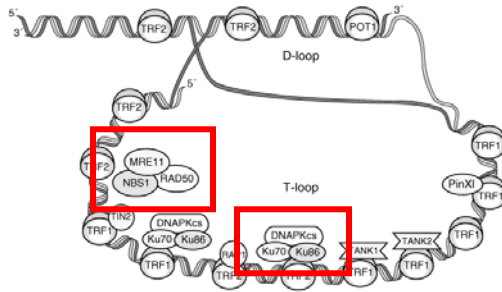
Telomeres are involved in immortalization



- Telomeres are found at the end of the chromosomes. Consists of multiple copies of a short sequence repeated. Are protected by telomere capping proteins, and by looping of the DNA.

Telomeres get shorter and shorter with each cell division!!

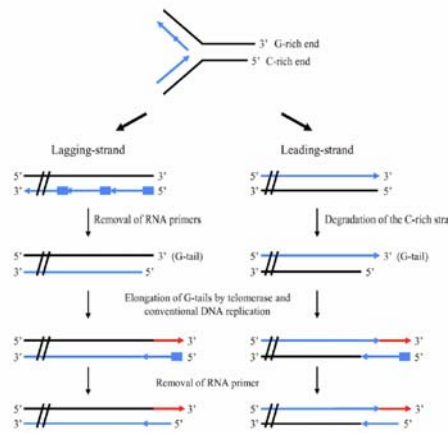
Human Telomere Structure



Telomeric repeats are bound by two proteins: TRF1 and TRF2.

In addition to interactions with proteins involved in telomere maintenance, proteins involved in DNA damage response and repair are also found associated with the telomere complex.

Telomere Replication



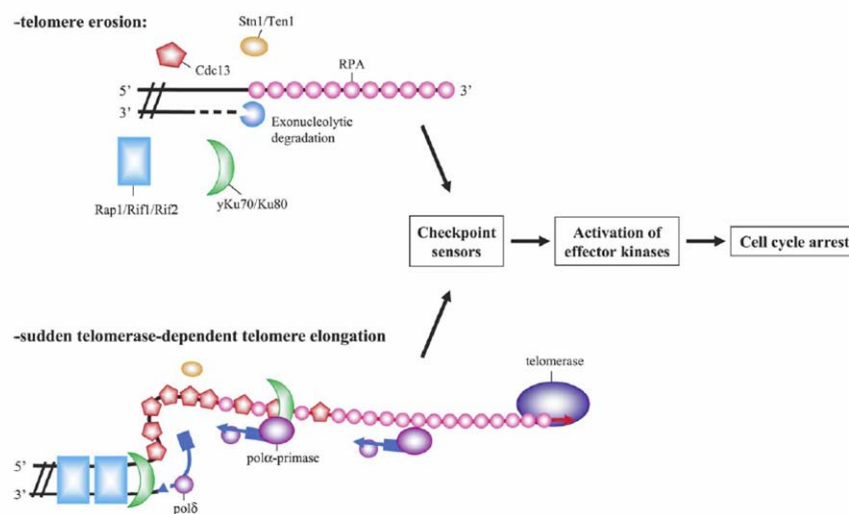
- DNA polymerases during S-phase cannot completely replicate the entire telomere
- This results in shortening of the telomeres during each cell cycle
- For the maintenance of telomere length there is a specialized ribonucleoprotein complex consisting of
 - a telomerase RNA (hTERC) that serves as template
 - a telomerase catalytic subunit (hTERT) (reverse transcriptase)

!! In normal human cells, telomerase levels are insufficient!!
= loss of telomere length

Viscardi et al., (2005) Biochimie 87:613-624

Loss of telomere length is associated with chromosomal damage

- Telomeres can become so short that they can no longer “loop” or be capped.
- Unprotected chromosomal DNA ends are unstable and may fuse.
- Fused chromosomes can become fragmented, and will not separate properly during cell division.



Viscardi et al. (2005) Biochimie 87:613-624

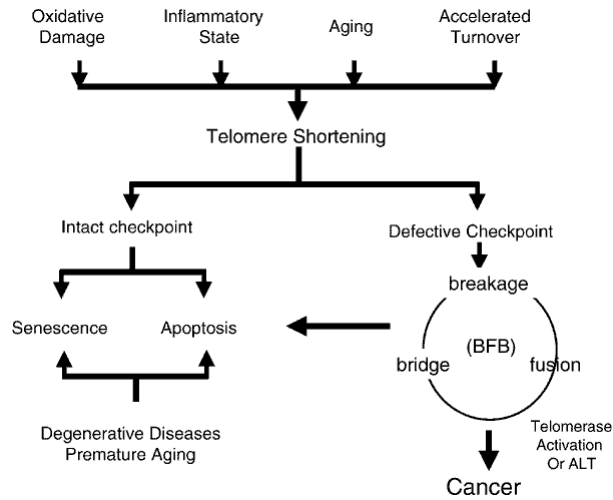
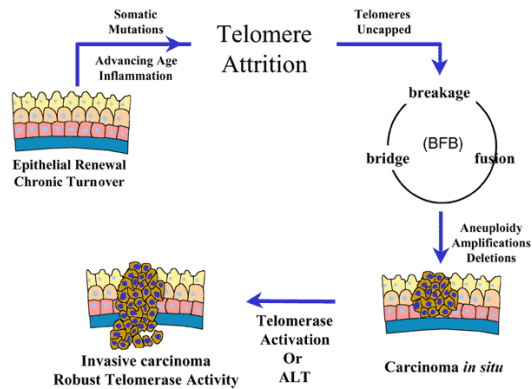
Telomeres are implicated in the episodic instability of DNA in cancer progression

• Robust telomerase activity is observed in over 80% of all human cancers.

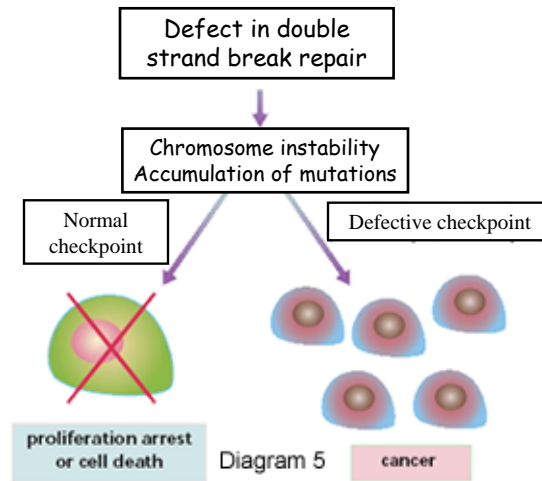
• However, despite this, telomere lengths are short.

• WHY???

• In early stages of neoplastic transformation, there is attrition of the telomere leading to....



DNA damage, repair and cell cycle checkpoints



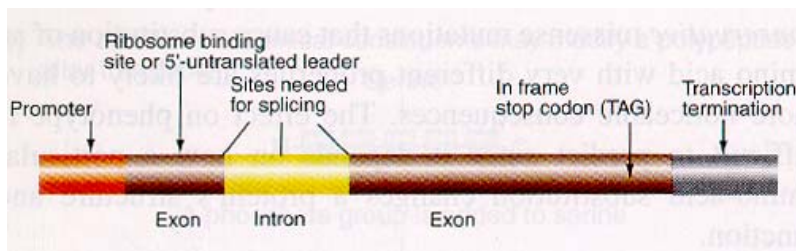
Types of Mutations

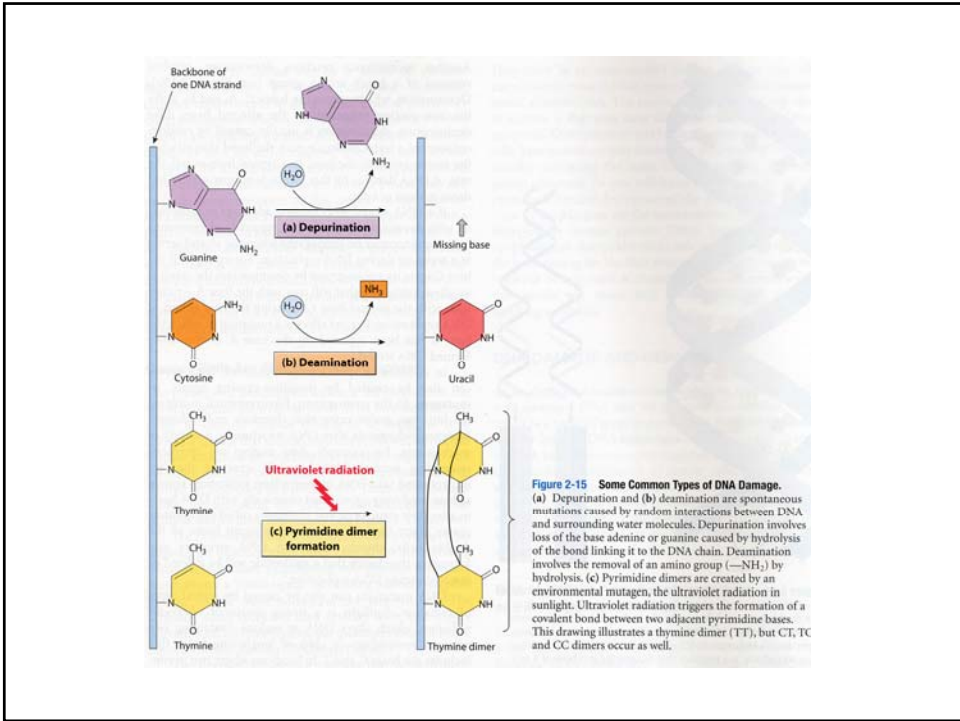
- Point mutations
 - Transitions (purine-purine; pyrimidine-pyrimidine)
 - Transversions (purine-pyrimidine)
- Deletions/insertions
 - Result from intercalating agents and thymidine dimers resulting from UV exposure.
- Chromosomal translocations
 - Double strand breaks (ionizing radiation)

Results of Point Mutations

Wildtype mRNA	5'	GCU	GGA	GCA	CCA	GGA	CAA	GAU	GGA	3'
Wildtype polypeptide	N	Ala	Gly	Ala	Pro	Gly	Gln	Asp	Gly	C
Silent mutation		GCU	GGA	GCC	CCA	GGA	CAA	GAU	GGA	
		Ala	Gly	Ala	Pro	Gly	Gln	Asp	Gly	
Missense mutation		GCU	GGA	GCA	CCA	AGA	CAA	GAU	GGA	
		Ala	Gly	Ala	Pro	Arg	Gln	Asp	Gly	
Nonsense mutation		GCU	GGA	GCA	CCA	GGA	UAA	GAU	GGA	
		Ala	Gly	Ala	Pro	Gly	Stop			
Frameshift mutation		GCU	GGA	GCC	ACC	AGG	ACA	AGA	UGG	A
		Ala	Gly	Ala	Thr	Arg	Thr	Arg	Trp	

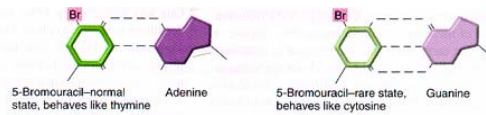
Mutations in coding and non-coding sequences can affect expression and activity





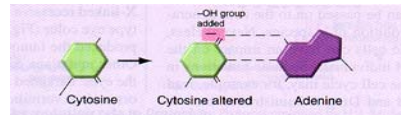
How mutagens alter DNA sequence

1. Replace a base



2. Alter base structure/properties

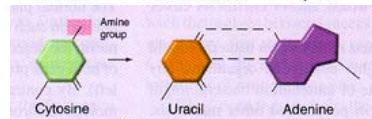
a. Add a hydroxyl group



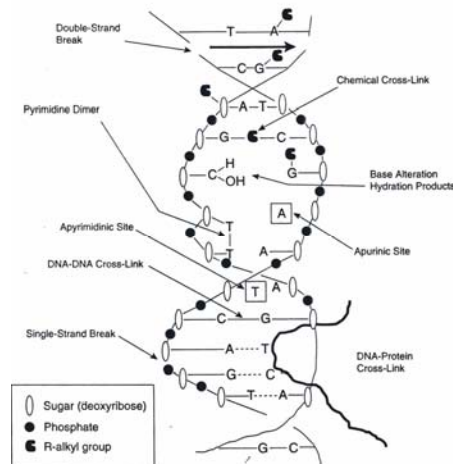
b. Add ethyl/methyl groups



c. Remove amine group



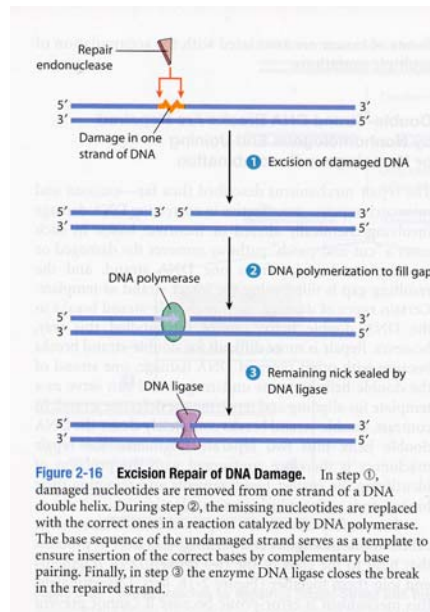
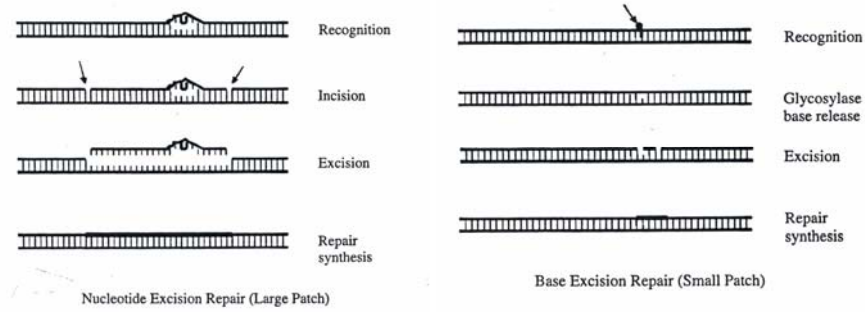
Potential Outcomes of Radiation and Chemical Exposure



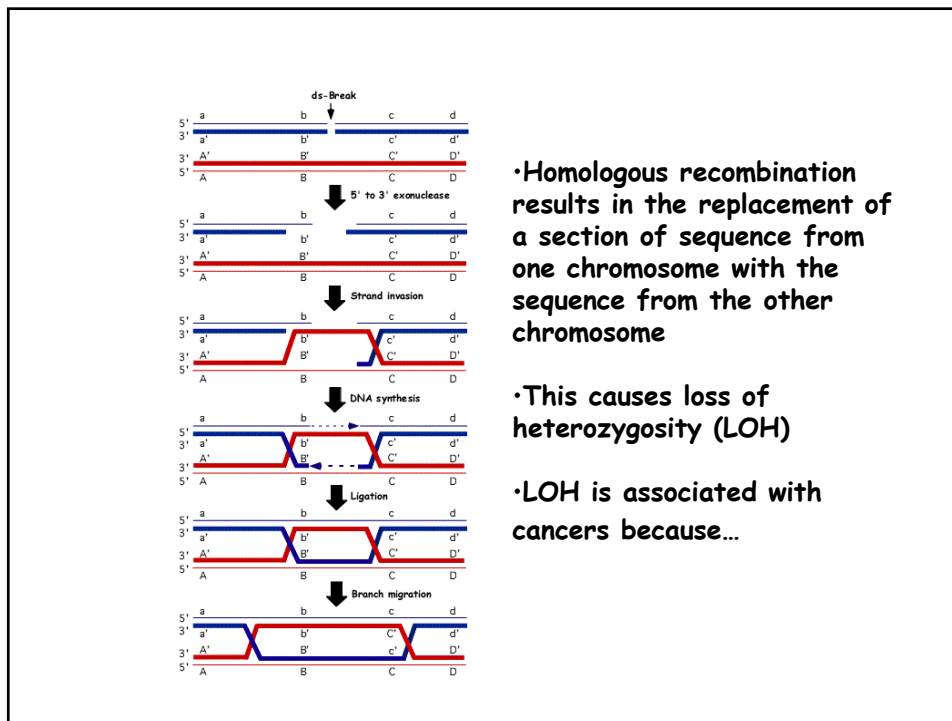
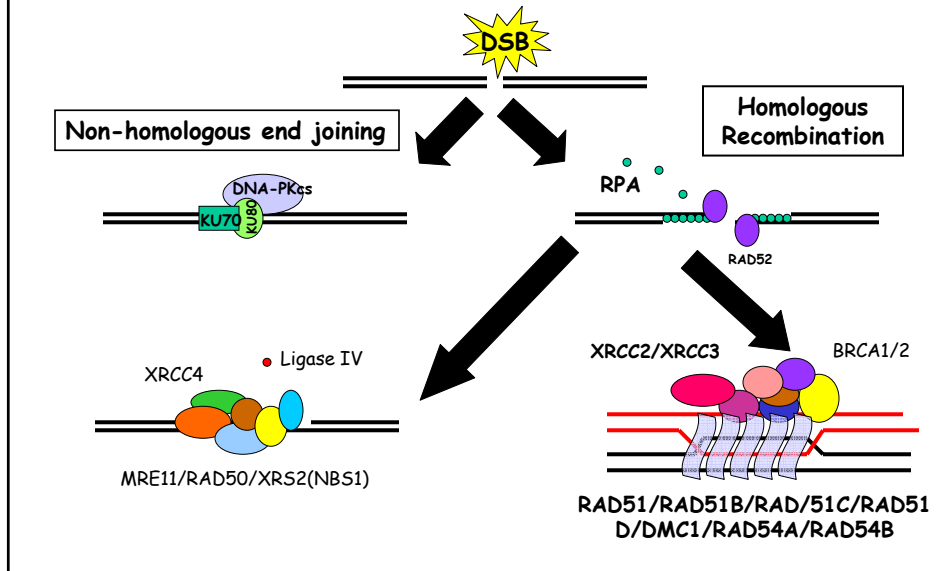
DNA Repair

- Several mechanisms of DNA repair.
 - Excision repair, mismatch repair, double strand break repair, post replication repair
- Repair process is not 100% effective.
- Some lesions (ie. Double strand breaks) are difficult to repair.
- Errors (=mutations) result from ineffective repair!
- Too much DNA damage will lead to **apoptosis**.

Repair can result in mutation (or how the road to hell is paved...)



Repair of Double Strand Breaks



Apoptosis and Cancer

How cellular and/or DNA
damage alters cell fate

Cellular Reactions to Adverse Stimuli

- Adaptation
- Reversible Injury
- Irreversible Injury/Death

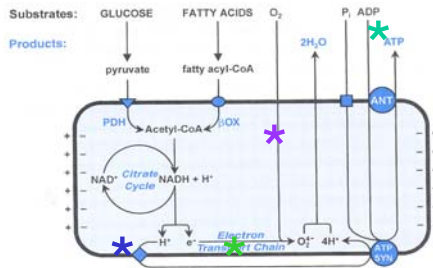
Cellular Adaptation: New/altered state that preserves the viability of the cell

- HYPERTROPHY= increase in cell mass
- ATROPHY = decrease in cell mass
- HYPERPLASIA = increased number of cells
- METAPLASIA = transformation of one cell type for another

Biochemical Pathways Important for Mediating Cell Injury/Death

- Oxygen-Derived Free Radicals
- ATP depletion
- Alterations in Ca^{2+} homeostasis
- Defects in membrane permeability

Mitochondrial ATP Synthesis



Inhibit hydrogen delivery:
arsenate, ethanol

Inhibit electron transport:
cyanide, dinitroaniline and diphenylether herbicides

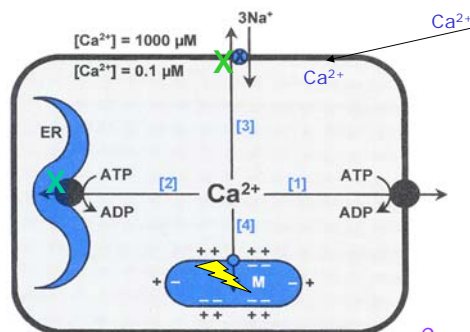
Inhibit oxygen delivery:
CNS depressants, cocaine

Inhibit ADP phosphorylation:
valinomycin, DDT

Mechanisms for Maintaining Ca²⁺ Concentration in the Cytoplasm

Inhibit Ca²⁺ export:
acetaminophen, chloroform

Agents that cause sustained Ca²⁺ elevation:

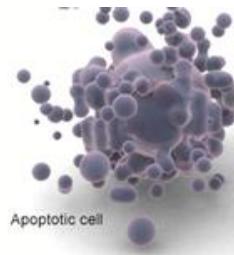
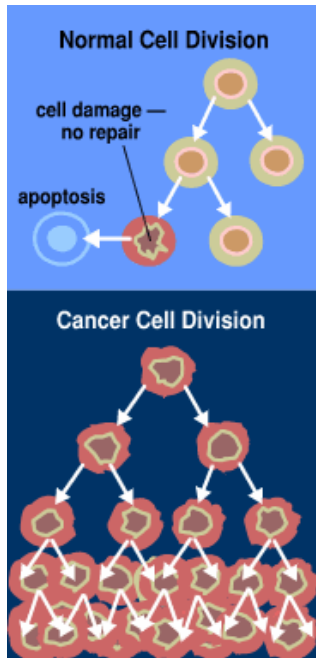
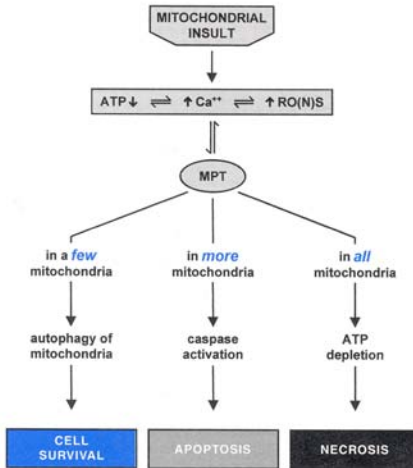


Induce Ca²⁺ influx into the cytoplasm: **methymercury, phospholipases in snake venom**

Inhibit ATP synthesis

Cause hydrolysis of NAD(P)⁺ in mitochondria:
alloxin, NO

Mitochondrial Damage and Cellular Fate



TWO MORPHOLOGICAL PATTERNS OF CELL DEATH

NECROSIS

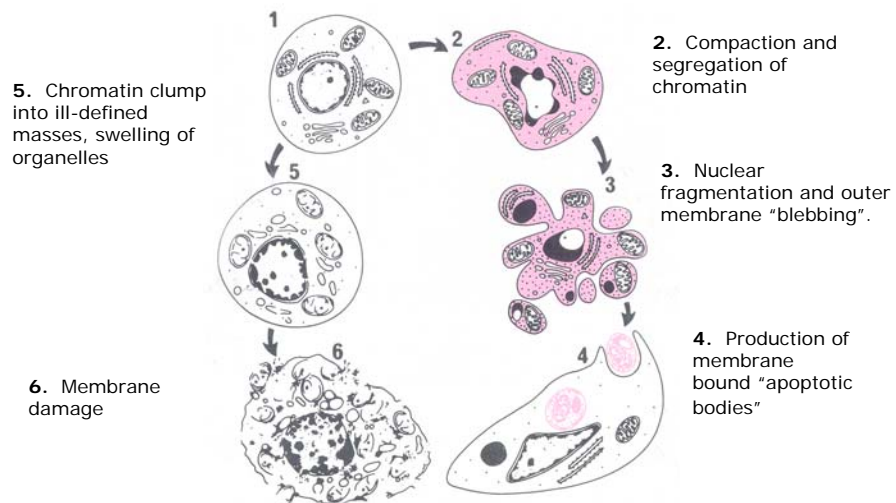
- ❖ Groups of cells
- ❖ Denaturation/
❖ coagulation of proteins
- ❖ Breakdown of cellular membranes
- ❖ Cell rupture

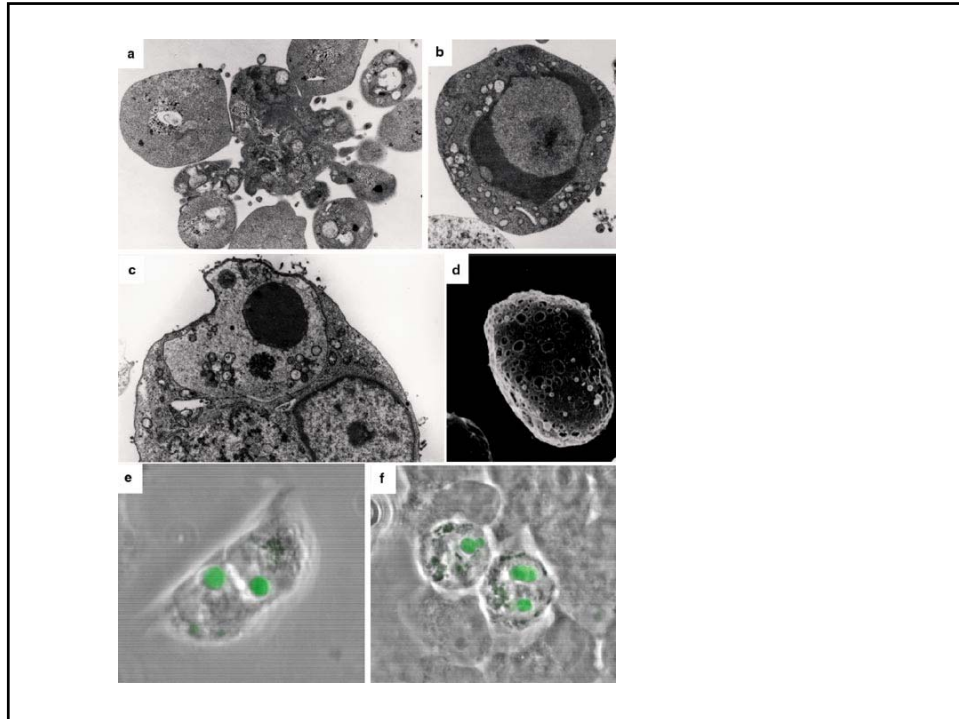
APOPTOSIS

- ❖ Single/small clusters of cells
- ❖ Chromatin condensation
- ❖ Fragmentation
- ❖ Elimination of unwanted cells

Necrosis

Apoptosis

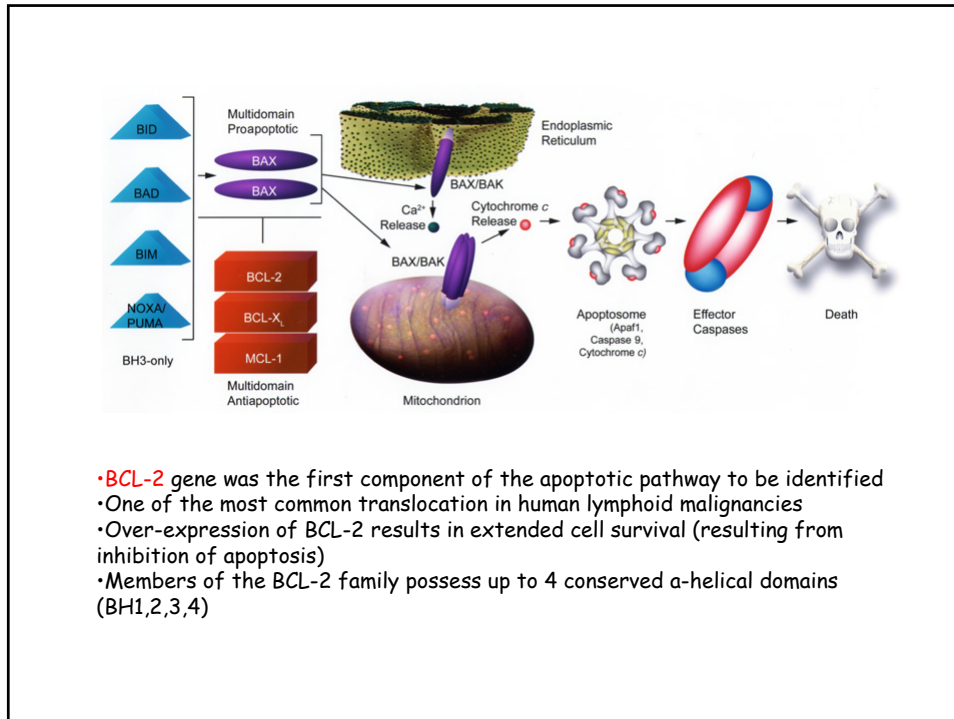




DNA Fragmentation During Cell Death



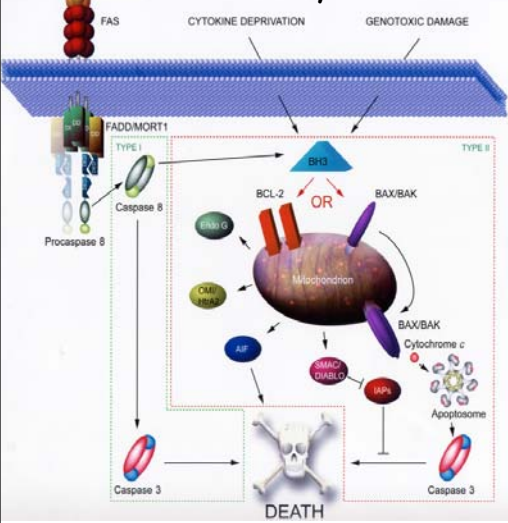
- A= DNA from undamaged cell
- B= DNA from cell undergoing apoptosis (DNA laddering)
- C= DNA from cell undergoing necrotic death



What are caspases?

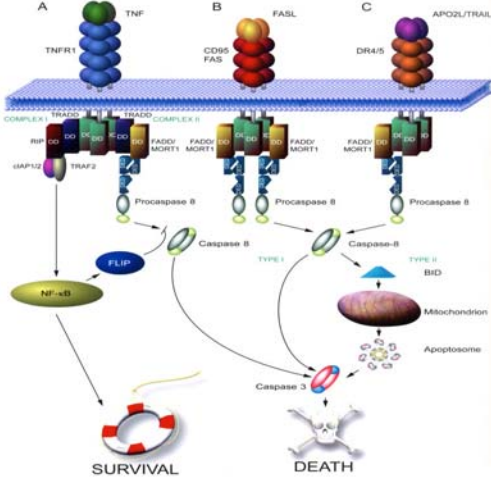
- Family of proteases whose functions range from inflammation to apoptosis.
- In apoptosis, caspases mediated the coordinated proteolysis of critical cell structures.
- caspases are initiators (ie. caspases 8 & 10) or effectors (ie. caspases 3 & 7).

BCL family members link mitochondria dysfunction to apoptosis



Whether a cell lives or dies is determined by the balance between the pro- and anti-apoptotic members of the BCL family

Death Receptors initiate the apoptotic process

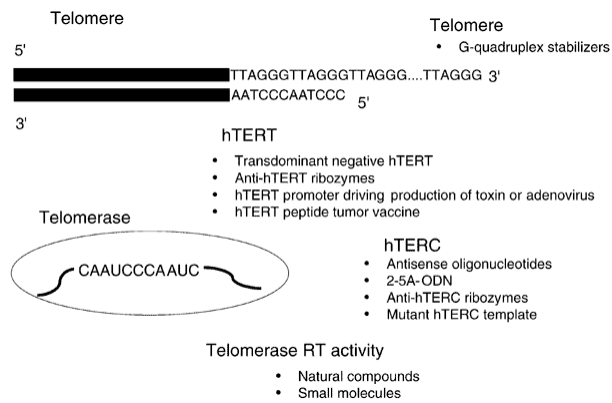


How will the horror cease????

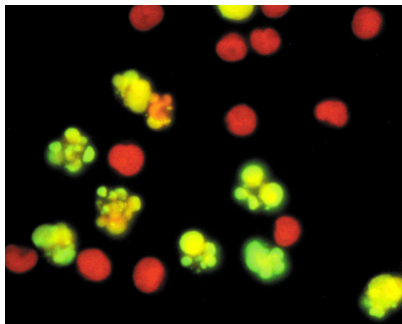
- Inhibitors of Apoptosis (IAPs) are a family of proteins containing a zinc finger binding region.
- Members of this family include
 - XIAP, cIAP-1 and cIAP-2 which bind to and inhibit caspases
 - Survivin, which functions in the control of cytokinesis.

Options for cancer therapy

Telomeres are potential targets of cancer therapy



Some chemotherapeutic agents induced apoptosis



- Lymphoma cells in culture exposed to chemo agent induces apoptosis.

Apoptosis Signaling

