Mutagenesis

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Mutagenesis Overview

1. DNA as the Target

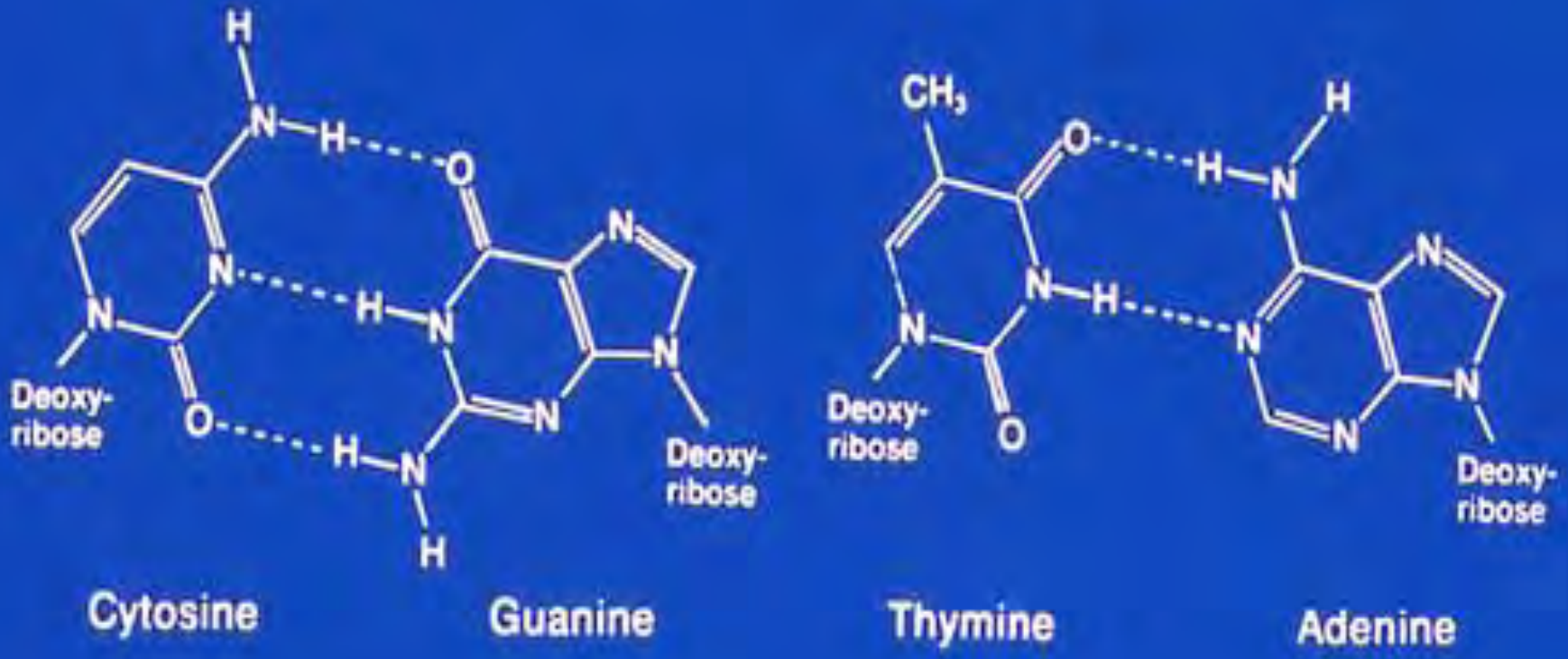
2. DNA Damage (The Mutagenesis Paradigm)
    A. Types of Damage
    B. DNA Damage Assays (How You Detect Damage)
    C. DNA Damage Response (How Cell Detects)

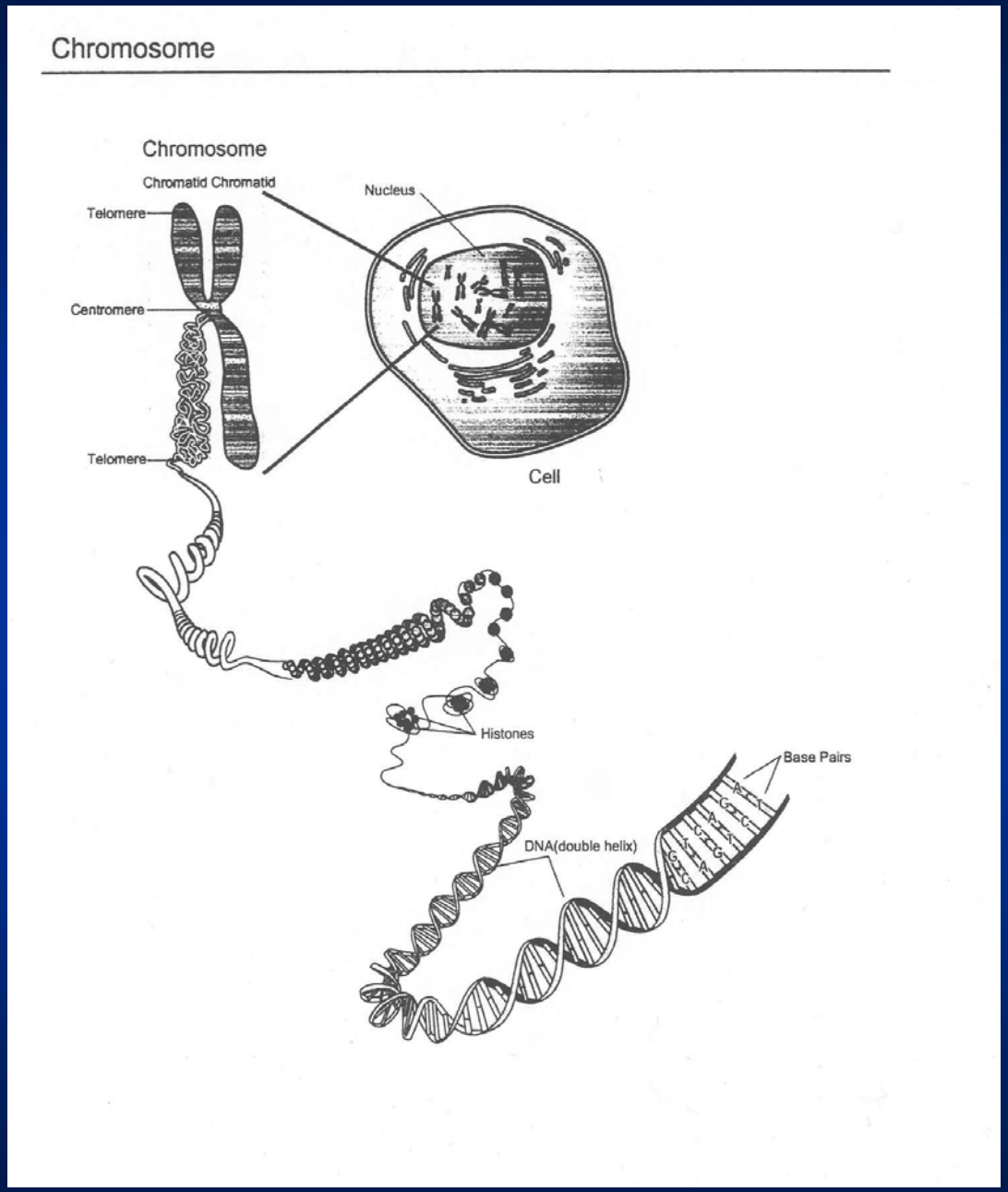
3. DNA Repair (Types and Mechanisms)

4. Mutations
    A. Types, Mechanisms, Consequences
    B. Types of Mutagens

5. Mutagenesis and Carcinogenesis

The Bases and the Phosphodiester Backbone Are the Main Sites at Which Damage Results in Mutation

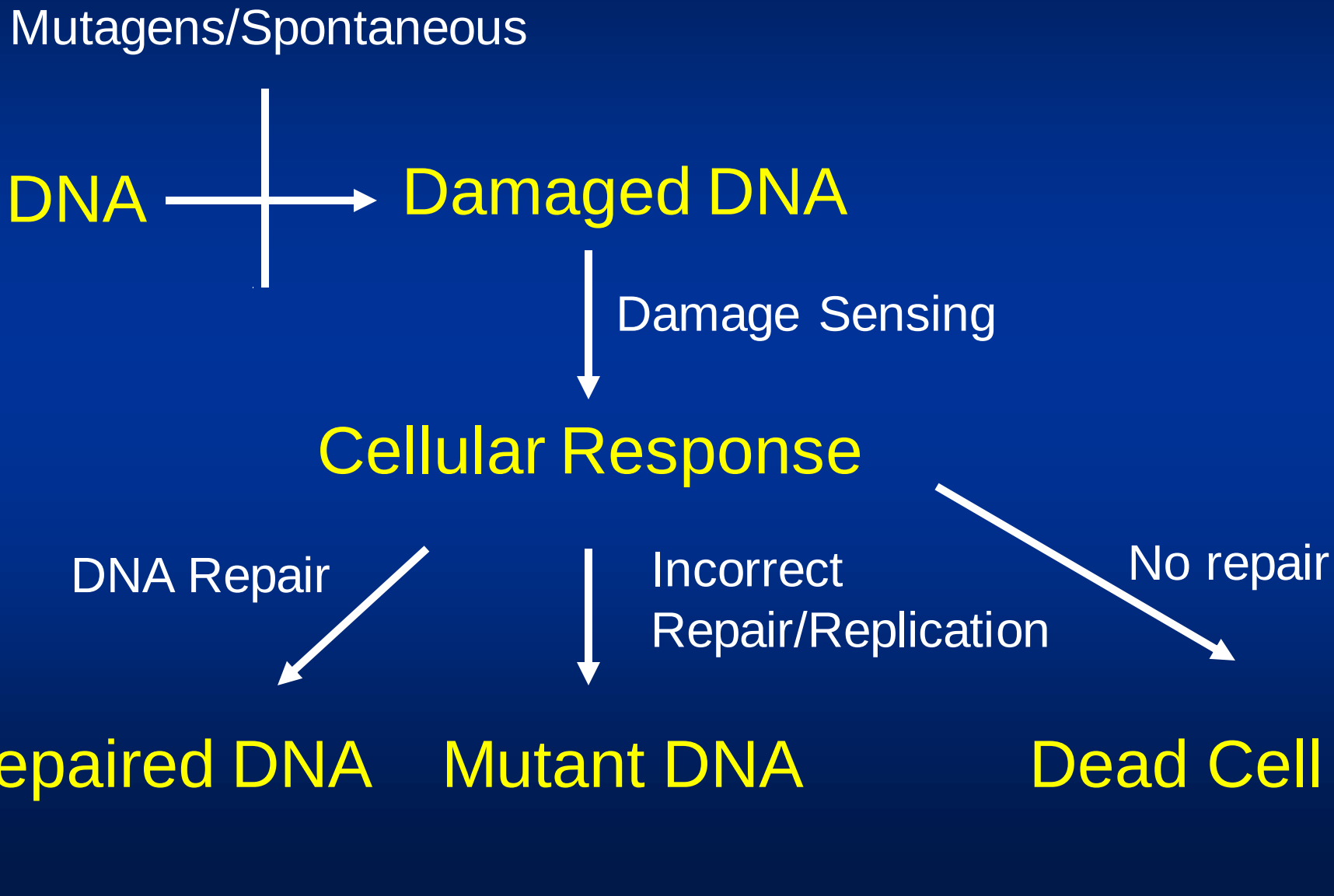




DNA Is Most Susceptible to Mutation in the Open State

DNA exists in a variety of states, from highly compact as in sperm, to single stranded as during replication. DNA can be most vulnerable to damage while in the single-stranded state.

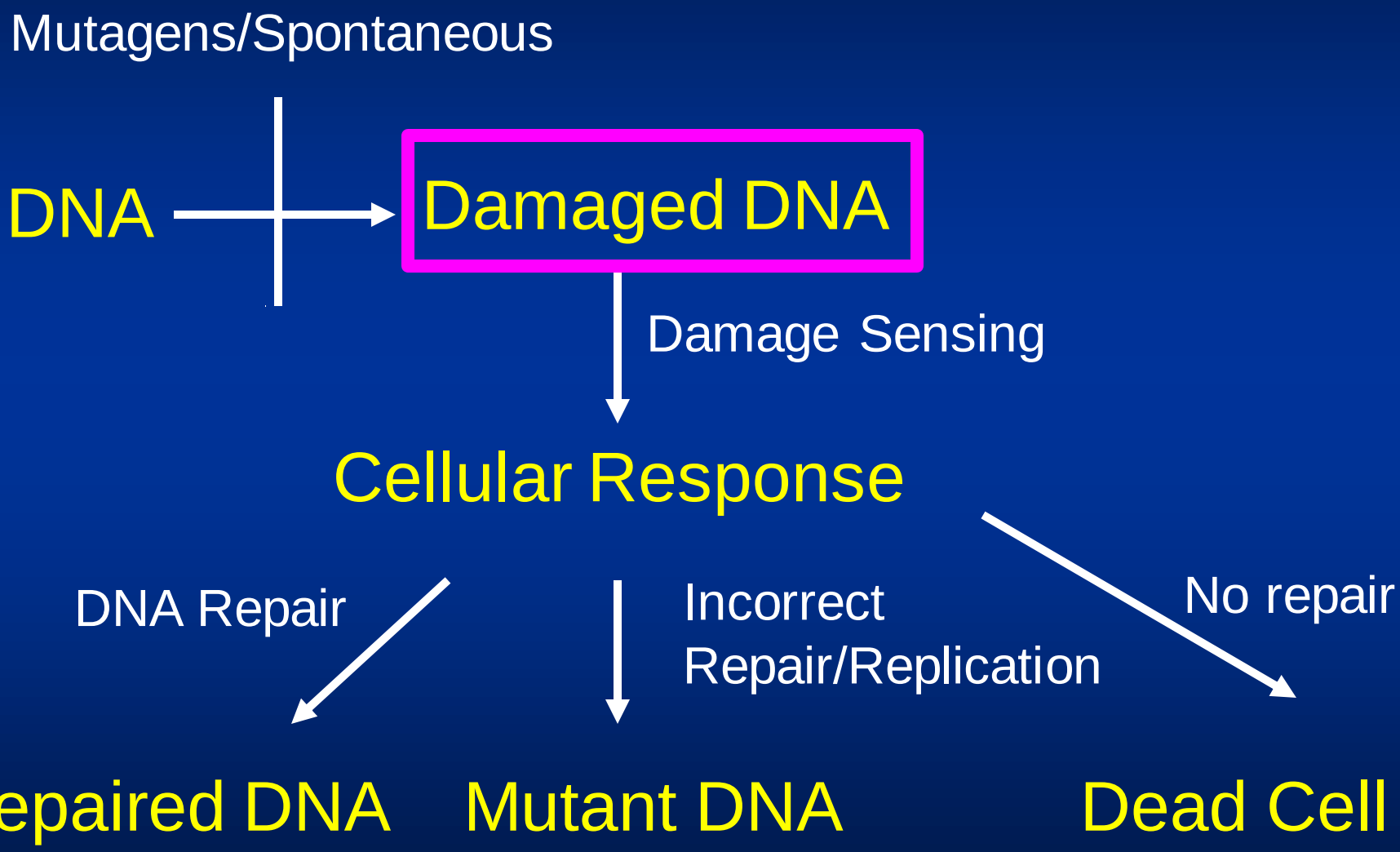
Mutagenesis Paradigm



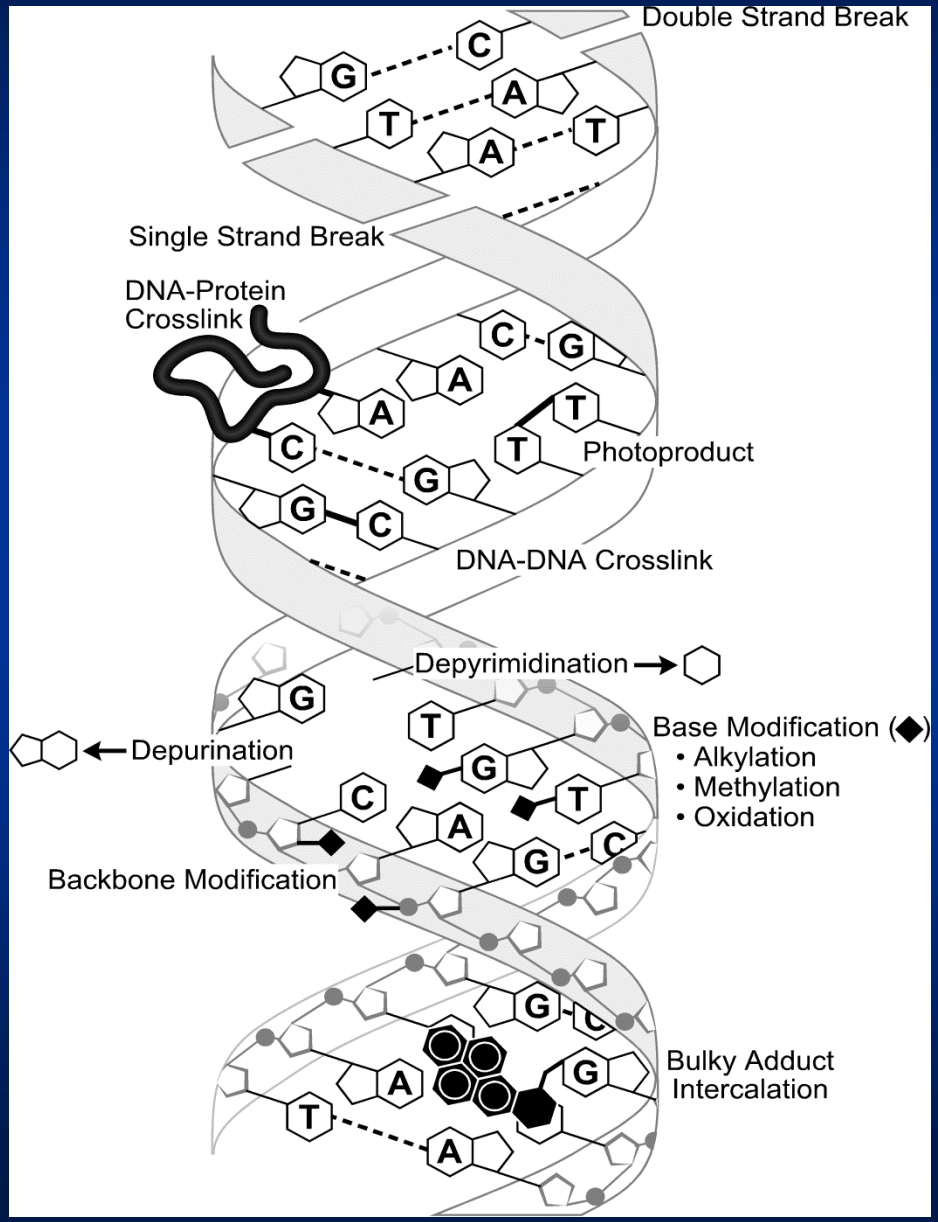
DNA Damage vs. Mutation

- Mutagens do not make mutations.
- Mutagens make DNA damage.
- DNA damage does not change DNA sequence.
  - DNA adducts (molecules bound to DNA)
  - DNA strand breaks or damaged bases
- Mutations are changes in nucleotide sequence.
- Cells make mutations (~200 DNA repair/replication proteins in humans).
- Mutagenesis is a cellular process requiring enzymes and/or DNA replication.

Mutagenesis Paradigm



Types of DNA Damage

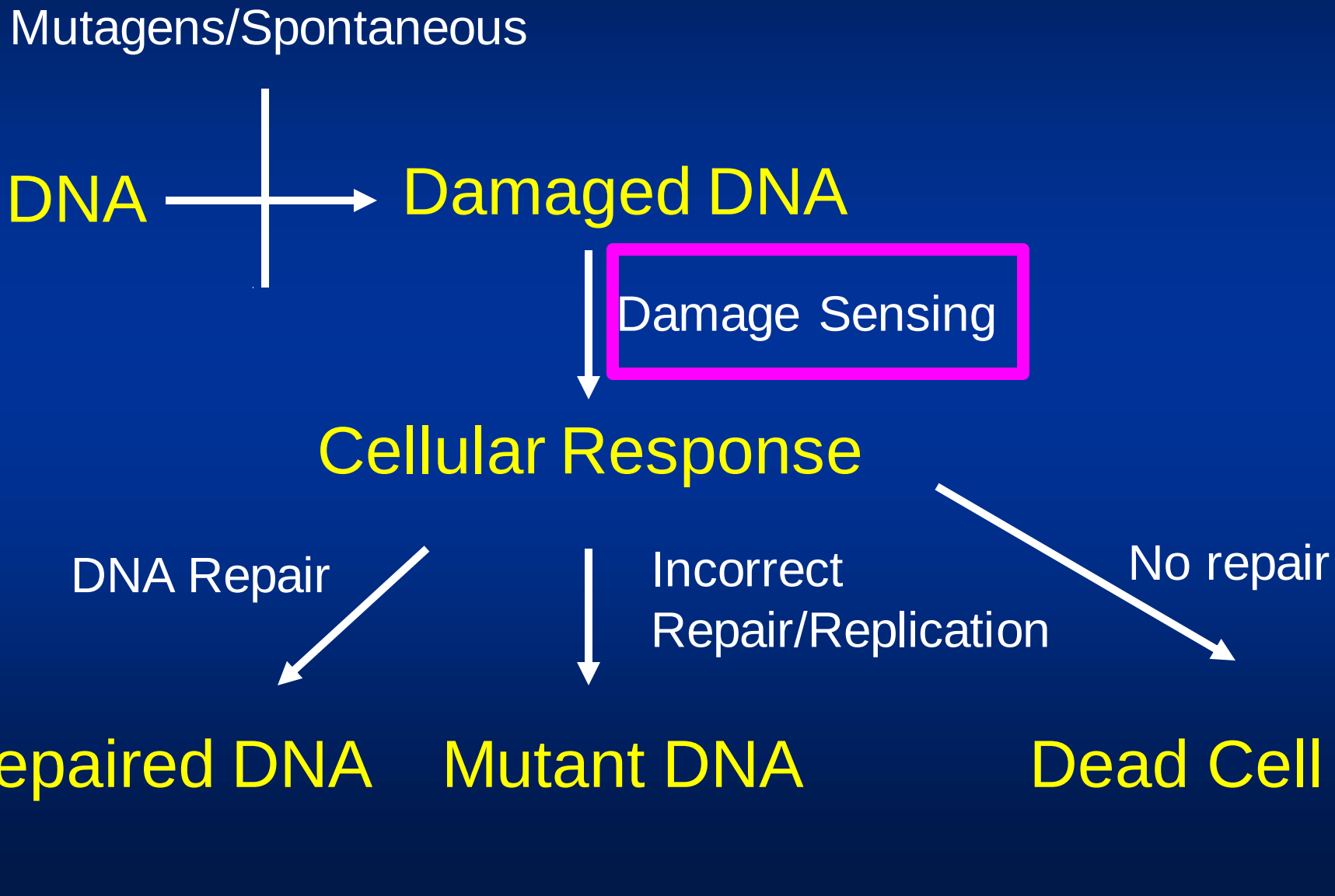


DT Shaughnessy & DM DeMarini (2009) Types and Consequences of DNA Damage, pg 21, in *Chemoprevention of Cancer and DNA Damage,* Ed. Knasmuller, DeMarini, Johnson, Gerhauser

Methods to Detect DNA Damage

1. ^{32}P-Postlabeling (bulky adducts)
2. Single Cell Gel Electrophoresis (comet assay; strand breaks & damaged bases
3. Alkaline Elution (strand breaks)
4. Unscheduled DNA Synthesis (UDS)
5. Antibody Methods (Specific Bulky Adducts)
6. LC-MS/GC-MS for specific adducts

Mutagenesis Paradigm

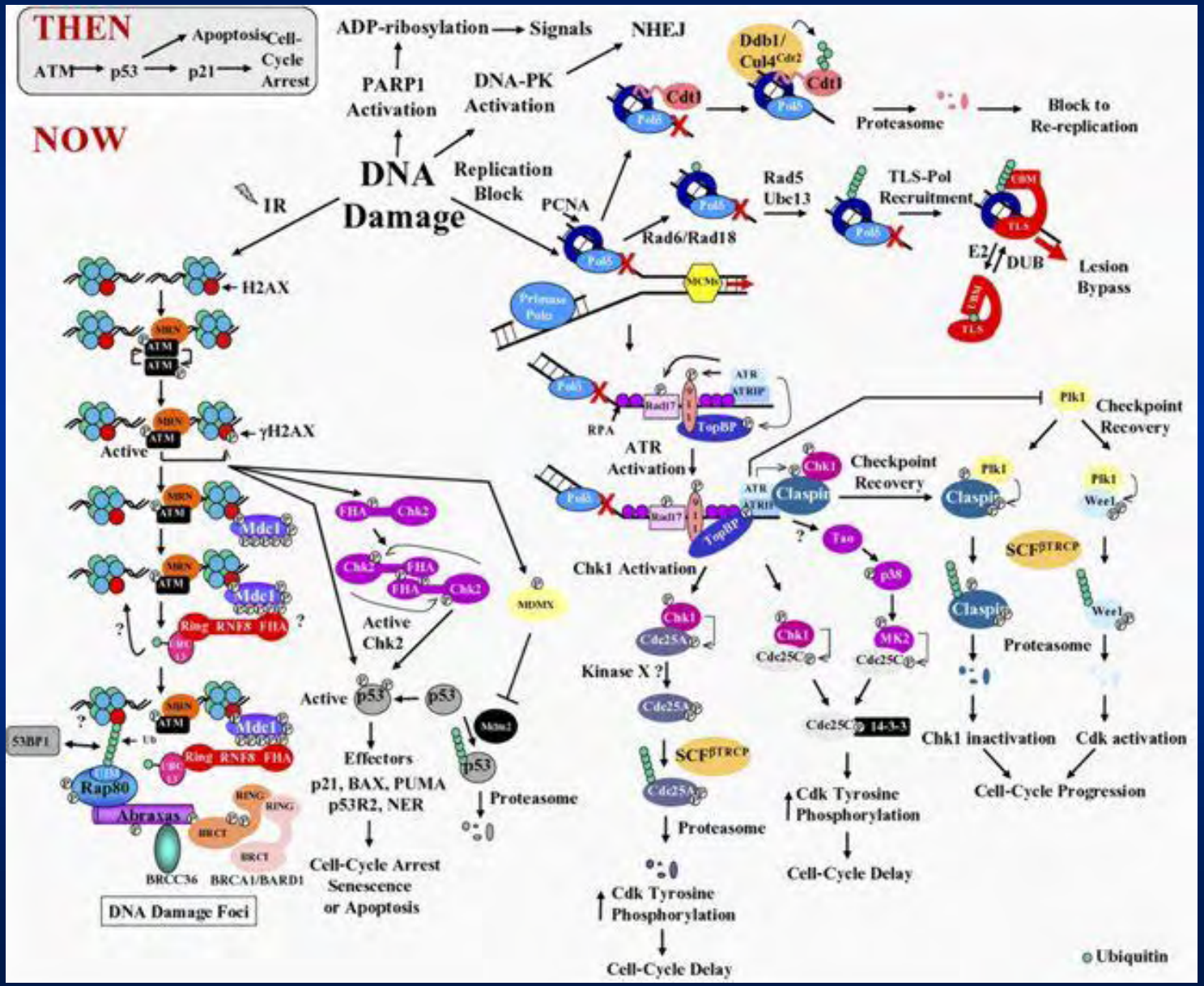


DNA Damage Response (DDR)
[A Ciccia & SJ Elledge, Mol Cell 40:179, 2010]

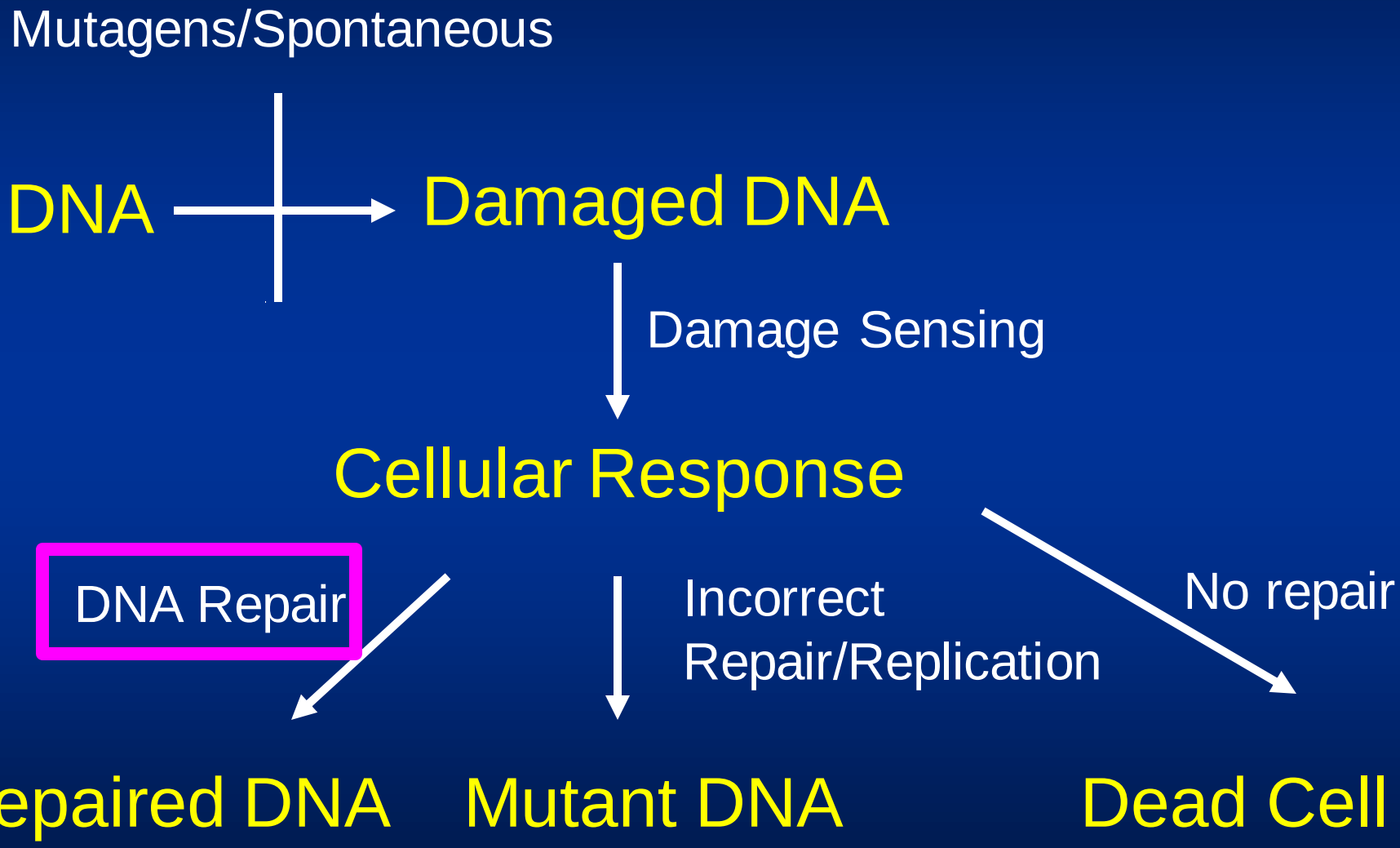
- DDR is a single-transduction pathway that senses DNA damage and replication stress and initiates a response.
- The response can be a wide variety of repair processes (some of which may lead to mutation) or cell death.
- More than 200 proteins involved in DDR.
- Many diseases associated with mutations in the DDR genes.

The DNA Damage Response: 10 Years After

[Harper & Elledge, Mol Cell 14:739, 2007]



Mutagenesis Paradigm



Types of DNA Repair

Direct - photoreactivation (removal of UV-dimers, not in humans); alkyl transfer

Base Excision – non-bulky adducts; alkyl groups; oxidized, reduced or fragmented bases

Nucleotide Excision – any covalent base modification, but mostly bulky adducts

Mismatch – Incorrect base pairs are corrected

SOS – inducible, error prone, mostly bacteria

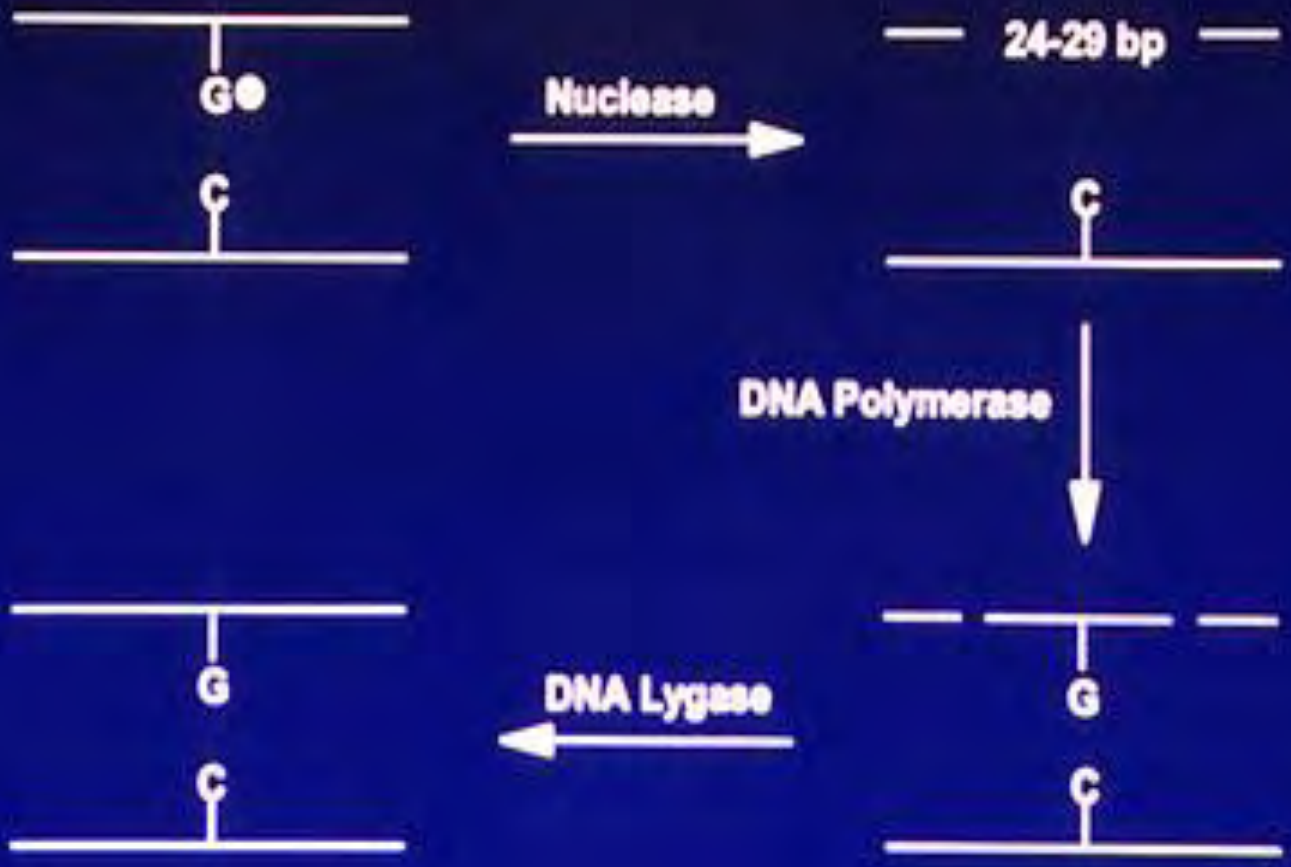
Blunt-End (Non-homologous Recombinational) Repair – rejoining of broken chromosomes

Homologous Recombinational Repair – repair by-pass of chromosomes

Diseases Due to Mutations in DDR Genes

Disease	DDR Pathway	Gene	Phenotype
XP	NER	*XPA-G*	Skin cancer
Werner Syn	BER	*WRN*	Aging
Familial BC	HR	*BRCA*	Breast cancer
SCID	NHEJ	*RAG*	Immuno
Colon CA	MMR	*MYH*	Colon cancer
Ataxias	SSB	*APTX*	Neuro
AT	DSB	*ATM*	Leukemia

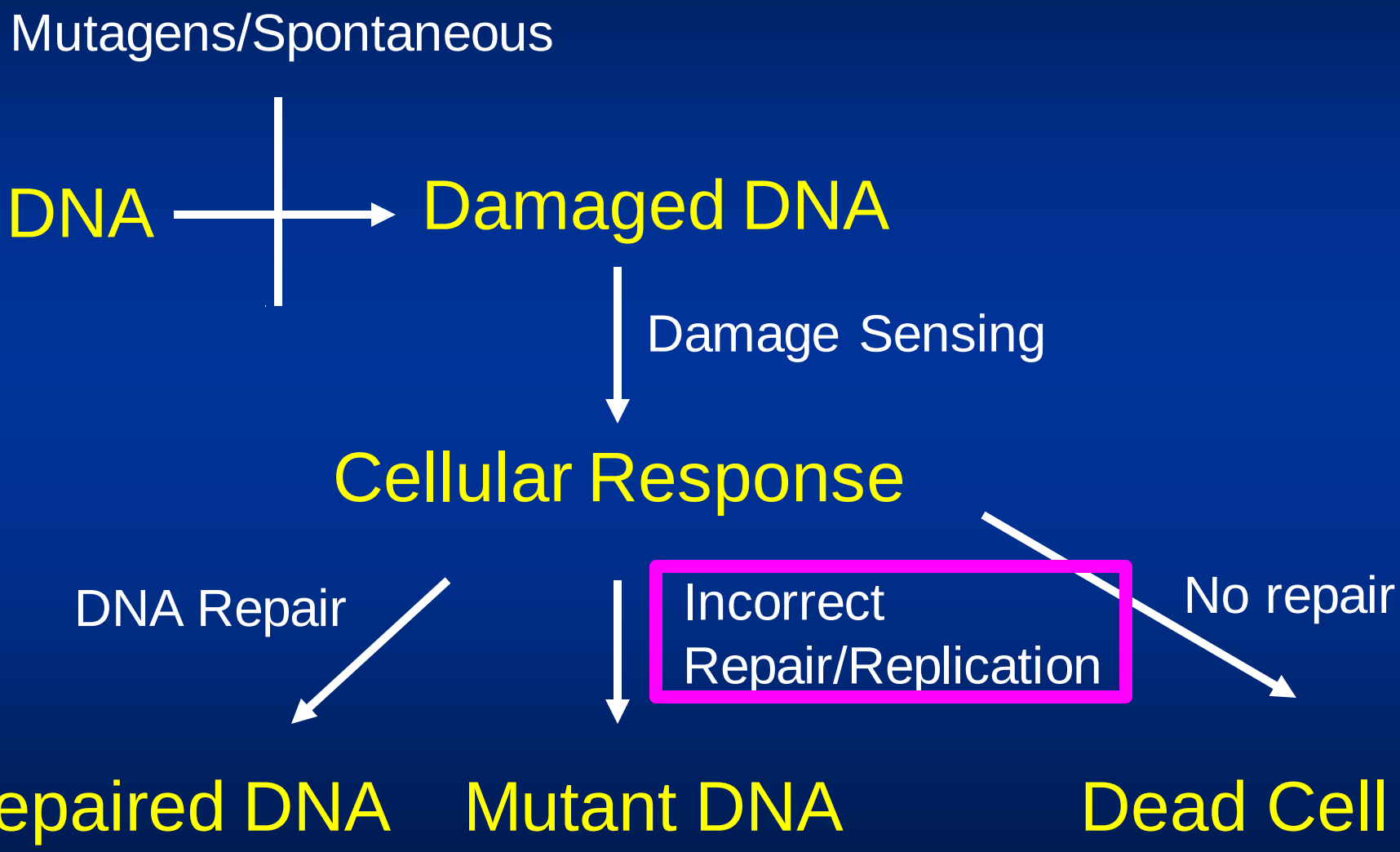
Nucleotide-Excision Repair



Relationship Between DNA Damage, DNA Repair, and Mutation

- Some types of DNA damage are repaired efficiently, and other types are not.
- Some regions of DNA (certain stretches of DNA sequence) are repaired quickly, whereas others are repaired slowly—if at all.
- The location of mutations in DNA (i.e., changes in DNA sequence) are coincident with sites of slow or no DNA repair of DNA damage.

Mutagenesis Paradigm




Mutation
Somatic Cells
Germ Cells
Cancer, Aging
Birth Defects
Genetic Diseases

Mutations and Diseases

P53, RAS,
Chrom. Ab.

Somatic →

Cancer

HPRT

CF

Trisomy 21

Germ →

Lesch-Nyhan

Cystic Fibrosis

Down's Syndrome

Possible Human Germ-Cell Mutagens

[DeMarini, Environ Mol Mutagen 53:166, 2012]

Agent	Rodent			Human		
		Mutagenic			Mutagenic	
	Carcino	Somatic	Germ	Carcino	Somatic	Germ
Ionizing Radiation	+	+	+	+	+	?+
Chlorambucil	+	+	+	+		?
Chlornaphazine	+	+	+	+		?
Cyclophosphamide	+	+	+	+	+	?
Melphalan	+	+	+	+	+	?
Myleran	+	+	+	+	+	?
Tobacco Smoke	+	+	+	+	+	?+
Air Pollution	+	+	+	+	+	?+

Types of Mutations & Assays to Detect Them

Gene Mutation: mutations within a gene, generally base-substitutions or small deletions/insertions, e.g., frameshifts. Generally called point mutations. [Salmonella, CHO/*Hprt*, *Tk*$^{+/-}$]

Chromosome Mutation: mutations spanning more than one gene, usually large deletions/insertions or inversions, recombinational events, copy-number variants (CNVs), e.g., chromosome aberrations or some micronuclei. [Chromosome aberrations, some micronuclei]

Genomic Mutation: change in chromosome number, e.g., aneuploidy. [Chromosome aberrations, some micronuclei]

How Cells Can Make Mutations: Trans-lesion DNA Polymerases

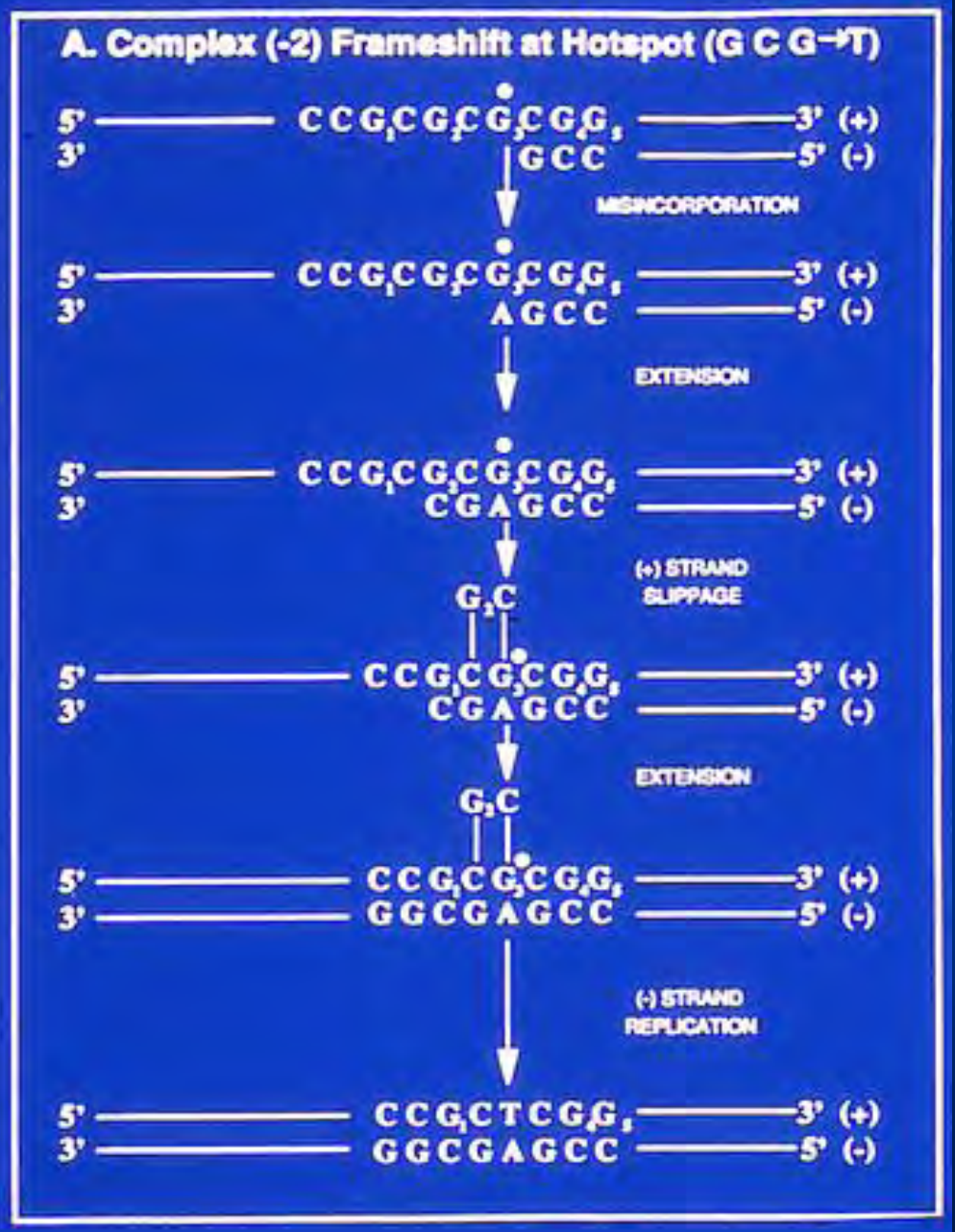
[J.E. Sales, CSH Perspect Biol 5:a012708, 2013]

- DNA polymerases replicate DNA using one strand of the DNA as a template for making the new strand.
- ~15 different DNA polymerases in human cells.
- ~7 are involved in trans-lesion synthesis or DNA damage-tolerant or error-prone synthesis.
- These polymerases replicate past the DNA damage, but some have a high probability of inserting an incorrect nucleotide opposite the damage base.

Mutational Mechanism

[DeMarini et al., Genetics 149:17, 1998]

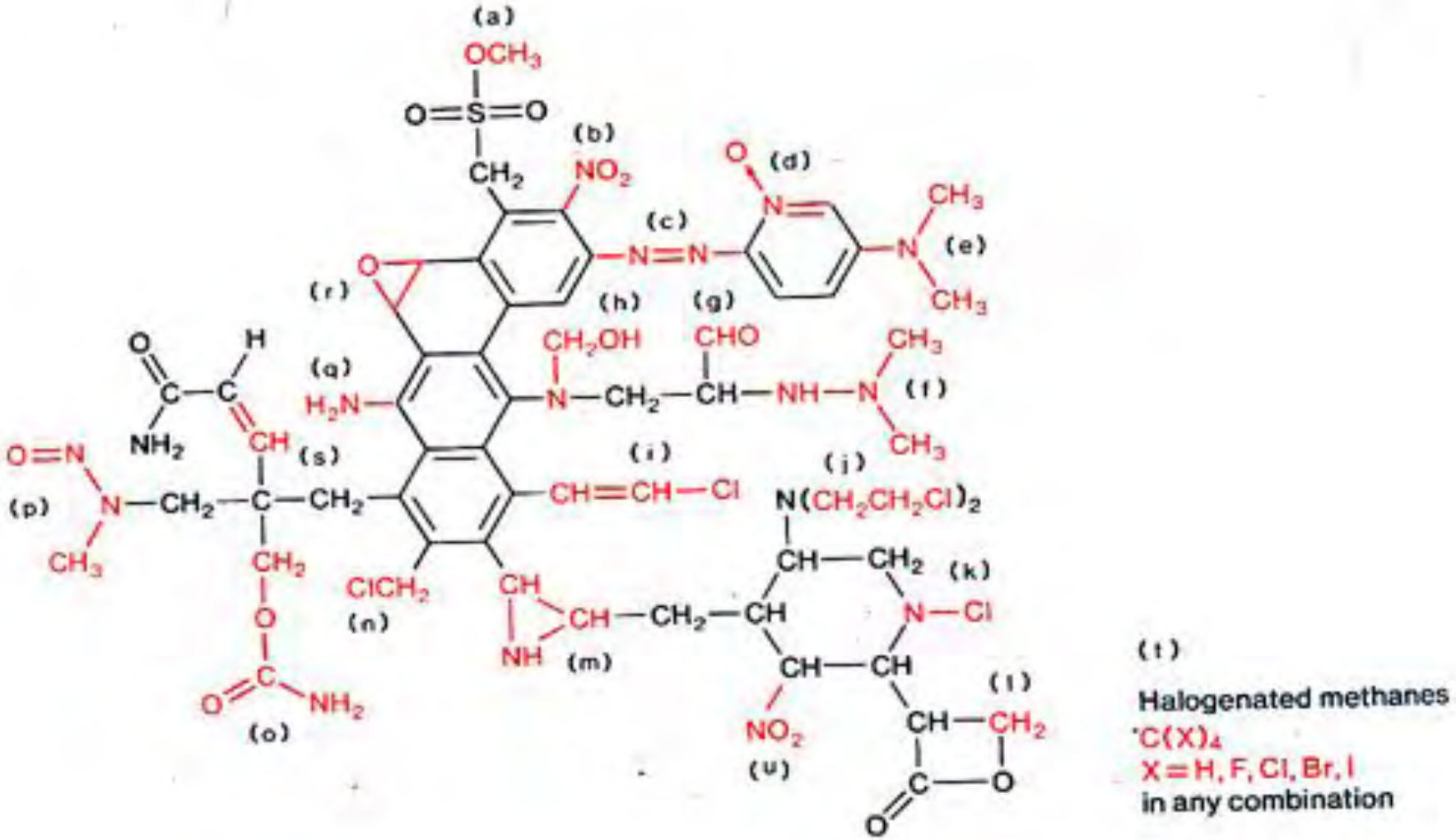
Example of a mutational mechanism in *Salmonella* TA98 involving a base substitution and a 2-base deletion induced by 4-aminobiphenyl



Environmental Mutagens

1. Physical
   a. Ionizing Radiation – X-rays
   b. Ultraviolet Light – sunlight, tanning salons
2. Chemical
   a. Naturally Occurring – mycotoxins, free radicals
   b. Pyrolysate Products – food mutagens, combustion emissions
   c. Synthetic Emissions – drugs, pesticides
3. Biological (e.g., viruses)

Structural Alerts for Mutagenicity
(Ashby et al., Mutat. Res. 223:73-103, 1989)



Genetic Toxicity Assays

1. Primary DNA Damage
   A. DNA Adducts (^{32}P-Postlabeling, Antibody)
   B. DNA Breaks (Comet)
   C. DNA Repair (UDS, Challenge Assays)

2. Gene Mutation
   A. Bacteria (*Salmonella*/Ames, *E. coli*)
   B. Mammalian Cells (CHO/*Hprt*, Mouse Lymphoma/*Tk*$^{+/-}$)
   C. Transgenic Mice (BigBlue, MutaMouse)

Genetic Toxicity Assays (c*ont.*)

3. Chromosomal Mutation
   A. Chromosomal aberrations in rodent or human cells
   B. Aberrations or micronuclei in vitro or in mouse bone marrow

4. Toxicogenomics
   A. Gene expression patterns (microarray)
   B. Protein expression patterns (proteomics)

Mutation Spectra in *Salmonella* and Humans

[DeMarini, Mutat Res 450:5, 2000]

Mutagen	Mutation in SAL and Human	Gene / Disease
Sunlight	GC ➔ AT, CC ➔ TT	*P53* / Skin Cancer
Cigarette Smoke & Smoky Coal	GC ➔ TA	*P53* / Lung Cancer
AFB	GC ➔ TA	*P53* / Liver Cancer
Heterocyclic Amines	Frameshifts	APC / Colon Cancer

SOURCES OF HARMONIZED PROTOCOLS & TEST GUIDELINES

[D.A. Eastmond et al., Mutagenesis 24:341-349, 2009]

The Standard Battery

1. *Salmonella* and/or *E. coli* Gene Mutation Assays
2. In vitro Cytogenetics or Mouse Lymphoma *Tk*$^{+/-}$ Assays
3. Mouse Bone Marrow Micronucleus or Chromosomal Aberrations Assays

Conclusions

1. Mutagens make DNA damage (adducts, strand breaks). Cells make mutations (change in DNA sequence)

2. DNA damage is detected by the cell's DNA Damage Response (DDR) system, which may either (a) repair the damage, (b) process the damage into mutations, or (c) cause cell death.

3. Cells generally make mutations by errors in DNA replication and/or DNA repair.

Conclusions (*cont.*)

4. Mutations can lead to cancer and other diseases if they occur in somatic cells or to hereditary diseases if in germ cells.

5. Although there are no "officially declared" human germ-cell mutagens, many likely exist.

6. Standard assays are required for regulatory purposes to detect agents that cause DNA damage or mutations: (a) *Salmonella* for gene mutation & (b) flow cytometry, cytogenetics, *Tk*$^{+/-}$, or mouse bone marrow CA/MN for chromosomal mutation.

Conclusions (*cont.*)

7. A variety of agents cause DNA damage that can be processed by the cell into mutations: viruses, ionizing radiation and UV light, some drugs, dietary components, and natural and manufactured chemicals .

8. Mutational mechanisms are somewhat similar across species due to the evolutionary conservation of DNA replication and repair processes. Thus, a particular mutagen produces similar types of DNA damage in most species, and that damage is processed into similar types of mutations across species.

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Gracias