DNA damage
mutagenicity and genotoxicity

DNA:

- **principal molecule for life of the cell**
- **structure and function carefully checked**
- **changes rapidly repaired**
- **irreversible changes -> cell death** *(apoptosis)*

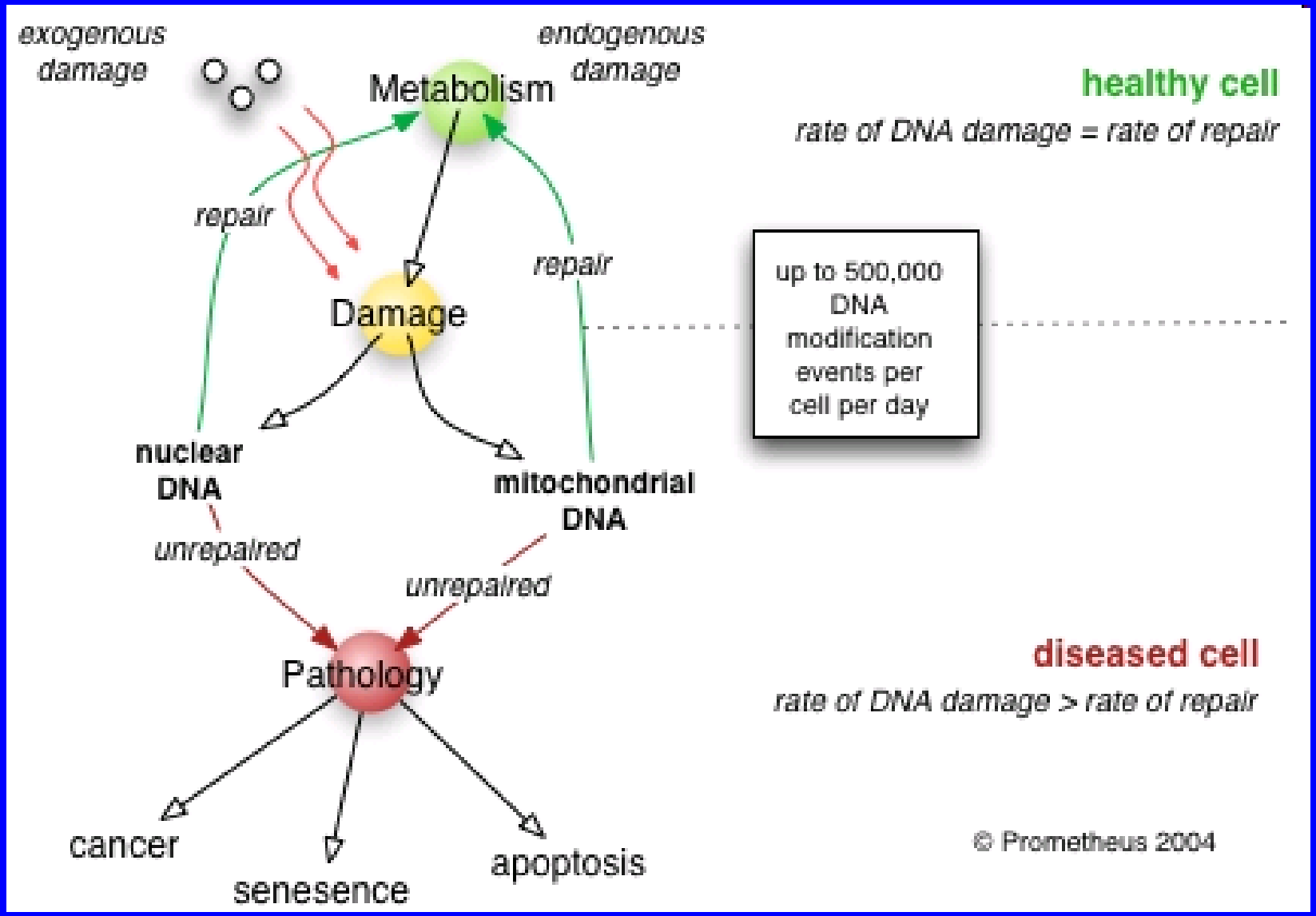
Mutagenesis - MUTATIONS

- changes in the sequences of deoxynucleotides

- natural mutations (billions of nucleotides/day)
: variability in genoms; reparations

- chemical-induced mutagenesis

DNA damage


exogenous damage
endogenous damage
Metabolism
repair
repair
Damage
nuclear DNA
mitochondrial DNA
unrepaired
unrepaired
Pathology
cancer
senesence
apoptosis
healthy cell
rate of DNA damage = rate of repair
up to 500,000 DNA modification events per cell per day
diseased cell
rate of DNA damage > rate of repair
© Prometheus 2004

DNA repair

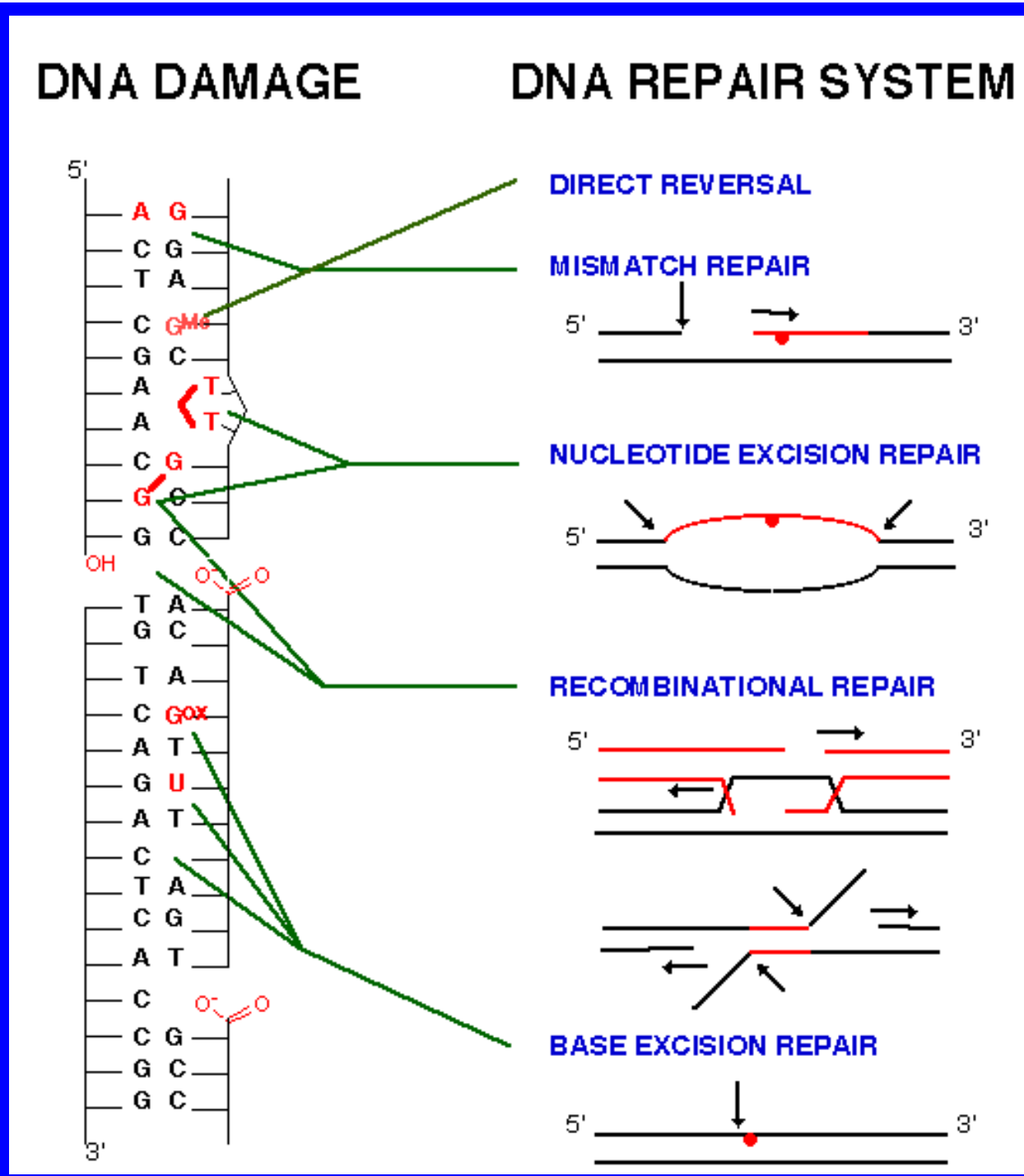
Damage of DNA is carefully controlled

constitutively expressed proteins

Changes in DNA

induction of reparation enzymes ("SOS-repair")

= biomarker of DNA damage


DNA DAMAGE
DNA REPAIR SYSTEM
5'
A G
C G
T A
C G
Me
G C
A T
A T
C G
G C
G C
OH
O
O
T A
G C
T A
C G
ox
A T
G U
A T
C
T A
C G
A T
C
O
O
C G
G C
G C
3'
DIRECT REVERSAL
MISMATCH REPAIR
5'
3'
NUCLEOTIDE EXCISION REPAIR
5'
3'
RECOMBINATIONAL REPAIR
5'
3'
BASE EXCISION REPAIR
5'
3'

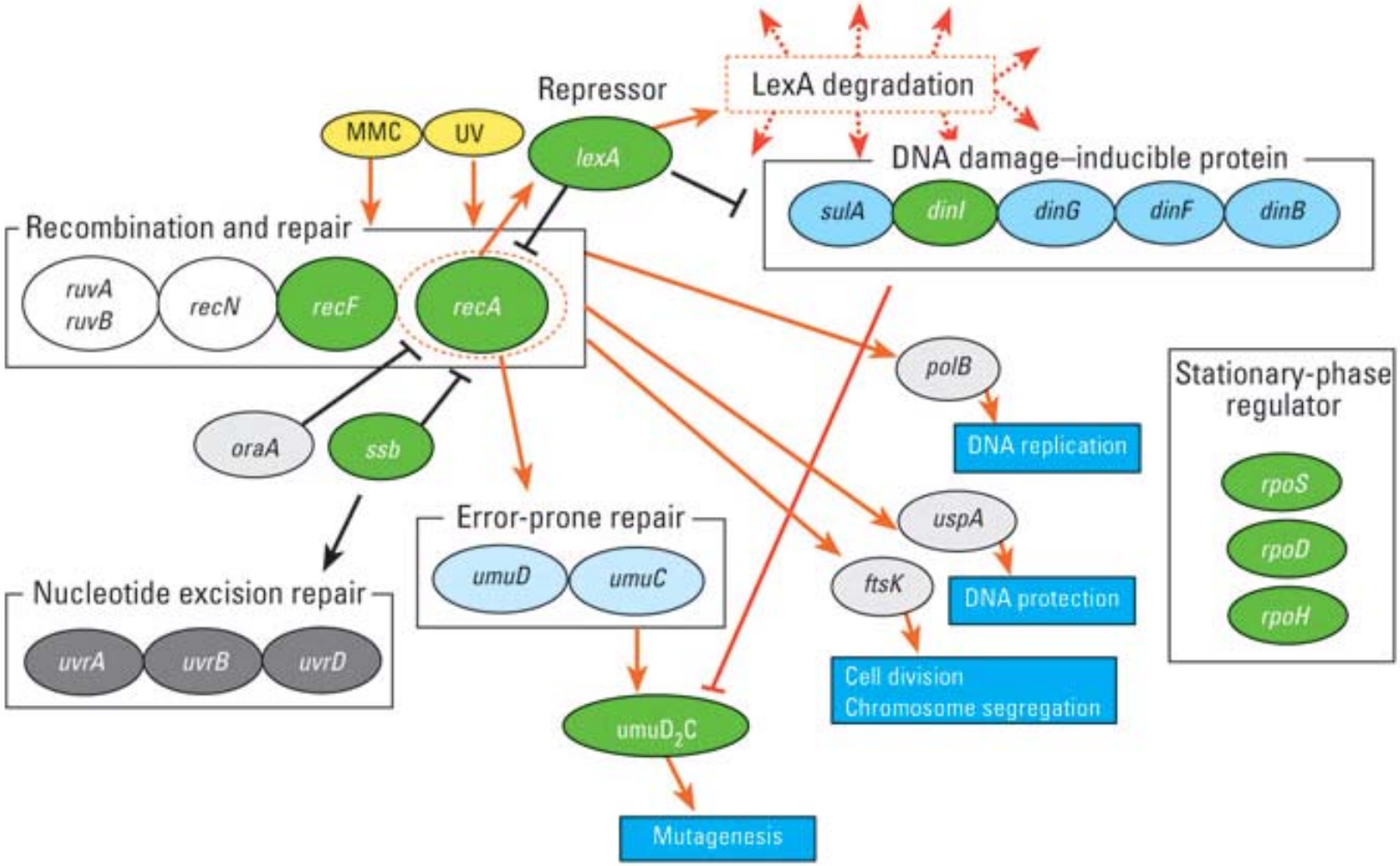


Figure 3. A literature-based linkage map between genes in the SOS response in *E. coli*. The map represents inducible genes/proteins in the SOS response for repair from DNA damage. Black lines indicate pathways in the normal repair process and red lines with arrows activation/induction due to an exposure to damaging agents. Recombination and repair, DNA damage–inducible protein, nucleotide excision repair, error-prone repair, and stationary-phase regulator have family molecules in each box. Green circles are genes used for the analysis.

Induced mutations

MUTAGENS

- ionizing radiation and UV

- chemicals

  Base analogs - inserted into the DNA strand during replication in place of the substrates.

  Agents reacting with DNA - structural changes leading to miscopying of the template strand

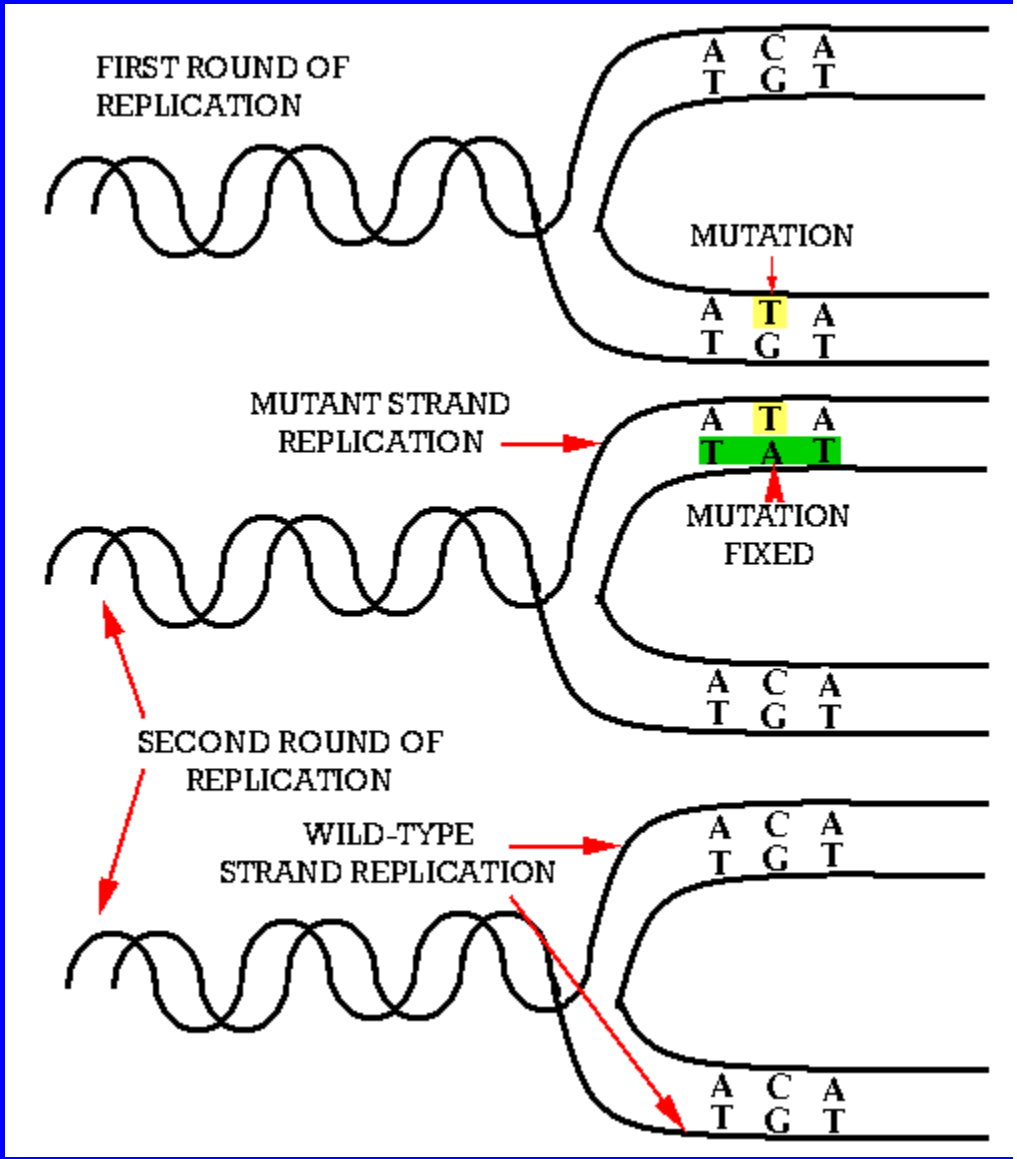
  Indirect mutagens - affect cells that synthesize chemicals with direct mutagenic effect

Point mutations
BASE - EXCHANGE:

- Silent mutations:
  - code for the same amino acid.

- Missense mutations:
  - code for a different amino acid.

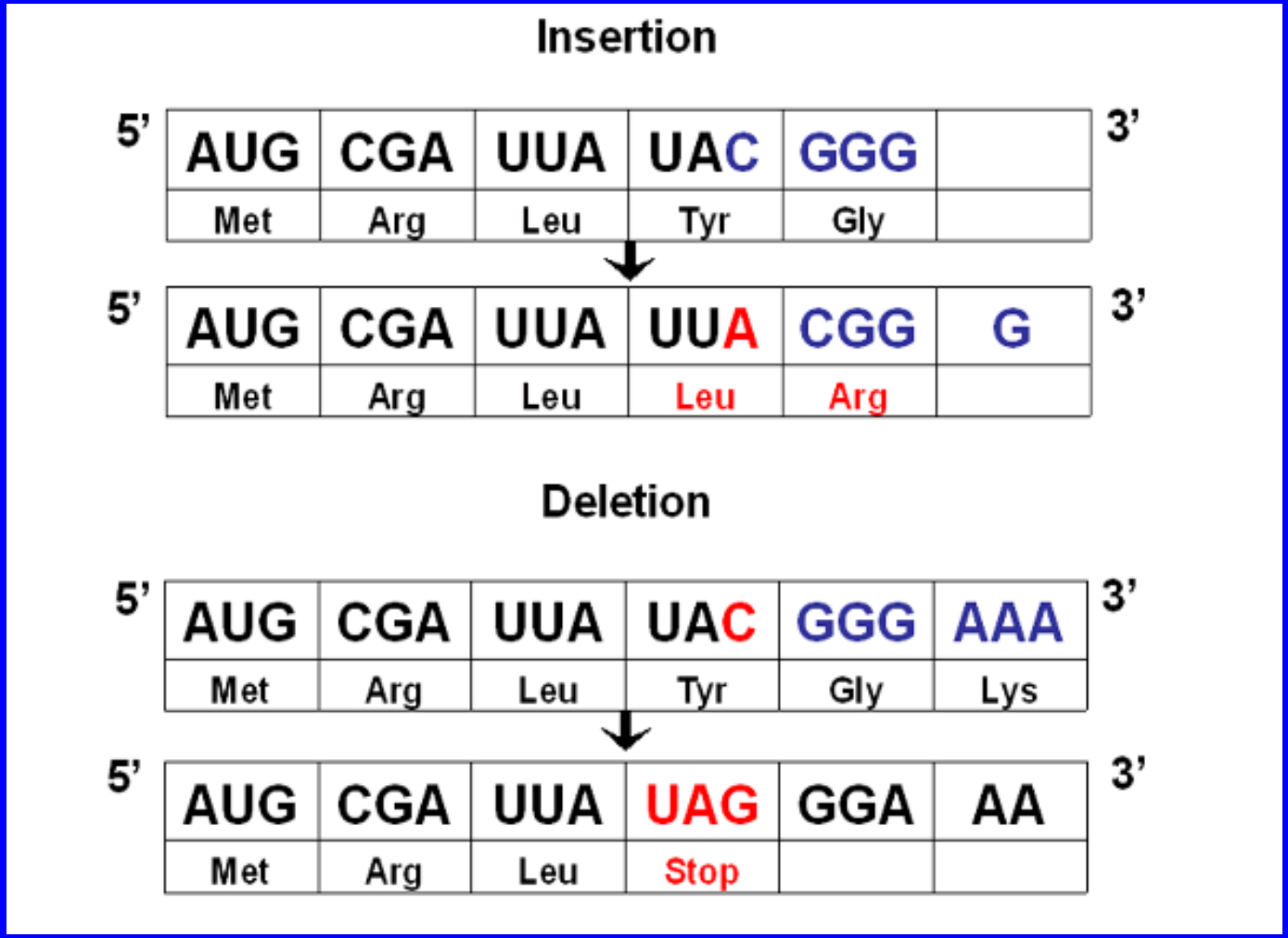
- Nonsense mutations:
  - which code for a stop



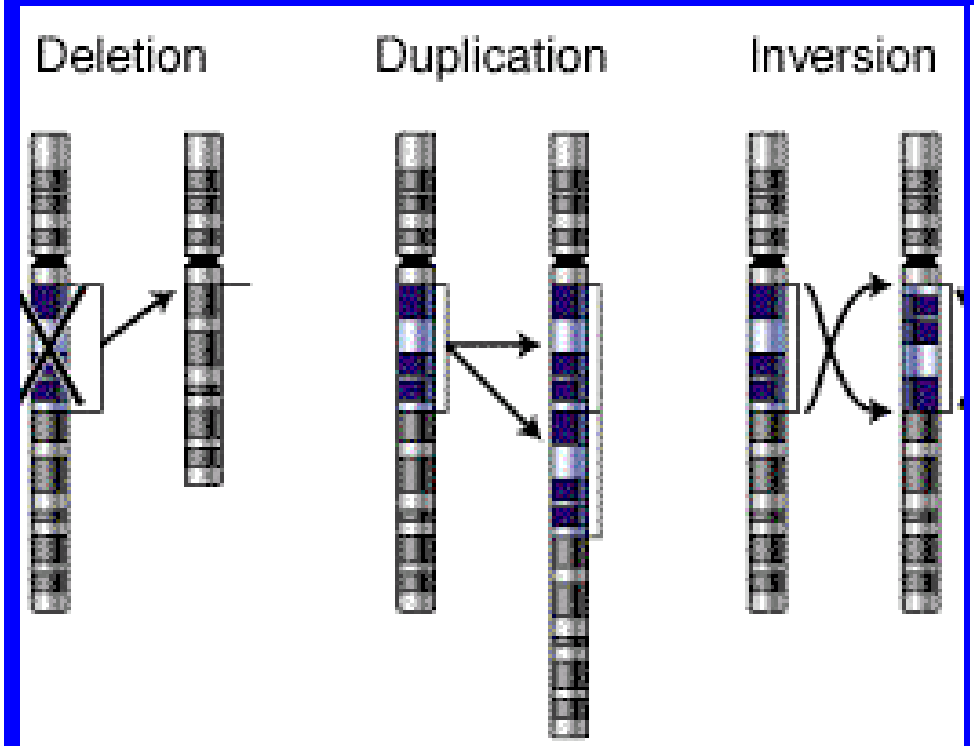
Point mutation

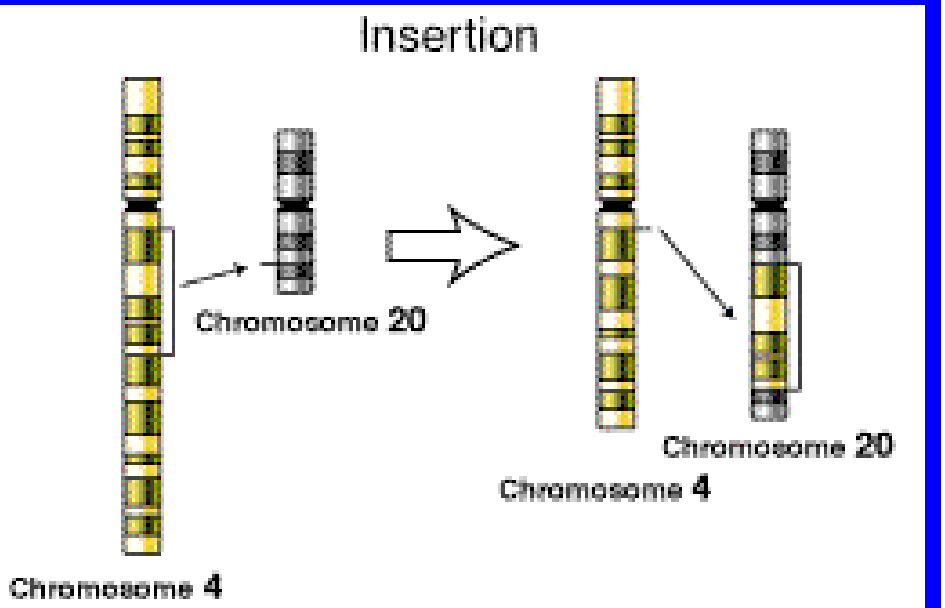
INSERTION
DELETION

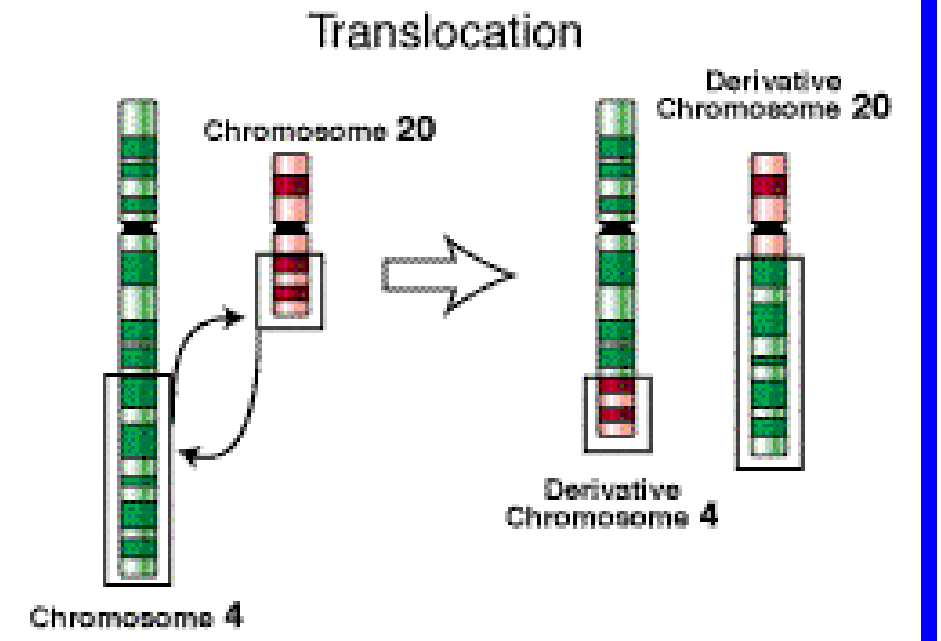
Change of the reading frame



Large scale mutations / chromosomal







Physical factors & DNA damage

Ionizating radiation

- direct interaction with hydrogen atoms in water (and bases)

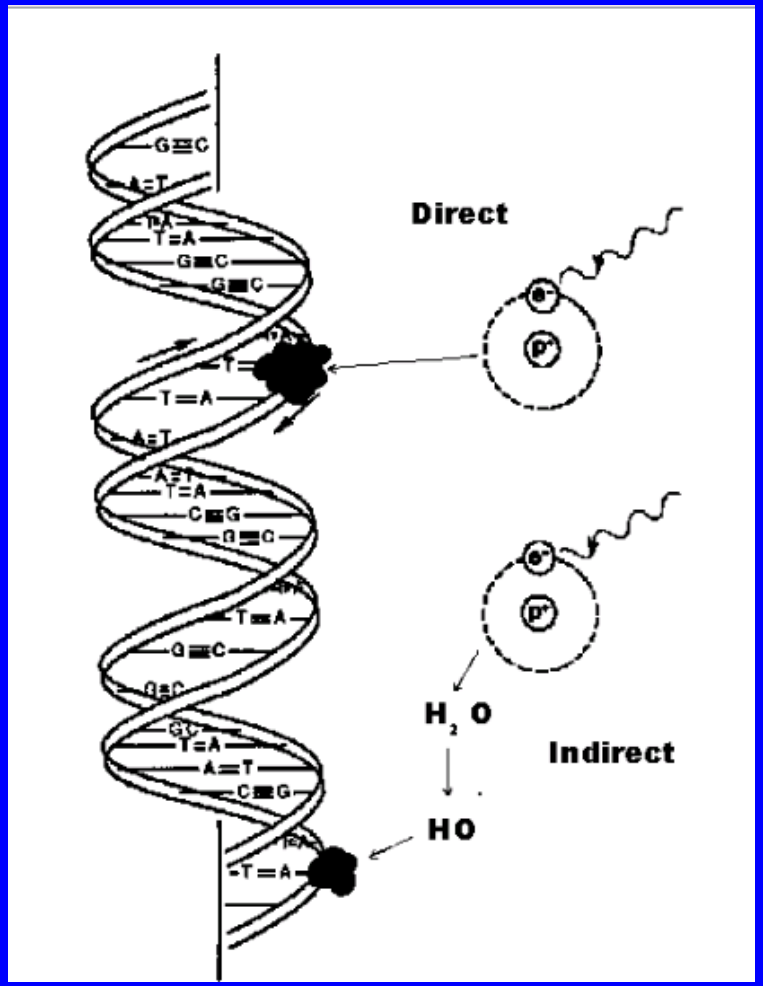
  -> OH* radicals; H_2O_2, O_2-

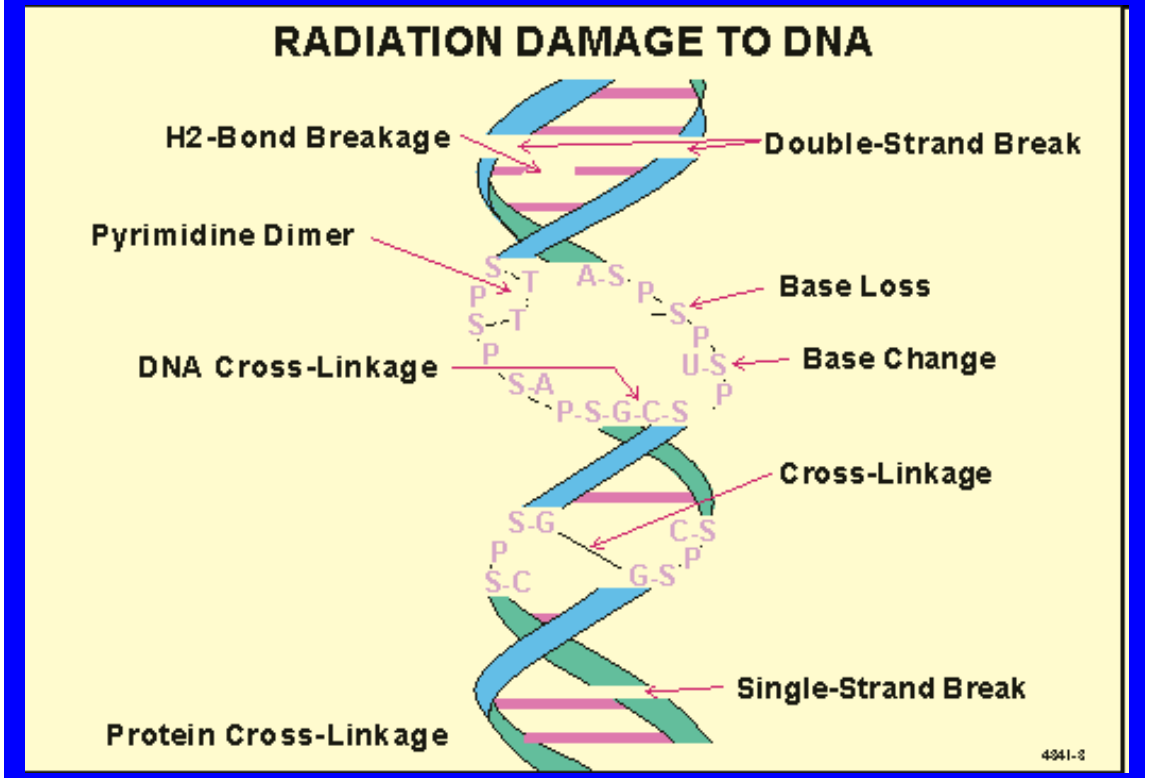
- oxidation of bases; dimerization ...

UV radiation

- interaction with aromatic cycles (bases)
- base dimerization (T=T)

Ionizing radiation effects on DNA



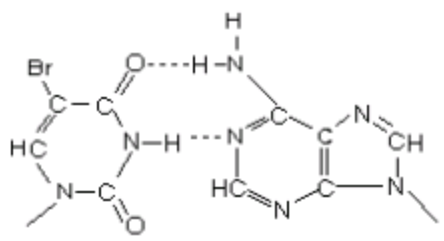


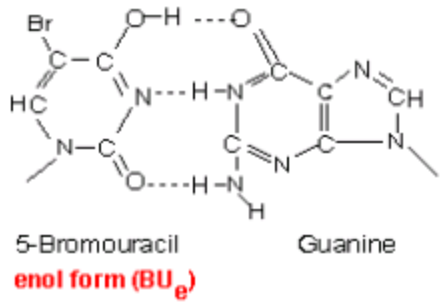
Chemical induced DNA damage

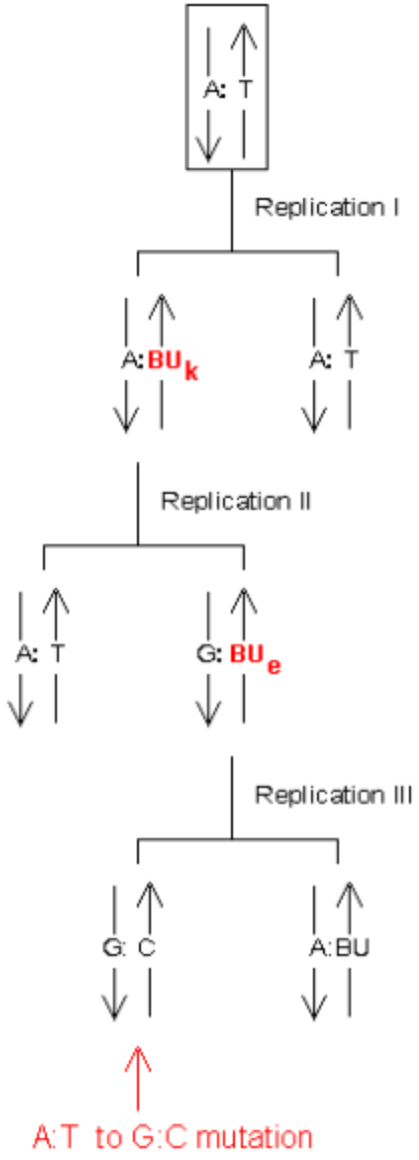
Bases analogs

- incorporation into DNA during replication

(5-Br-Uracil: AT -> GC)





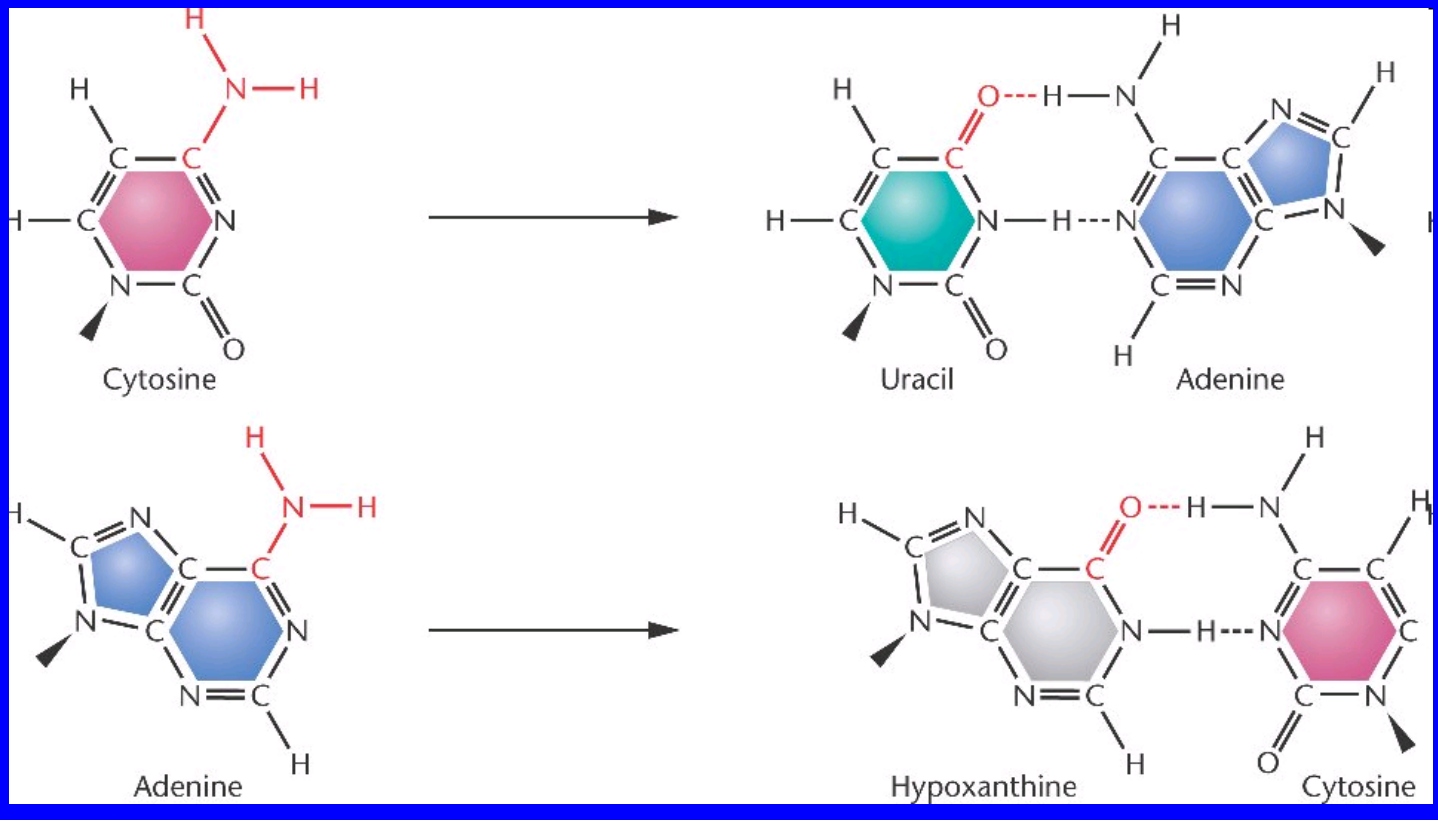


Chemical induced DNA damage

HNO_2, HSO_3^-,Hydroxylamine, Methoxyamine

deamination of bases

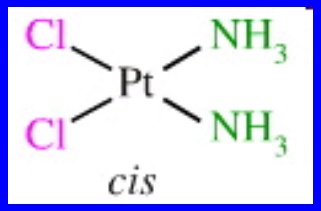
(GC -> AT)



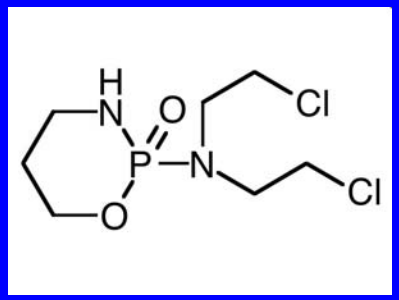
Chemical induced DNA damage

Alkylsulphates, N-nitroso-alkyles, cis-platinum

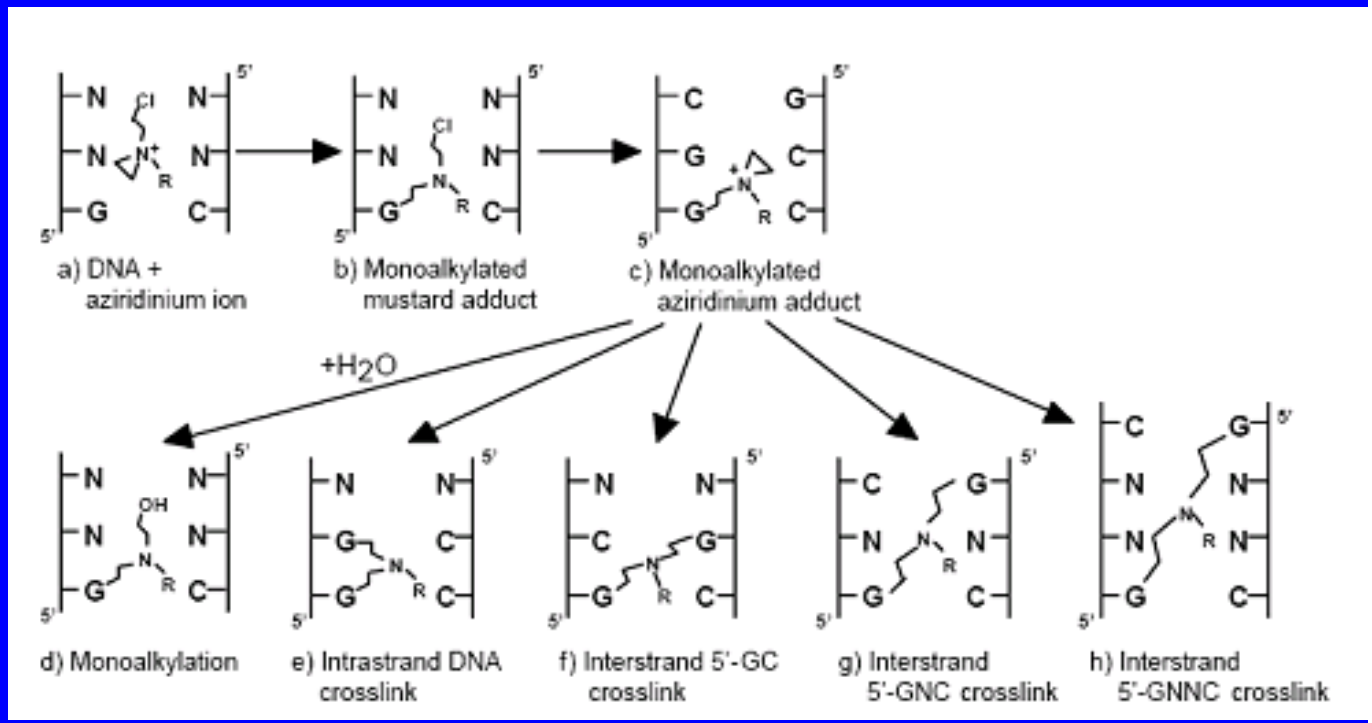
- alkylation of bases; crosslinks of dsDNA



cisplatin



cyclophosphamide



Chemical induced DNA damage

INTERCALATION & ADDUCT FORMATION

Polycyclic aromatic hydrocarbons (PAHs) & derivatives (N-acetyl-2-aminofluorene (AAF), benzo[a]pyrene)

Mycotoxins (aflatoxins) aduct formation with DNA (*biomarkers*)

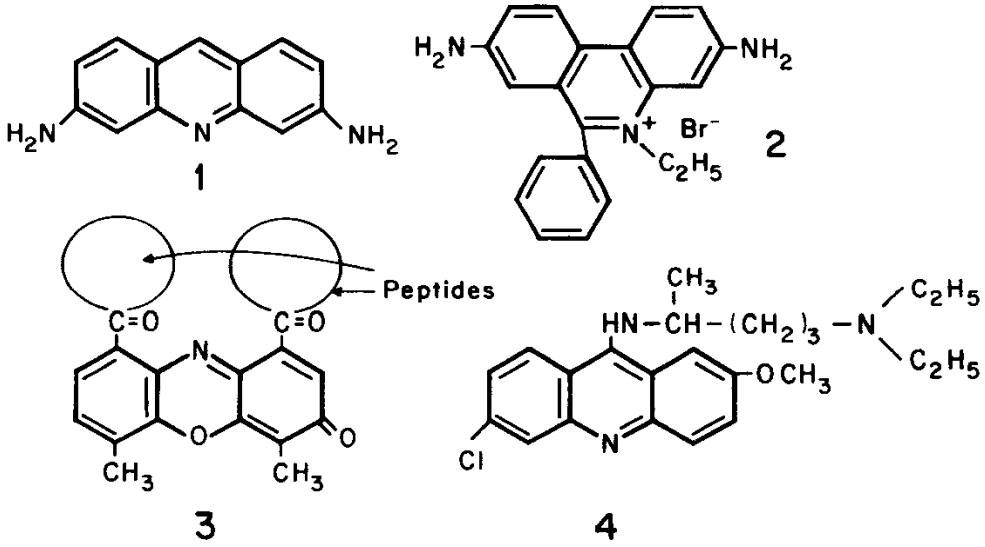
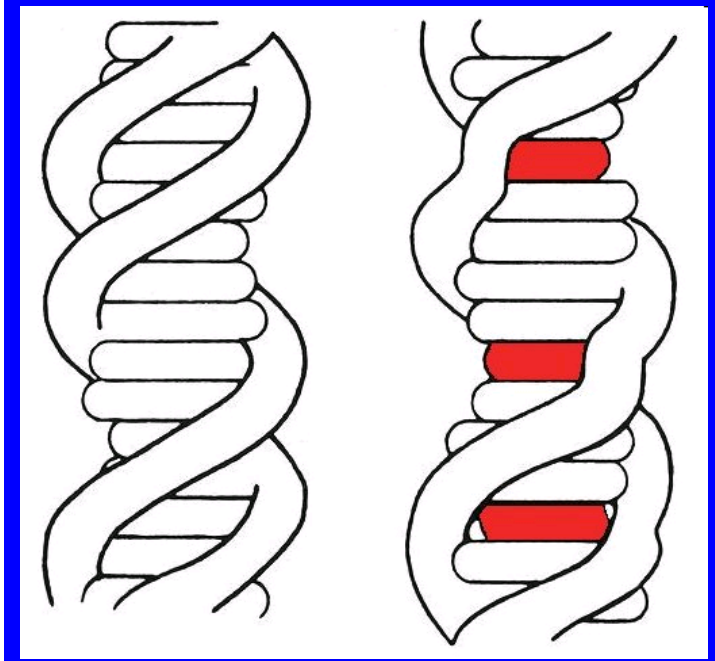
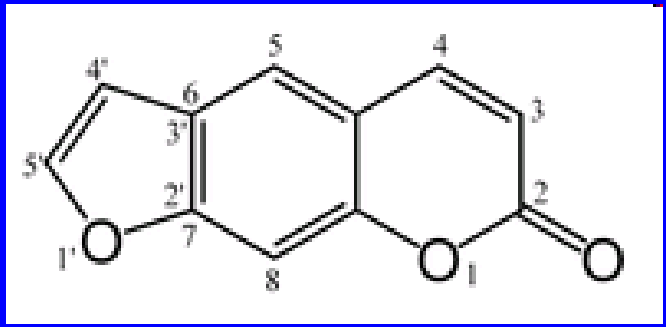
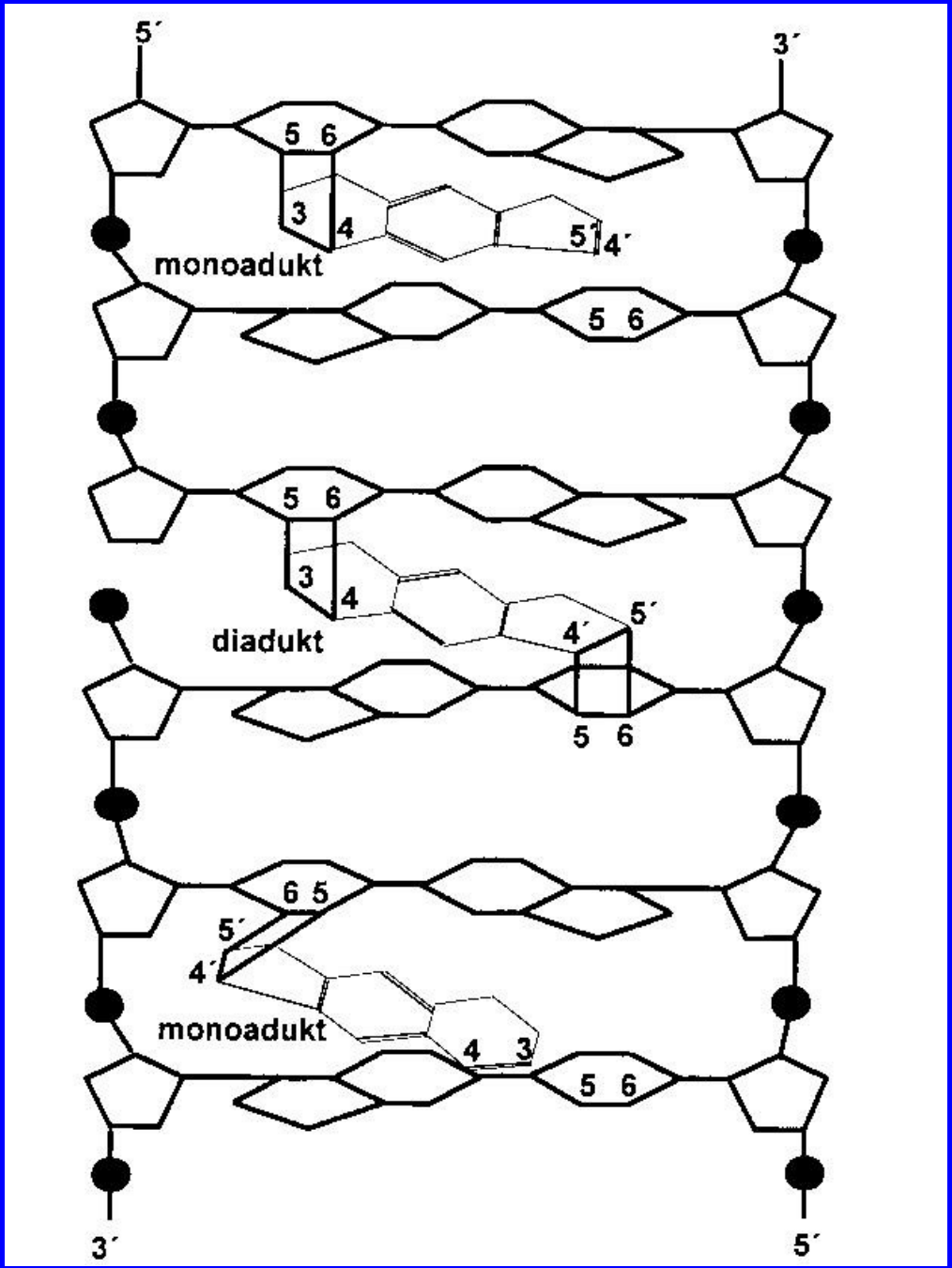


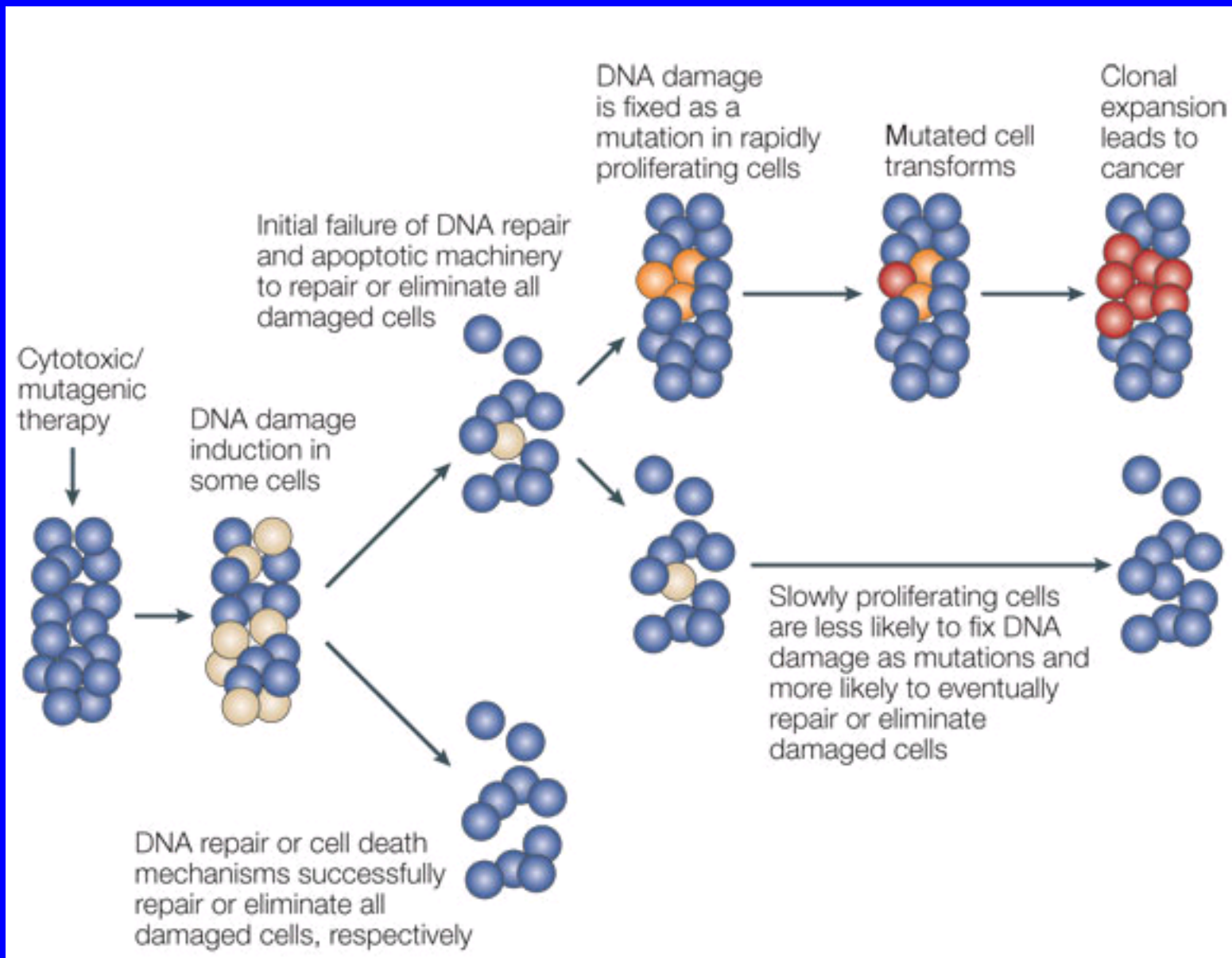
Chart 5.8. Examples of intercalating agents. Key: 1, acriflavine; 2, ethidium bromide; 3, actinomycin; 4, quinacrine.



Psoralen
DNA intercalation

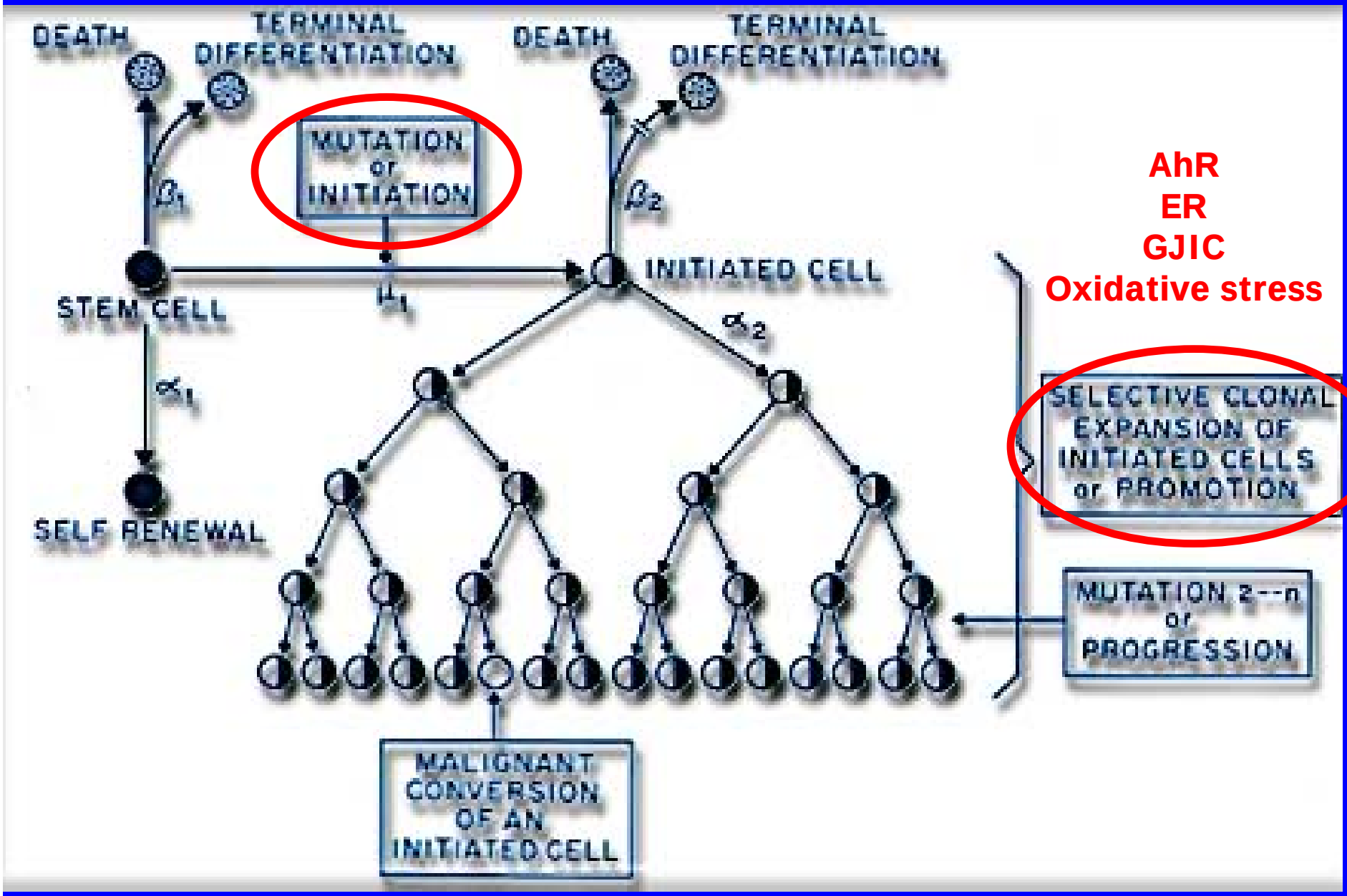






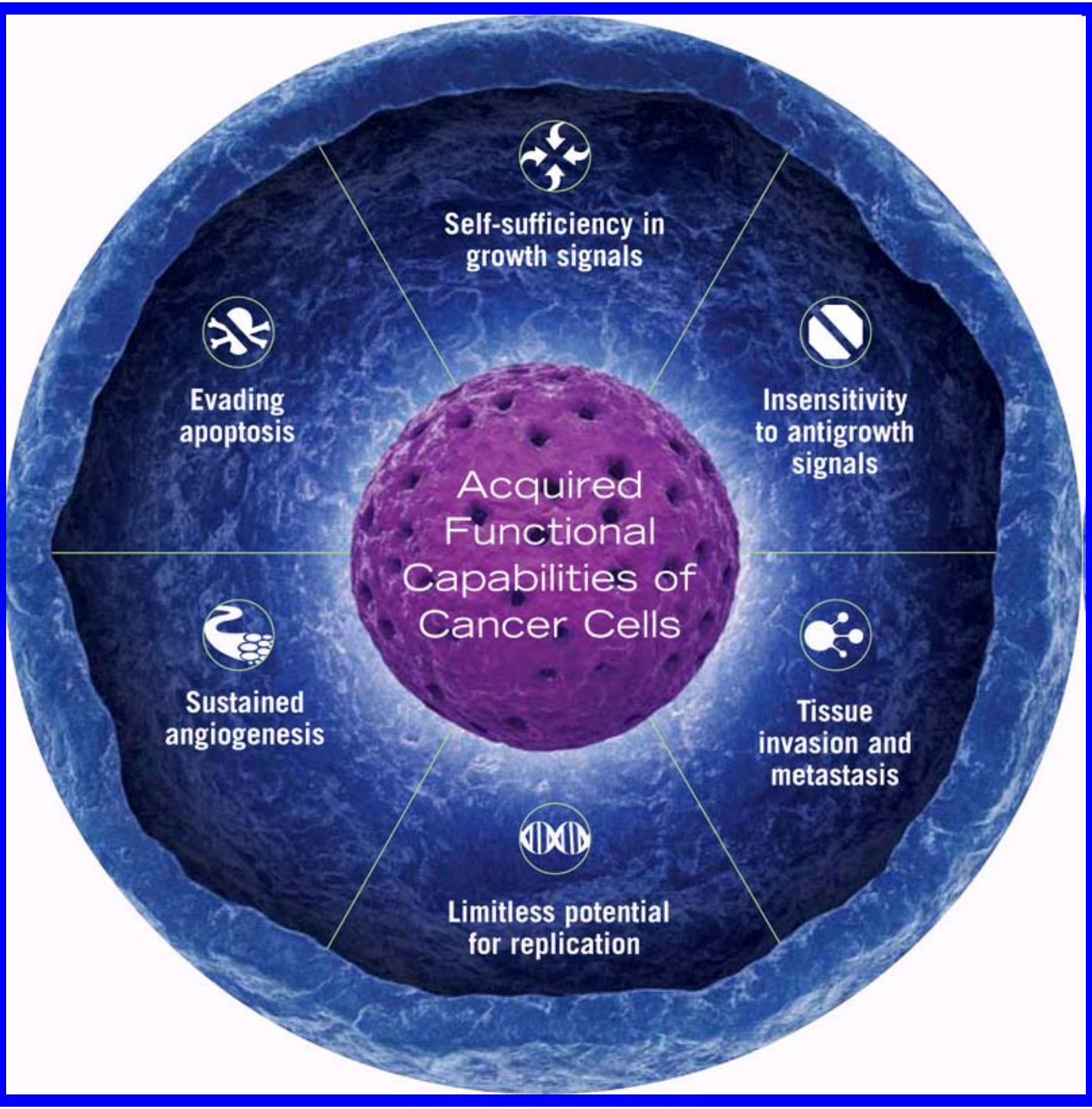
Nature Reviews | Cancer

IMPORTANT PROCESSES IN CANCEROGENESIS



AhR
ER
GJIC
Oxidative stress

Trosko and Ruch 1998, Frontiers in Bioscience 3:d208


Self-sufficiency in growth signals
Insensitivity to antigrowth signals
Tissue invasion and metastasis
Limitless potential for replication
Sustained angiogenesis
Evading apoptosis
Acquired Functional Capabilities of Cancer Cells

Does **chemically-induced genotoxicity** results in in vivo effects

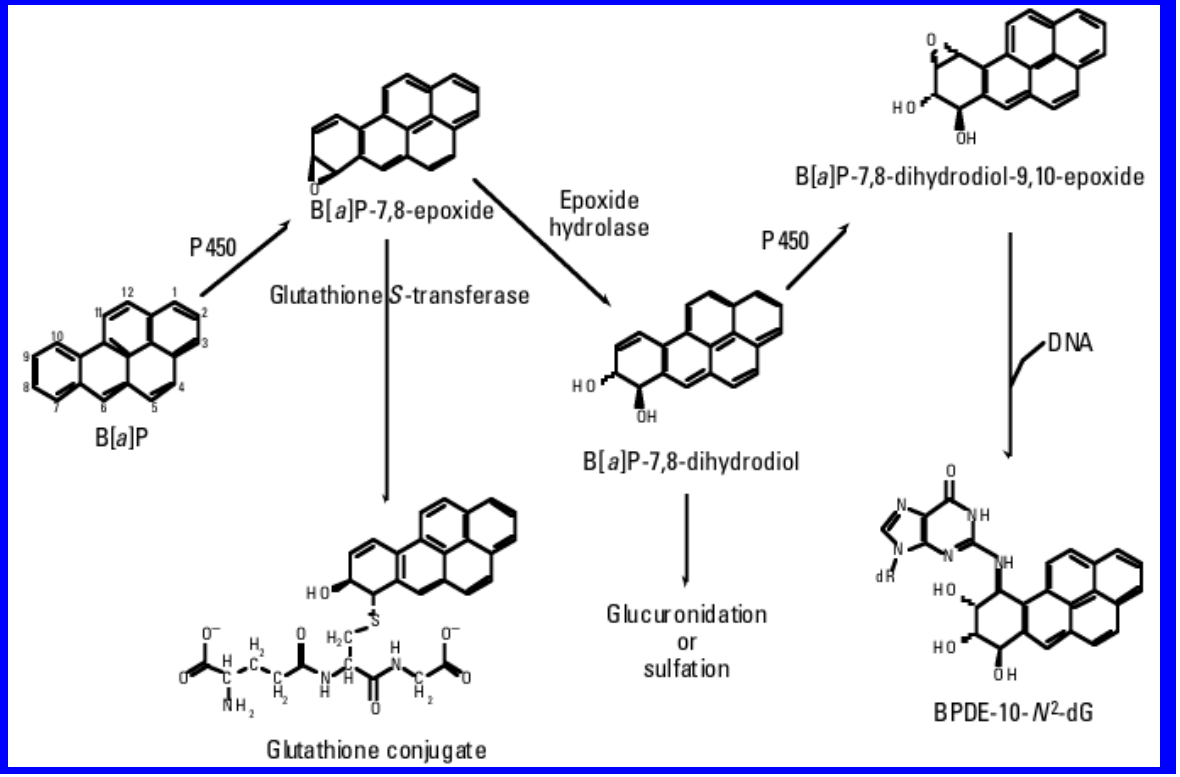
- adducts from mitochondrial DNA ?
- distance between „source of radicals“ and nuclear DNA ?
- protection mechanisms (mutation -> death/apoptosis)

Rubin (2002) *Oncogene* 21:7392

Thilly (2003) *Nature Genetics* 34(3):255

Mutations are not caused by chemicals

Chemicals only allow „unveil“ previously existing mutations in nuclear DNA *(non-genotoxic events cause cancer !!!)*



Redox homeostasis & oxidative stress

Redox homeostasis

- natural levels of oxidants (O_2) and antioxidants in each cell

Disruption of redox homeostasis

-> depletion of oxygen: metabolism disruption, acidosis in tissues, cell necrosis

rare: INSIDE TUMORS

-> overproduction of oxidants:

= oxidative stress

GENERAL MECHANISM OF TOXICITY

Overproduction of oxidants

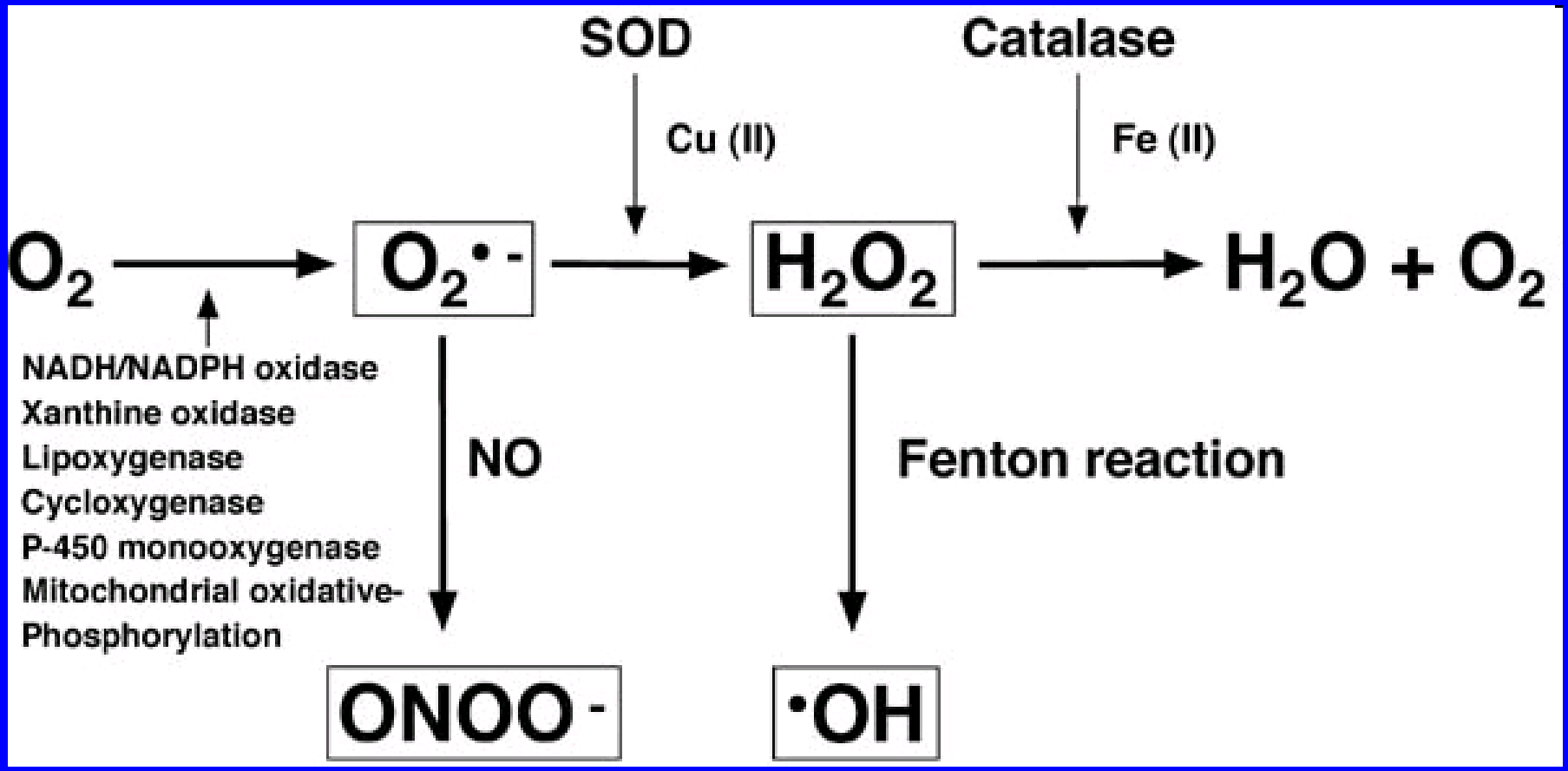
<u>Oxygen</u> – principal molecule in living organisms

Oxygen increase or reactive derivatives -> toxicity

ROS = Reactive Oxygen Species: *Sources*

- production in mitochondria (byproducts)
- redox-cycling (quinones of xenobiotics)
- Fenton-reaction (metals)
- oxidations mediated via MFO (CYP)
- depletion of antioxidants (reactive molecules)

Reactive Oxygen Species (ROS)



SOD = Superoxide dismutase

Reduction of molecular oxygen to superoxide radical

$$O2 + e^- \rightarrow {}^{\bullet}O2^-$$

Dismutation of superoxide radical

$$2\ {}^{\bullet}O_2^- + 2\ H^+ \rightarrow H_2O_2 + O_2$$

Transition metal catalyzed reaction (Fenton reaction)

$${}^{\bullet}O_2^- + Me^{n+} \rightarrow Me^{(n-1)+} + O_2$$

$$Me^{[n-1]+} + H_2O_2 \rightarrow Me^{n+} + OH^- + {}^{\bullet}OH$$

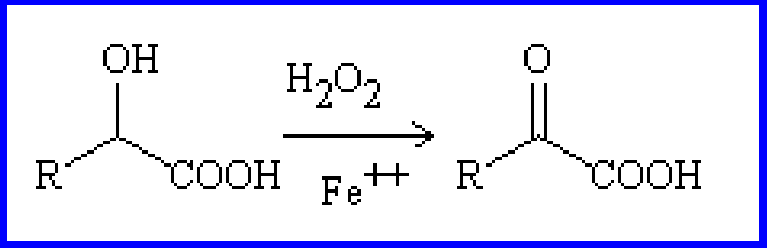
Haber-Weiss reaction

$${}^{\bullet}O2^- + H_2O_2 \rightarrow O_2 + OH^- + {}^{\bullet}OH$$

Me = metal (e.g. Fe^{3+}/Fe^{2+})

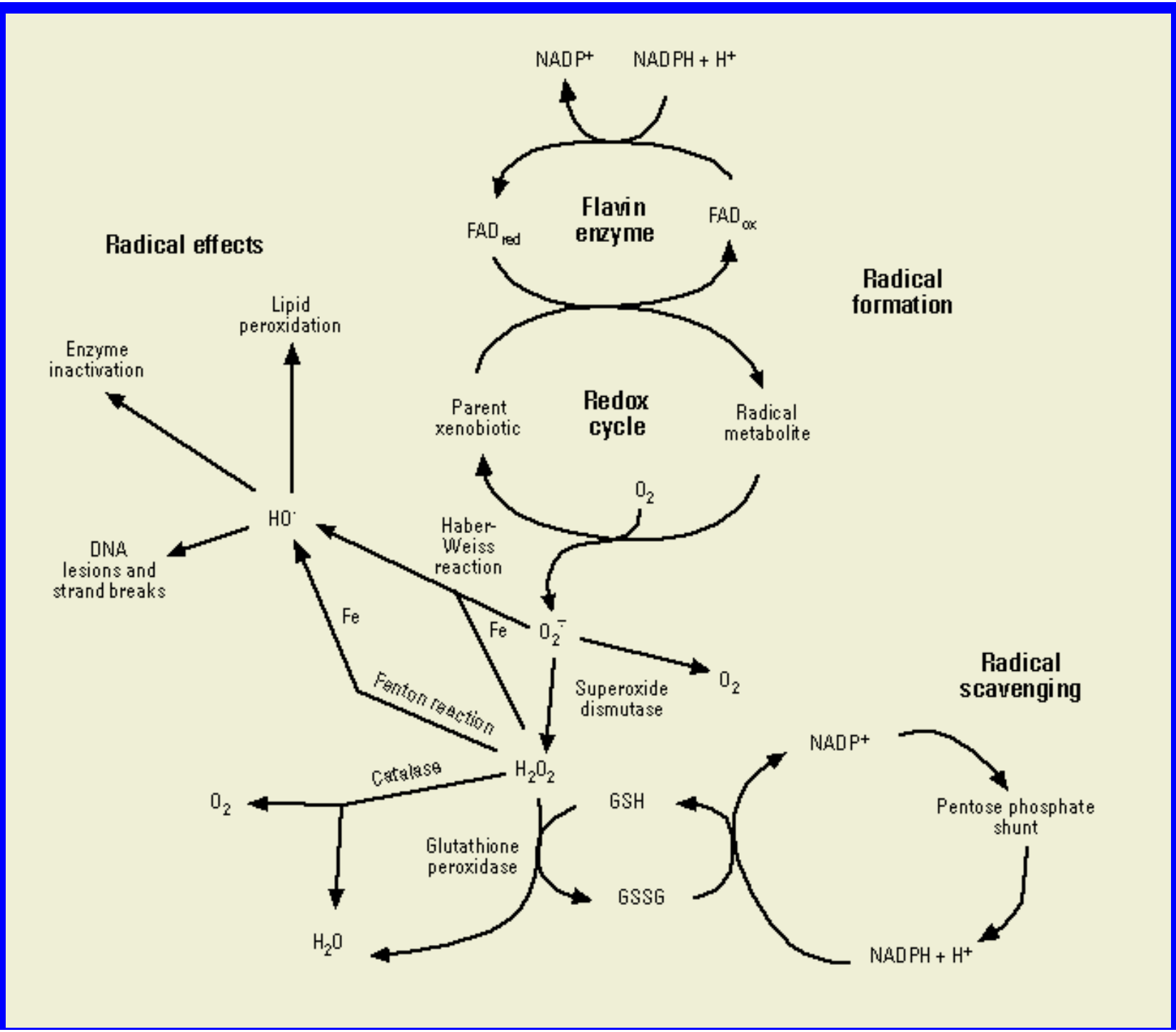
${}^{\bullet}O_2^-$ = superoxide radical (superoxide anion)
${}^{\bullet}OH$ = hydroxyl radical
OH^- = hydroxyl anion
H_2O_2 = hydrogen peroxide

Fenton reaction



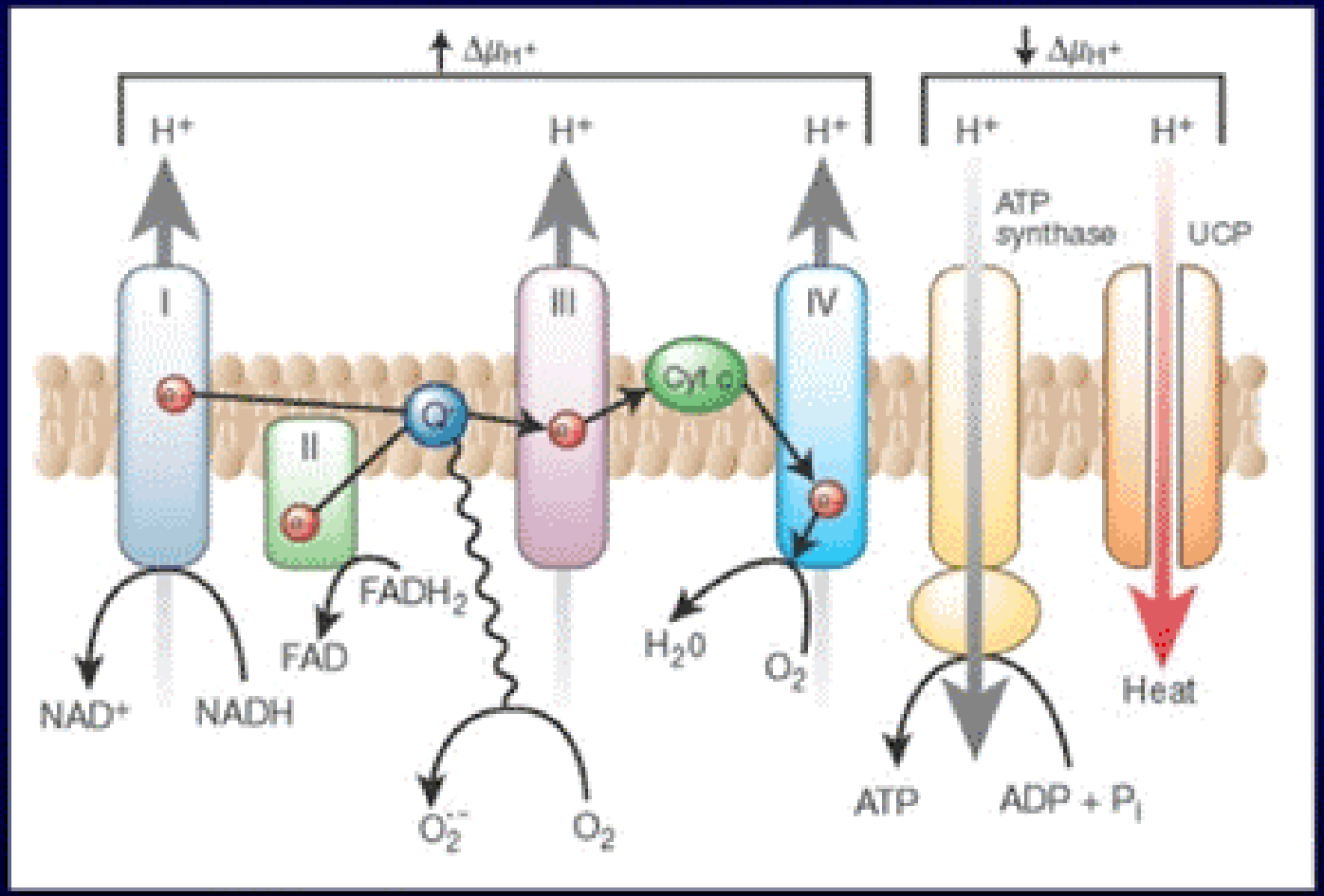
Reactive Oxygen Species (ROS)

ROS	Antioxidant	Rate constant, $M^{-1} \cdot sec^{-1}$
Superoxide anion of oxygen	carnosine	$5.0 \cdot 10^{-5}$
	carnosine	$0.8 \cdot 10^{-5}$
	ascorbate	$2.7 \cdot 10^{-5}$
	α-tocopherol	$2.0 \cdot 10^{-5}$
Singlet oxygen	carnosine	$3 \cdot 10^{-7}$
	imidazole	$2 \cdot 10^{-7}$
	ergothioneine	$2 \cdot 10^{-7}$
	NaN_3	$44 \cdot 10^{-7}$
Hydroxyl radical	carnosine	$(5\text{-}8) \cdot 10^{-9}$
		$9 \cdot 10^{-9}$


NADP+
NADPH + H+
Flavin enzyme
FAD red
FAD ox
Radical effects
Radical formation
Lipid peroxidation
Enzyme inactivation
Parent xenobiotic
Redox cycle
Radical metabolite
O2
HO·
Haber-Weiss reaction
DNA lesions and strand breaks
Fe
Fe
O2-
O2
Fenton reaction
Superoxide dismutase
Radical scavenging
Catalase
H2O2
O2
GSH
NADP+
Pentose phosphate shunt
Glutathione peroxidase
GSSG
H2O
NADPH + H+

ROS & mitochondria

Glucose-Derived ROS: Mitochondrial Electron Transport System



Examples of chemical-induced oxidative stress

- **Metals:**

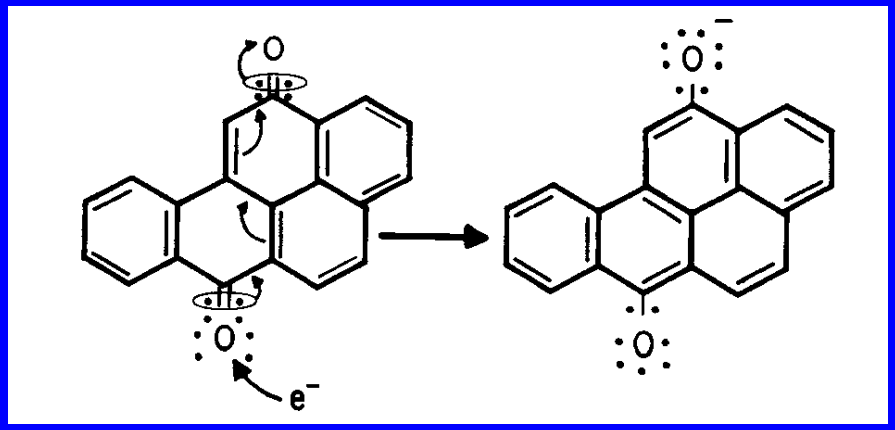
  fenton reaction -> OH*

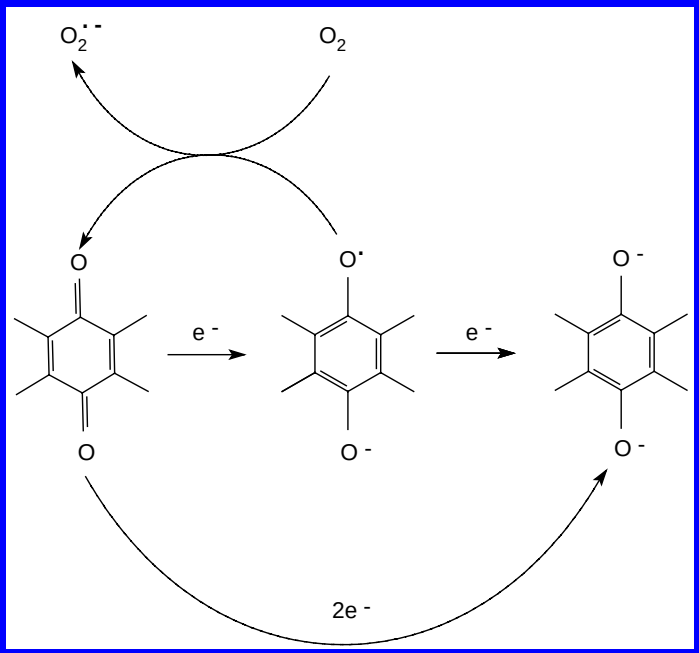
- **Redox-cycling chemicals:**

  oxy-PAHs

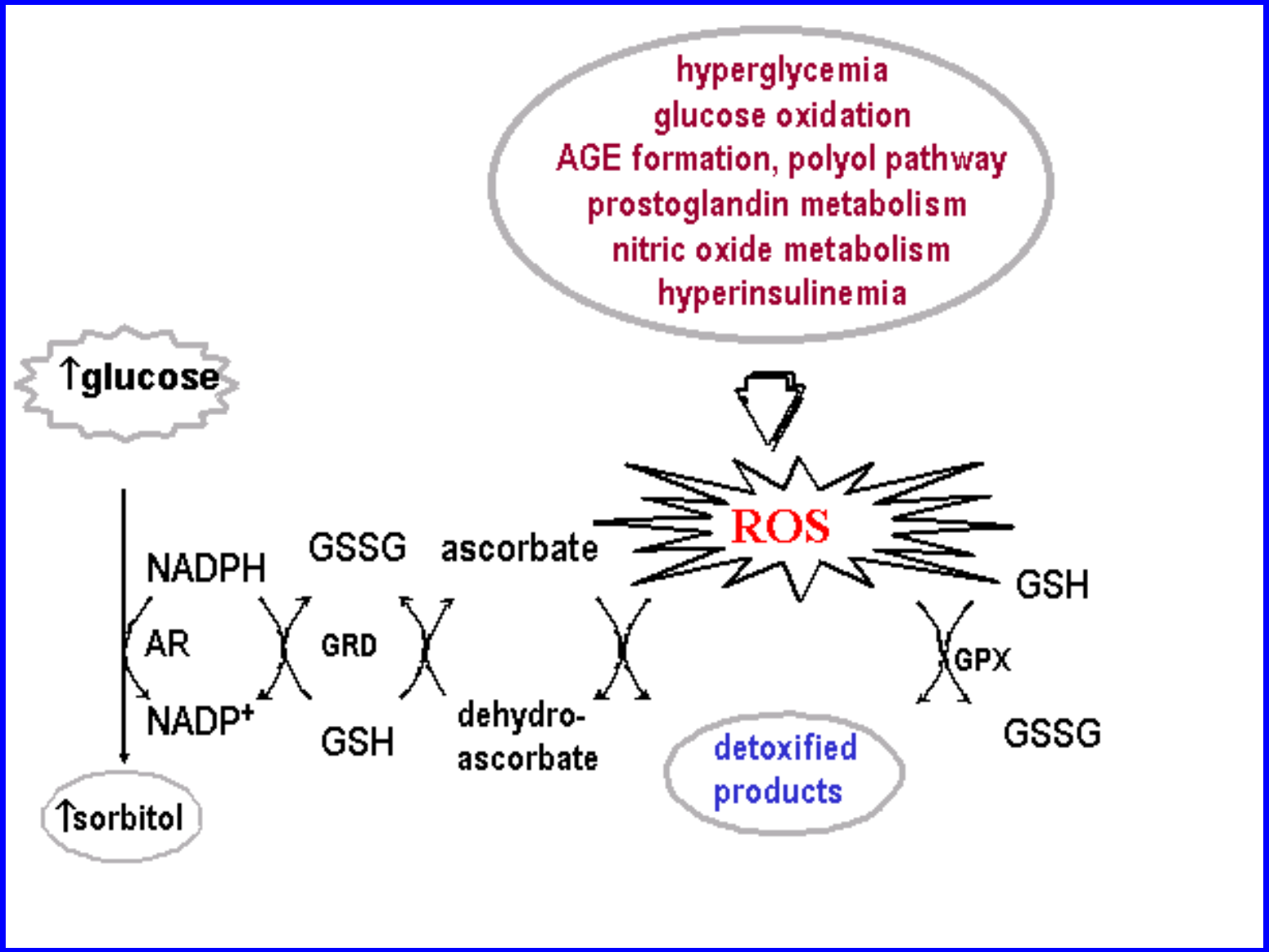
- Depletion of GSH:

  reactive molecules, GST-conjugation, metals: SH oxidation ...





Antioxidant depletion
GSH (glutathione)



Biomarkers of oxidative damage

BIOMARKER	AVAILABILITY	FREQUENTLY USED ASSAYS
Lipid Peroxidation		
F_2-isoprostanes	Plasma, urine	GC/MS, HPLC-MS/MS
Oxidized low-density lipoprotein (oxLDL)	Plasma, serum	ELISA
Malondialdehyde (MDA)	Plasma, serum, saliva, urine, exhaled breath condensate	Colorimetry, spectrophotometry, HPLC +fluorescence, GC/MS
Protein Oxidation		
Protein carbonyls	Plasma, serum	ELISA
DNA Oxidation		
8-hydroxy-2-deoxyguanosine (8-OHdG)	Plasma, serum, urine	HPLC-EC, HPLC-MS/MS*, GC/MS, Comet assay*