DNA damage
mutagenicity and genotoxicity

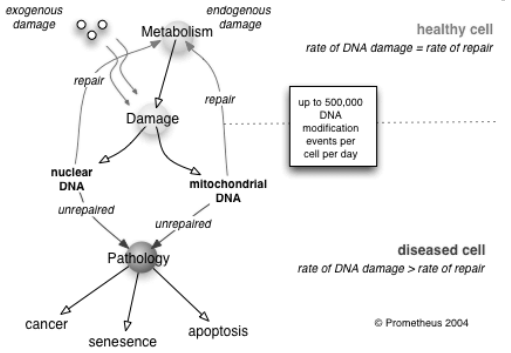
DNA:
- **principal molecule for life of the cell**
- **structure and function carefully checked**
- **changes rapidly repaired**
- **irreversible changes -> cell death** *(apoptosis)*

Mutagenesis - MUTATIONS
- changes in the sequences of deoxynucleotides

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

- chemical-induced mutagenesis

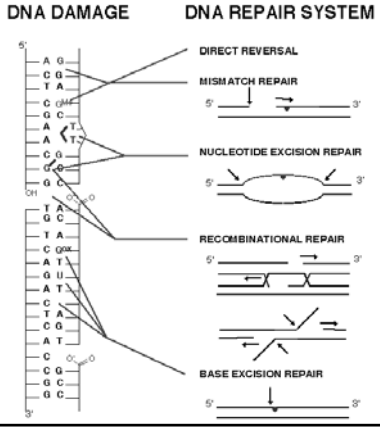
DNA damage



DNA repair

Damage of DNA is carefully controlled
constitutively expressed proteins

Changes in DNA
induction of reparation enzymes ("SOS-repair")
= biomarker of DNA damage



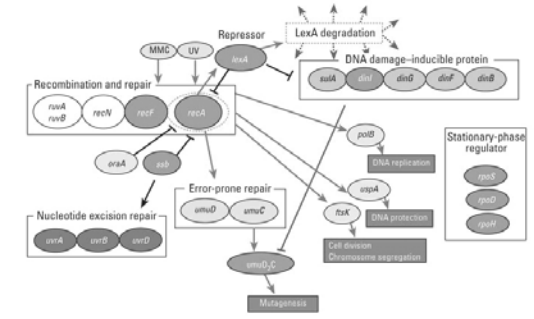


Figure 3. A literature-based linkage map between genes in the SOS response in *E. coli*. The map represents inducible genes/proteins in the SOS response for repair from DNA damage. Black lines indicate pathways in the normal repair process and red lines with arrows activation/induction due to an exposure 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

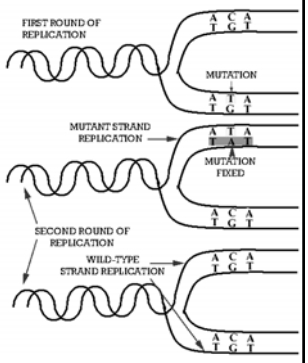
Indirect mutagens - affect cells that synthesize chemicals with direct mutagenic effect

Point mutations
BASE - EXCHANGE:

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

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

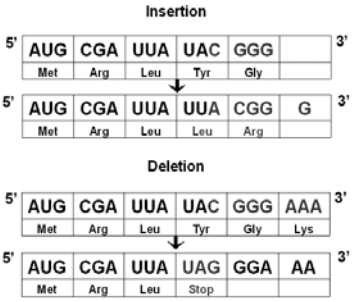
•Nonsense mutations:
- which code for a stop



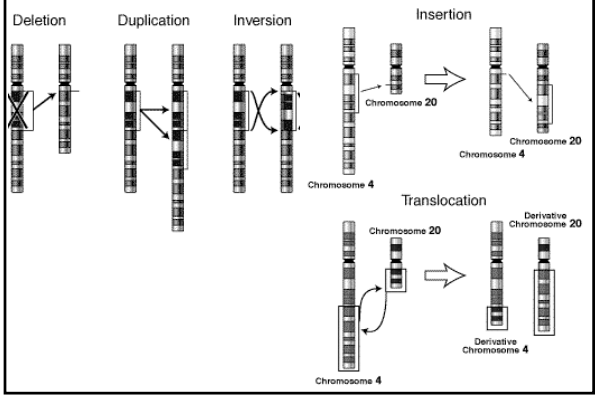
Point mutation

INSERTION
DELETION

Change of the reading frame



Large scale mutations / chromosomal



Physical factors & DNA damage

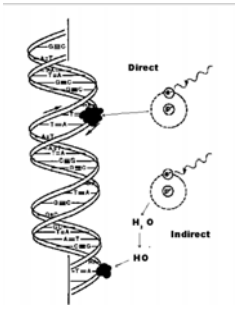
Ionizating radiation

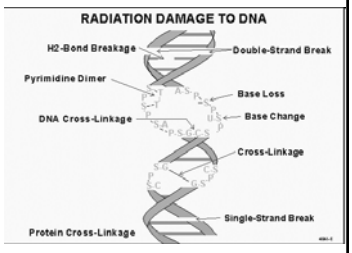
- direct interaction with hydrogen atoms in water (and bases)
  -> OH* radicals; H_2O_2, O_2-
- oxidation of bases; dimerization ...

UV radiation

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

Ionizing radiation effects on DNA



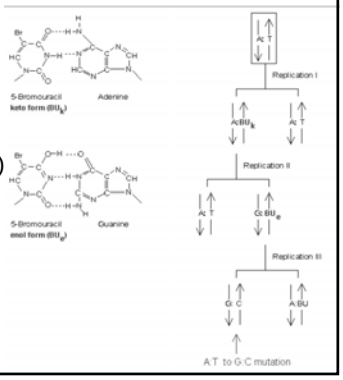


Chemical induced DNA damage

Bases analogs

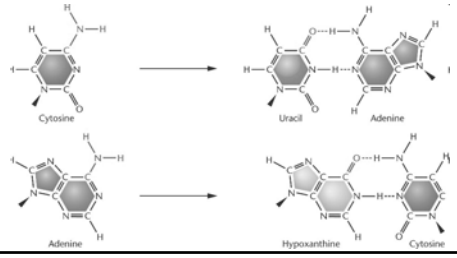
- incorporation into DNA during replication

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



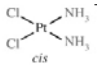
Chemical induced DNA damage

HNO_2, HSO_3^-,Hydroxylamine, Methoxyamine
deamination of bases
(GC -> AT)

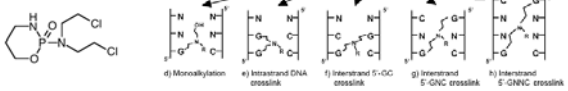


Chemical induced DNA damage

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*)

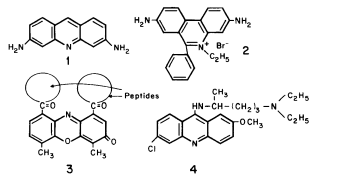
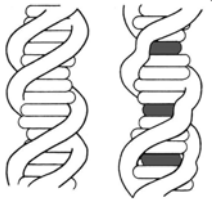
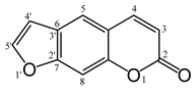
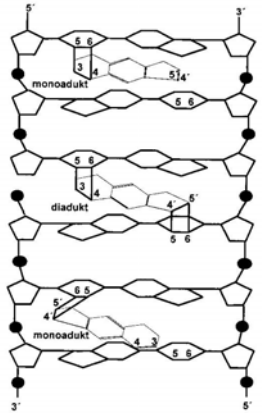


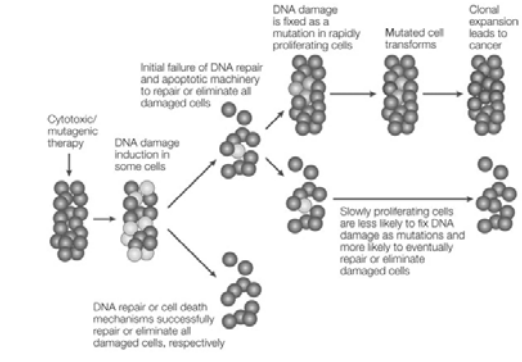
Chart 5.8. Examples of intercalating agents. Key: 1, acriflavine; 2, ethidium bromide; 3, actinomycin; 4, quinacrine.



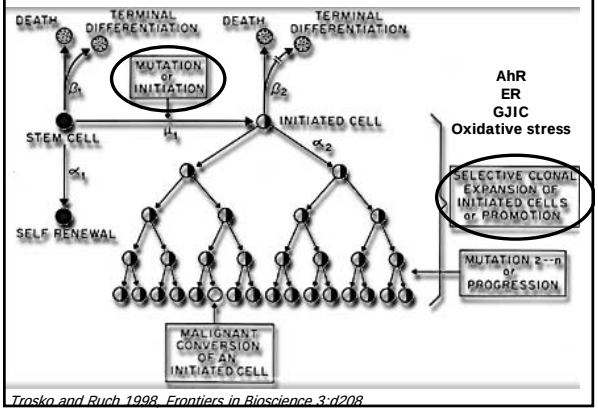
Psoralen
DNA intercalation



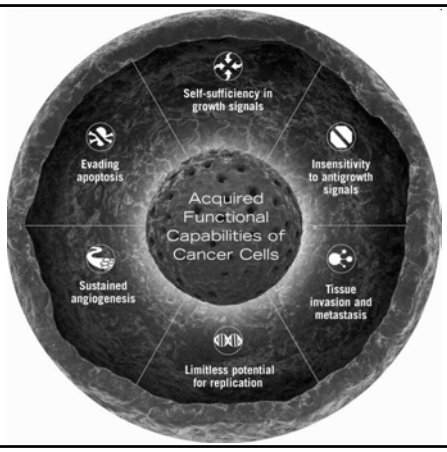




IMPORTANT PROCESSES IN CANCEROGENESIS

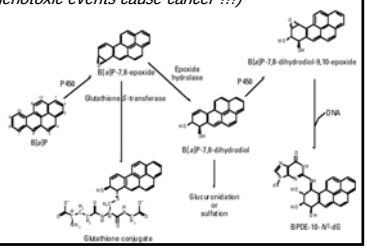


Trosko and Ruch 1998, Frontiers in Bioscience 3:d208



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

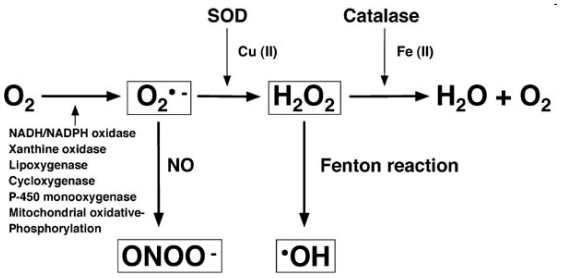
-> overproduction of oxidants:
= oxidative stress
GENERAL MECHANISM OF TOXICITY

Overproduction of oxidants

Oxygen – 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)

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

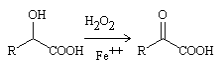
$Me^{(n-1)+} + H_2O_2 \rightarrow Me^{n+} + OH^- + {}^{\bullet}OH$

Haber-Weiss reaction

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

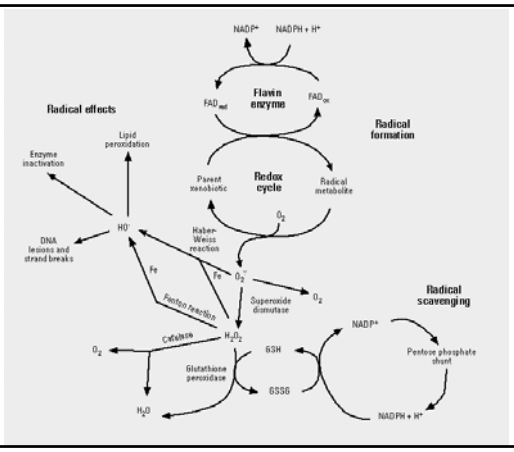
Me = metal (e.g.Fe^{3+}/Fe^{2+})
${}^{\bullet}O_2^-$ = superoxide radical (superoxide anion)
${}^{\bullet}OH$ = hydroxyl radical
OH^- = hydroxyl anion
H_2O_2 = hydrogen peroxide

Fenton reaction

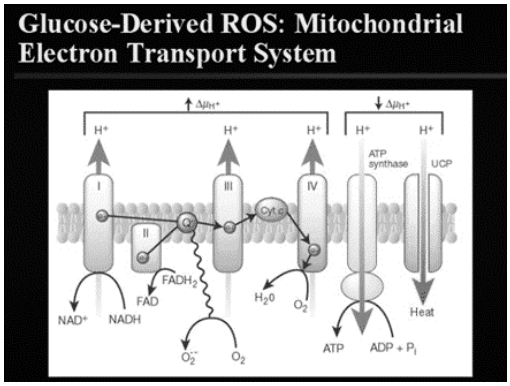


Reactive Oxygen Species (ROS)

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	$(5\text{-}8) \cdot 10^{9}$
		$9 \cdot 10^{9}$

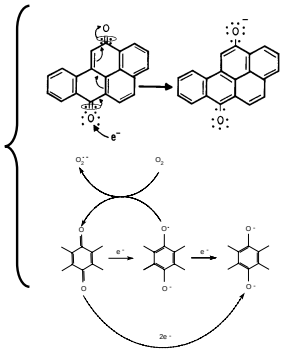


ROS & mitochondria

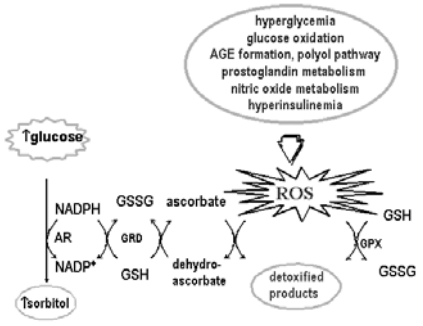


Examples of chemical-induced oxidative stress

- **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*