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

-> overproduction of oxidants: depletion of antioxidants, oxidation of biological molecules (membranes, proteins, DNA ...)
-> disruption of signalls (GSH), carcinogenesis, health problems, necrosis ...

= oxidative stress

Overproduction of oxidants

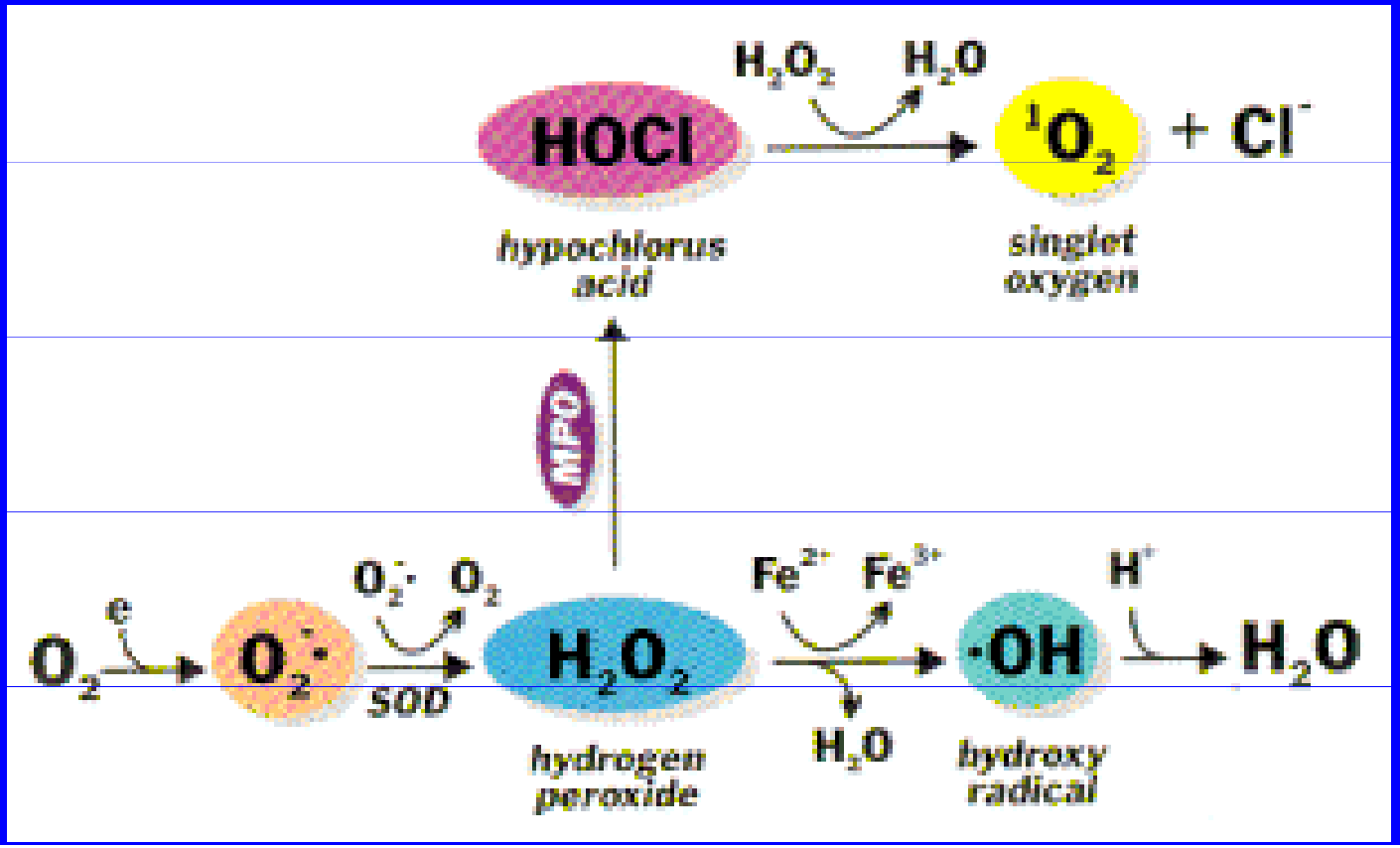
Oxygen – principal molecule in living organisms

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)

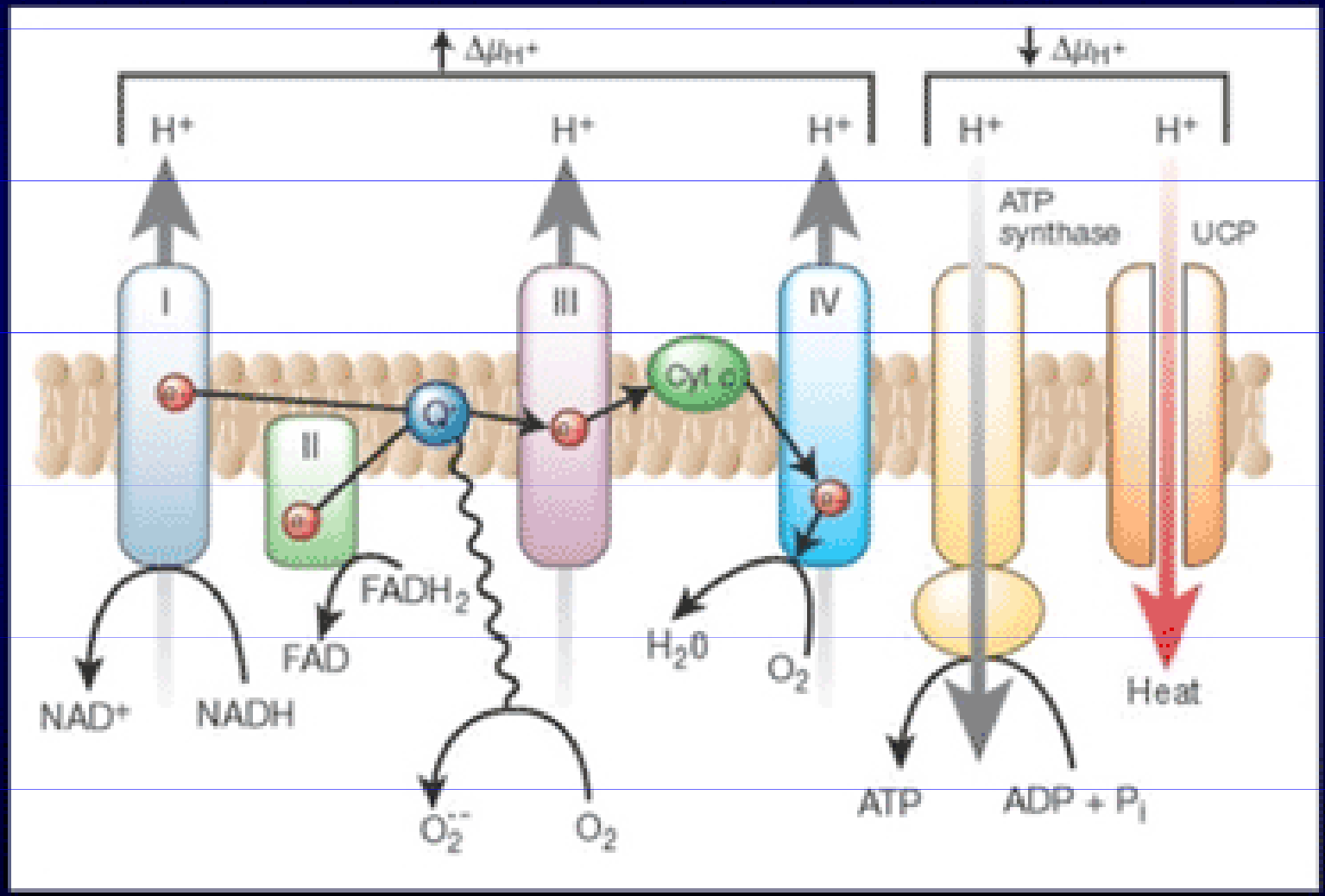
Reactive Oxygen Species (ROS)



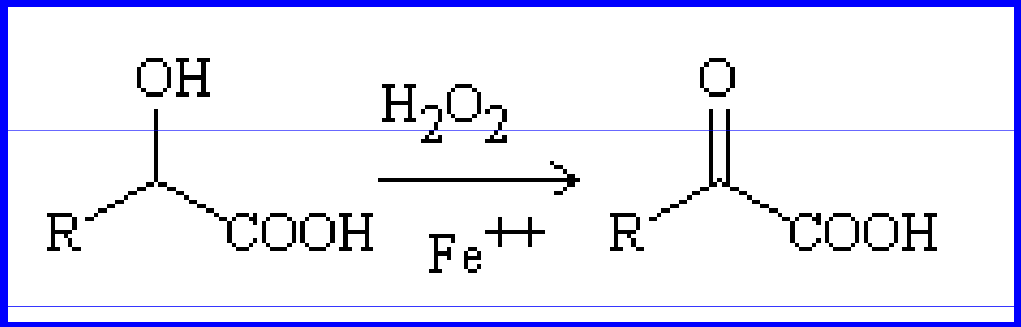
Druh	Symbol	Poločas života (s) při 37 °C
Superoxid	$O_2^{\bullet -}$	1×10^{-6}
Hydroxylový radikál	$OH^{\bullet}$	1×10^{-9}
Alkoxylový radikál	$RO^{\bullet}$	1×10^{-6}
Peroxylový radikál	$ROO^{\bullet}$	1×10^{-2}
Singletový kyslík	O_2	1×10^{-6}
Molekulární kyslík	O_2	$> 10^{2}$

ROS & mitochondria

Glucose-Derived ROS: Mitochondrial Electron Transport System

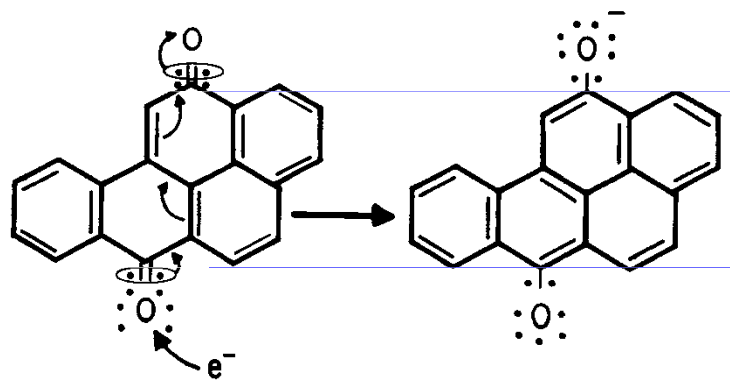


Fenton reaction

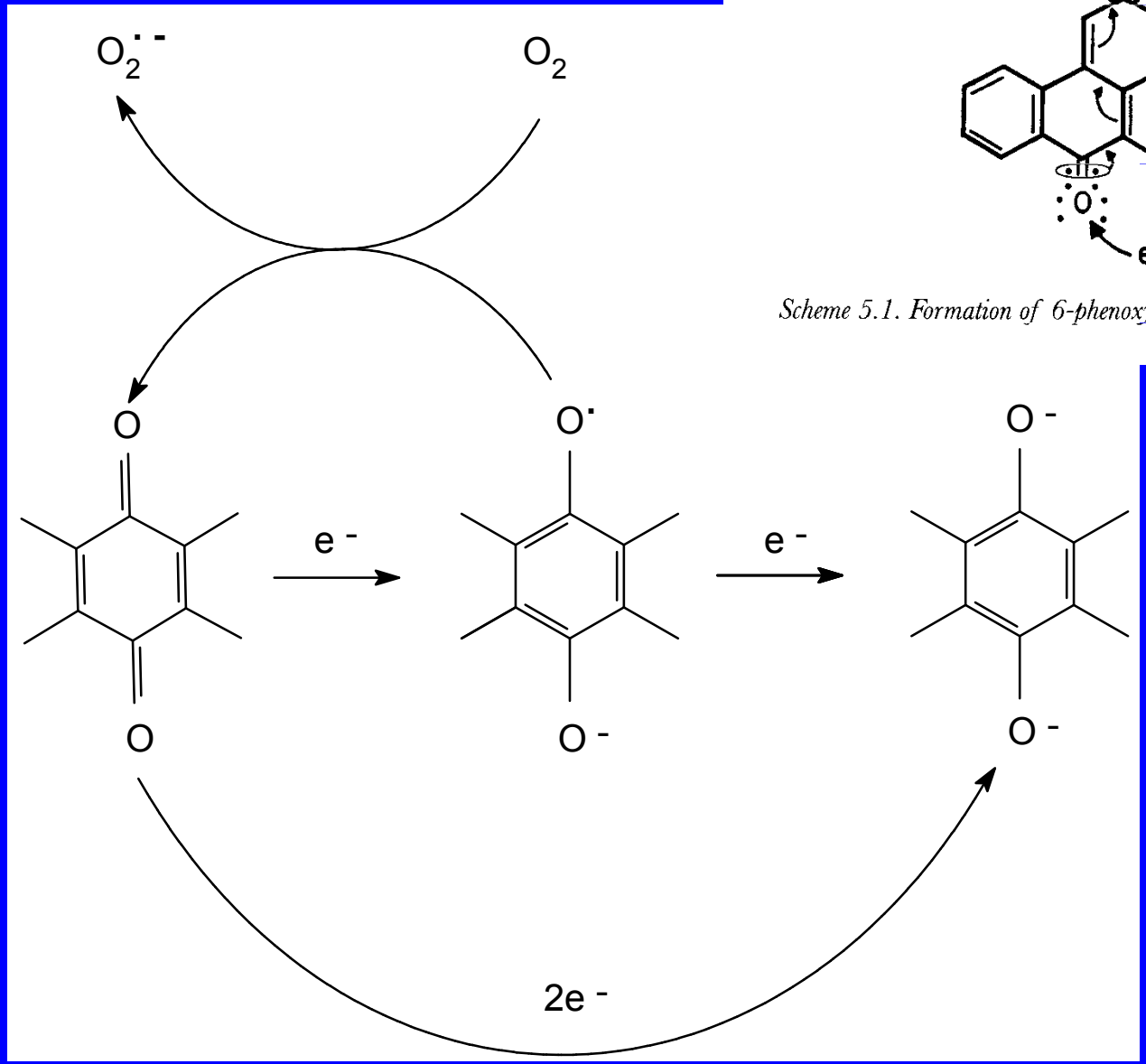


$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH + OH^-$$

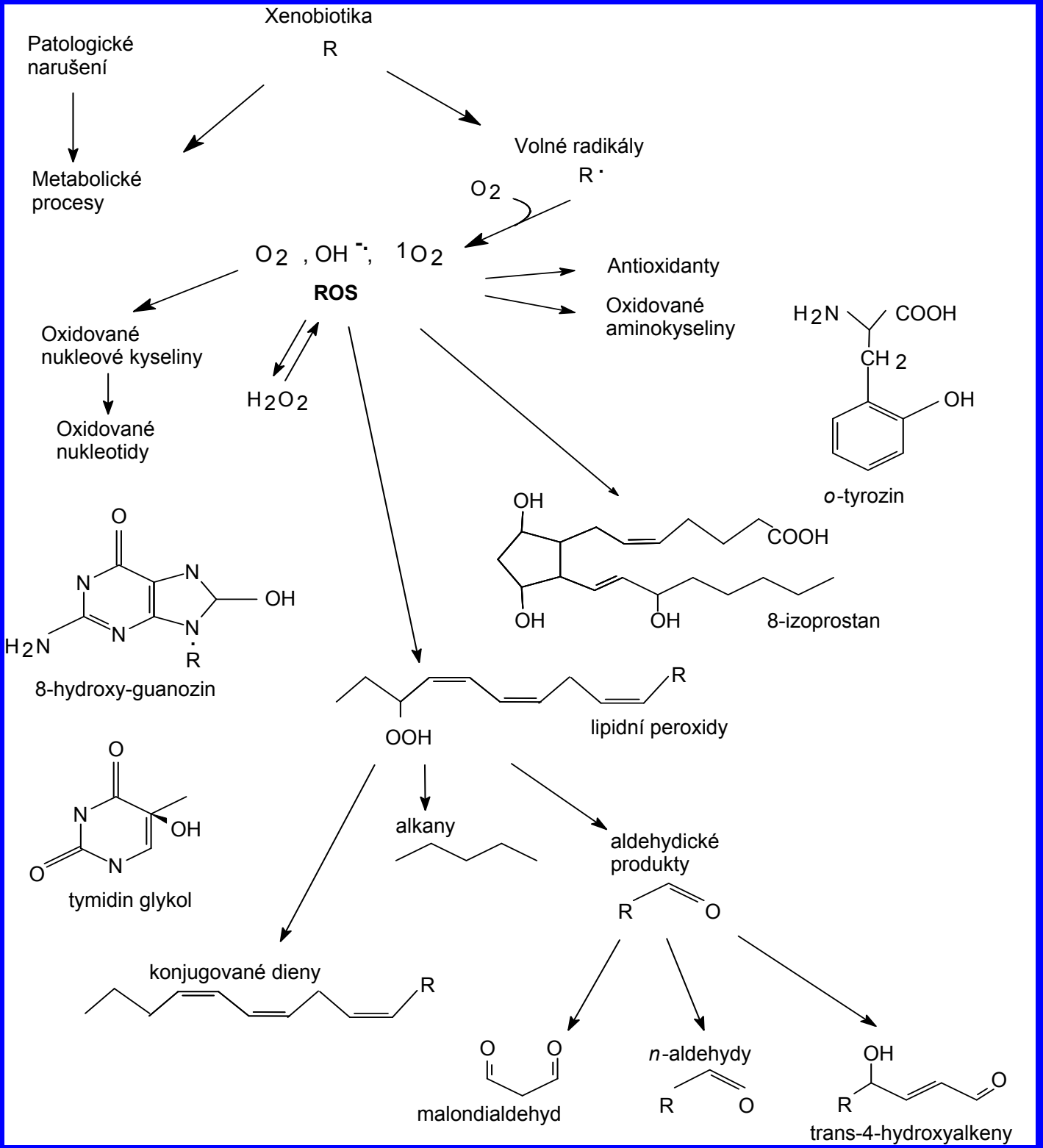
Redox-cycling and ROS formation



Scheme 5.1. Formation of 6-phenoxy radical from benzo[a]pyrene-6,12-quinone (see Chapter 3).



Toxicity of ROS



Examples of chemical-induced oxidative stress

- Metals: fenton reaction -> OH*

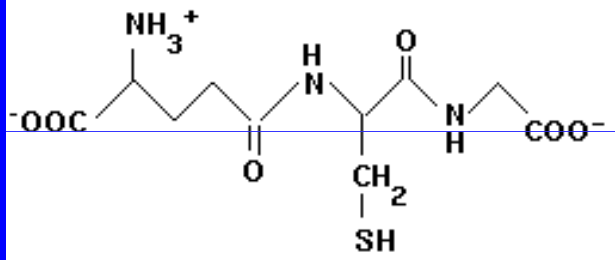
- Depletion of GSH:

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

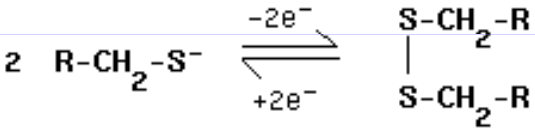
- Redox-cycling chemicals: oxy-PAHs

GSH
and its depletion

Glutathion: Glu–Cys–Gly



Reduktion von Glutathion in die Disulfidform:



1. Scavenge-Reaktionen

O_2 + G–SH ⟶ G–$S_{ox.}$

O_2 + G–SH + 2 H* ⟶ G–S–S–G + H_2O_2

2 OH + 2 G–SH ⟶ G–S–S–G + 2 H_2O

H_2O_2 + G–SH ⟶ G–S–S–G + 2 H_2O

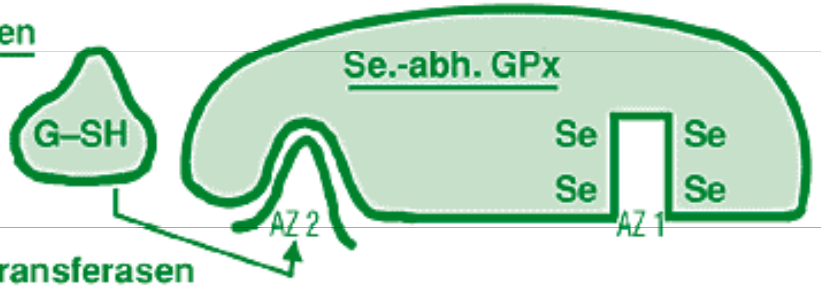
2. Gemischte Disulfide

2 G–SH ⇌ gemische Disulfide ⇌ G–S–S–G

- Coenzym A
- 1-Cystein
- Serum-Albumin
- Cristallin
- Ascites-Tu.-Zellen

3. Enzymreaktionen

Se.-abh. GPx



Glutathion-S-Transferasen

Glutathion-Reduktase

4. Konjugationsmoleküle für Xenobiotika

Biomarkers of oxidative damage

Poškození	Produkt	Stanovení	Citace
fosfolipidy	**MDA**	TBARS assay, HPLC, HPLC s UV-detekcí	Draper et al. 1993, Bird et al. 1983, Selim 1977
DNA	**8-OH-dG**	HPLC, metoda s využitím imunoafinitní izolace	Degan et al. 1991, Loft et al. 1992
proteiny	**o-Tyr**	spektrofotometricky, HPLC, MS	Deneshvar et al. 1997, De Zwart et al. 1998

Vysvětlivky:

MDA	malondyaldahyd
8-OH-dG	8-hydroxy-2^{l}-deoxyguanozin
o-Tyr	orthotyrozin
TBARS	reaktivní látky s kyselinou thiobarbiturovou
HPLC	vysokotlaková kapalinová chromatografie
MS	hmotnostní spektrometrie

DNA damage
mutagenicity and genotoxicity

DNA:

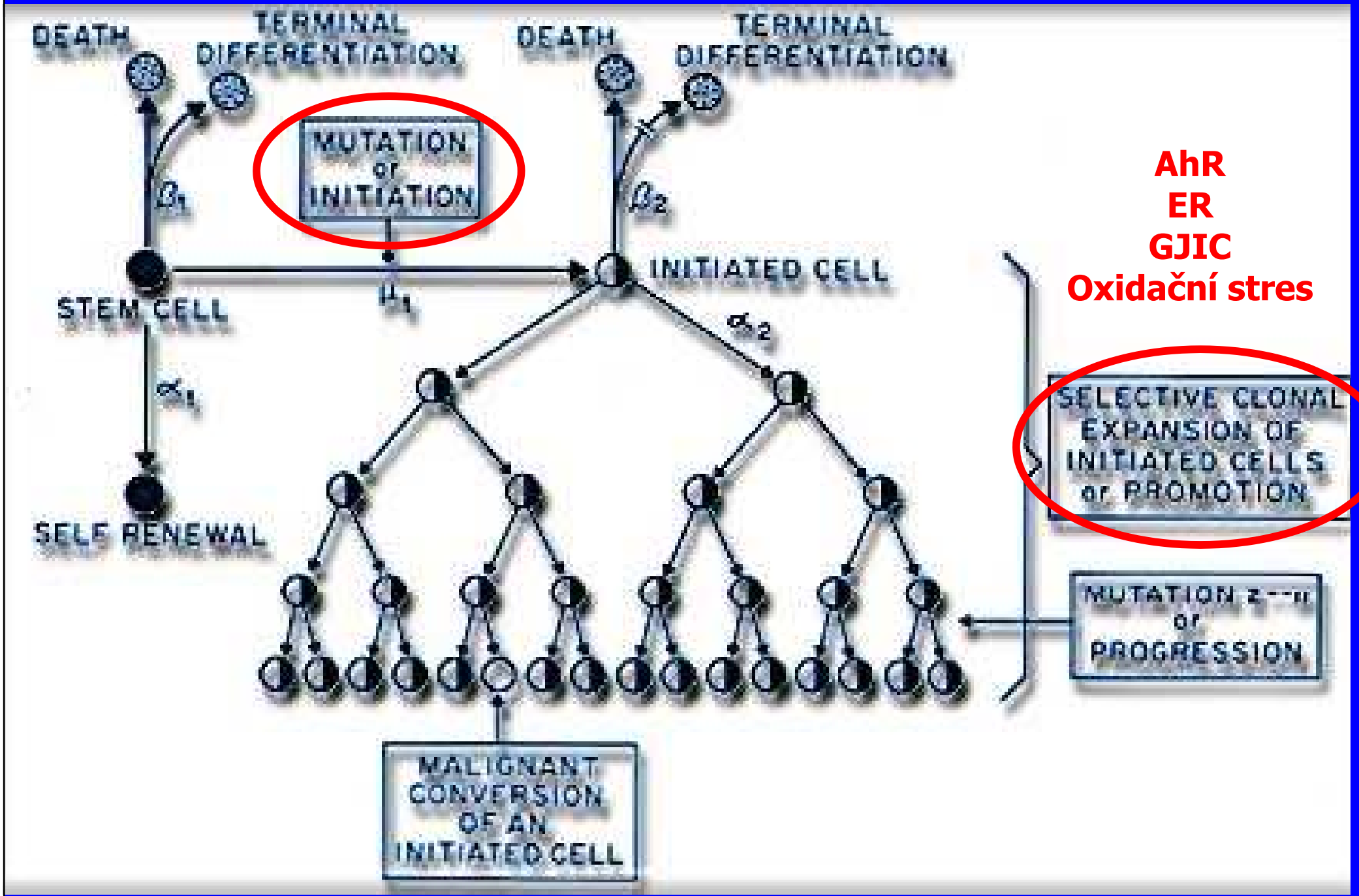
- **principal molecule for life of the cell**
- **structure and function carefully checked**
- **changes rapidly repaired**
- **irreversible changes -> cell death** *(apoptosis)*

Mutagenesis

- changes in the sequences of deoxynucleotides
  - deletions/insertions: changes in reading frame
  - exchanges of nucleotides: changes in aminoacids

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

IMPORTANT PROCESSES IN CANCEROGENESIS



AhR
ER
GJIC
Oxidační stres

Trosko and Ruch 1998, Frontiers in Bioscience 3:d208

Chemical induced DNA damage

Bases analogs

- incorporation into DNA during replication (5-Br-Uracil: AT -> GC)

HNO_2, HSO_3^-, Hydroxylamine, Methoxyamine-

- deamination of bases (GC -> AT)

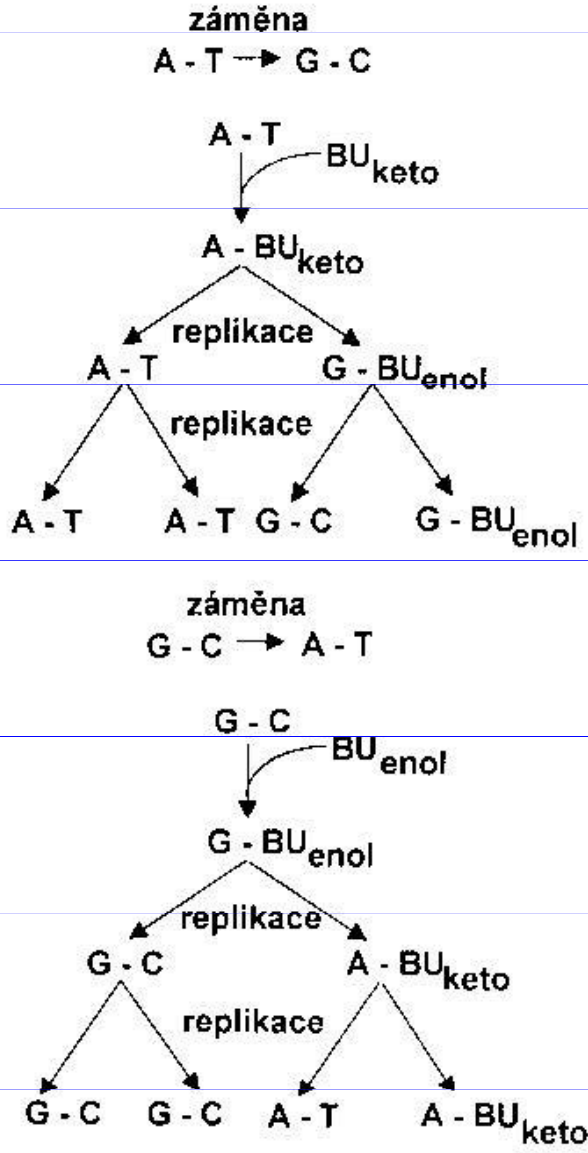
Alkylsulphates, N-nitroso-alkyles, cis-platinum

- alkylation of bases; crosslinks of dsDNA

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

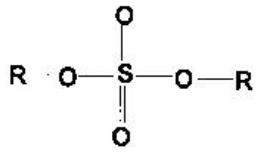
Mycotoxins (aflatoxins)

- require metabolic activation by CYPs
- aduct formation with DNA (*biomarkers*)



Obr. 371

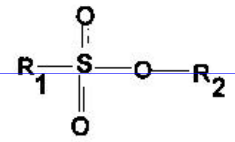
Záměny párů bází v DNA pod mutagenním účinkem brómuracilu



dialkylsulfát

Příklad: dimetylsulfát

$R = -CH_3$



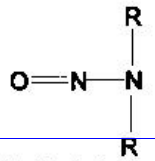
alkyl-alkan-sulfonát

Příklady: metylmetansulfonát

$R_1 = R_2 = -CH_3$

etylmetansulfonát

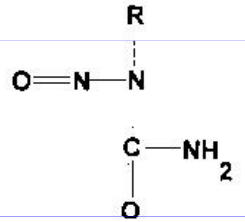
$R_1 = -CH_3$ $R_2 = -C_2H_5$



dialkylnitrózamin

Příklad: dimetylnitrózamin

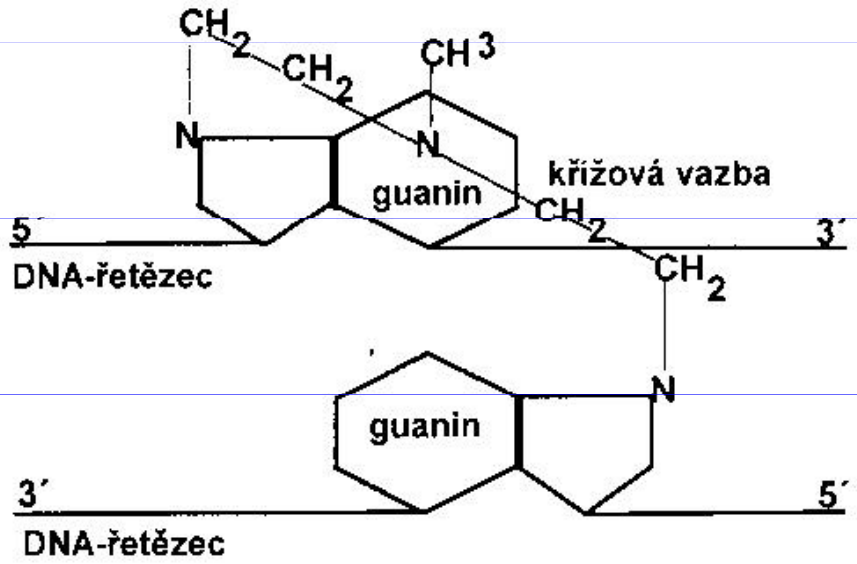
$R = -CH_3$

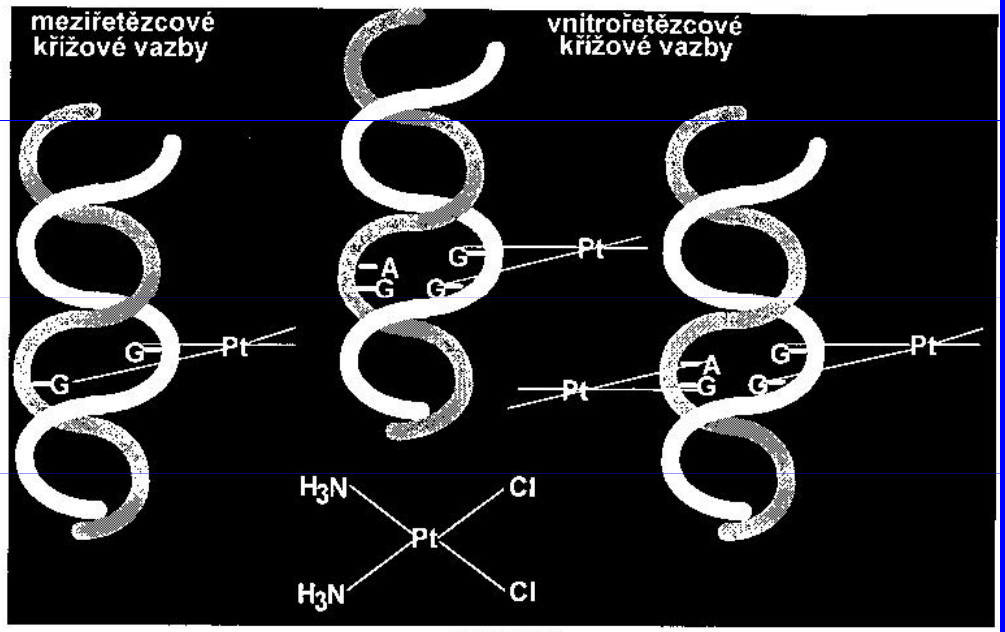


N-alkyl-nitrózomočovina

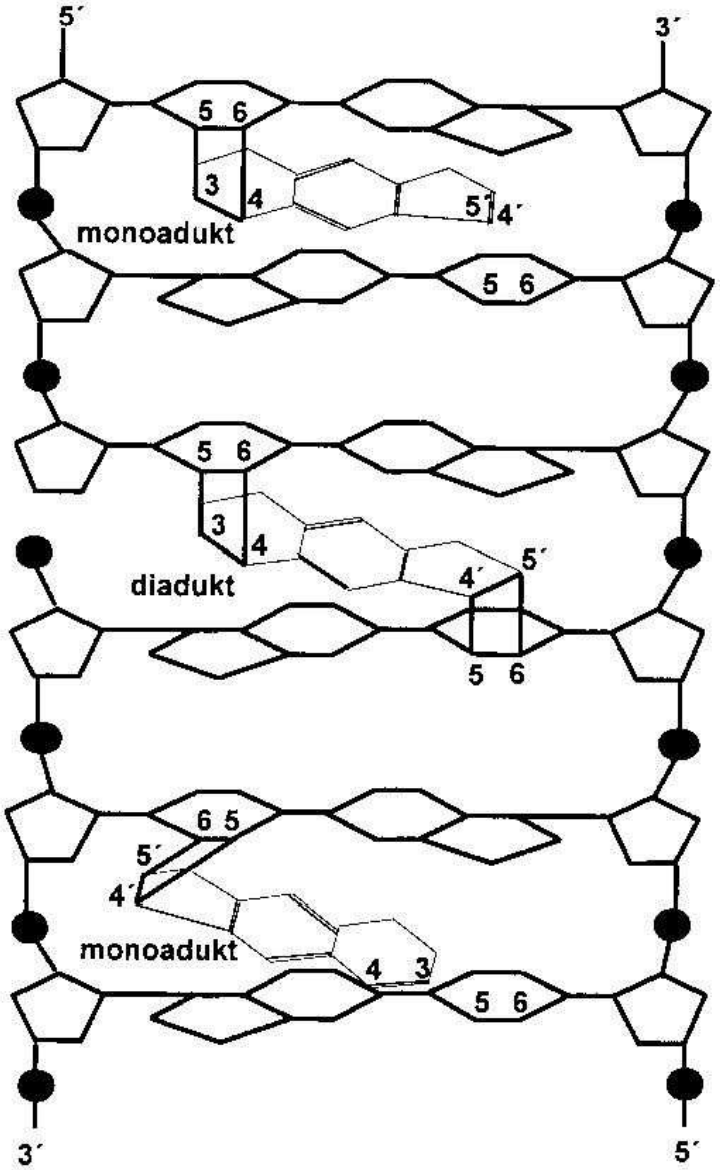
Příklad: N-metyl-N-nitrózomočovina

$R = -CH_3$





Obr. 375
Křížové vazby tvořené cis-platinou



Obr. 377
Interakce psoralenu s DNA za tvorby monoaduktů a diaduktů

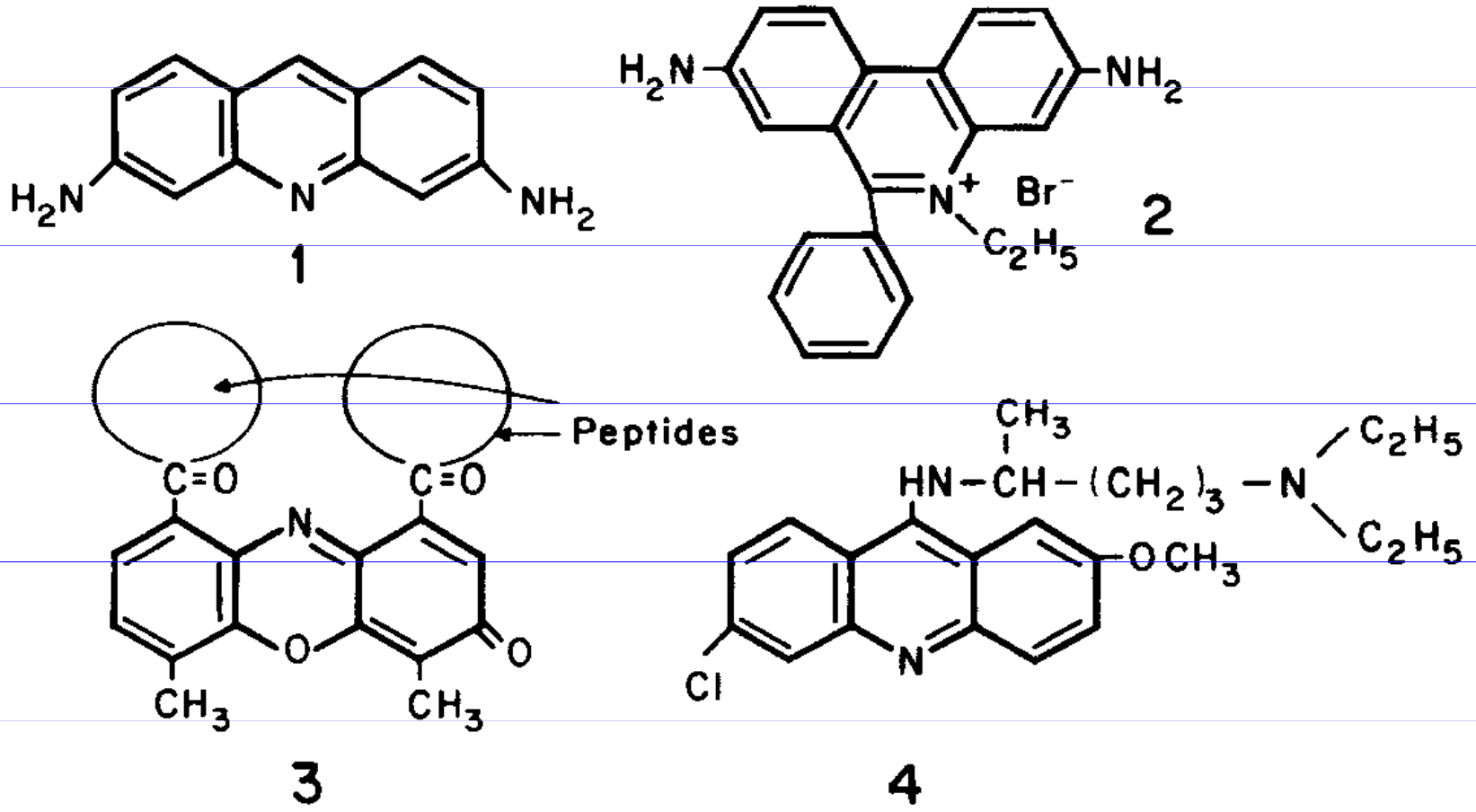
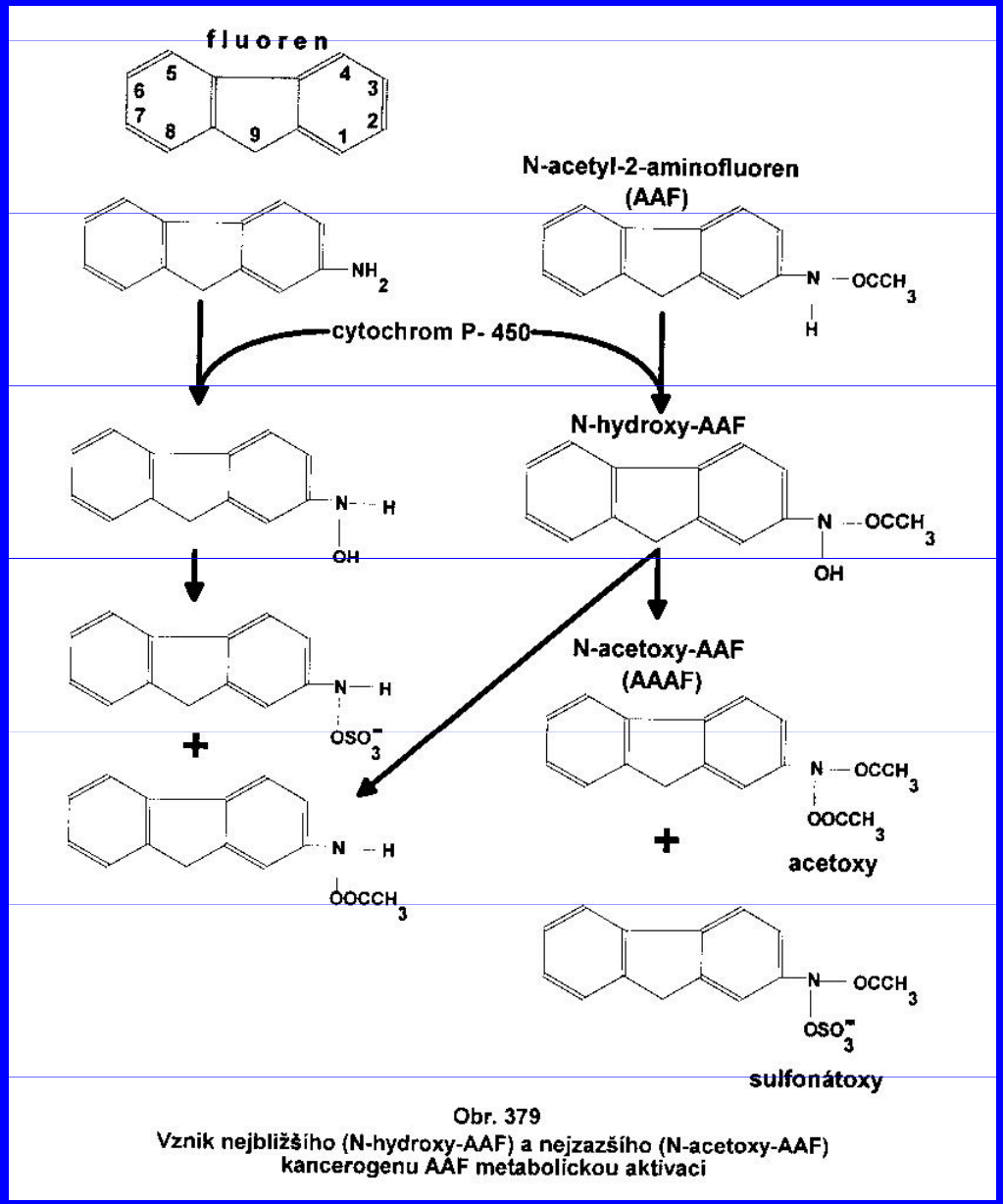
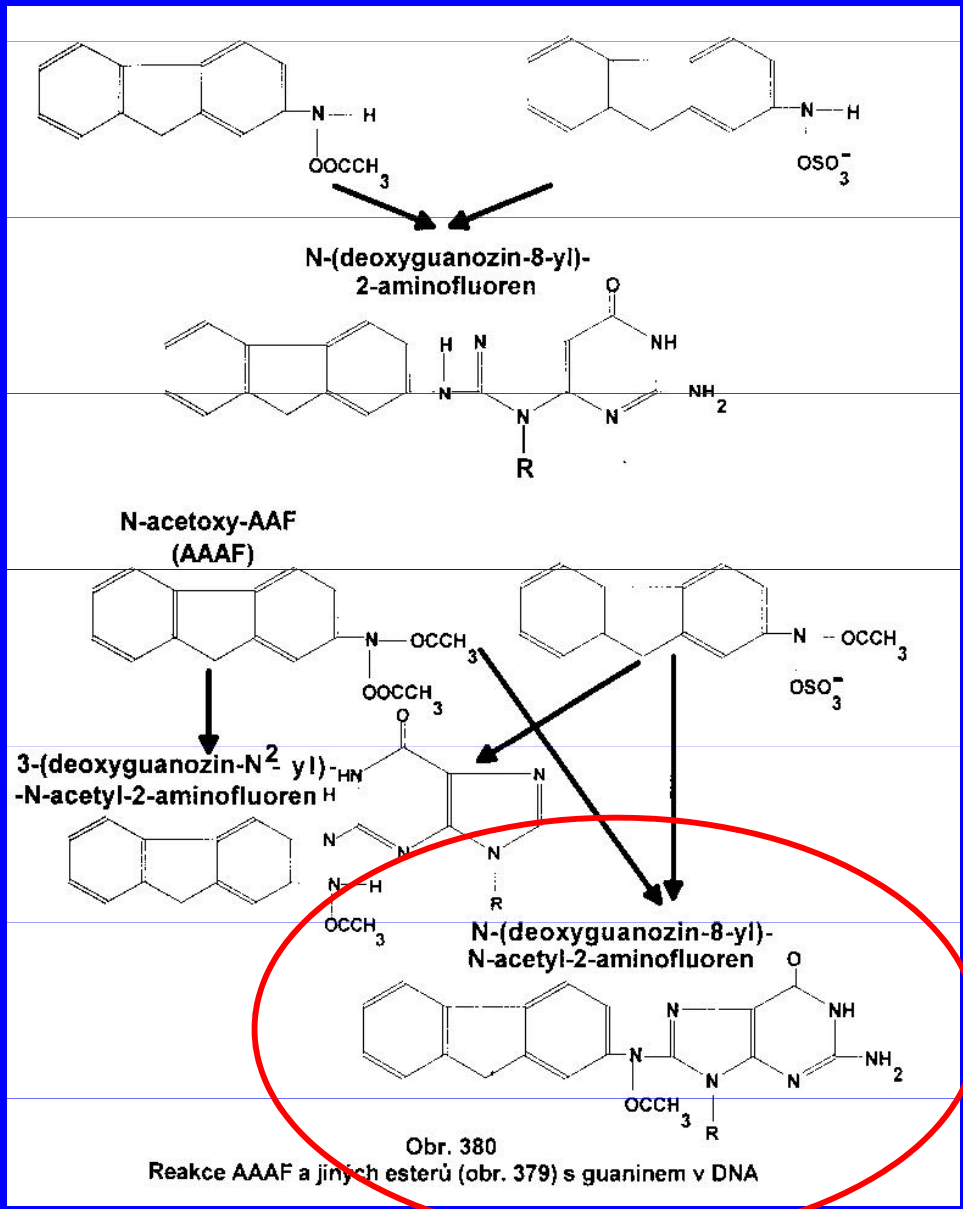


Chart 5.8. Examples of intercalating agents. Key: 1, acriflavine; 2, ethidium bromide; 3, actinomycin; 4, quinacrine.

Metabolic activation of PAH and DNA-aduct formation

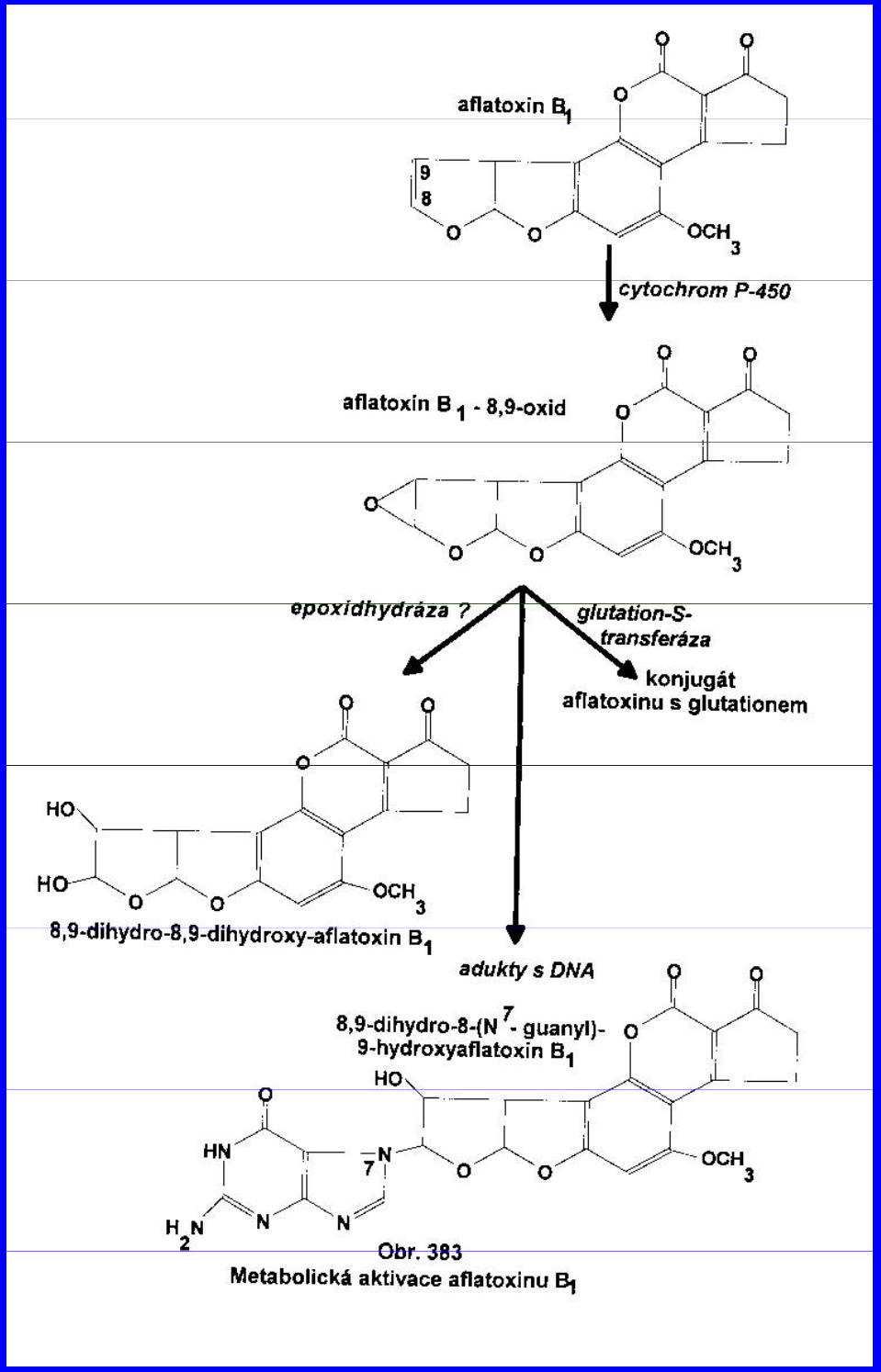


Obr. 379
Vznik nejbližšího (N-hydroxy-AAF) a nejzazšího (N-acetoxy-AAF) kancerogenu AAF metabolickou aktivací



Obr. 380
Reakce AAAF a jiných esterů (obr. 379) s guaninem v DNA

Metabolic activation of aflatoxin and formation of DNA-aducts



Obr. 383
Metabolická aktivace aflatoxinu B_1

Does **chemically-induced genotoxicity** has effects in vivo ?

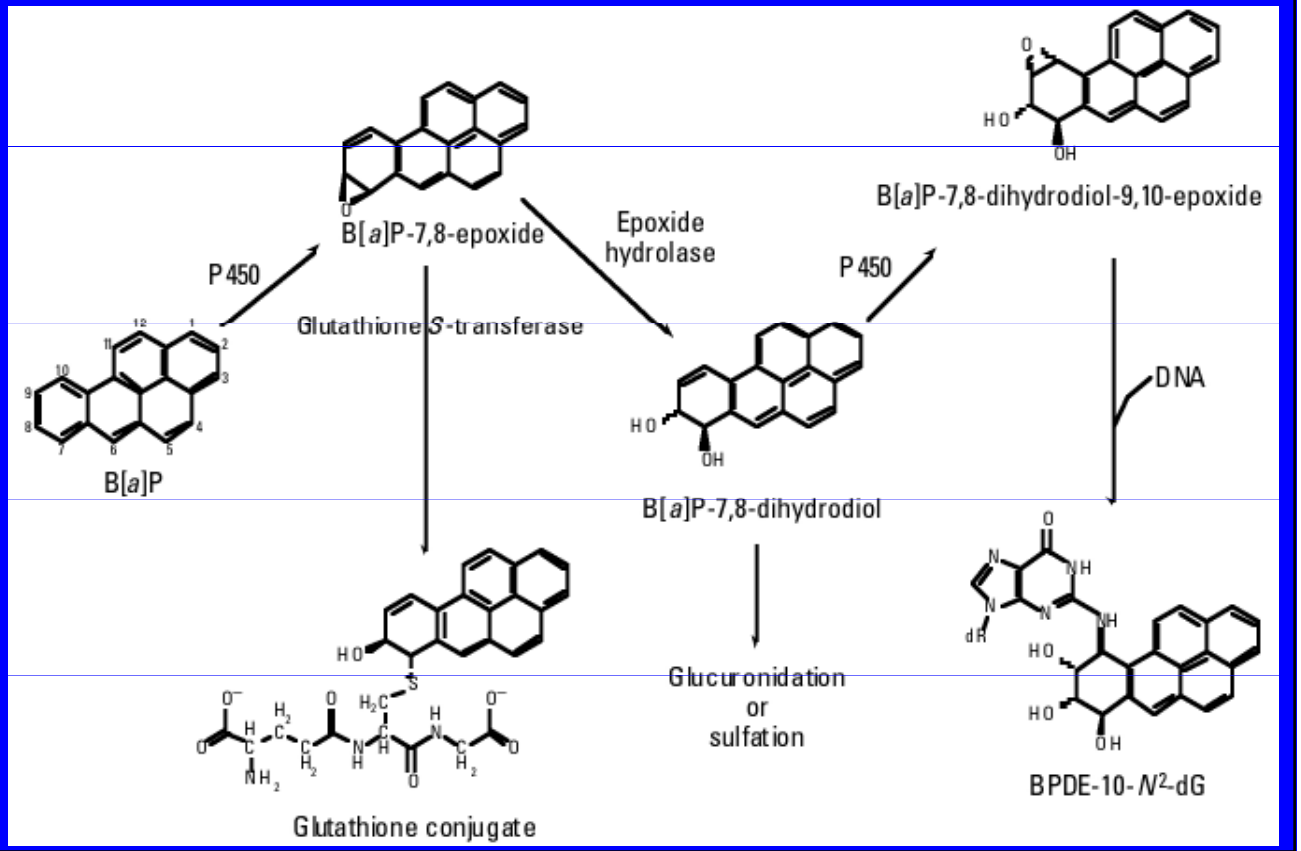
- adducts from mitochondrial DNA ?
- distance between „source of radicals" and nuclear DNA ?
- protection mechanisms (mutation -> death)

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 !!!)*



Physical factors & DNA damage

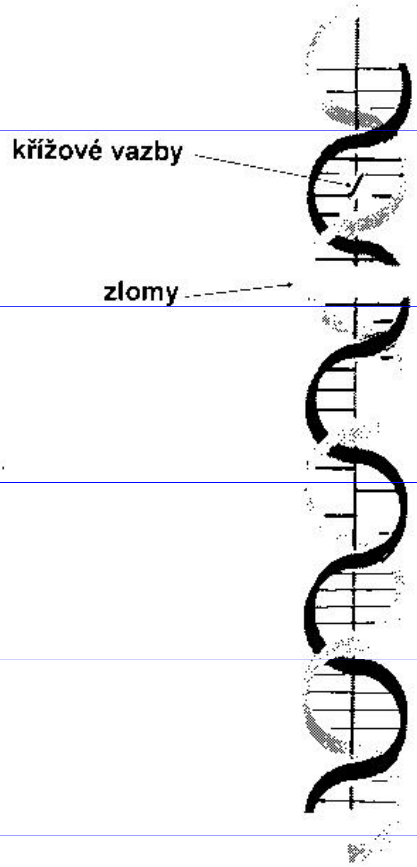
Ionizating radiation

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

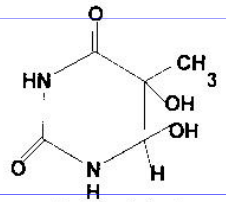
UV radiation

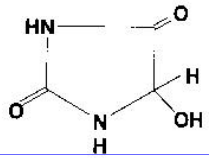
- interaction with aromatic cycles (bases)
- base dimerization (T=T)

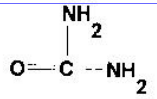
Ionizing radiation effects on DNA

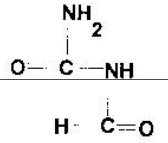


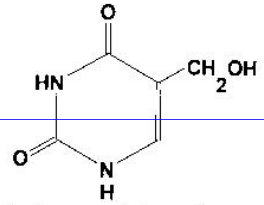
Obr. 384
Účinek ionizujícího záření na DNA

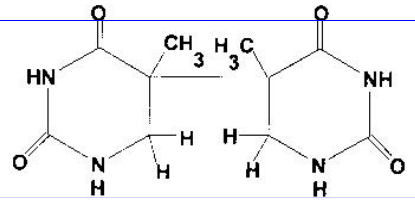

tyminglykol

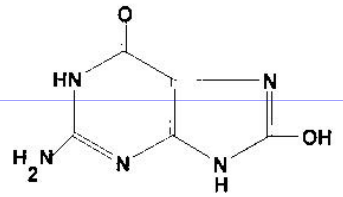

5-hydroxyhydantoin


močovina


N-formamidomočovina


5-hydroxymetyluracil


C(5)-C(5)-dihydrodimer tyminu


8-hydroxyguanin

Obr. 385
Změny bází vyvolané ionizujícím zářením za přítomnosti kyslíku

DNA repair

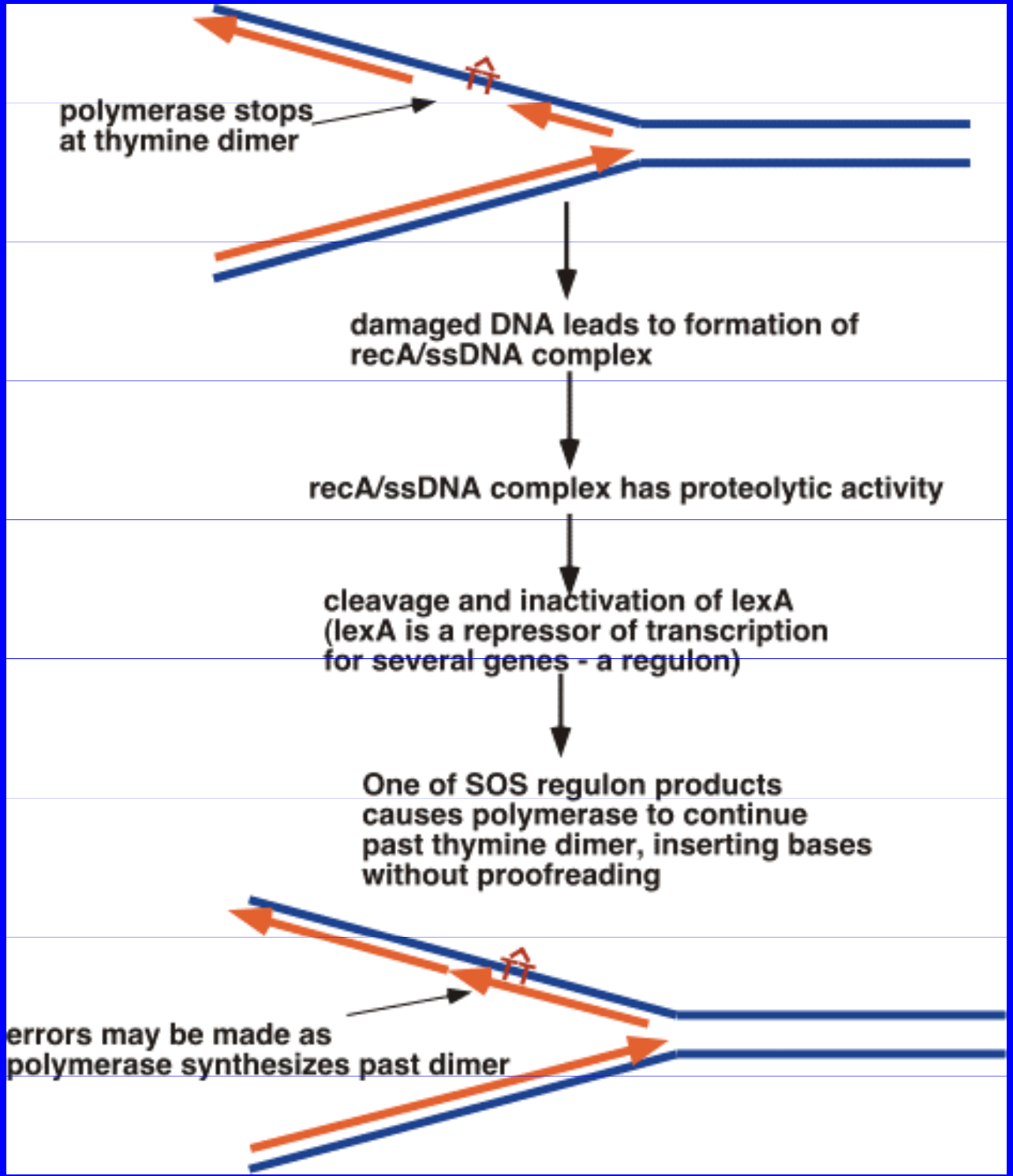
Damage of DNA is carefully controlled

constitutively expressed proteins

Changes in DNA

induction of reparation enzymes ("SOS-repair")

= biomarker of DNA damage


polymerase stops
at thymine dimer
damaged DNA leads to formation of
recA/ssDNA complex
recA/ssDNA complex has proteolytic activity
cleavage and inactivation of lexA
(lexA is a repressor of transcription
for several genes - a regulon)
One of SOS regulon products
causes polymerase to continue
past thymine dimer, inserting bases
without proofreading
errors may be made as
polymerase synthesizes past dimer