



Centrum pro výzkum
toxických látek
v prostředí

BIOMARKERS AND TOXICITY MECHANISMS

05 – Mechanisms - DNA

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Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

DNA

- principal molecule for life
- structure and function carefully checked
- changes rapidly repaired
- irreversible changes → cell death
(*physiologically by apoptosis*)

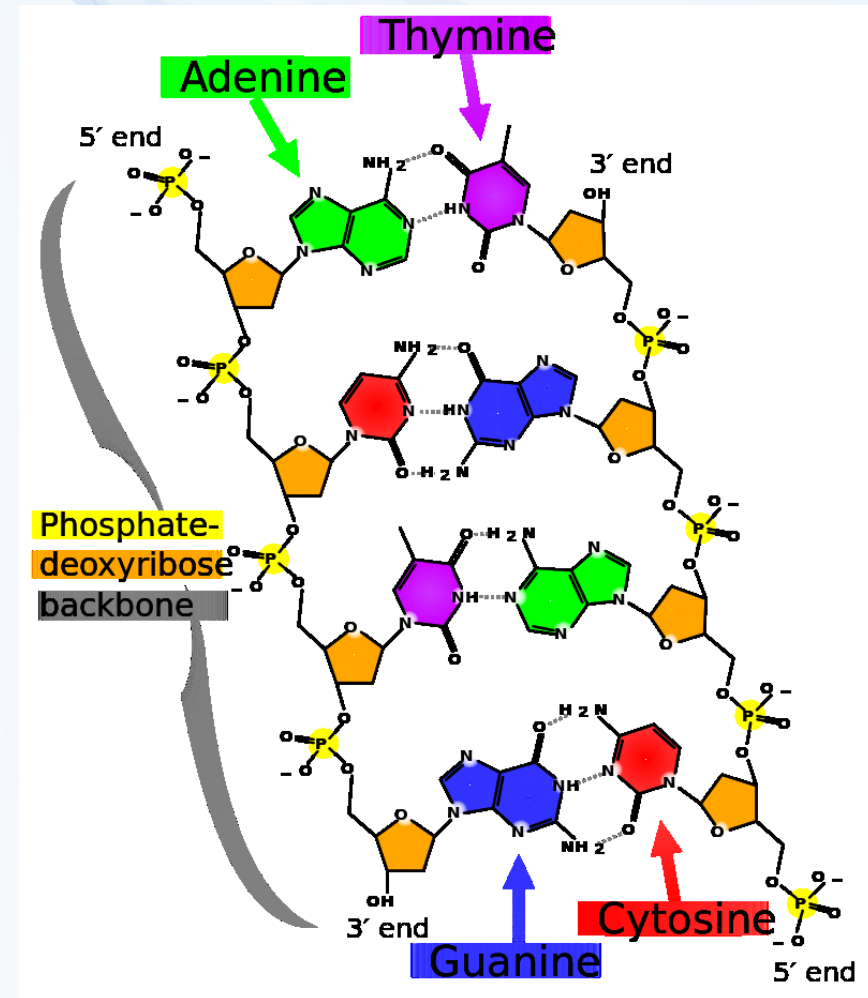
Mutagenesis → MUTATIONS

→ variability and evolution
or → damage to DNA
(structure or coding)

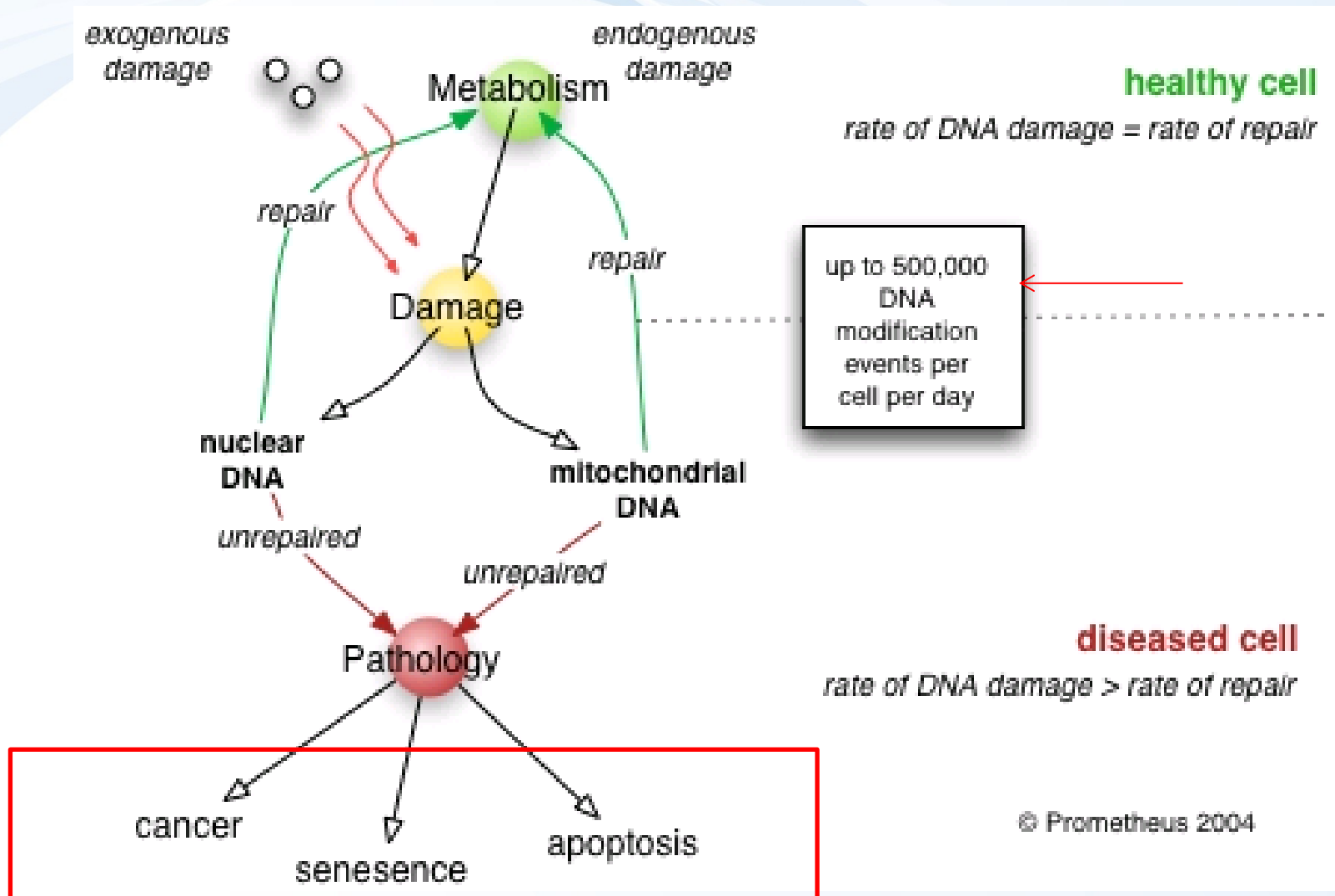
... naturally

billions of nucleotides/day
→ most are repaired

... stress-induced → toxicity



DNA damage and its effects



DNA repair

Damage of DNA is carefully controlled
constitutively expressed repair systems

Sudden changes in DNA

→ **induction** of additional repair enzymes
(e.g. "SOS-repair" in bacteria - biomarker of DNA damage)

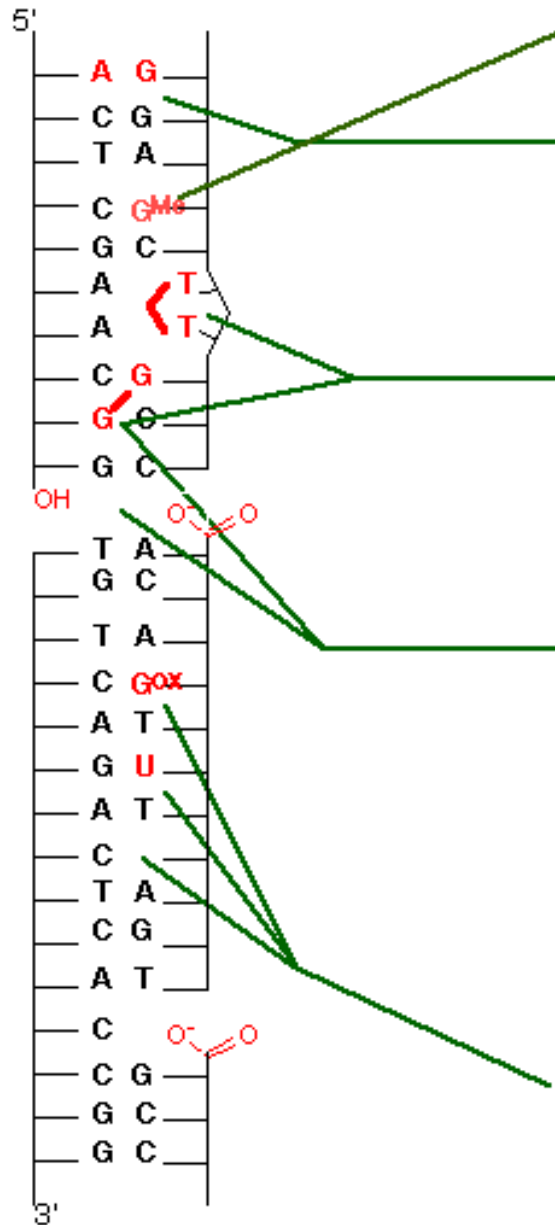


Various types of molecular changes in DNA ... and corresponding repair systems

Note!
 • Not all nucleotides are affected in the same rate
(mutations occur only at specific sites due to physicochemical properties)

- Most common patterns:
- **G** - the most frequent target *(highly nucleophilic character)*
 - T=T at the same strand
 - G=G crosslinks

DNA DAMAGE



DNA REPAIR SYSTEM

DIRECT REVERSAL

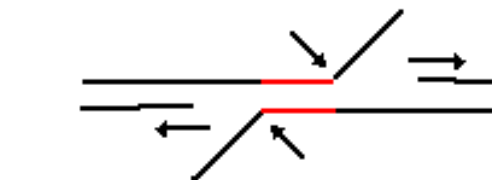
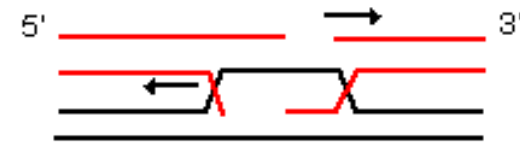
MISMATCH REPAIR



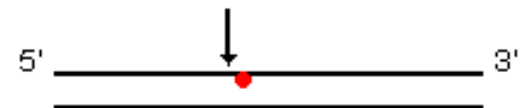
NUCLEOTIDE EXCISION REPAIR



RECOMBINATIONAL REPAIR



BASE EXCISION REPAIR



Complex system of **SOS repair** proteins induced in *E. coli* by DNA damage

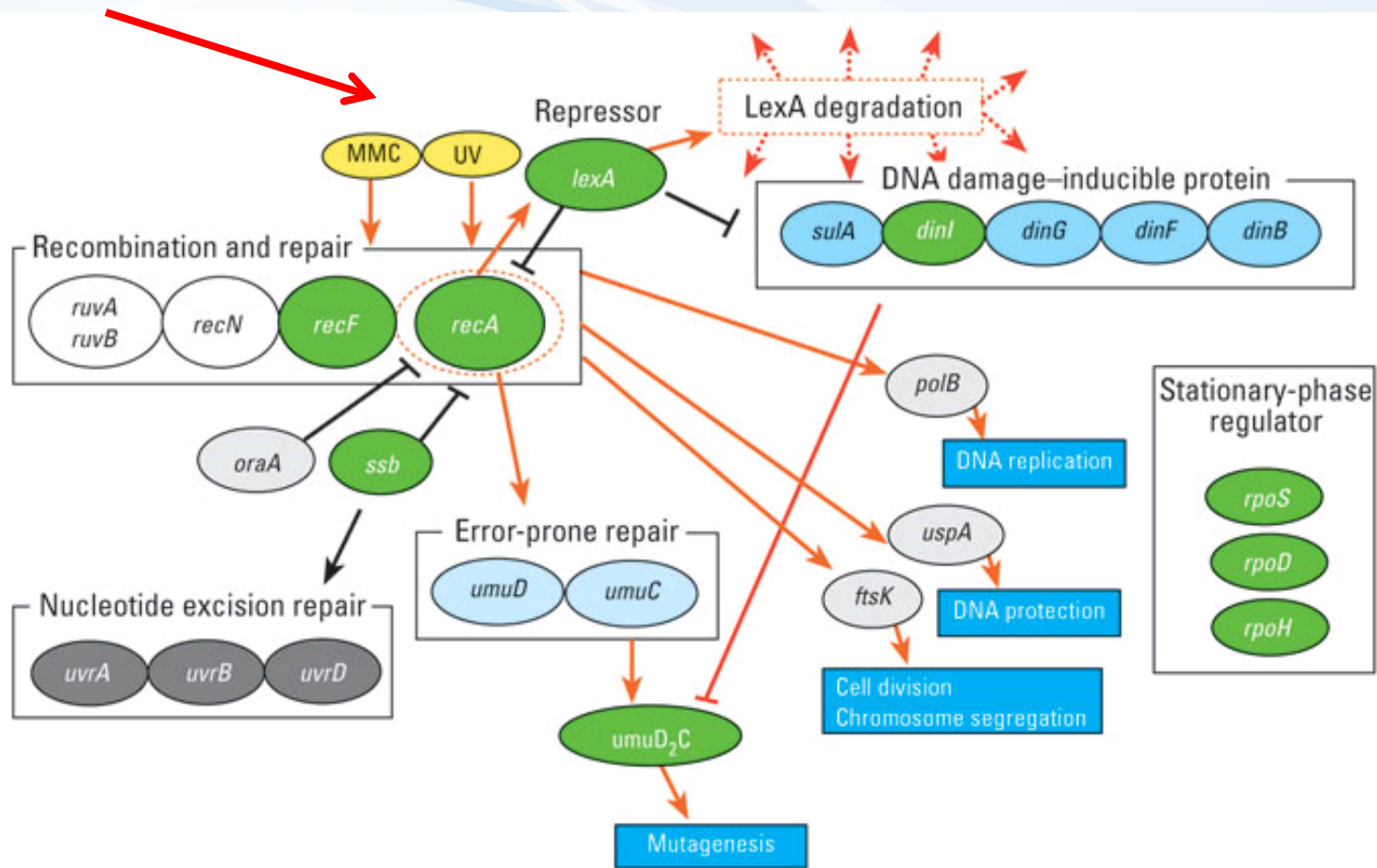


Figure 3. A literature-based linkage map between genes in the SOS response in *E. coli*. The map represents inducible genes/proteins in the SOS response for repair from DNA damage. Black lines indicate pathways in the normal repair process and red lines with arrows activation/induction due to an exposure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



TYPES of mutations

POINT mutations

Base exchanges

Deletions / Insertions

→ *Impacts of point mutations*

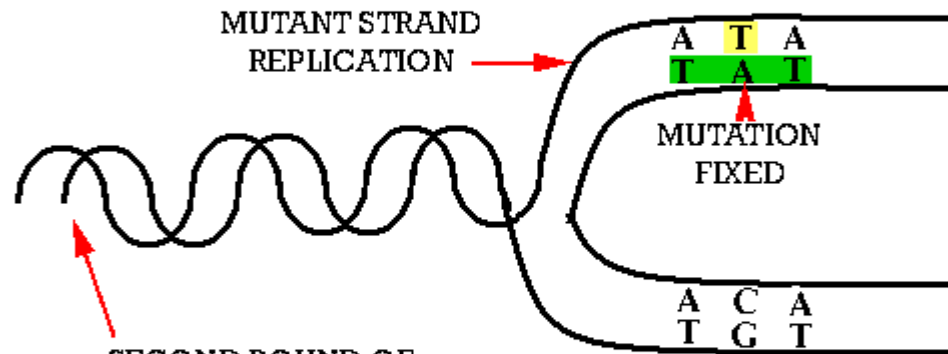
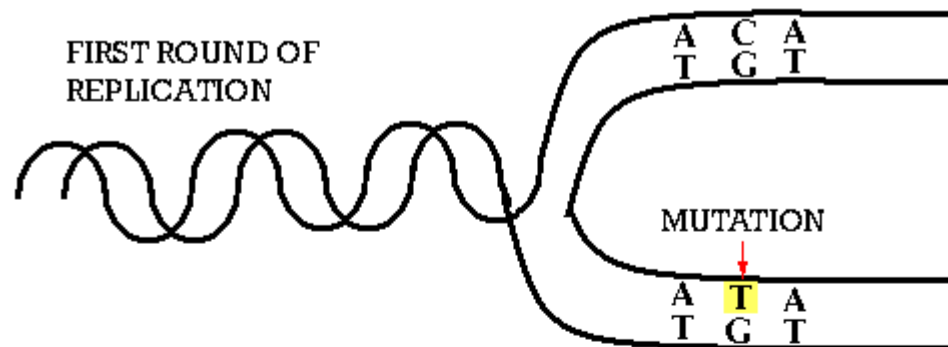
(a) silent, (b) missense, (c) nonsense, (d) frameshift

CHROMOSOMAL mutations

→ *large scale impact*

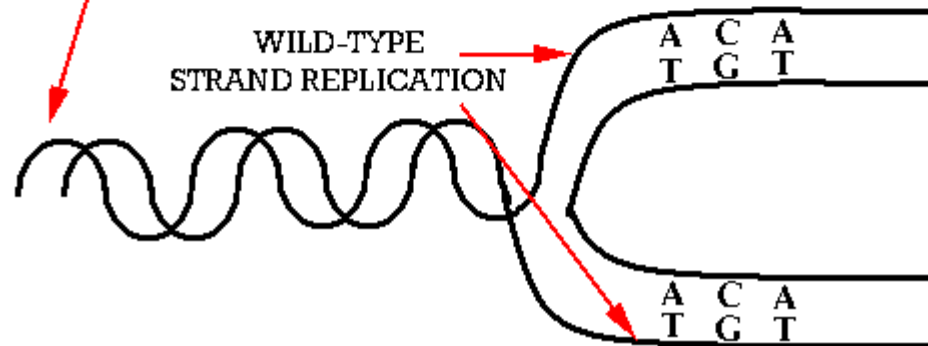


BASE – EXCHANGE



SECOND ROUND OF REPLICATION

WILD-TYPE STRAND REPLICATION



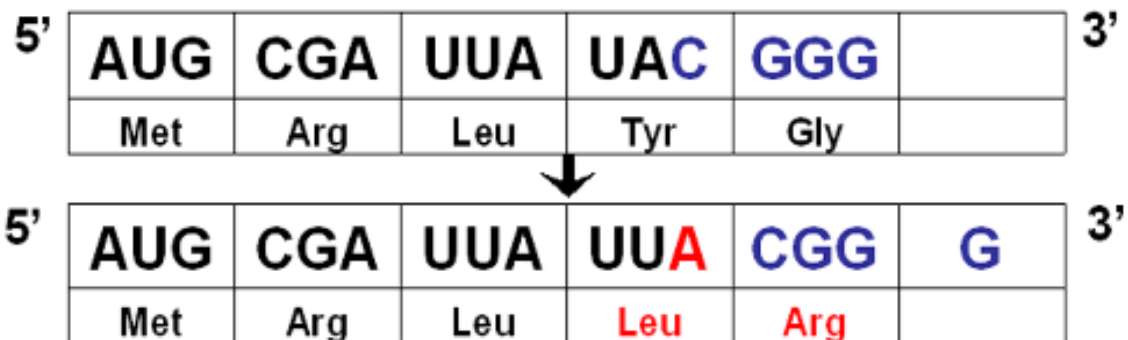
→ Mutation fixed in 50% of cells after the first replication



INSERTION DELETION

→ reading frame shifts

Insertion

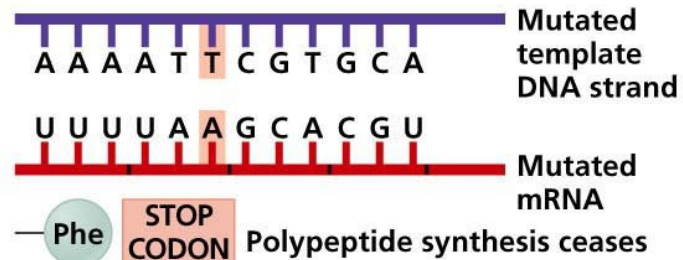
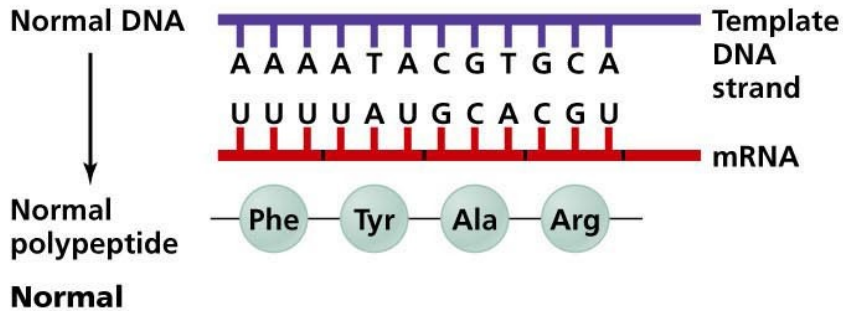


Deletion

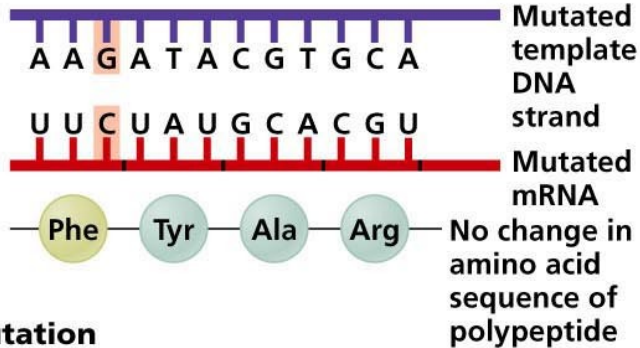


Impacts of point mutations

→ (a) silent, (b) missense, (c) nonsense, (d) frameshift



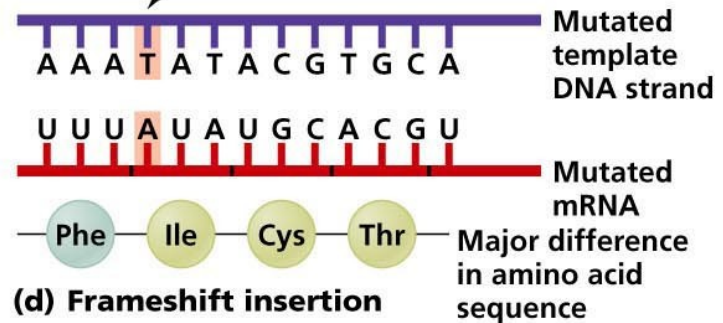
(c) Nonsense mutation



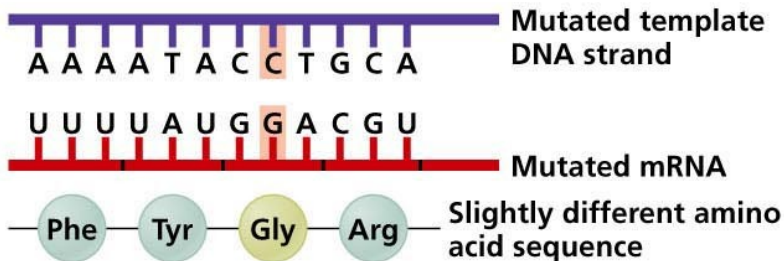
(a) Silent mutation

Frameshift mutations

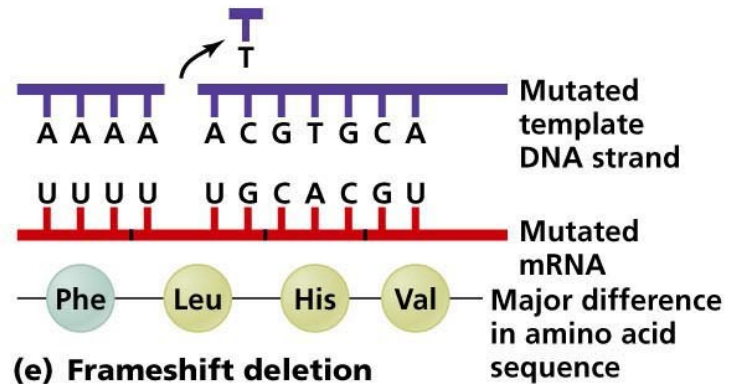
Insertion



(d) Frameshift insertion



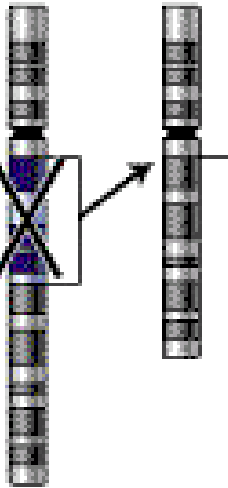
(b) Missense mutation



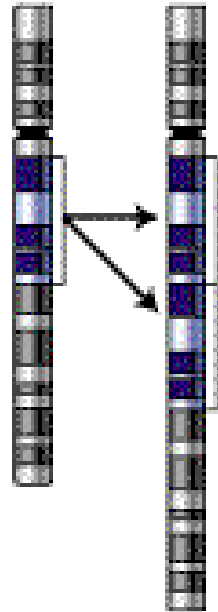
(e) Frameshift deletion

Large – chromosomal mutations

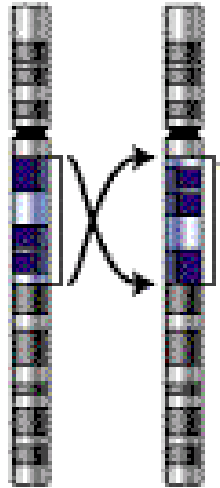
Deletion



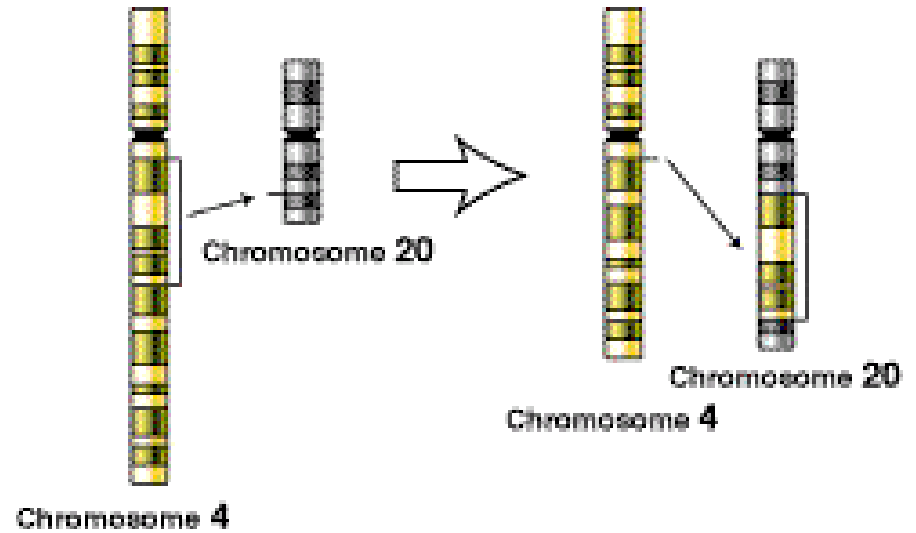
Duplication



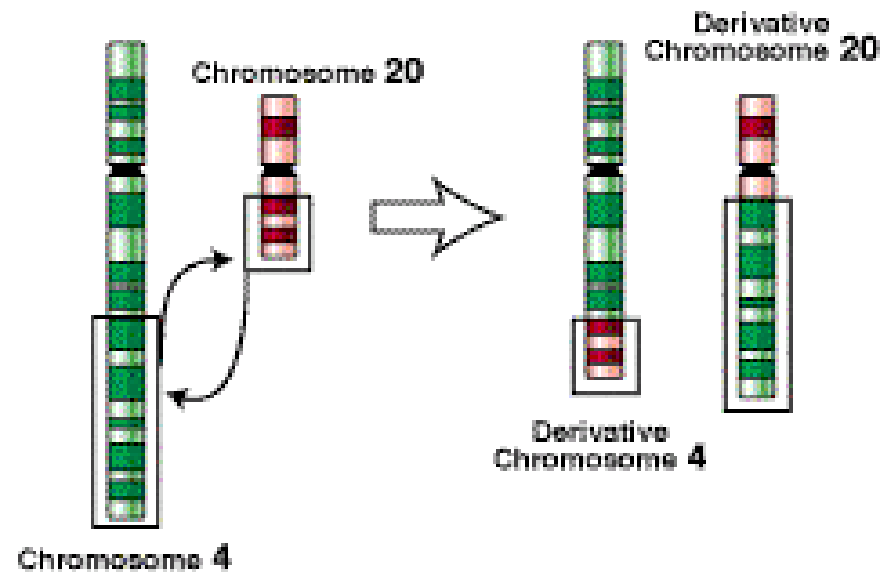
Inversion



Insertion



Translocation



What are the agents inducing mutations? MUTAGENS

PHYSICAL FACTORS

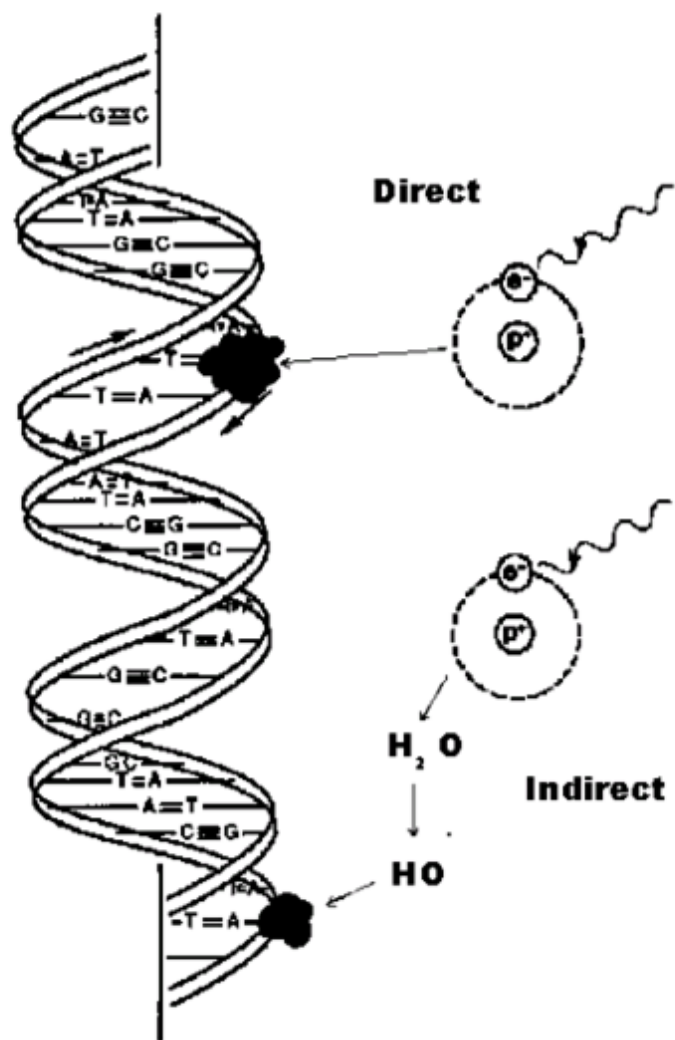
Ionizing radiation

- direct interactions with NA
- interactions with water
 - formation of OH*
 - (and other oxygen radical species – ROS)
- *Various impacts on bases and strands*

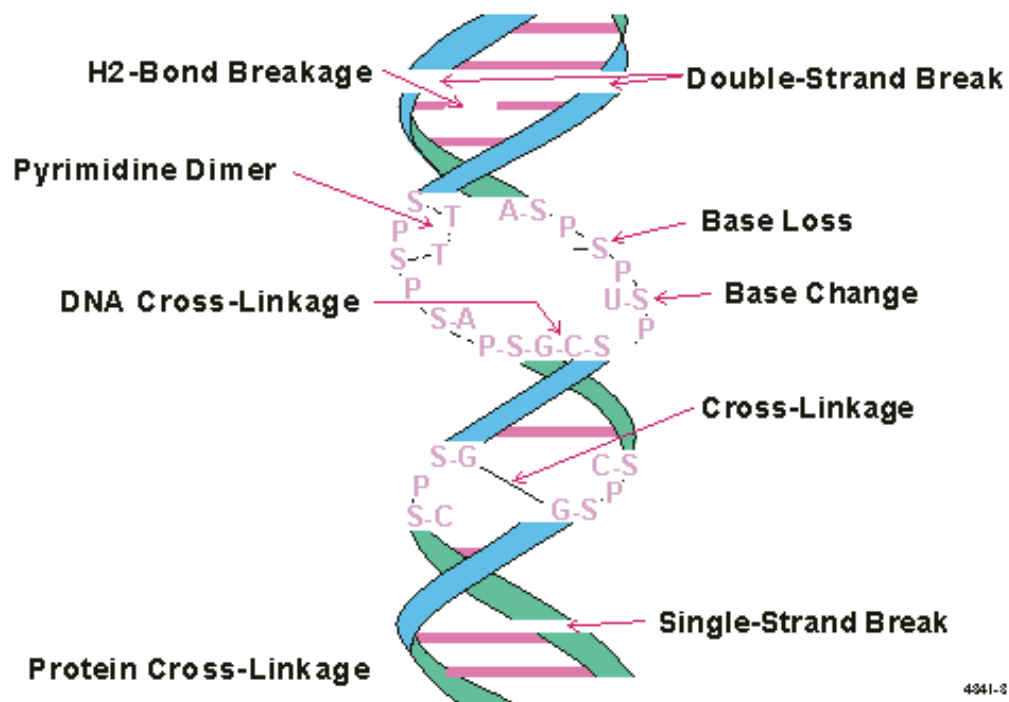
UV radiation

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

Ionizing radiation effects on DNA



RADIATION DAMAGE TO DNA



4341-3



What are the agents inducing mutations? MUTAGENS

CHEMICALS

1) Small electrophilic molecules

(attracted by nucleophilic/basic sites ... e.g. in DNA)

2) Other reactive molecules

- * alkylating and arylating agents – covalent adducts
- * specifically intercalating agents

3) Base analogs

inserted during replication instead of nucleotides

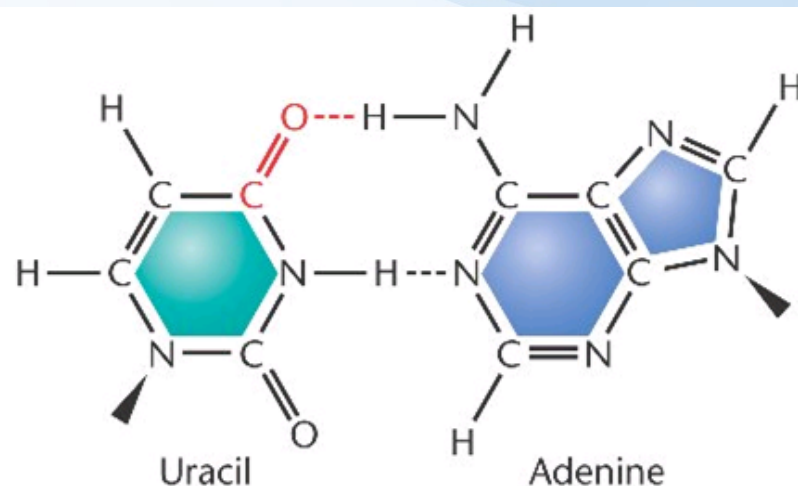
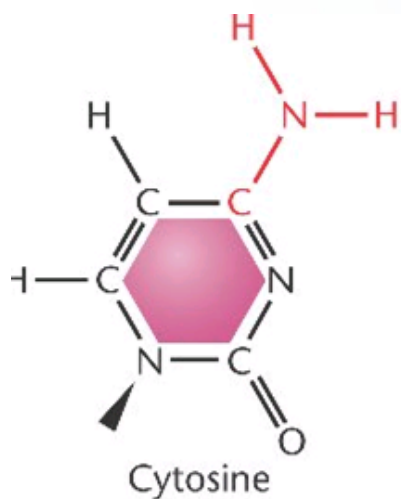
*Some compounds may require “**activation**” by metabolism
pro-mutagen (pro-carcinogen) → mutagen (carcinogen)*



Small molecules → deamination of bases

HNO_2 , HSO_3^- Hydroxylamine (HO-NH_2), Methoxyamine ($\text{CH}_3\text{-O-NH}_2$)

Example: oxidation (**deamination**)
→ CG to → TA shift

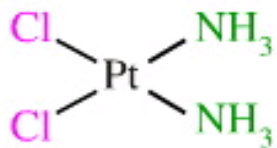


ALKYLating compounds

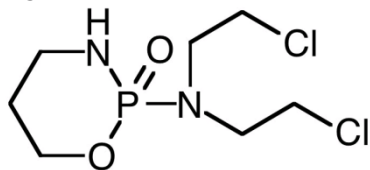
Covalent binding to NA (alkylation of bases, crosslinks in dsDNA)

Alkylsulphates, Nitro-urea, N-nitroso-alkyles, cis-platinum

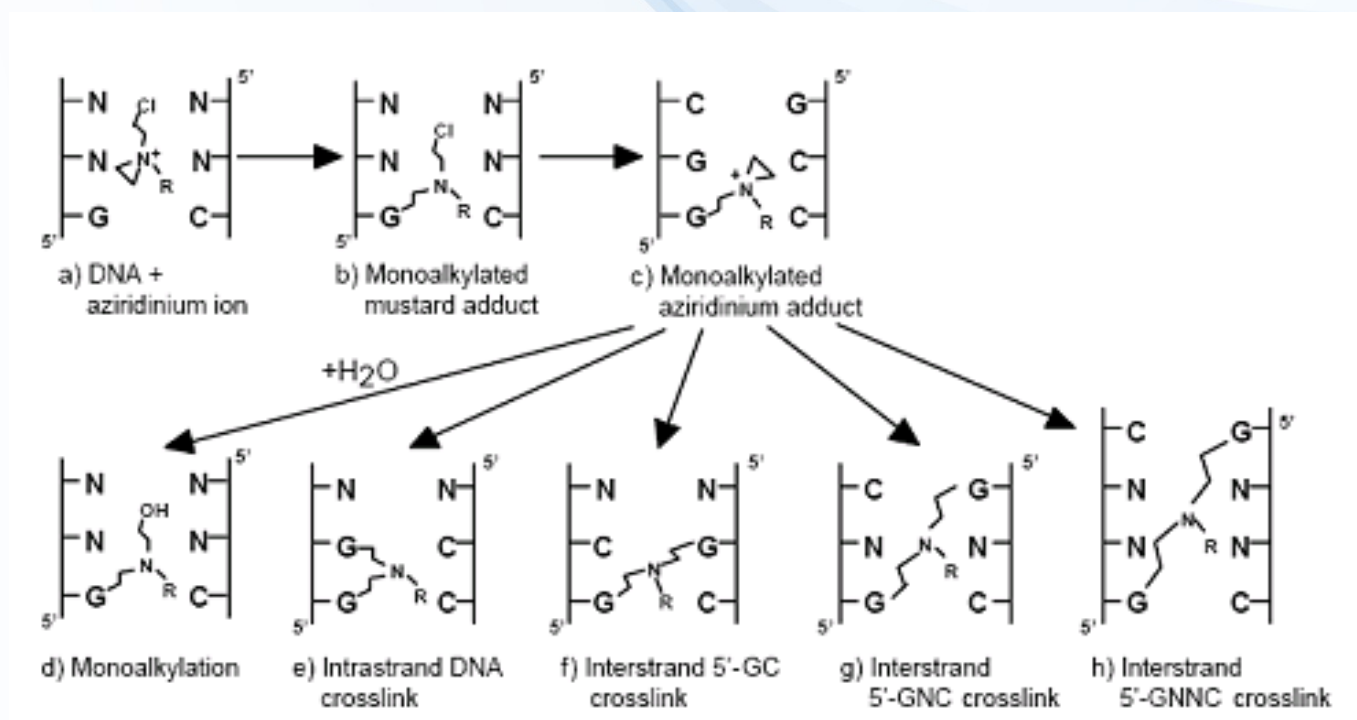
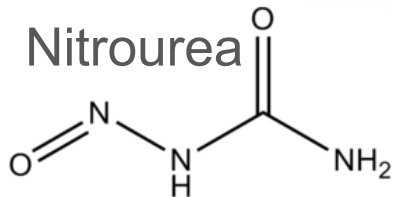
cisplatin



cyclophosphamide



Nitrourea



ARYLating compounds

Covalent binding, aromatic „adducts“ with bases
(see also discussion at biomarkers)

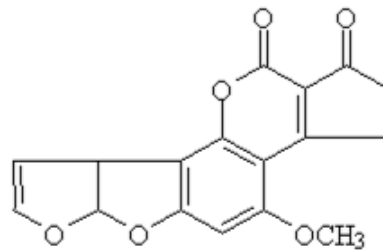
Mycotoxins (Aflatoxins) – requires activation

PAHs (benzo[a]pyrene) – requires activation

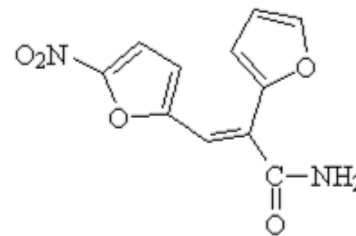
PAH derivatives

- **2-AA**, **2-AF** (grill products)
- **NQO** – model mutagen in experiments

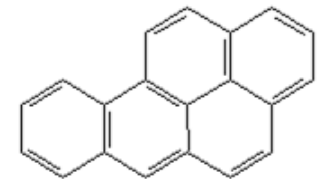
... many others



Aflatoxin B₁ 312.27



AF-2 (furylfuramide) 248.19



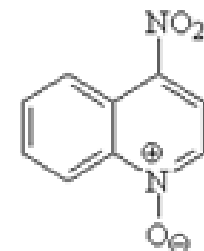
benzo[a]pyrene
(B[a]P) 252.31



2-aminoanthracene
(2-AA) 193.24



2-aminofluorene
(2-AF) 181.23

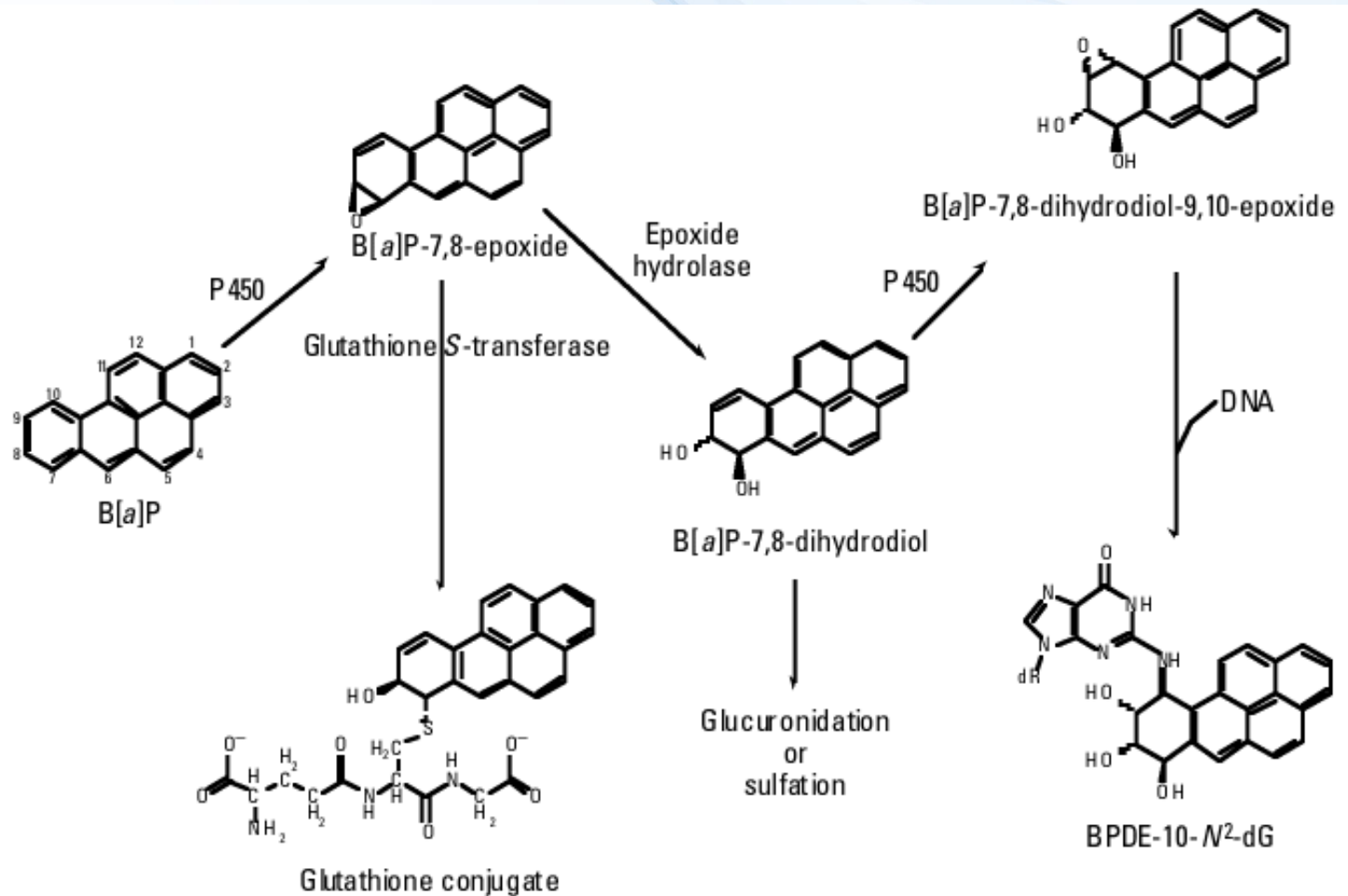


4-nitroquinoline-1-oxide
(NQO) 190.15



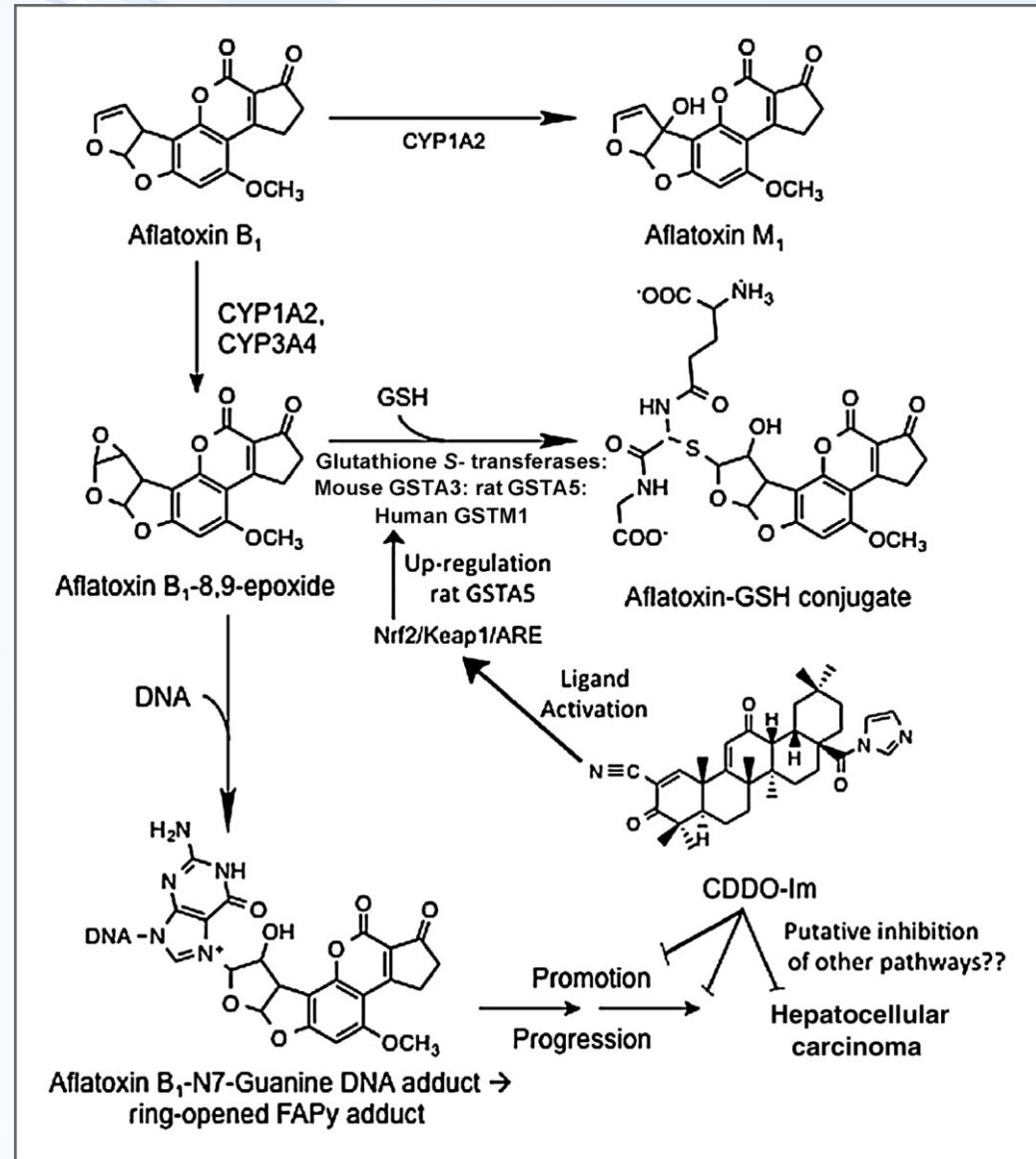
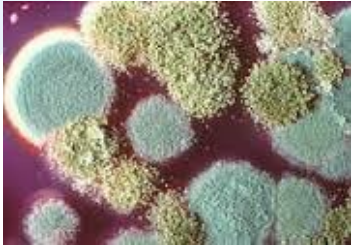
Bioactivation of benzo[a]pyrene → genotoxicity

BaP is oxidized to epoxides and OH-derivatives during detoxification (CYP450)
→ increased reactivity (including binding to bases ... primarily G or A)
(*Similar bioactivation e.g. at aflatoxin*)



Bioactivation of aflatoxin → genotoxicity

AFLATOXIN sources



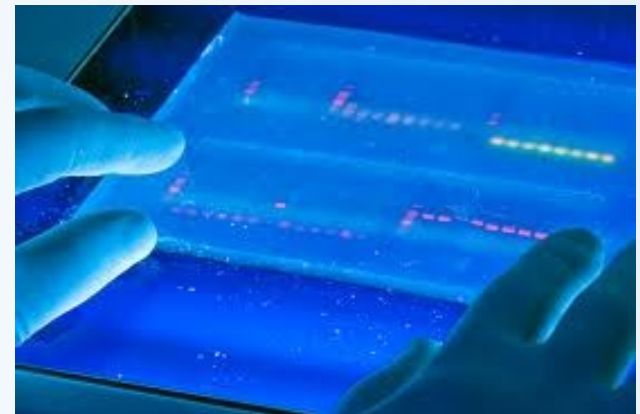
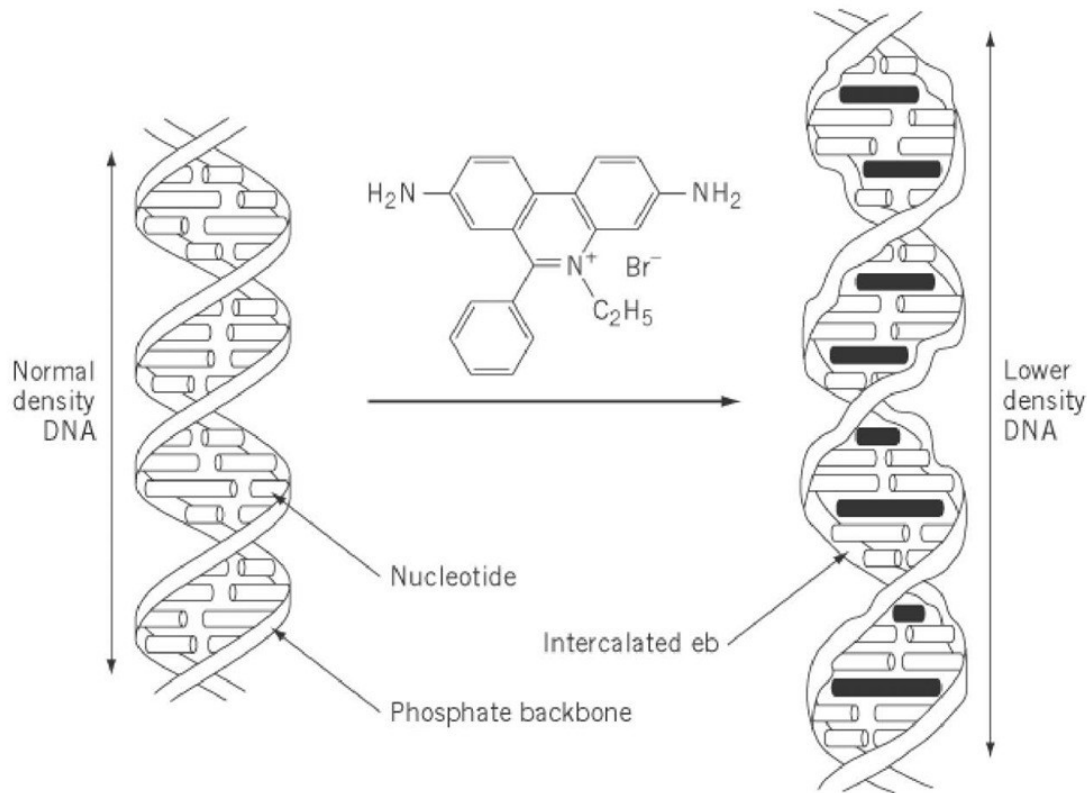
Intercalating agents

INTERCALATORS

Compounds with characteristic structures “fitting” into DNA
→ both noncovalent and covalent intercalation

Example 1 – ETHIDIUMBROMIDE

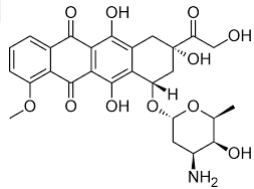
- experimental dye – visualization of DNA
- intercalation → sharing of electrons with bases → high fluorescence



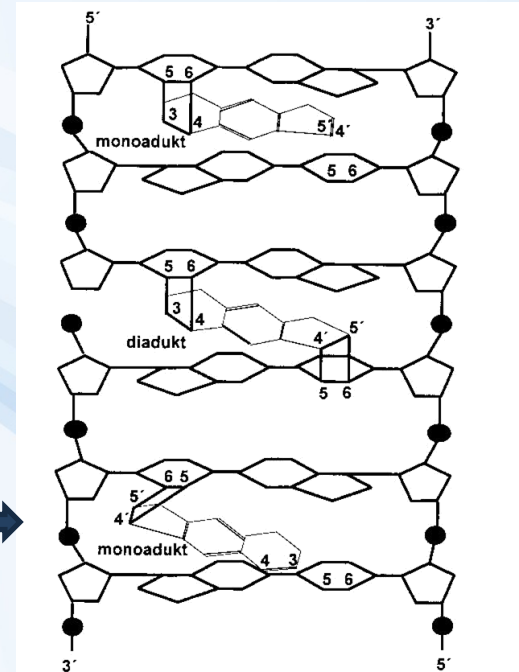
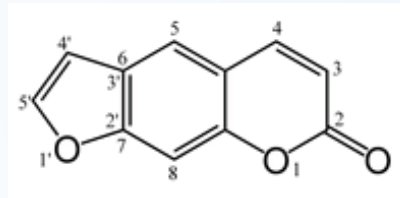
Intercalating agents

Other intercalator examples

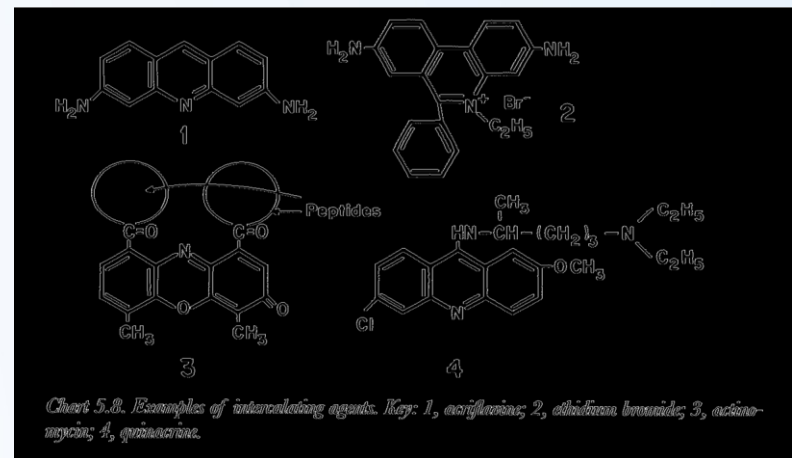
-Anticancer drug - doxorubicin



- Psoriasis treatment – psoralen →



-Experimental research compnds (e.g. acriflavine) →



Base analogs

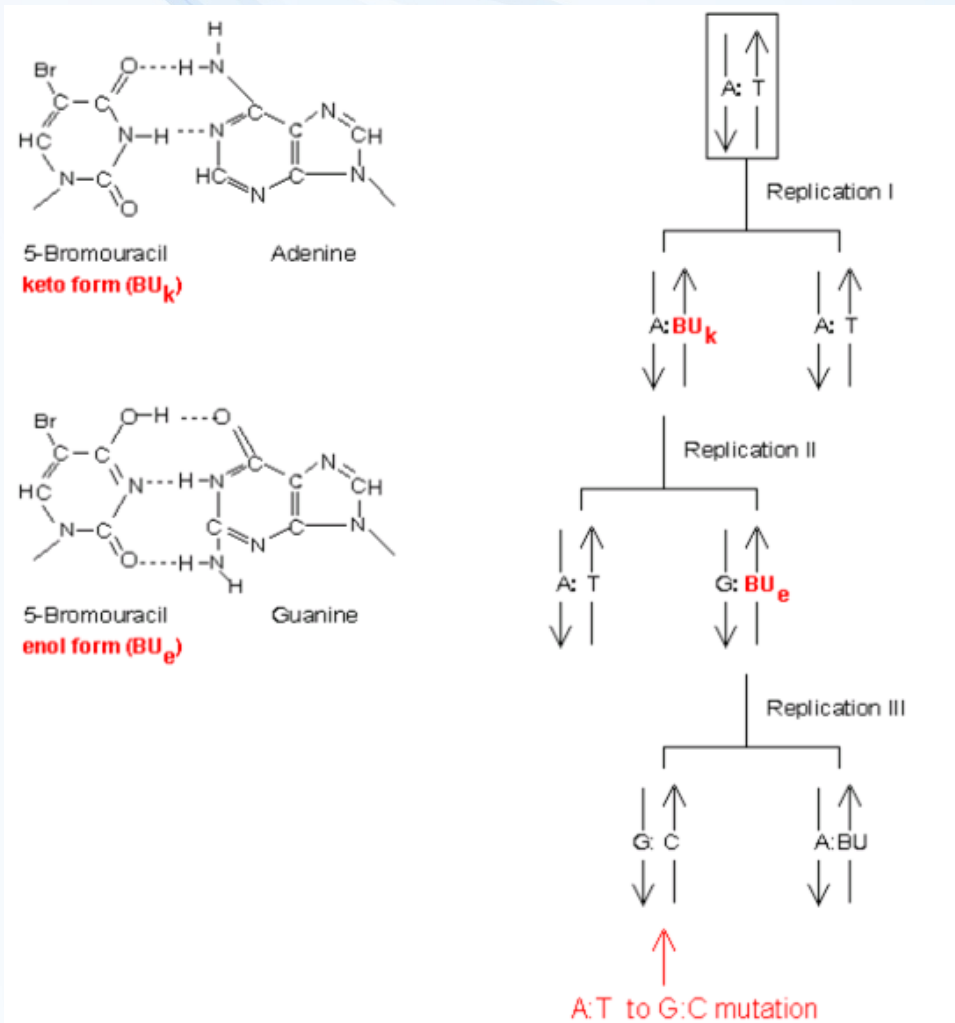
Structure similarity with natural bases

- Incorporation into DNA during replication
- Base exchange mutations

Example

5-Br-Uracil (anticancer drug)

AT → GC shift



Mutations (alleles) and evolution

