Centrum pro výzkum toxických látek v prostředí

BIOMARKERS AND TOXICITY MECHANISMS

03 – Mechanisms - DNA

Luděk Bláha, PřF MU, RECETOX
www.recetox.cz

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

EVROPSKÁ UNIE esf

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OP Vzdělávání pro konkurenceschopnost

UNIVERSITAS MASARYKIANA BRUNENSIS

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

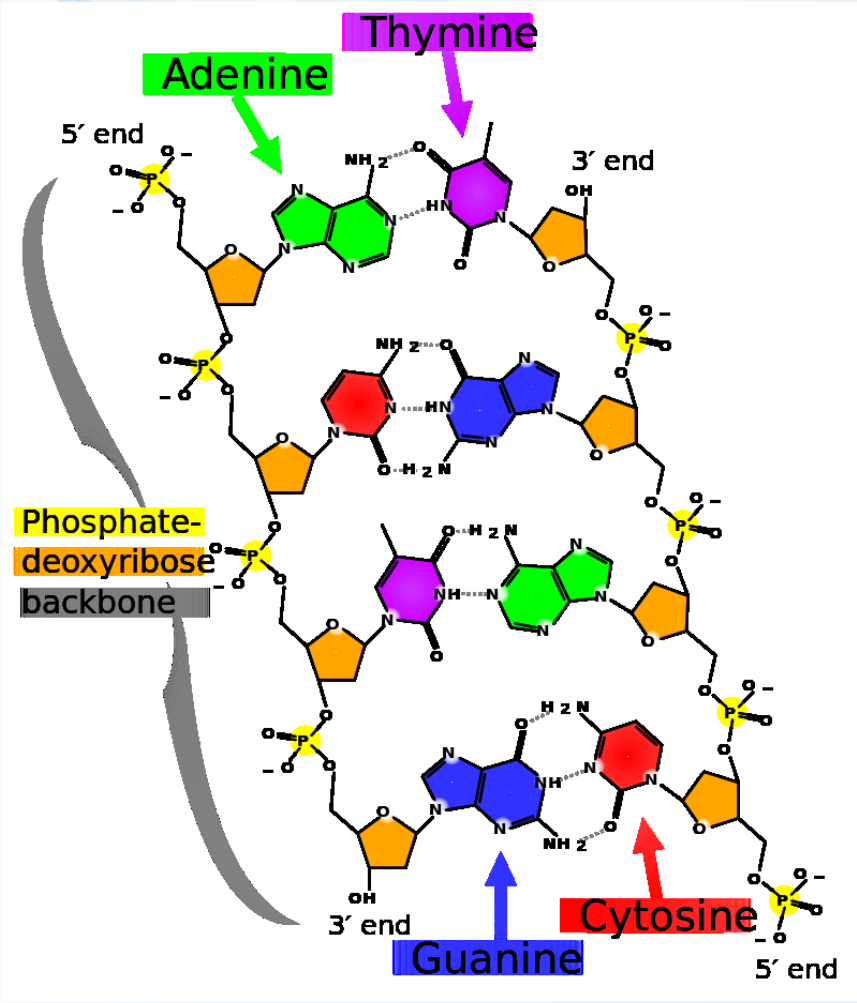
→ variability and evolution

or → damage to DNA
(structure or coding)

… natural mutagenesis

billions of nucleotides/day
→ most are repaired

… stress-induced → toxicity



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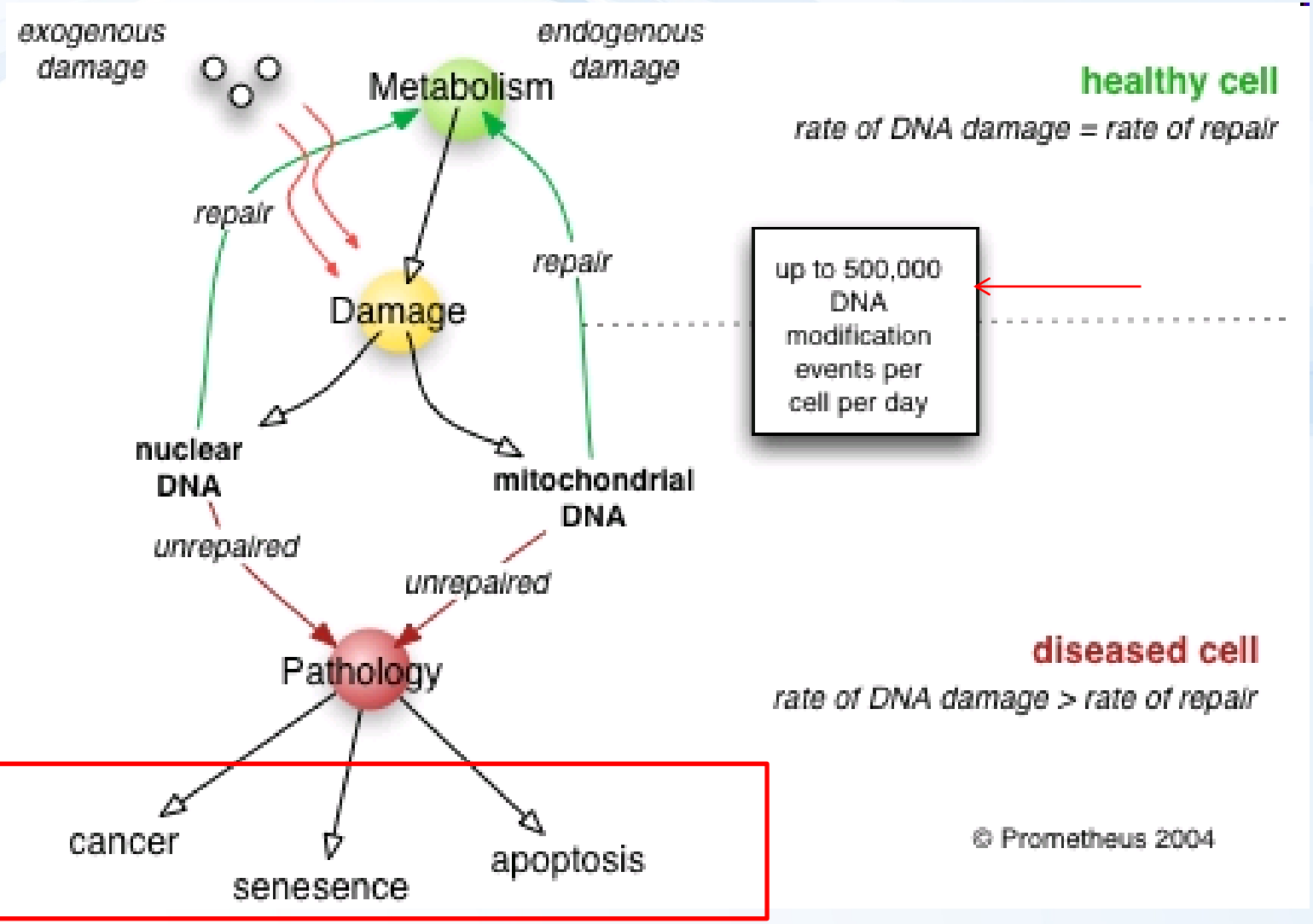
DNA damage and its effects



Cellular changes → Health and evolutionary consequences

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

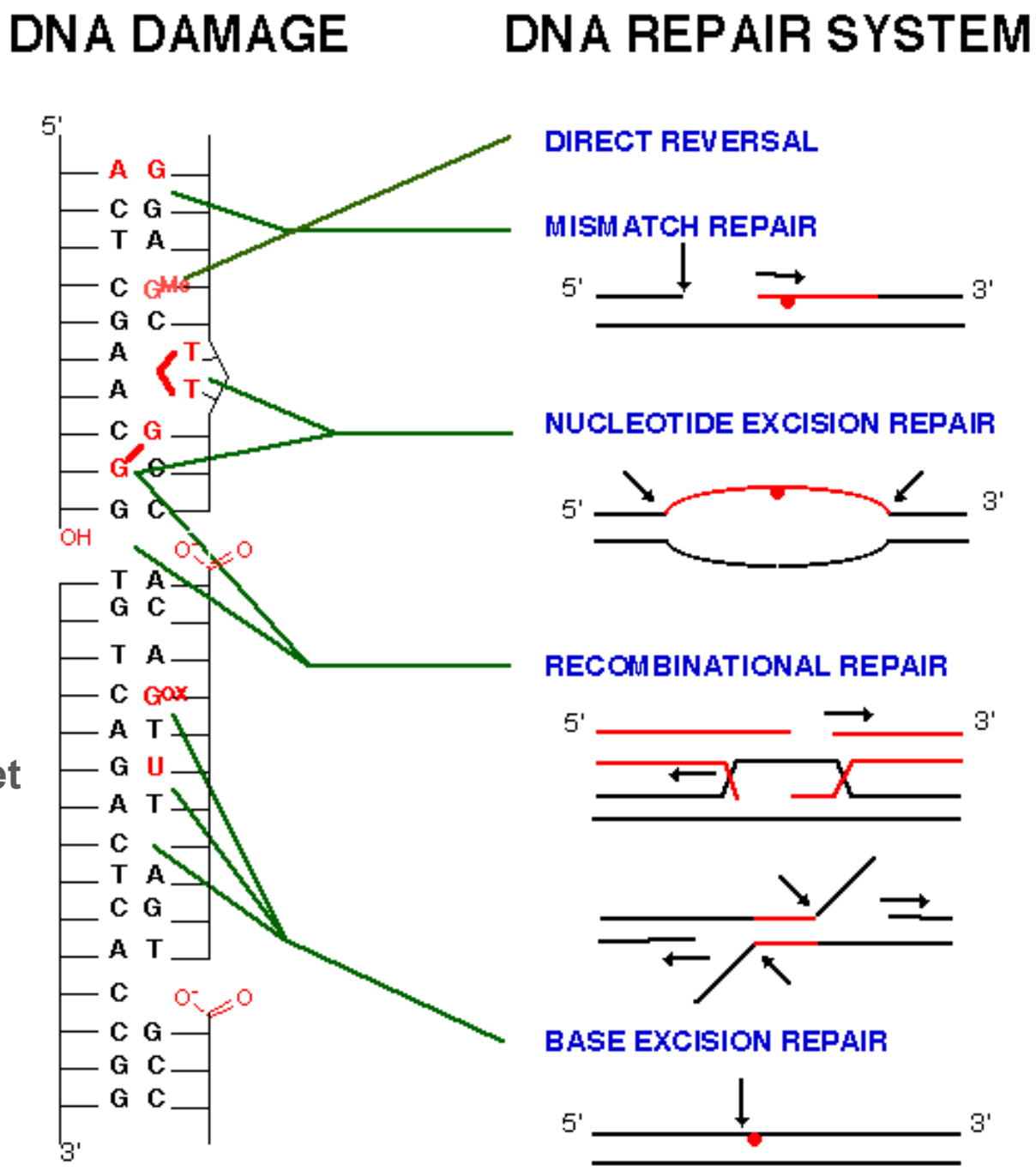
Note!

•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



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Example:

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

(induction and/or elevated levels of SOS-repair also used as a „biomarker of genotoxicity“)

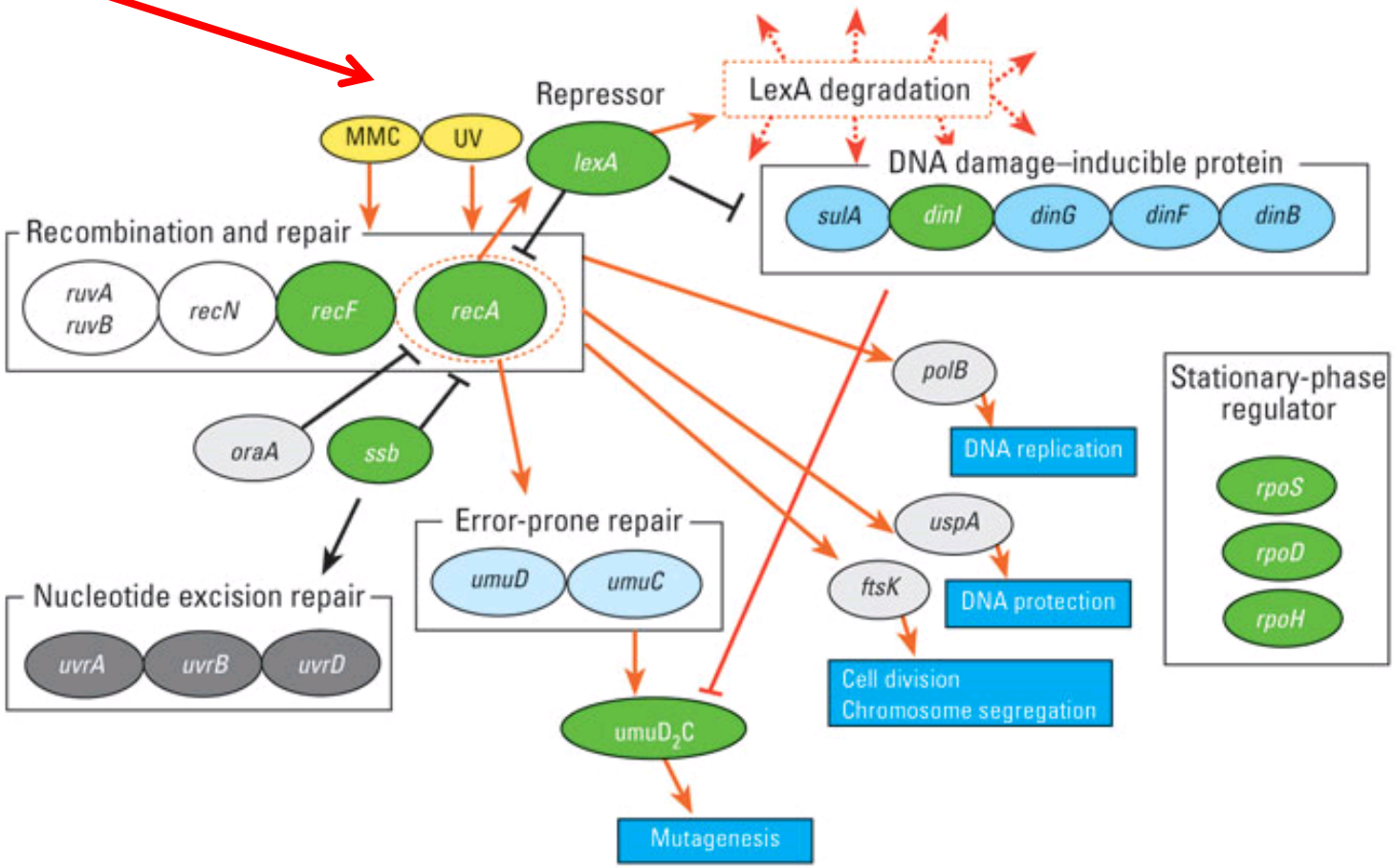
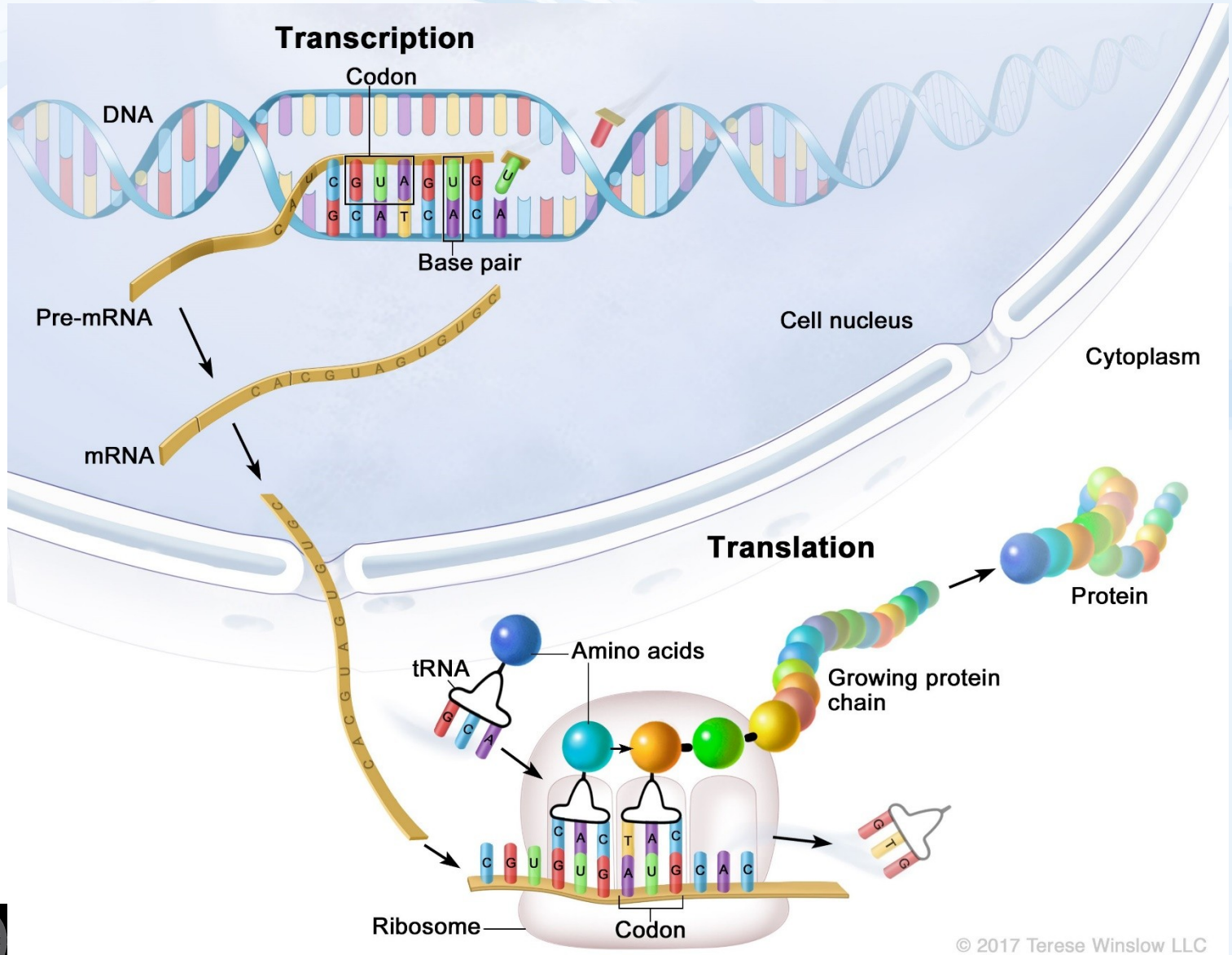


Figure 3. A literature-based linkage map between genes in the SOS response in *E. coli*. The map represents inducible genes/proteins in the SOS response for repair from DNA damage. Black lines indicate pathways in the normal repair process and red lines with arrows activation/induction due to an exposure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

How is the information stored in DNA decoded and used



TYPES of mutations

POINT mutationts

Base exchanges

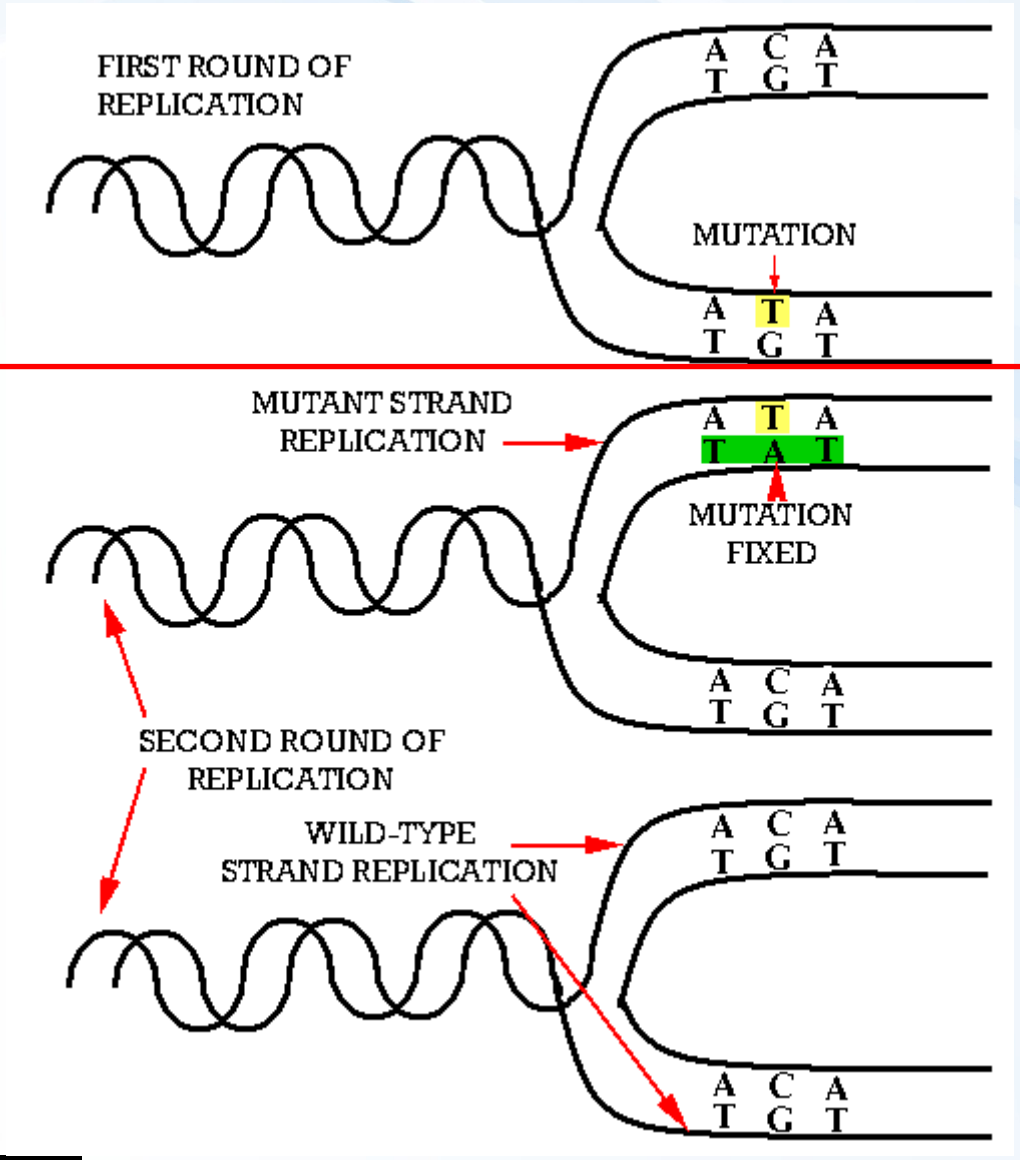
Deletions / Insertions

→ *Impacts of point mutations*
(a) silent, (b) missense, (c) nonsense, (d) frameshift

CHROMOSOMAL mutations

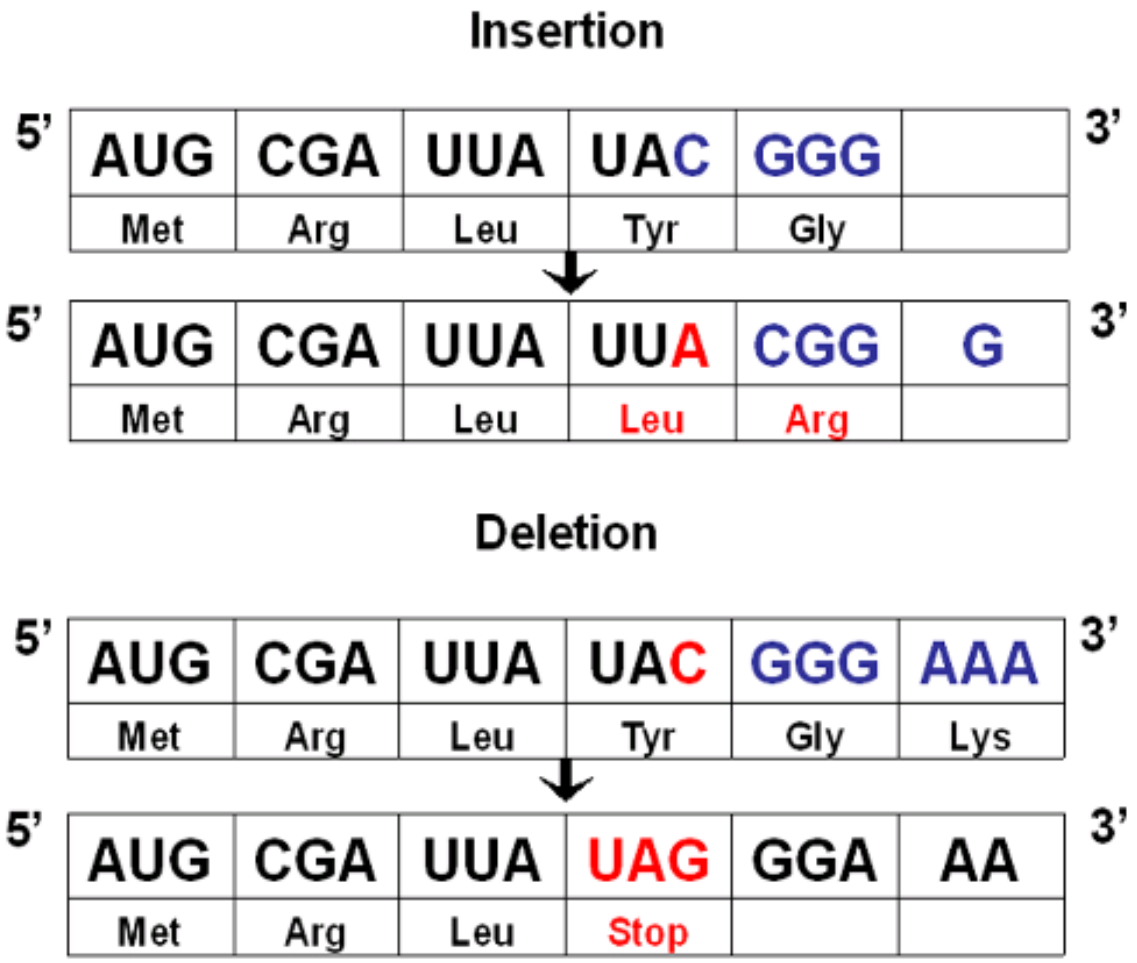
→ *large scale impact*

BASE – EXCHANGE



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

INSERTION
DELETION → shifts in reading frame



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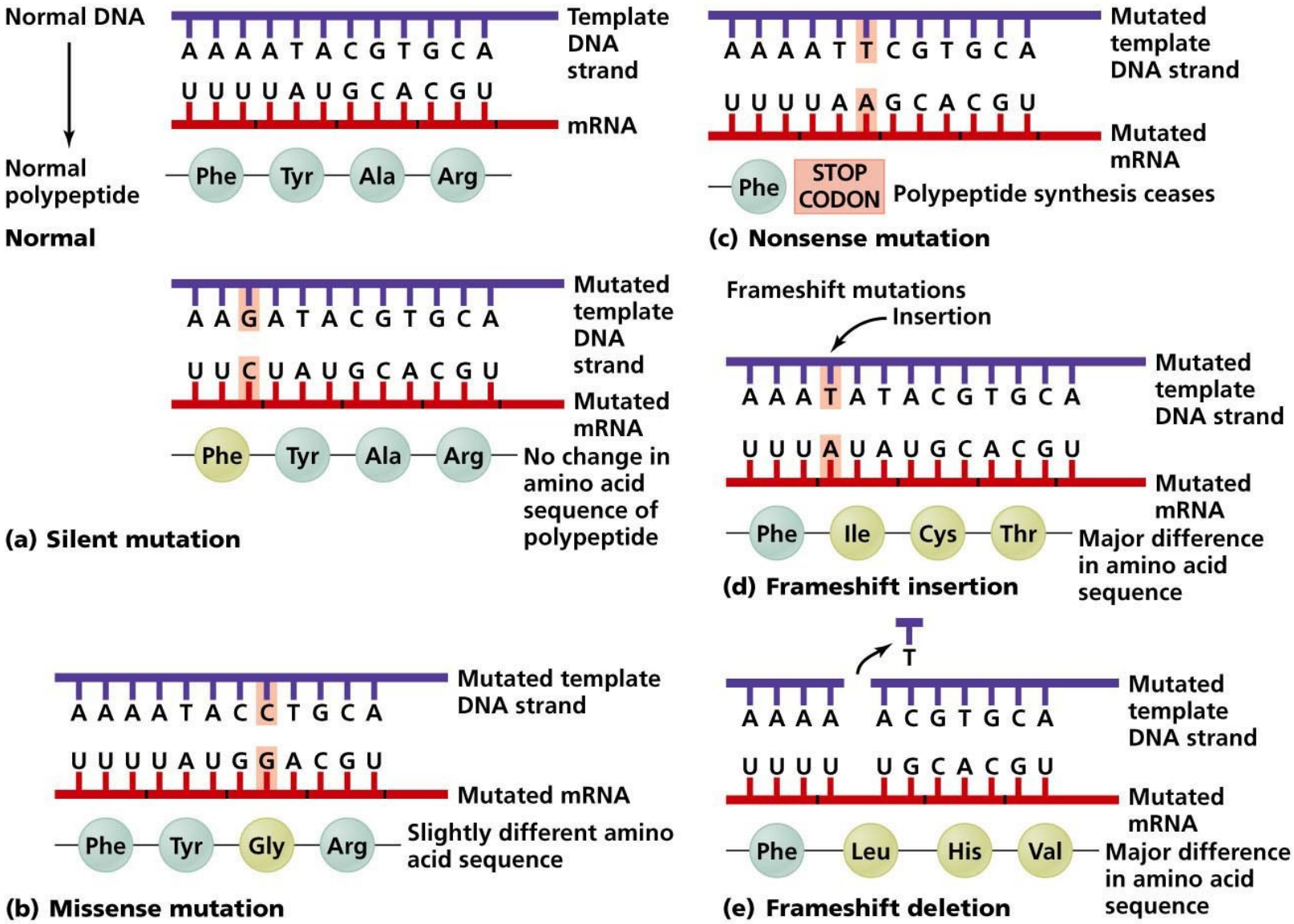
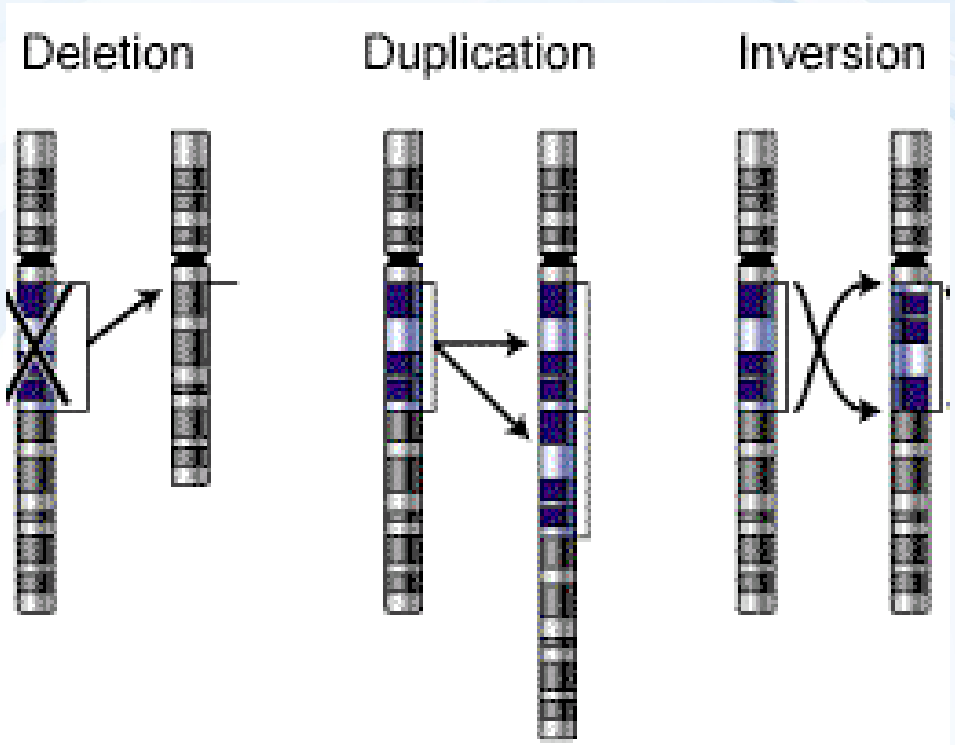
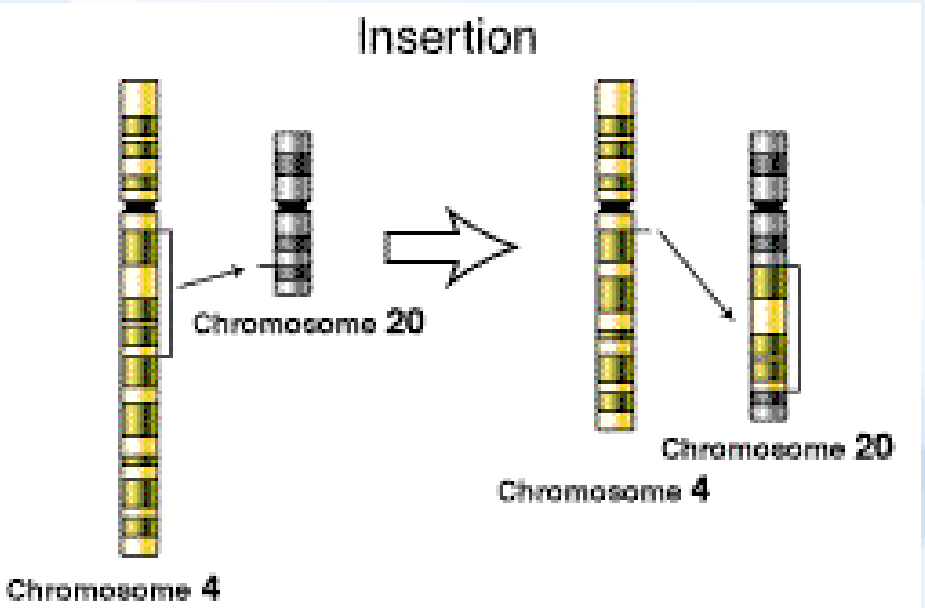
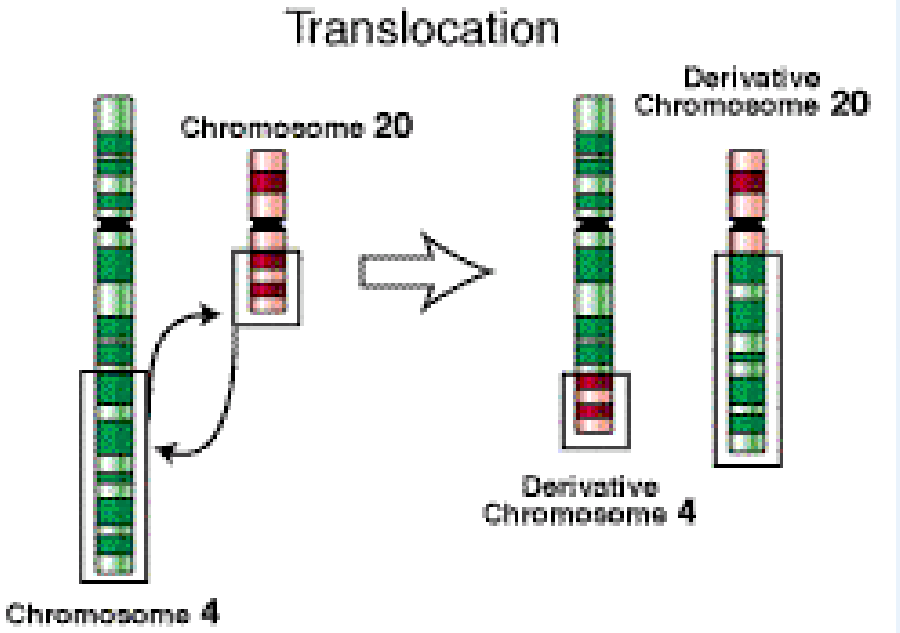
Impacts of point mutations
→ (a) silent, (b) missense, (c) nonsense, (d) frameshift



Large – chromosomal mutations







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What are the agents inducing mutations? MUTAGENS

PHYSICAL FACTORS

Ionizating radiation

- direct interactions with NA
- interactions with water

→ formation of OH*

(and other oxygen radical species – ROS)

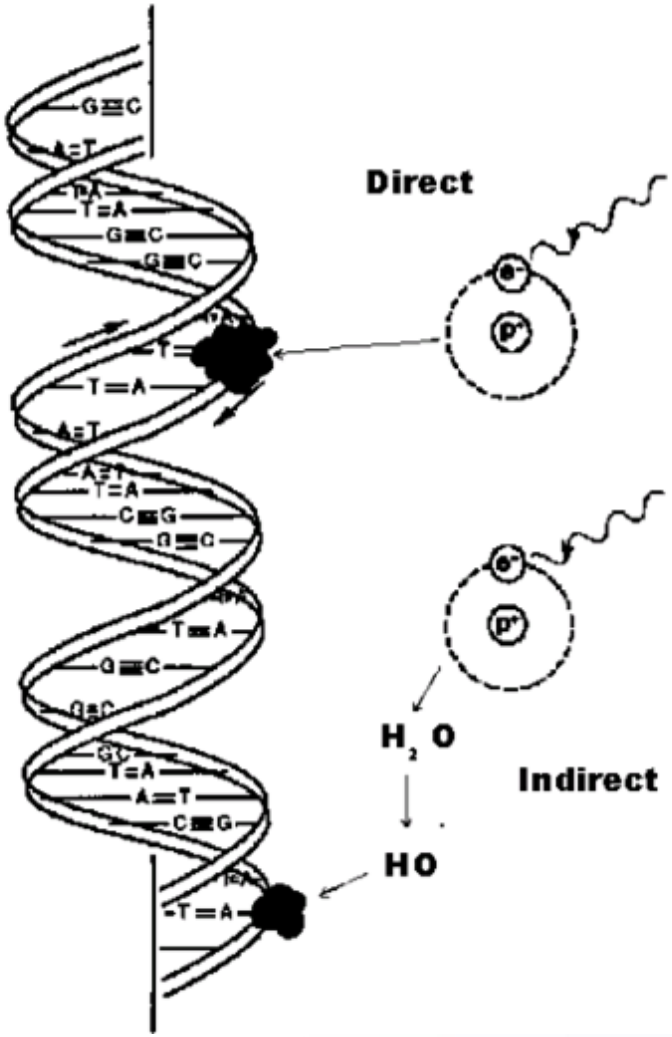
→ *Various impacts on bases and strands*

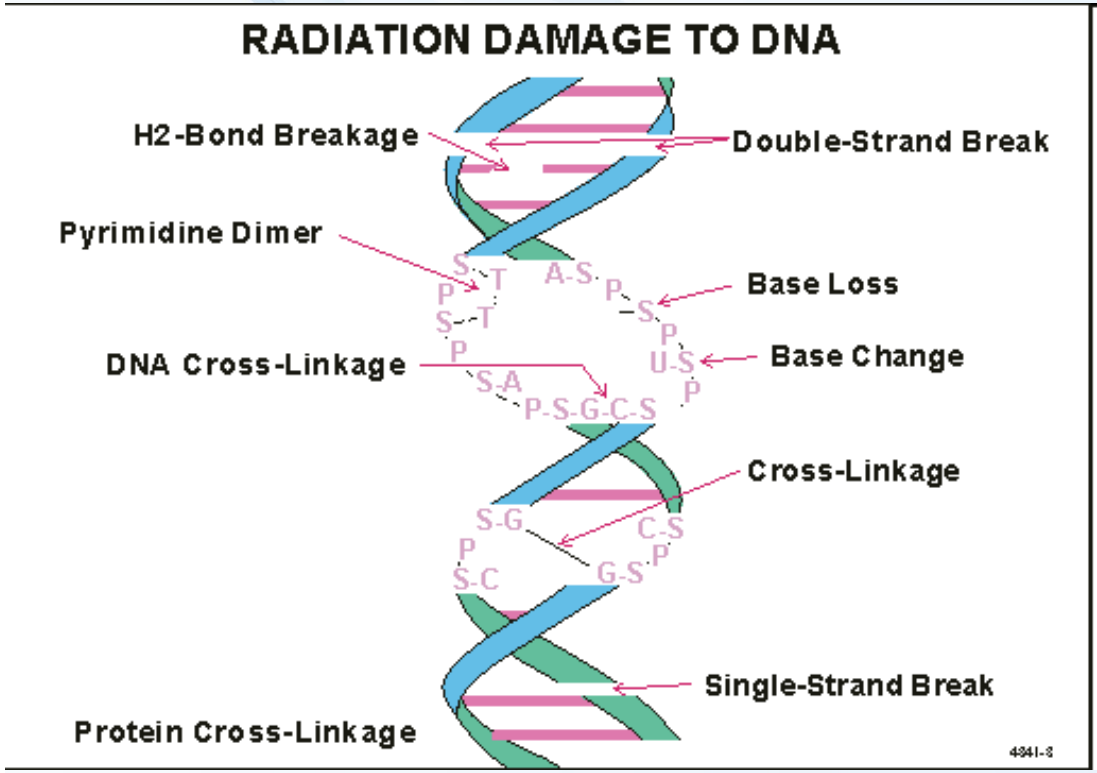
UV radiation

- interaction with aromatic cycles (bases)

→ base dimerization (T=T)

Ionizing radiation effects on DNA





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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

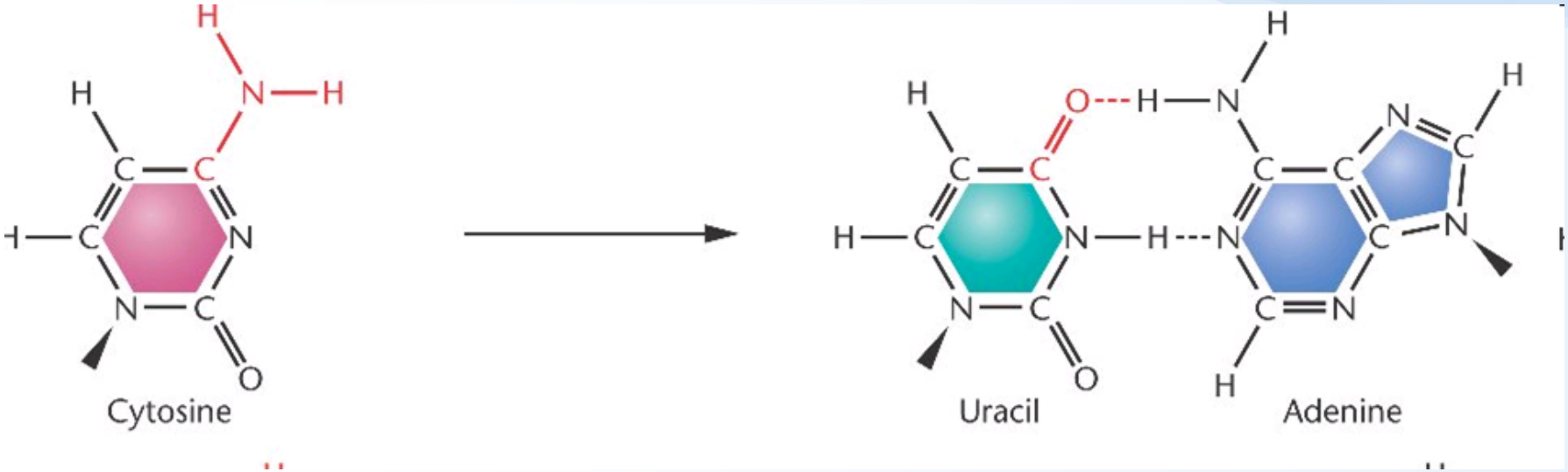
inserted during replication instead of nucleotides

Some compounds may require "activation" by metabolism
pro-mutagen (pro-carcinogen) → mutagen (carcinogen)

Small molecules → deamination of bases

HNO_2, HSO_3^- Hydroxylamine (HO-NH2), Methoxyamine (CH3-O-NH2)

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



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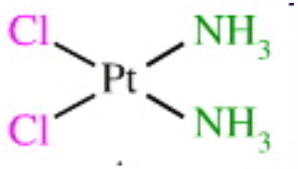
ALKYLating compounds

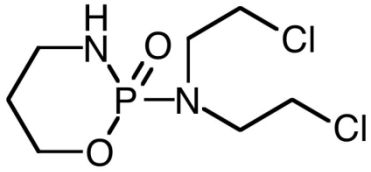
Covalent binding to NA (alkylation of bases, crosslinks in dsDNA)

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

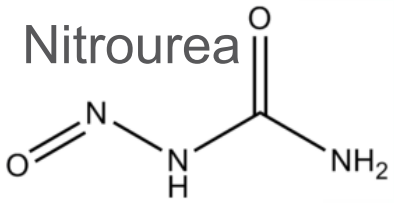
cisplatin

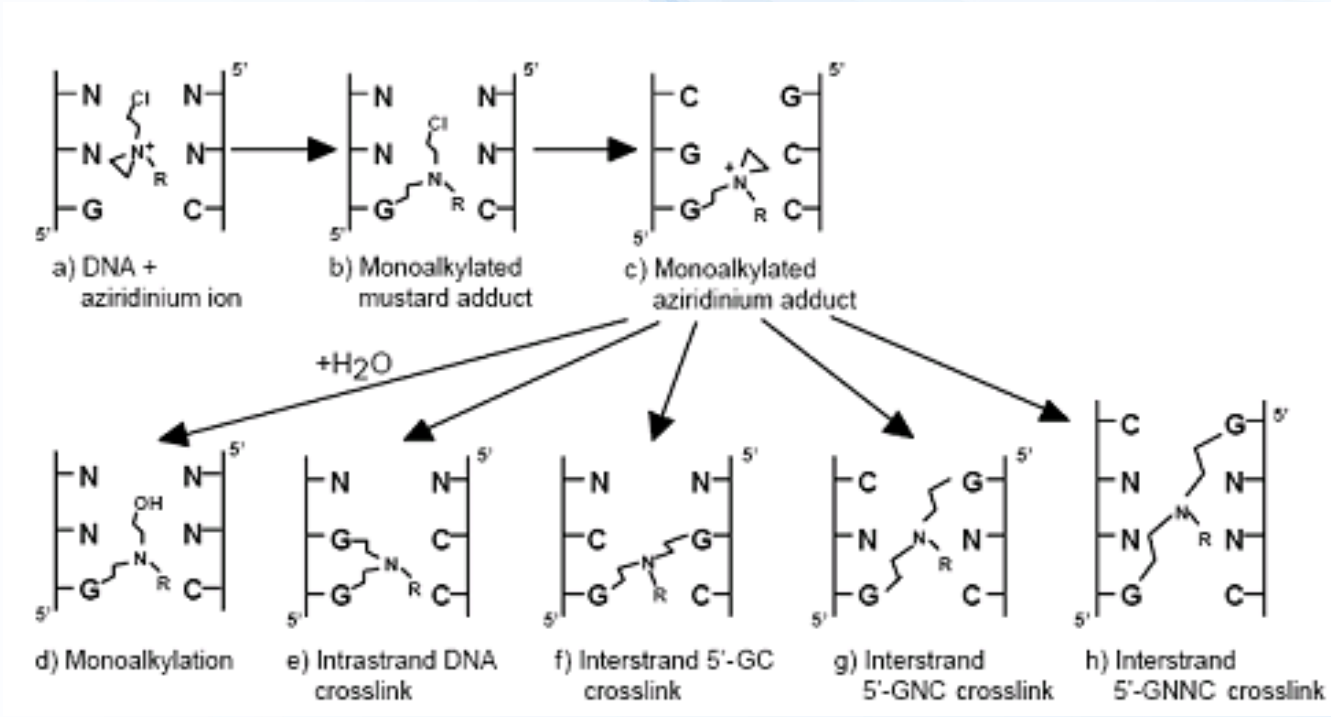


cyclophosphamide



Nitrourea





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ARYLating compounds

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

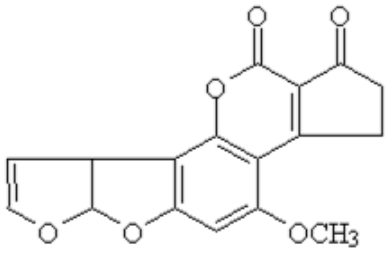
Mycotoxins (Aflatoxins) – *requires activation*

PAHs (benzo[a]pyrene) – *requires activation*

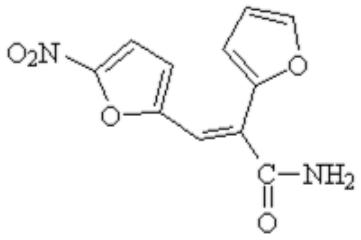
PAH derivatives

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

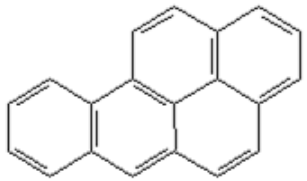
... many others

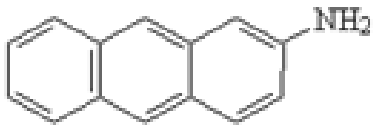


Aflatoxin B1 312.27



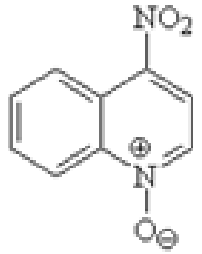
AF-2 (furylfuramide) 248.19


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

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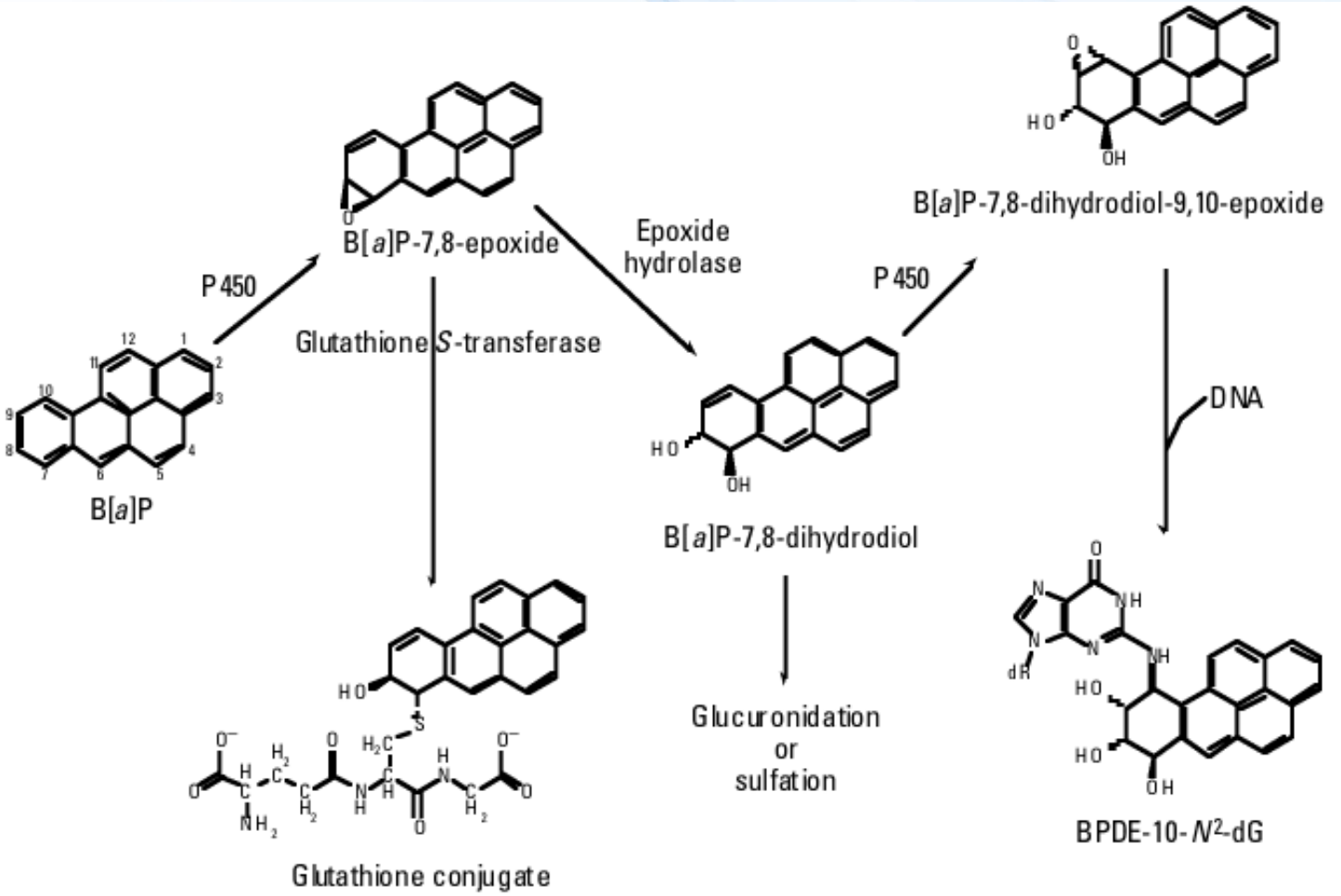
Bioactivation of benzo[a]pyrene → genotoxicity

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)



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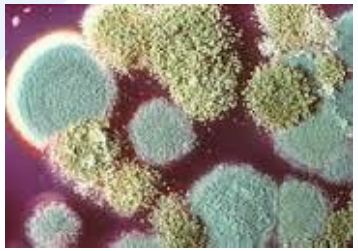
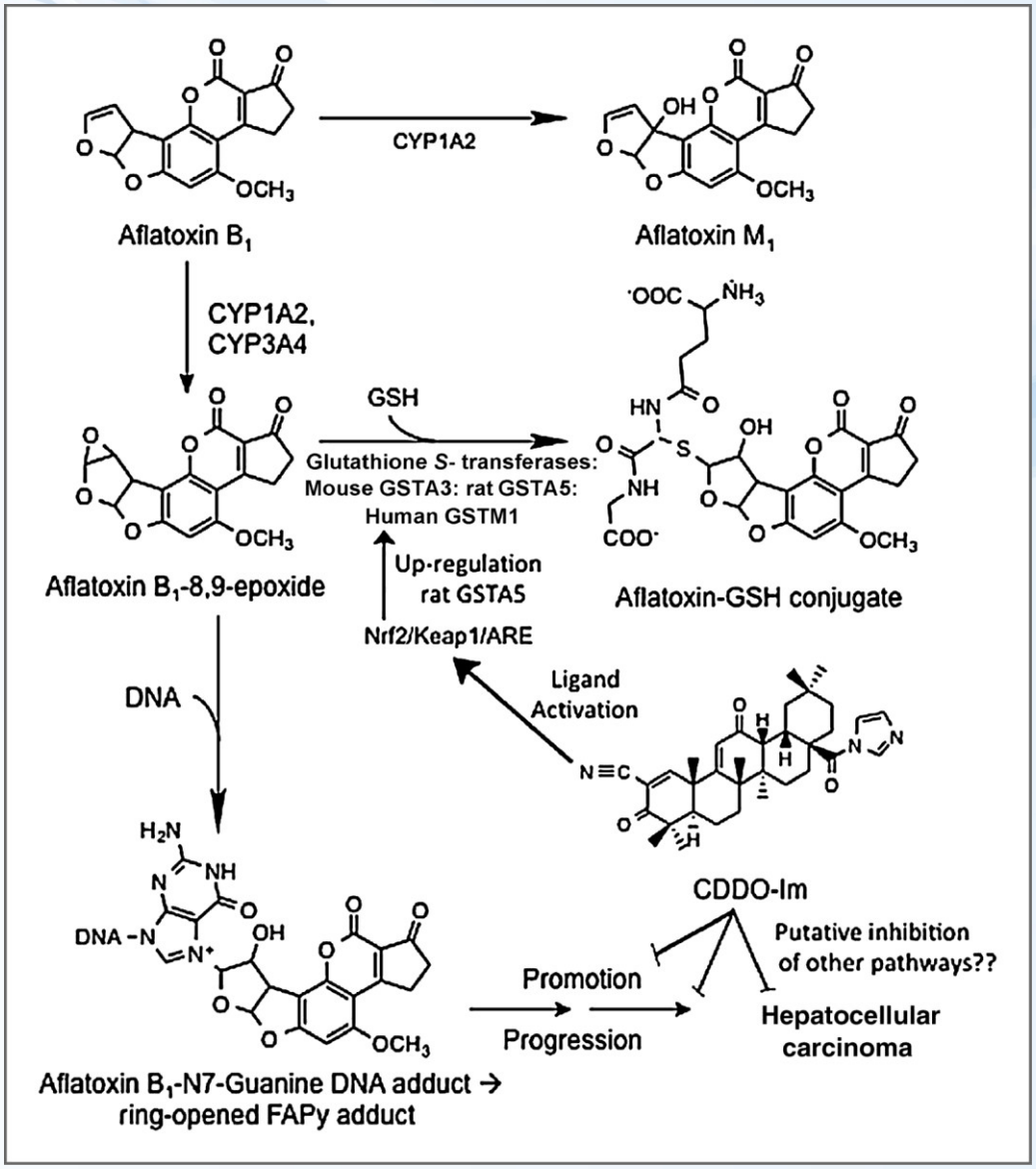
Bioactivation of aflatoxin → genotoxicity

AFLATOXIN sources





Intercalating agents

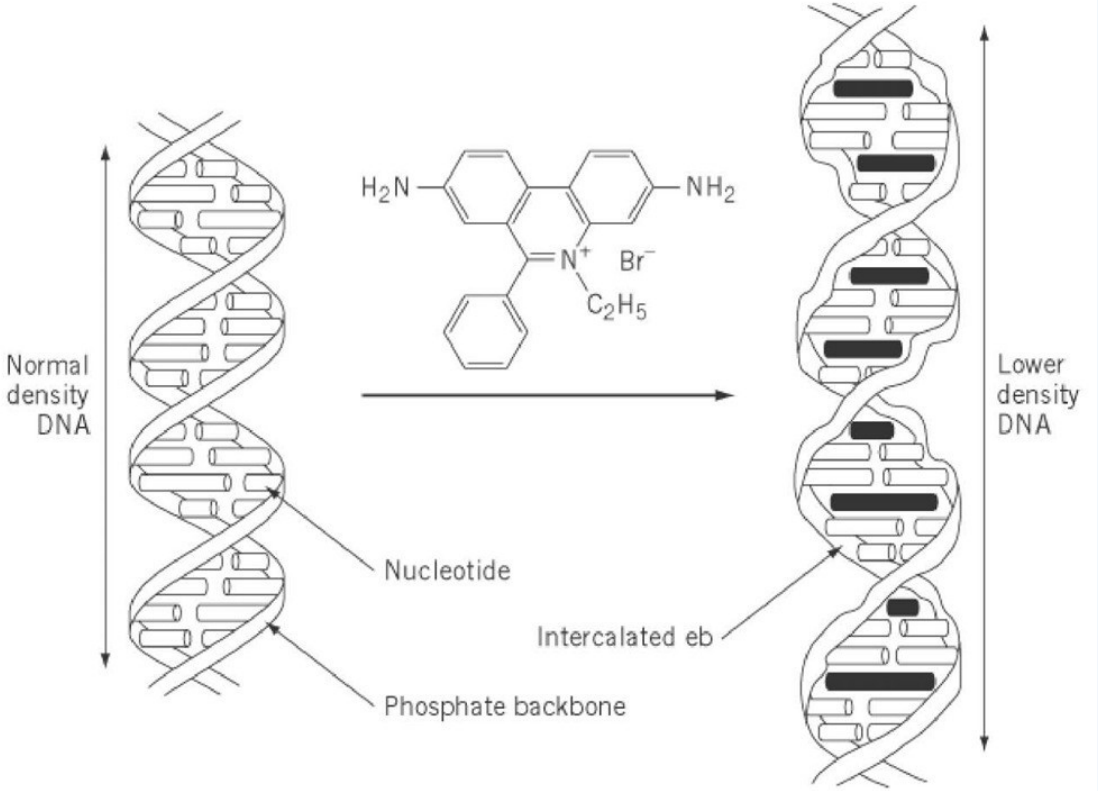
INTERCALATORS

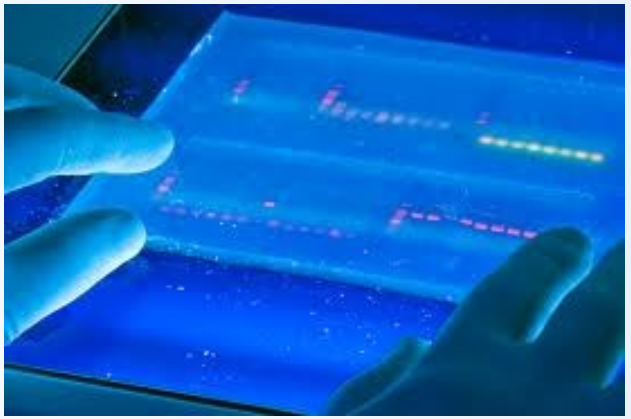
Compounds with characteristic structures “fitting” into DNA

→ both noncovalent and covalent intercalation

Example 1 – ETHIDIUMBROMIDE

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

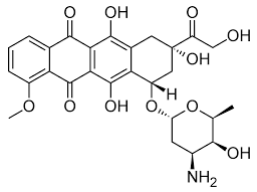




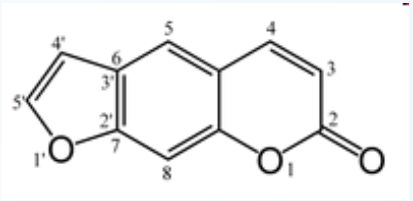
Intercalating agents

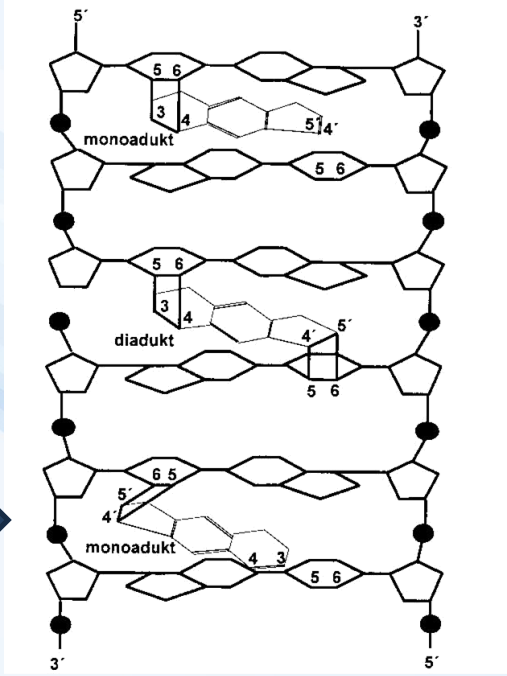
Other intercalator examples

-Anticancer drug - **doxorubicin**



- Psoriasis treatment **– psoralen** →





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

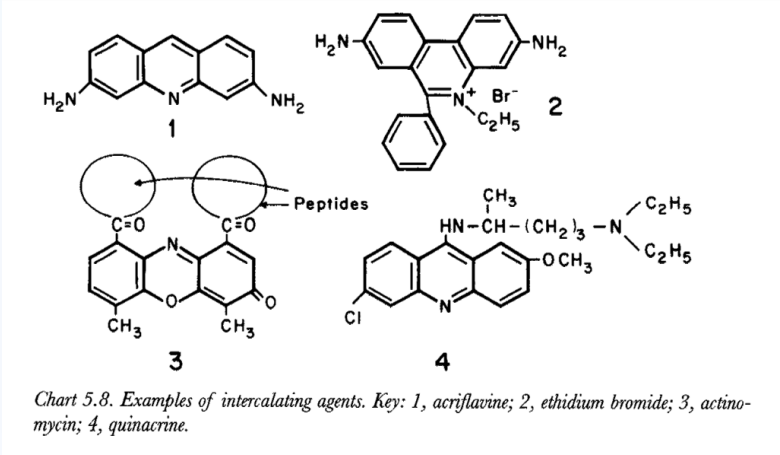


Chart 5.8. Examples of intercalating agents. Key: 1, acriflavine; 2, ethidium bromide; 3, actinomycin; 4, quinacrine.

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Base analogs

Structure similarity with natural bases

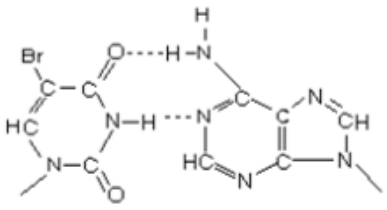
→ Incorporation into DNA during replication

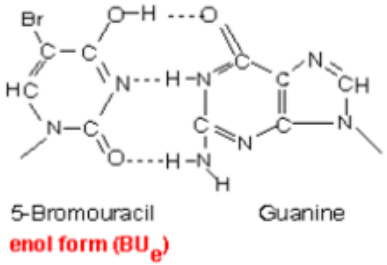
→ Base exchange mutations

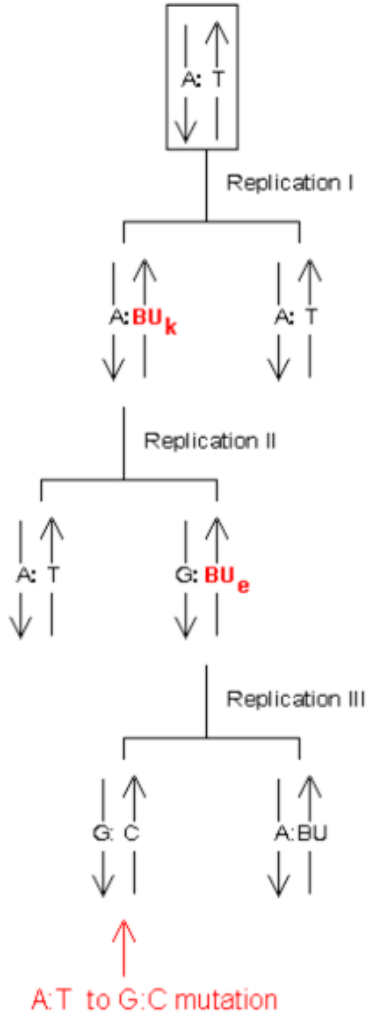
Example

5-Br-Uracil (anticancer drug)

AT → GC shift







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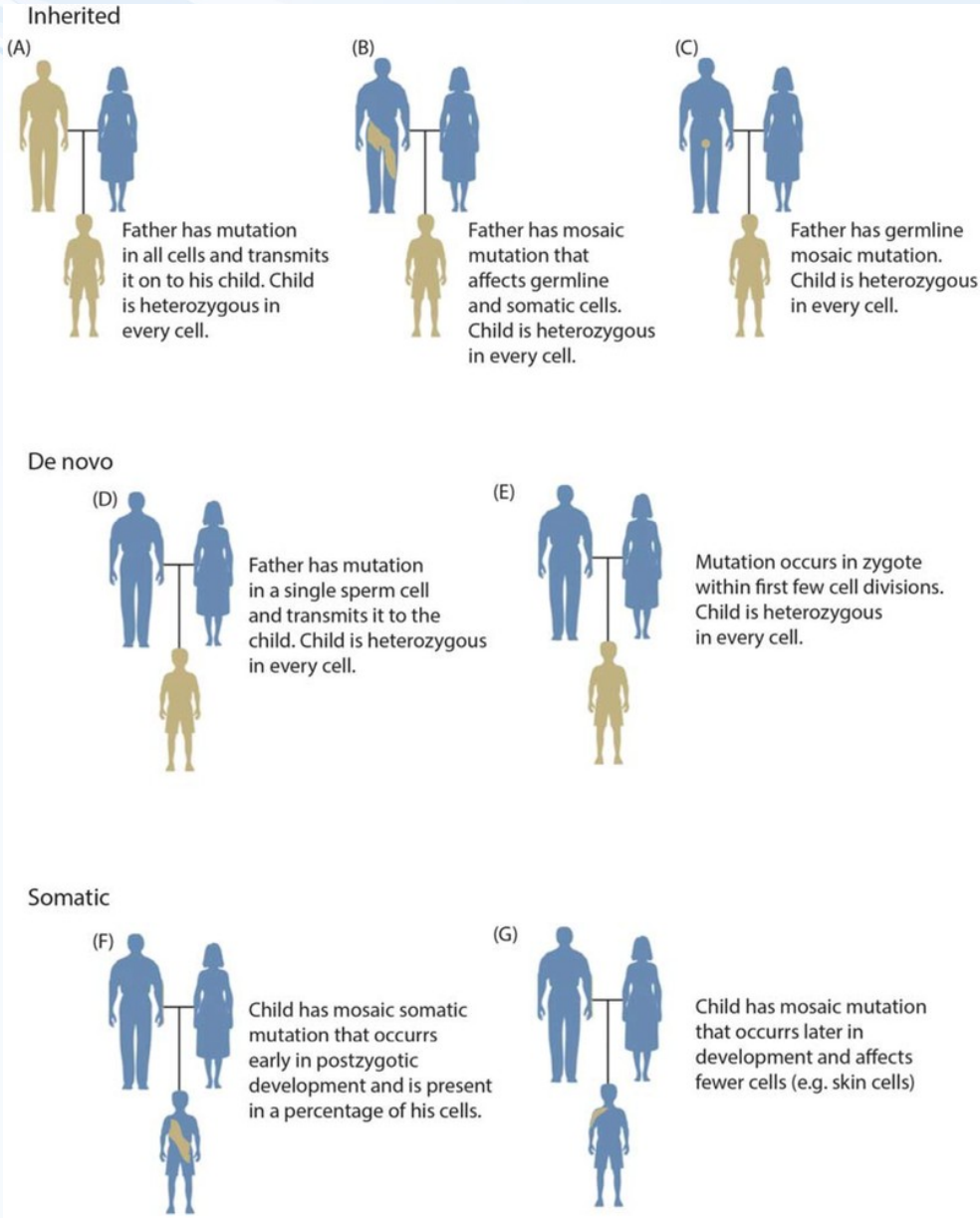
Wrap-up: Mutations and genotoxicity

- **Mutations can be:**
  - **Inherited (inheritable) or somatic**
- **Impacts of mutations**
  - Lethal
  - Non-lethal

Impacts of point mutations

- (a) silent … *silent*
- **(b) missense**
  - **Changes in protein structure and then function** – various effects - both adverse (disease incl. cancer; lower fitness) or beneficial (evolution)
- (c) nonsense and (d) frameshift
  - Usually lethal



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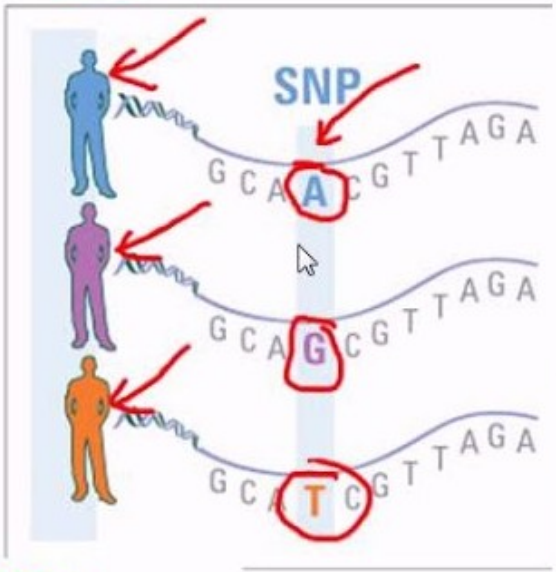
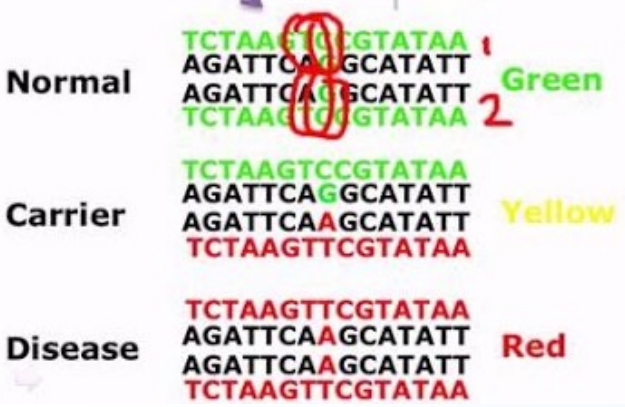
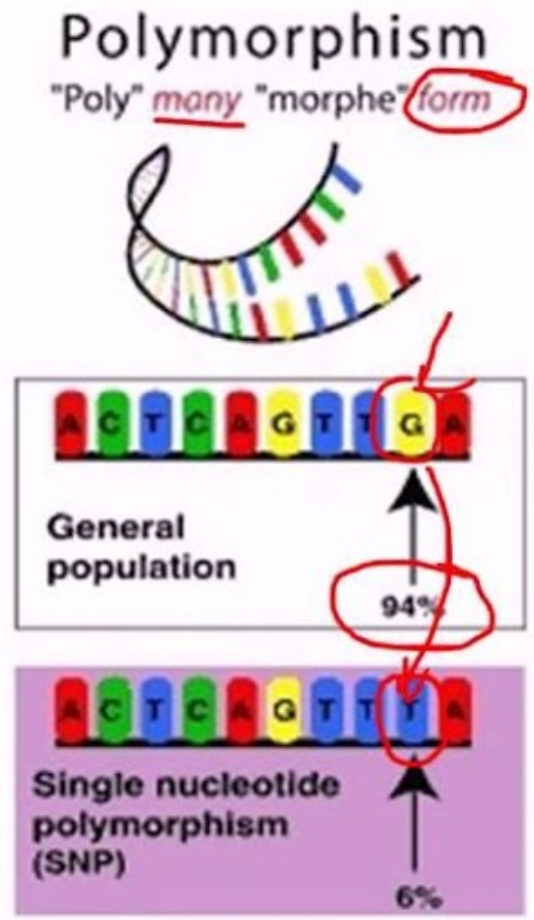
Single nucleotide polymorphism (SNP)

These are positions in a genome where some individuals have one nucleotide (e.g. a G) and others have a different nucleotide (e.g. a C).

Although each SNP could, potentially, have four alleles (because there are four nucleotides),







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Also further discussed at „biomarkers“ of susceptibility

Mutations (alleles) and evolution