Mutation

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Mutations

- A mutant gene is one that has a different sequence to the normal or wild type gene.
- This change is inheritable.
- There are a variety of mutations which may or may not cause a change in the phenotype. The vast majority of mutations are neutral, having no effect on the organism.

- DNA structure
  - Base-sugar-triphosphate
  - Double helix; A-T; C-G pairs
  - Chromosomes (with chromatin)

  Conserved and variable regions of code

Mutations

- In multicellular organisms for a mutation to be **inheritable** it must be present in the germline cells (meiotic).
- Mutations in somatic cells (mitotic) ONLY **will not be inherited** (**cancer**).
- Changes to the RNA code (errors in transcription) are not inherited.

Static Mutations

- Static mutations are those where the change in the code becomes a stable incorporation into the genome of the germline cells as well as all somatic cells in the organism (except RBC)
- This change is transferred to the next generation so the genome of the offspring is the same as the parent.

Static Mutations

- The classic mutations such as sickle cell anemia is much studied examples of this phenomenon.
- The ultimate expression of these mutations as a phenotype depends on the genetic information from both parents and **epigenetic** factors.

Dynamic Mutations

- Examples here are the trinucleotide repeats (TNR).
- The mutation increases (increasing number of repeats) in severity with each generation
- It also varies between tissues of the same organism.

Dynamic Mutations

- This leads to **genetic anticipation**.
- The following generation will be more affected
- The increase in the copy number of the repeat can occur at replication, repair or recombination – whenever DNA is being copied.

Mutation-Causes

- Incorrect base pairing due to tautomeric shifts
- Removal of nitrogenous bases
- Alteration of nitrogenous bases
- Addition or deletion of nucleotides
- Single strand breaks
- Double strand breaks
- Crosslinking—covalent linkage between bases

Types of Mutations

- **Transversion:** purine replaced by a pyrimidine or vice versa.
- **Transition:** pyrimidine for pyrimidine or purine for purine e.g. A→G,C→T
- **Silent mutations:** altered codon still codes for the same amino acid
- **Frameshift:** shifts the reading frame by adding or deleting base(s). Leads to a non-functional protein.

Types of Mutations

- **Neutral mutation:** altered codon codes for a functional similar amino acid → no effect on the functionality of protein.
- **Point mutation:** single base pair change, can be a substitution, deletion, or addition.
- **Missense:** altered codon for functionally different amino acid → these can be lethal
- **Nonsense:** mutation produces a stop codon → truncated protein → also dangerous.

Types of Mutations

- **Splice mutation:** produces a splice site or removes one (only in eukaryotes)

- **Temperature sensitive**: mutation causes a change the protein function which is temperature sensitive. Usually the protein functions normally at lower permissible temperatures (<30^{o}C) but is inactive at higher temperatures (>40^{o}C).

- **Leaky mutation:** A mutation which doesn't affect the organism under normal conditions. It will show up in "stressed" conditions.

Spontaneous Mutations

- Arise without mutagenic agents. DNA pol has proofreading function, can remove mismatched base
- Even if DNA pol misses a mismatch other systems can recognize and repair it.

Spontaneous Mutations

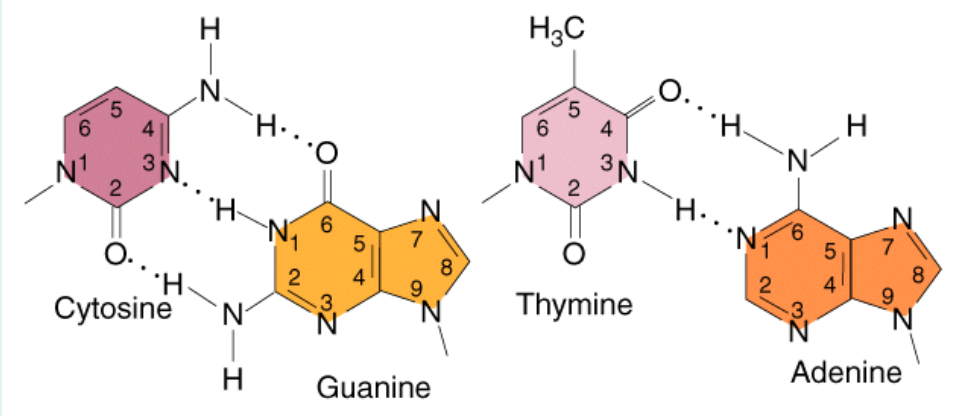
- Tautomeric shifts during replication.
- Depurination—if a purine base is lost from C-1 of deoxyribose, Enzymes specific for this type of mutation have evolved

- Deamination.
  - C→U
  - A→Hypoxanthine

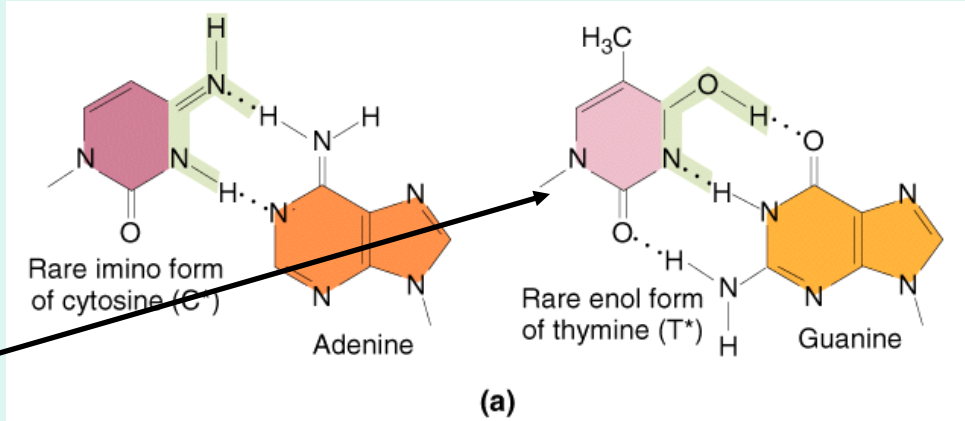
} Altered H-bonding

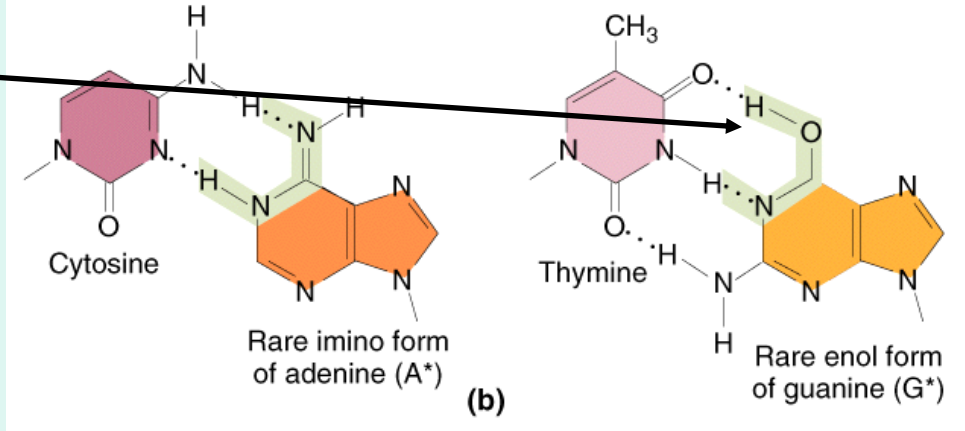
Tautomers and Mutation

Normal base pairing



Rare enol forms of thymine and guanine



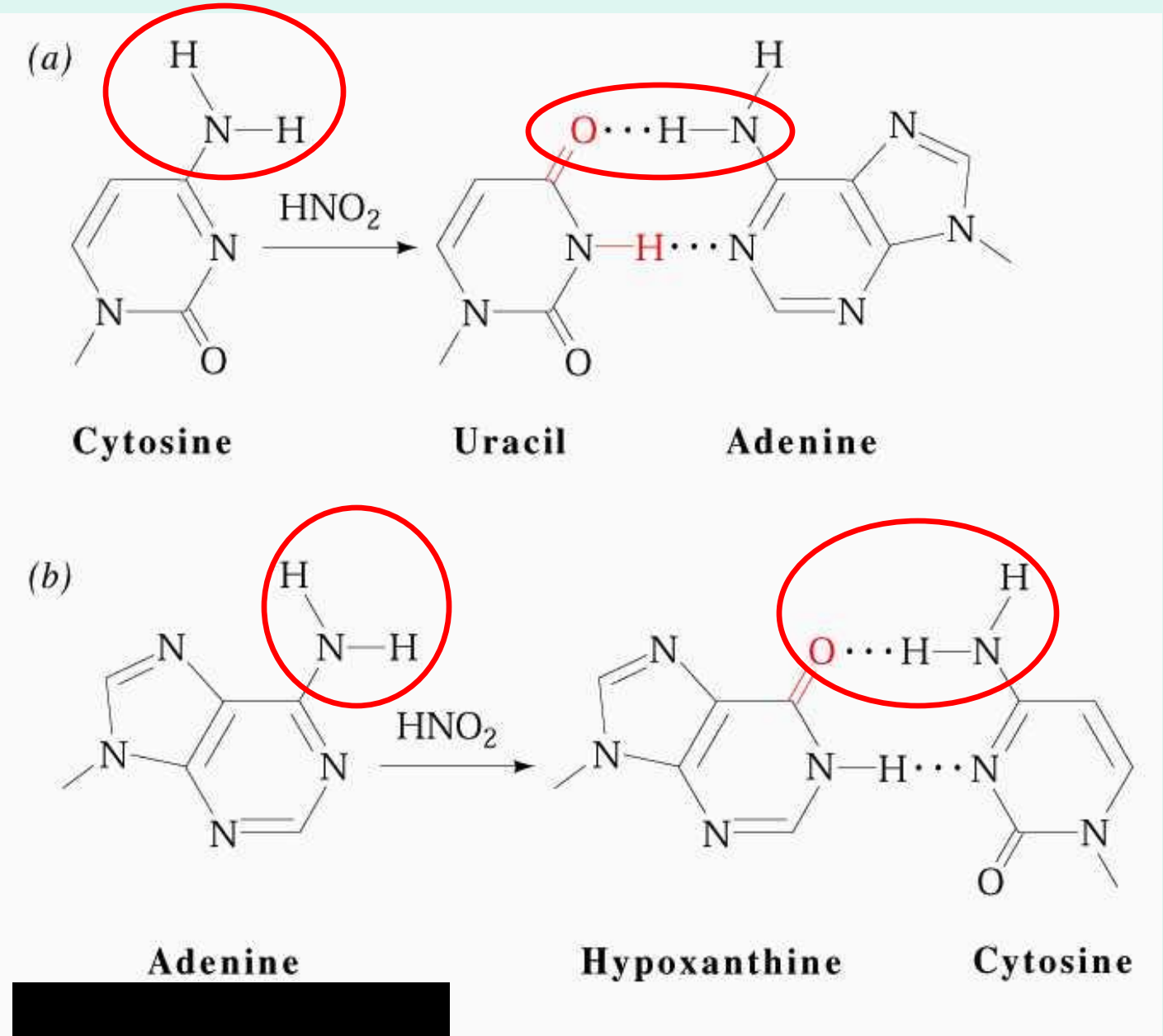


Back

Deamination of C and A

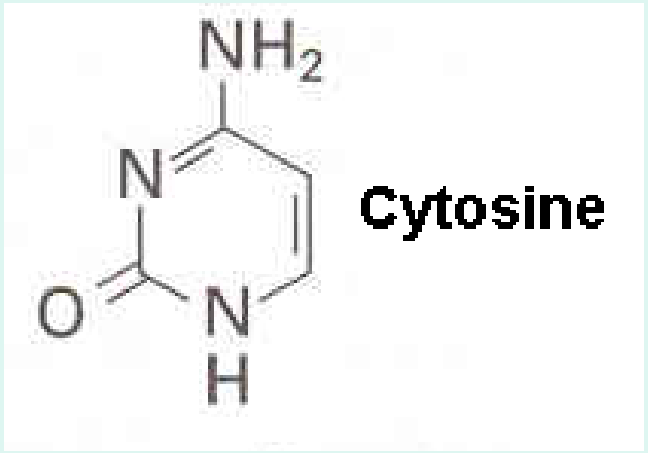
- C→U

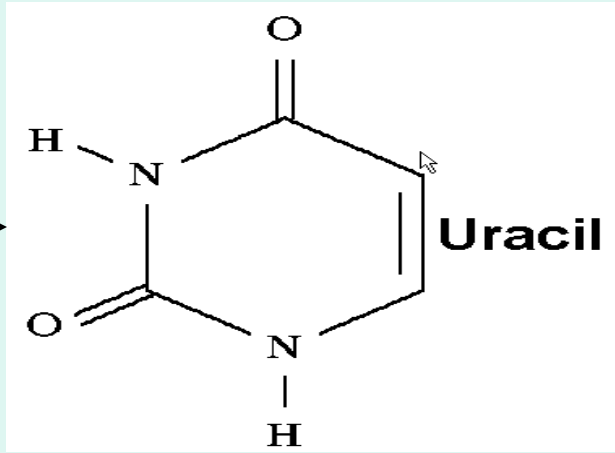
A→Hypox
anthe

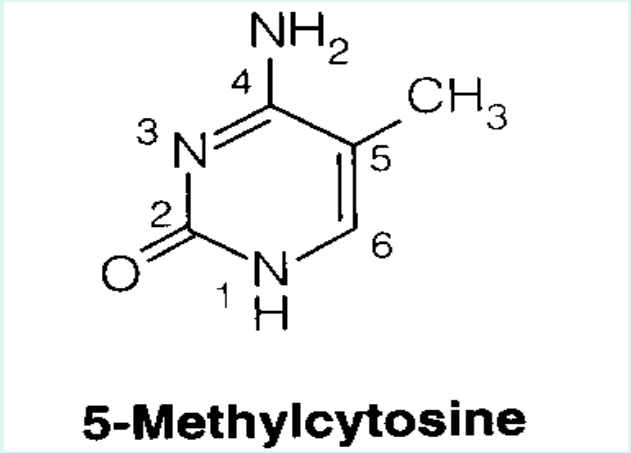


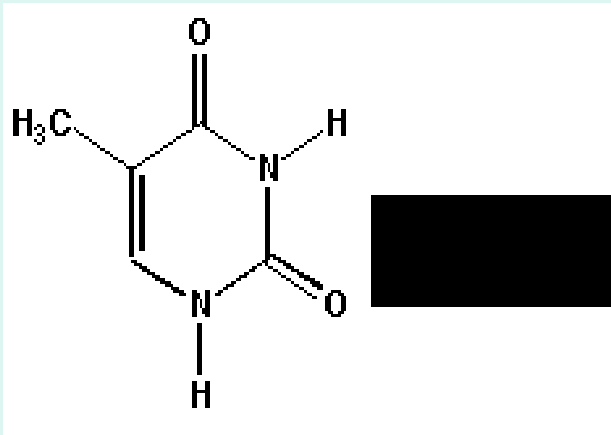
5 Methyl Cytosine Deamination

- Easily recognized and corrected
- What about 5-methyl cytosine?









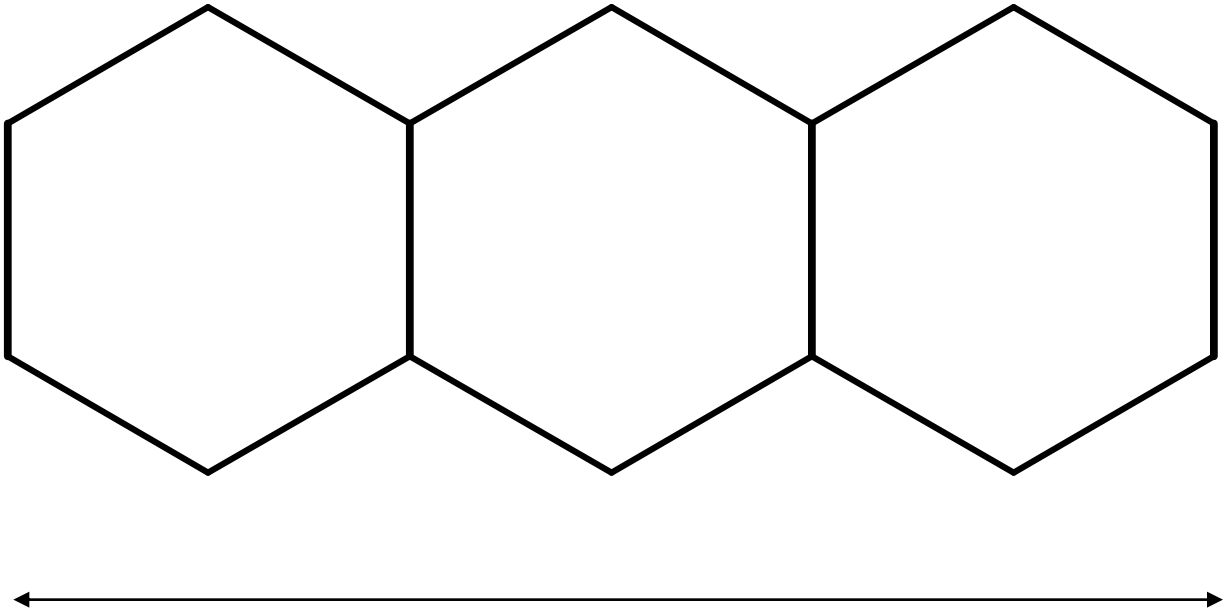
Mutagenesis.

- **Definition of mutagen:** a physical or chemical agent which causes mutation to occur at a higher frequency**.**
- **Natural or spontaneous mutations:** These are the mutations which occur at a normal background rate all the time. These mutations in the genome can arise naturally in the course of a cell's life.

Induced Mutagenesis.

- **Intercalators,** planar ring structures which slide in between the base pairs causing a disruption to the normal base stacking eg ethidium Bromide, acridine orange, actinomycin D.
- **Alkylating agents,** which methylate or ethylate bases and result in altered base pairing during replication e.g methylmethane sulfonate (MMS), nitrosamine.

Intercalators

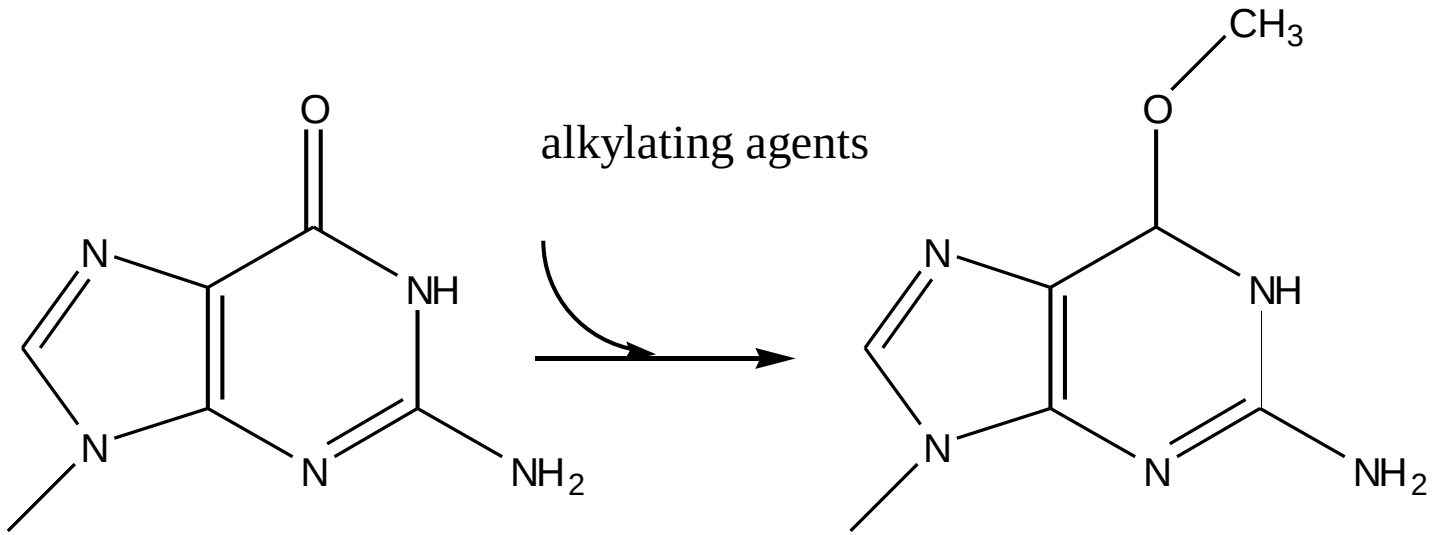


Distance of a base pair, fits in nicely
and separates the base stacking

Induced Mutagenesis.

- **Anti-cancer drugs,** used to treat brain tumours e.g. Temozolomide or temodal alkylates guanine residues at positions 6 and 7 and interferes with DNA replication.

Alkylating agents



Guanine

O^{6-}methylguanine

Other mutagen factors

- UV light (200-300nm)
- Thymine dimerization (T-T)
  - Cytosine hydration (C + H_2O)
- Ionizing radiation

  (x/ γ -rays, <10^{-10}m; α, β particles)
  - Single strand, double strand breaks, base changes
- Biotoxins (aflatoxin-B1)
- Viruses (HPV)

Types of DNA damage

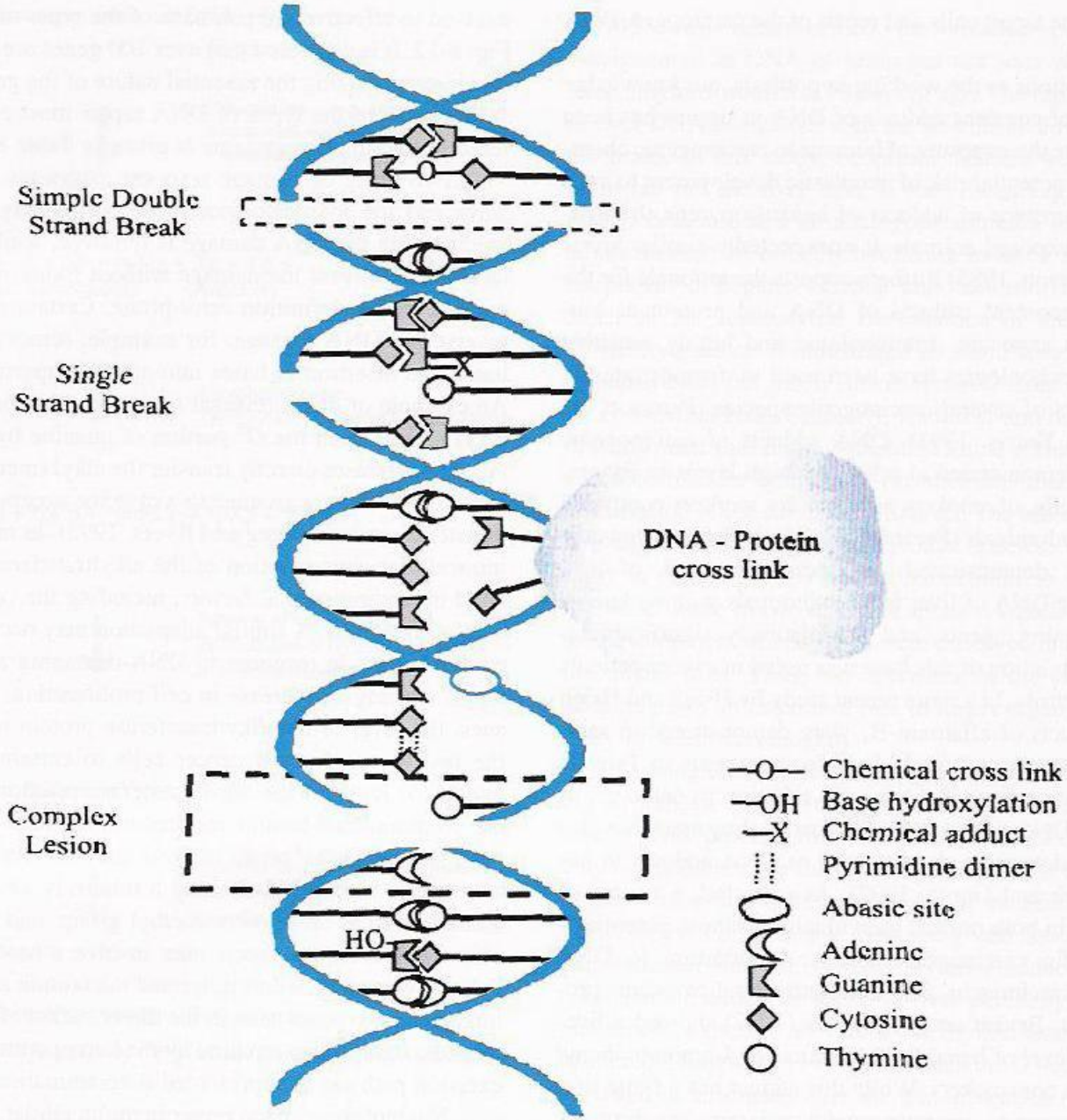


Figure 8-12. Schematic representation of chemical- and radiation-induced lesions in DNA.

Reactive oxygen species

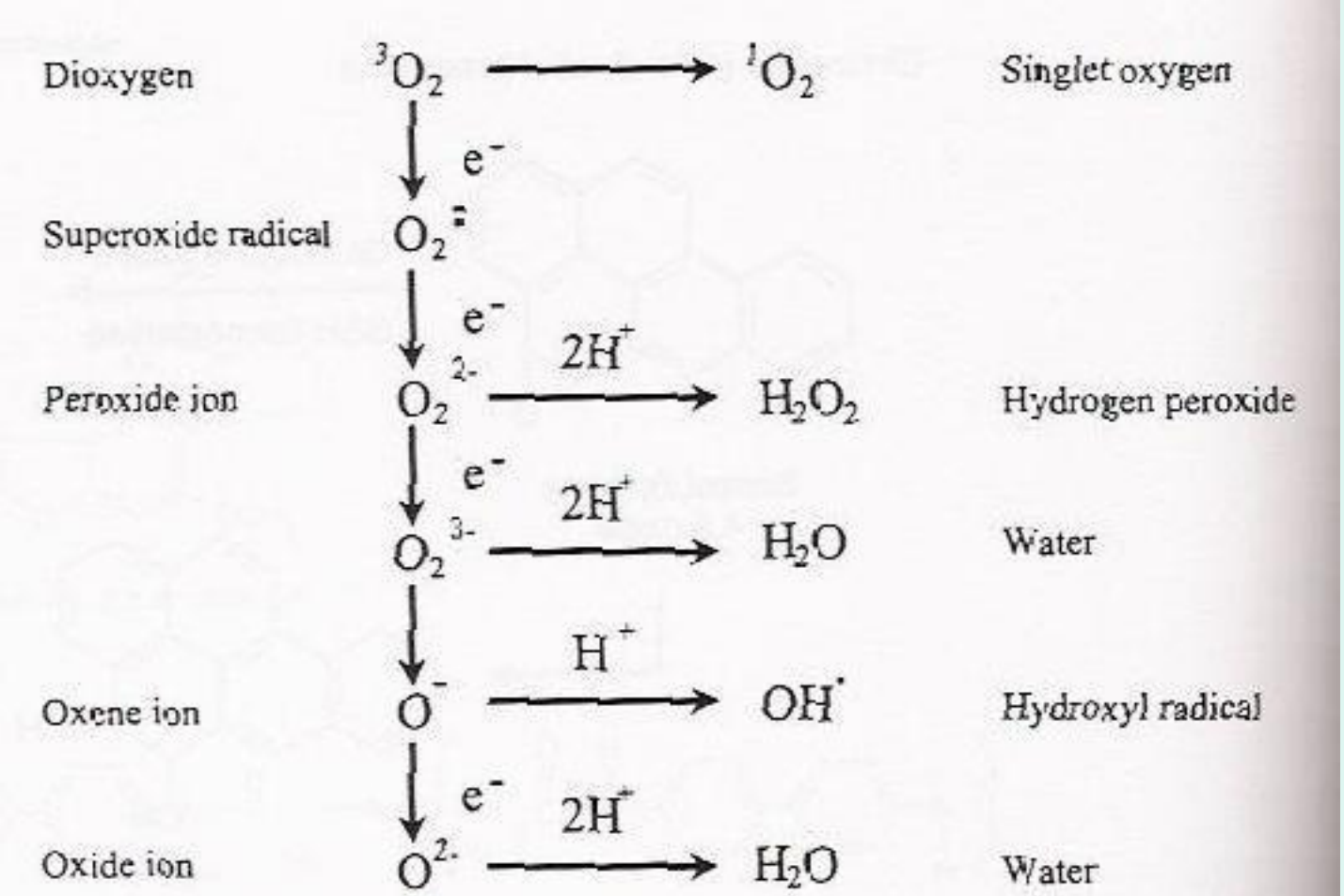


Figure 8-7. Sequential and univalent reduction of molecular oxygen indicating various species produced. [Modified from Martínez-Cayuela (1995), with permission of authors and publishers.]

Testing Mutagenesis:
the Ames test

- A quick screening test for potential **mutagenic compounds**.
- A strain of Salmonella which has a defect in the histidine biosynthetic pathway is plated out, as a lawn, on a medium containing minimal His *(just enough to keep the cells alive but not enough to sustain proliferation)*

Testing Mutagenesis:
the Ames test

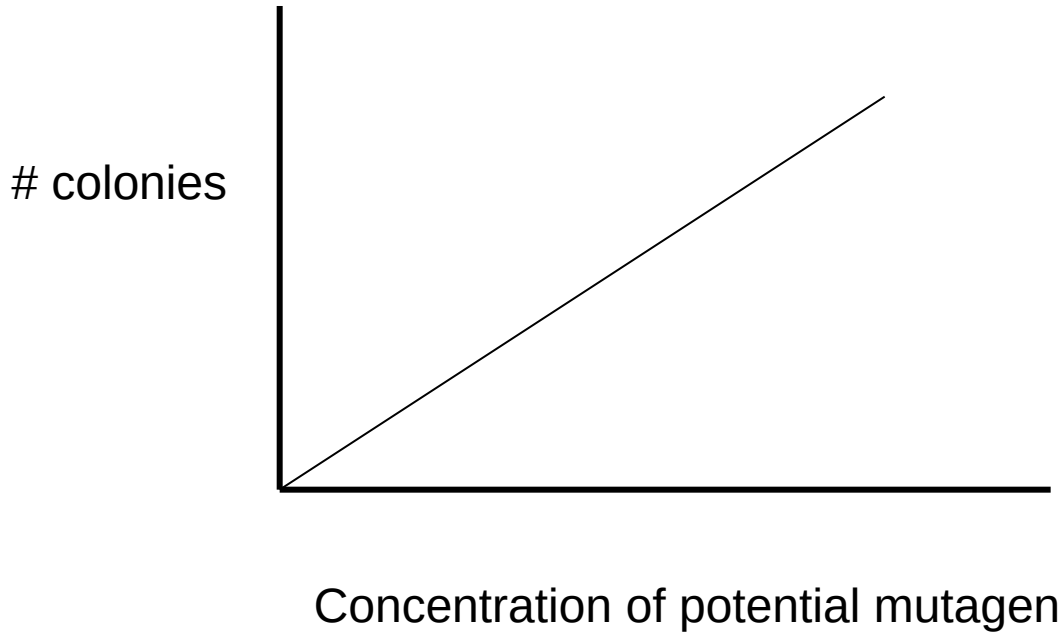
- The compound of interest is applied to a disc in the centre of the plate and the plate is incubated overnight.
- Different plates with increasing amounts of the compound are put up.
- Sometimes liver extract is applied also to check for cellular conversions

Testing Mutagenesis:
the Ames test

- If the compound is mutagenic it will cause a number of cells to revert to grow on the medium; *the other cells can't because of the His defect*.
- The more colonies forming around the disc the more mutagenic the compound.
- A non-mutagenic compound will have a few colonies scattered over the whole plate (spontaneous reversions).

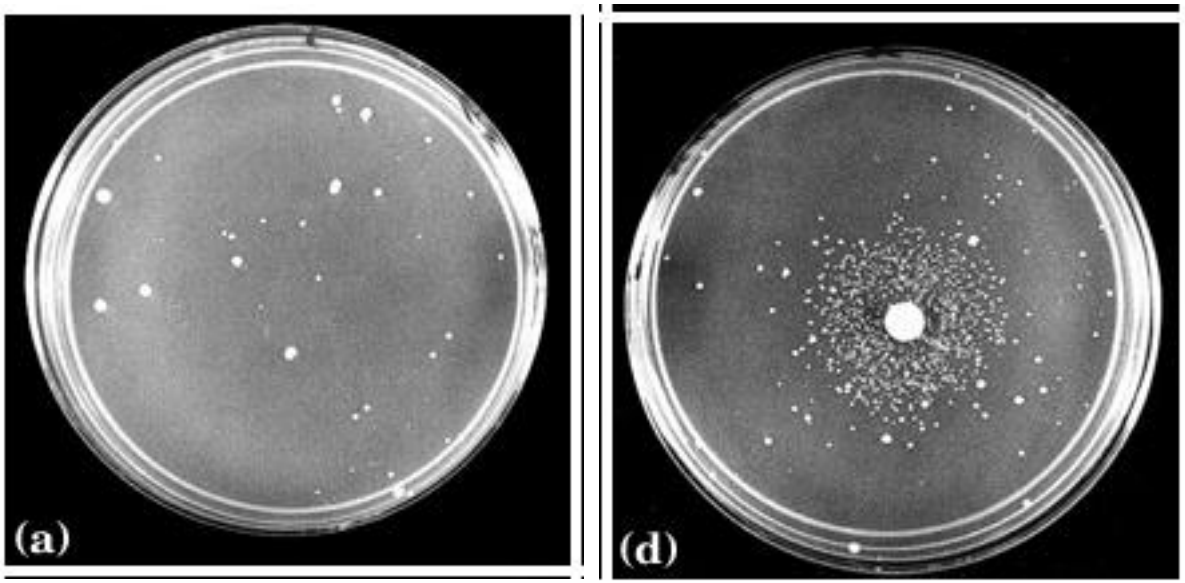
Mutagenic Response

- A mutagenic compound will typically have a linear dose response.



The negative control

A mutagenic compound



Rat liver extract is optionally added to simulate the effect of metabolism, as some compounds, like benzo[*a*]pyrene, are not mutagenic themselves but their metabolic products are.[3]

The bacteria are spread on an agar plate with small amount of histidine. This small amount of histidine in the growth medium allows the bacteria to grow for an initial time and have to mutate. **When the histidine is depleted only bacteria that have mutated to gain the ability to produce its own histidine will survive**. The plate is incubated for 48 hours. The mutagenicity of a substance is proportional to the number of colonies observed