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Bi4025en
Molecular Biology

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Lecture 8

- Molecular mechanisms of mutagenesis and recombination.

Source of genetic variability

- Mutations
- Recombination
- Transposition

Mutations

TABLE 14.1 **Major types of mutations and their distinguishing features**

Basis of classification	Major types of mutations	Major features
Origin	Spontaneous	Occurs in absence of known mutagen
	Induced	Occurs in presence of known mutagen
Cell type	Somatic	Occurs in nonreproductive cells
	Germ-line	Occurs in reproductive cells
Expression	Conditional	Expressed only under restrictive conditions (such as high temperature)
	Unconditional	Expressed under permissive conditions as well as restrictive conditions
Effect on function	Loss-of-function (knockout, null)	Eliminates normal function
	Hypomorphic (leaky)	Reduces normal function
	Hypermorphic	Increases normal function
	Gain-of-function (ectopic expression)	Expressed at incorrect time or in inappropriate cell types
Molecular change	Nucleotide substitution	One base pair in duplex DNA replaced with a different base pair
	Transition	Pyrimidine (T or C) to pyrimidine, or purine (A or G) to purine
	Transversion	Pyrimidine (T or C) to purine, or purine (A or G) to pyrimidine
	Insertion	One or more extra nucleotides present
	Deletion	One or more missing nucleotides
Effect on translation	Synonymous (silent)	No change in amino acid encoded
	Missense (nonsynonymous)	Change in amino acid encoded
	Nonsense (termination)	Creates translational termination codon (UAA, UAG, or UGA)
	Frameshift	Shifts triplet reading of codons out of correct phase

https://www.uky.edu/~tphillip/2FChapter14.ppt

Mutations

- Mutation is an inherited change in the genetic material of an organism.
- Consequence of replication failure or accidental DNA damage.
- Mostly without significant effect.
- Sometimes fatal.
- Sometimes bring an advantage (e.g. resistance of bacteria to antibiotics).
- Increase diversity among individuals of a given species.

Mutations

- Mutations occur randomly.
- More likely to cause harm than good.
- Responsible for thousands of human diseases, including cancer.
- The survival of the cell/organism depends on minimizing changes in DNA.

Mutations

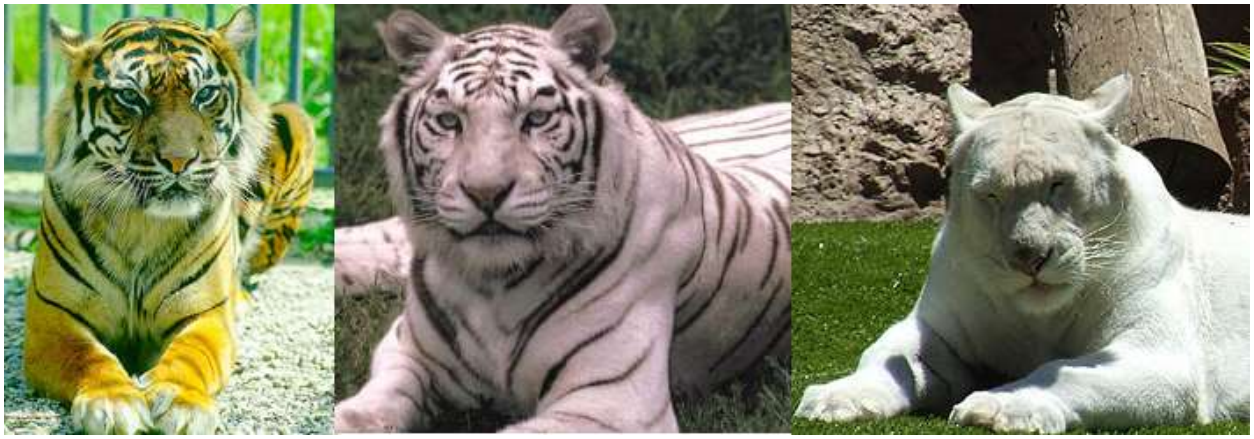
- Change in the primary structure of the nucleic acid:
  - at the gene level (base pair substitutions, insertions, deletions),
  - at the level of structure or number of chromosomes.
- Genotype can also be changed by new combinations of existing genetic variants – alleles, recombination.
- The basis of evolution, adaptation to the environment.
- Use in research (identification of genes, research of their function, regulation of expression, etc.)

Polymorphism

- A state where there are at least two genetic variants (alleles). Eventually, the occurrence of different forms among the members of a population or colony.
- We talk about a mutation if the percentage of the allele in a population less than 1%.
- Changes in gene expression that are phenotypically manifested, and which are not the result of changes in the DNA nucleotide sequence are referred to as epigenetic.

Standard x Mutant phenotype

- Standard allele - standard phenotype.
- Mutant allele - mutant phenotype (mostly recessive).



- Wild type tigers have orange fur and black stripes. One mutation prevents the deposition of the orange/brown pigment, and the result is a "white tiger" that still has dark stripes. A different mutation prevents any melanin (brown pigment) from forming at all, and the result is an albino tiger.

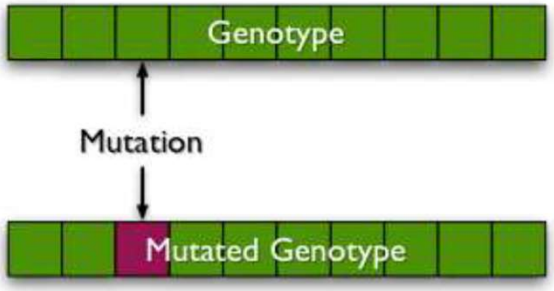
What does mutation affect?

- The sequence of the genes (and thus structure of their products) or regulatory areas in DNA.

- Chromosome structure (chromosome aberrations: duplication, deletion, inversion, translocation).

- Genome structure:
- Aneuploidy – change in the number of certain chromosomes.

- Euploidy – change in the number of chromosomal sets.



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Classification of Mutations

- Direction of mutations:

- Forward mutation - mutation changes wild type (ancestral) to mutant (derived).

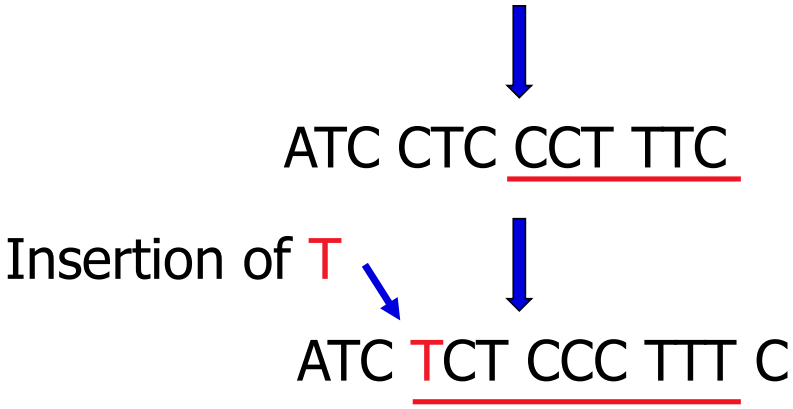
- „Reverse“ mutation – mutation changes mutant (derived) to wild type (ancestral).
  - Reversion to the wild type amino acid restores function.
  - Reversion to another amino acid partly or fully restores function.

- Suppressor mutation - partially or completely cancels out the effect of another so-called suppressor-sensitive mutation.
  - Intragenic suppressors occur on the same codon; e.g., nearby addition restores a deletion.
  - Intergenic suppressors occur on a different gene.

Intragenic suppressor mutations – type I

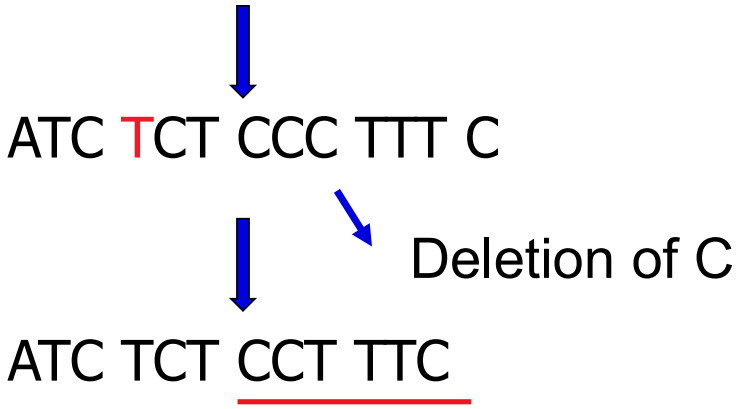
- Intragenic suppressor mutation suppresses suppressor-sensitive mutation at the same gene level (intragen).

- suppressor-sensitive mutation



- frameshift mutation (reading frame shift)

- suppressor mutation



- restoration of the original reading frame

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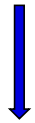
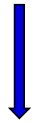
Intragenic suppressor mutations – type II

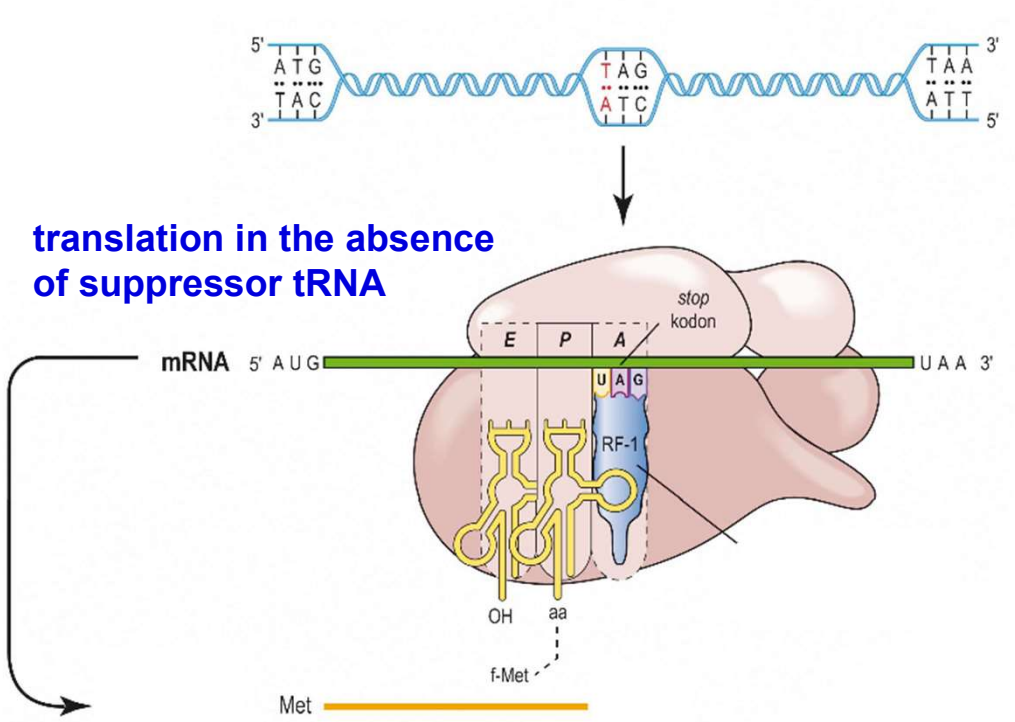
Serine	TCA	• Original codon.
		• Suppressor-sensitive (first) mutation.
	TAA	• Stop codon, loss of product function.
		• Suppressor-sensitive (second) mutation.
Tyrosine	TAT	• Codon for another amino acid, restoration of function (sometimes only partial).

Intergenic - Suppressor mutations

- Many function in mRNA translation.
- Each suppressor gene works on only one type of mutation - nonsense, missense, of frameshift mutation.
- Suppressor genes often encode tRNAs, which possess anti-codons that recognize stop codons and insert an amino acid.
- Three classes of tRNA nonsense suppressors, one for each stop codon (UAG, UAA, UGA).
  - tRNA suppressor genes coexist with wild type tRNAs.
  - tRNA suppressors compete with release factors, which are important for proper amino acid chain termination.
- Small number of read-through polypeptides are produced; tandem stop codons (UAGUAG) are required to result in correct translation termination.

Intergenic - Suppressor mutations

- The original mutated gene does not change, but the way of its mRNA translation is affected.
- The actual mutation is suppressor-sensitive (sus-).
- The codon turns into a nonsense codon or missense codon with altered sense.



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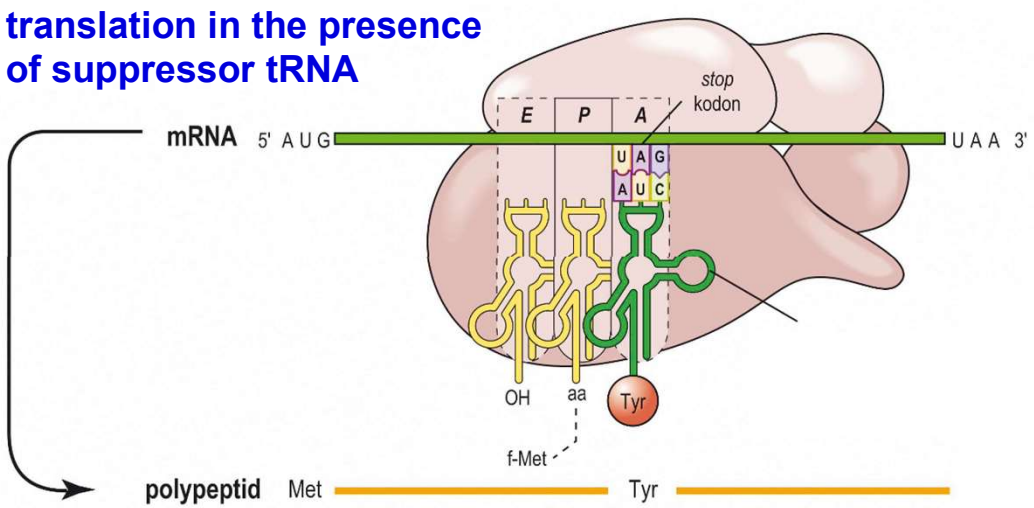
Intergenic - Suppressor mutations

- Suppressor mutation: mutation in the tRNA gene, which produces tRNA with altered antikodon.
- Gene suppressor = mutant allele of genu for tRNA (sup-).



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Classification of Mutations

- By type of affected cell:

- Somatic mutation
- Arise in the somatic cells.
- Passed on to other cells through the process of mitosis.
- Effect of these mutations depends on the type of the cell in which they occur & the developmental stage of the organism.
- In single-celled organisms, each mutation is duplicated during replication and passed on to the next cell generation.
- In multicellular mutations are passed on to the offspring only if they appear in the genome of germ line cells.

- Germline mutation
- They occur in the cells that produce gametes.
- Can provoke hereditary diseases.
- Passed on to future generations.
- In multicellular organisms, the term mutation is generally used for germ line mutations.

Classification of Mutations

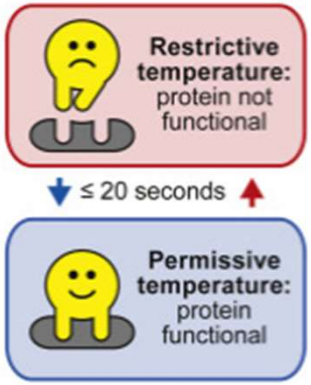
- According to the level at which it acts:
- Gene (point) – change of bases or sequence of bases at the gene level.
- Chromosome – change of sequence at the chromosome level.
- Genome – genomic mutations – change in the number of chromosomes.

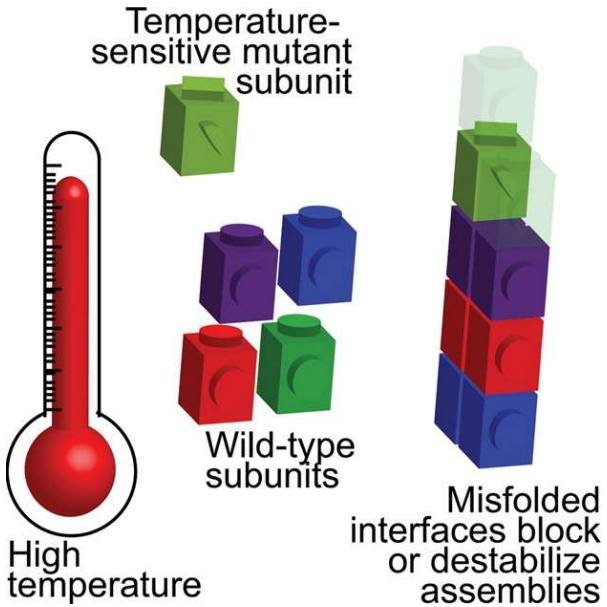
Classification of Mutations

- According to the effect on the viability of the organism:
- Vital mutations – compatible with survival.
- Lethal mutations – incompatible with survival.
- Conditionally lethal mutation – compatible with survival under certain conditions
  - ts ("temperature-sensitive") – lethal at elevated temperature.
  - sus ("suppressor-sensitive") – lethal without the presence of a suppressor.

Temperature sensitive mutation

- A conditional mutation that produces the mutant phenotype in one (restrictive or non-permissive) temperature range and the wild-type phenotype in another (permissive) temperature range.





- A conditional mutation that produces the mutant protein folds correctly only at a lower, permissive, temperature.
- At non-permissive temperature, the mutant protein is inoperative.
- Useful for experimentation (lethal mutations are difficult to study).

Bioessays 36: 836–846, DOI 10.1002/bies.201400062
Methods in Cell Biology, Volume 137, 2017, Pages 283-306.

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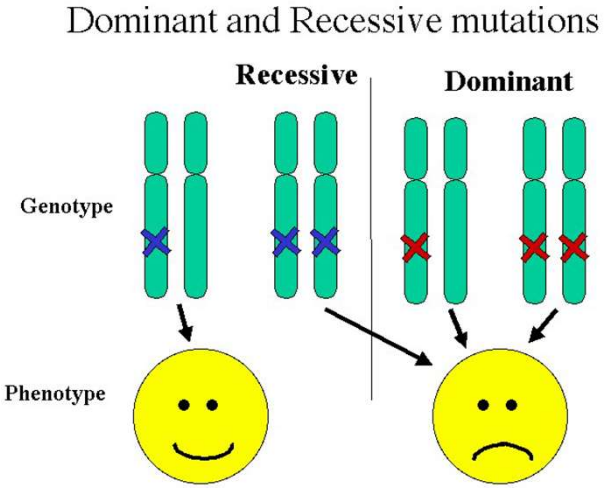
Classification of Mutations

- According to the degree of phenotypic manifestation (in diploid organisms):
- Dominant mutations – they manifest themselves even in heterozygous state.
- Recessive mutations – they manifest themselves only in a homozygous state, in a heterozygous state, the manifestation is masked by the dominant allele.



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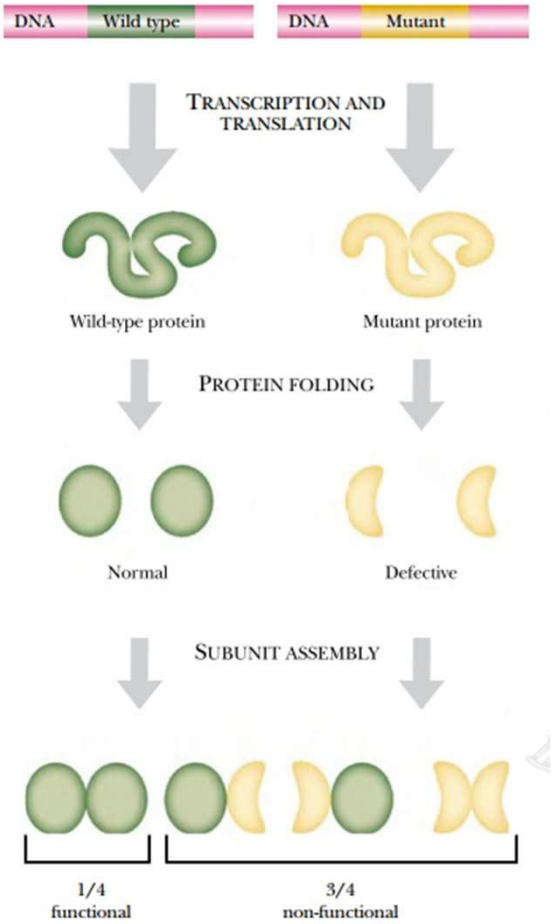
Dominant negative mutation



- Dominant negative mutations are those where the mutant protein loses its own function but, in addition, the defective protein interferes with the function of another protein.
- Thus a dominant negative mutation usually results from the presence of an altered, defective protein.
- Relate to genes coding for components of multimeric proteins.
- If the protein works as a dimer and in one both standard and mutant proteins appear in the cell, non-functional heterodimers are formed.
- Possibility of experimental use for targeted inhibition of proteins (research of their function).

https://www.brainkart.com/article/Dominant-Mutations-May-Be-Positive-or-Negative_13969/

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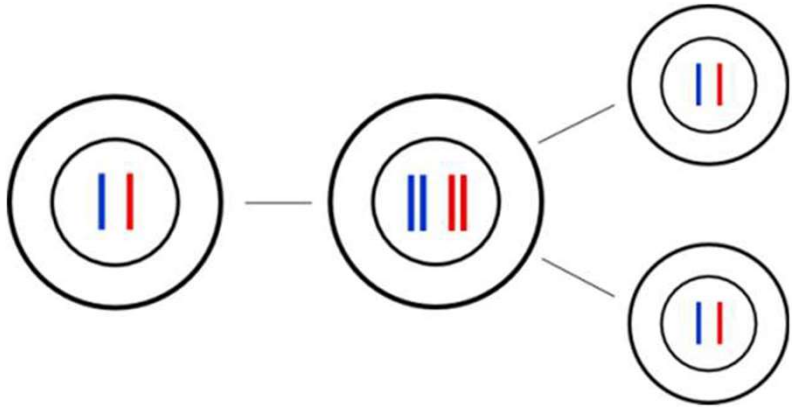
Diploid character of genome protects against adverse effect of mutations

- Diploid cell contains two complete sets of chromosomes, one from each parent.
- In diploid organisms, mammals, each gene has 2 copies.
- If one of them is damaged, the other can substitute the other one by providing the correct information.
- Therefore diploid state prevents defect caused by mutations, only, if the mutation is not dominant.



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Point mutations – molecular change

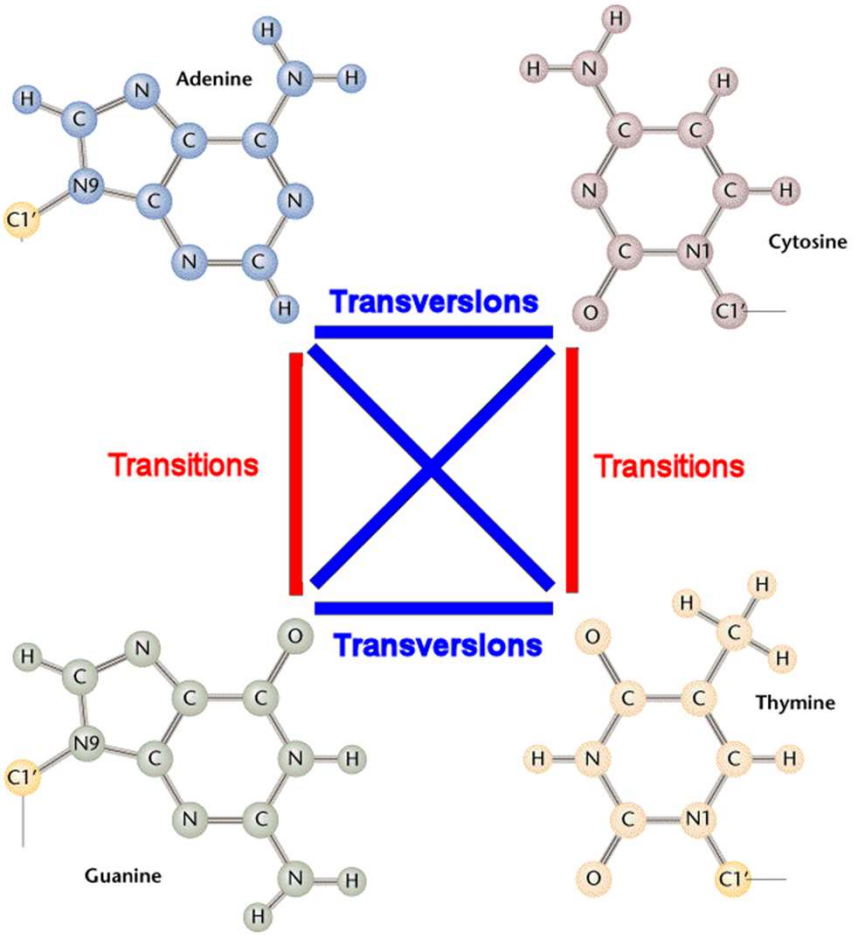
- **1. Base pair substitutions**

- Transitions
  - Convert a purine-pyrimidine to the other purine-pyrimidine.
  - 4 types of transitions; A <-> G and T <-> C
  - Most transitions results in synonymous substitution because of the degeneracy of the genetic code.

- Transversions
  - Convert a purine-pyrimidine to a pyrimidine-purine.
  - 8 types of transversions; A <-> T, A <-> C, G <-> T & G <-> C.
  - Transversions are more likely to result in nonsynonymous substitution.

- 2. **Base pair substitution, deletions and insertions**

Point mutations molecular change

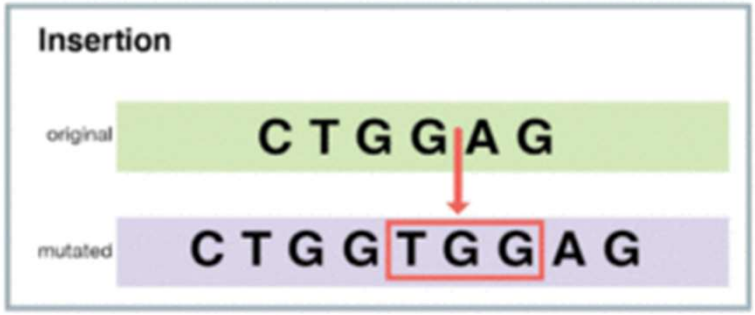


- Although there are twice as many possible transversions, because of the molecular mechanisms by which they are generated, transition mutations are generated at higher frequency than transversions.
- As well, transitions are less likely to result in amino acid substitutions (due to "wobble"), and are therefore more likely to persist as "silent substitutions" in populations as single nucleotide polymorphisms (SNPs).

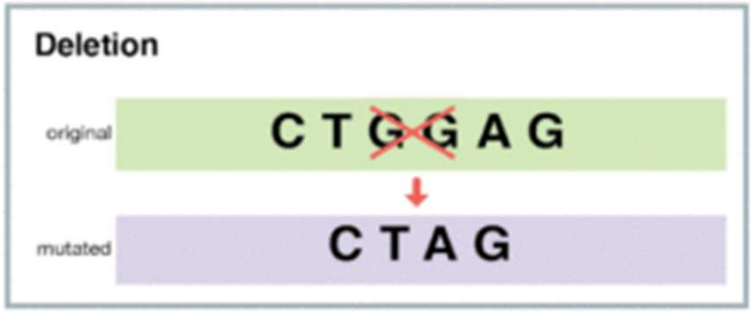
https://www.mun.ca/biology/scarr/Transitions_vs_Transversions.html

Point mutations molecular change

- Insertion
- Insertions are mutations in which extra base pairs are inserted into a new place in the DNA.



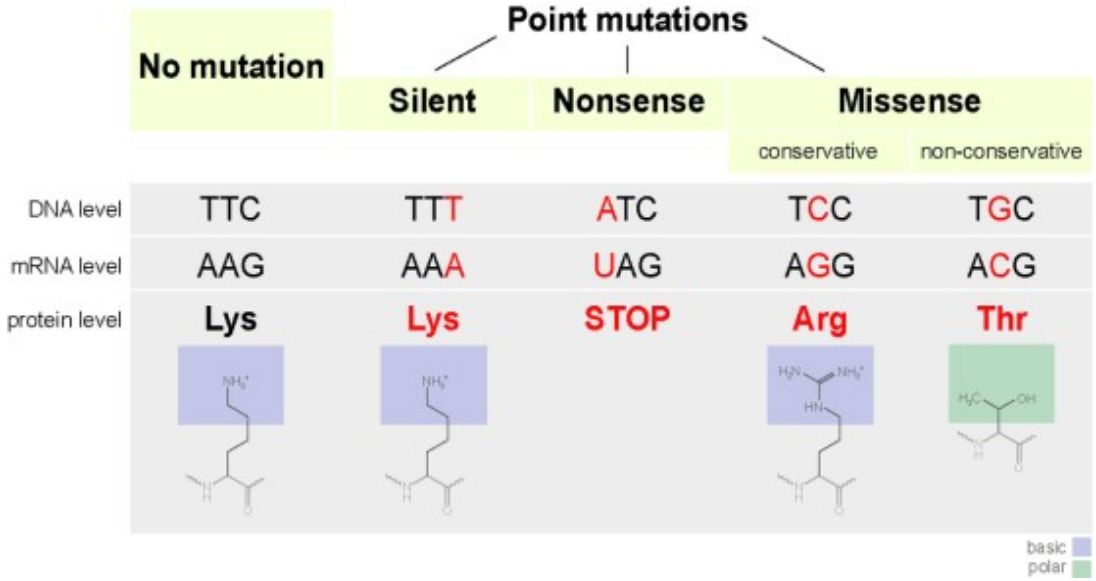
- Deletions
- Deletions are mutations in which a section of DNA is lost, or deleted.



https://evolution.berkeley.edu/dna-and-mutations/types-of-mutations/

Substitution point mutations

- Effect of translation:
- Substitution mutations change base pairs of a nucleotide sequence with different base pairs.
- The substitution may or may not give effects depending on the type of mutation.
- Silent mutation, missense mutation and nonsense mutation are three types of substitution mutations.



https://www.differencebetween.com/difference-between-substitution-insertion-and-deletion-mutations/

Substitution and point mutations

- Missense mutation
- Base pair substitution results in substitution of a different amino acid.

- Nonsense mutation
- Base pair substitution results in a stop codon (and shorter polypeptide).

- Neutral mutation
- Base pair substitution results in substitution of an amino acid with similar chemical properties (protein function is not altered).

- Silent mutation
- Base pair substitution results in the same amino acid.

- Frameshift mutations
- Deletions or insertions (not divided by 3) result in translation of incorrect amino acids, stops codons (shorter polypeptides), or read-through of stop codons (longer polypeptides).

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Substitution and point mutations

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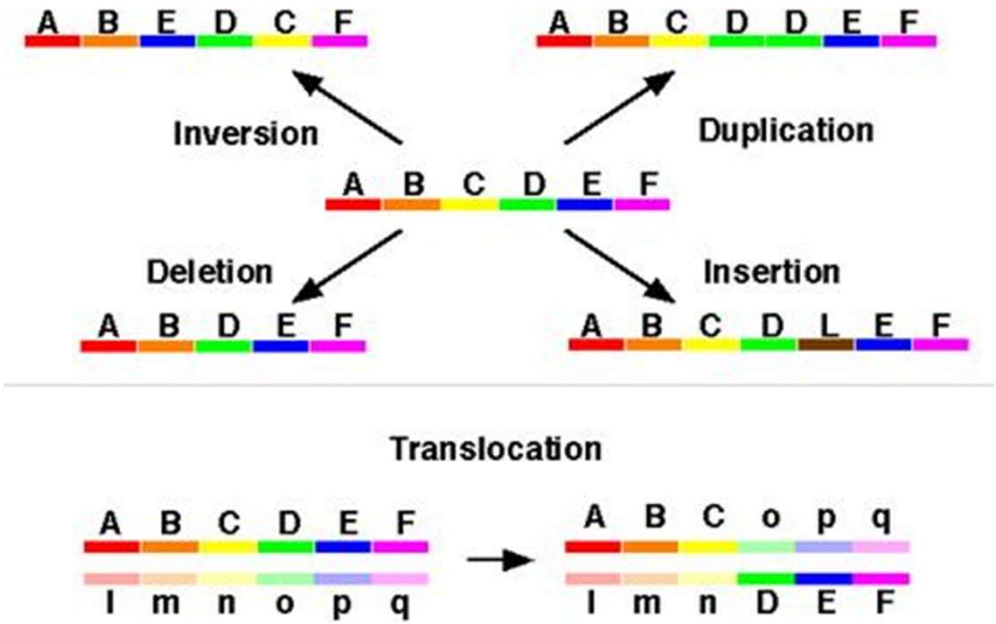
Additional type of Mutations

- null mutation - complete loss of function
  - often deletion of the gene or part of it.
- tight mutation - clear phenotypic expression
  - e.g. complete loss of ability to grow under certain conditionsor preventing the formation of a product of a given biochemical pathway.
- leaky mutation - partial activity of the gene product preserved
  - e.g. residual activity of the enzyme will allow cells to survive.
- frameshift mutation - changes the reading frame.
  - usually deletions or insertions.
- polar mutation - affects the expression of neighboring genes.
  - In operons.

Mutation and structural abberations



- Breakage of a chromosome can produce variety of arrangements.
- Chromosomal structural changes usually occur during meiosis , formation of egg or sperm cells, in early fetal development, or in any cell after birth.
- Pieces of DNA can be rearranged within one chromosome or transferred between two or more chromosomes.
- Some changes cause health problems, while others may have no effect on a person's health.

https://biologyboom.com/change-in-chromosome-structure/

https://medlineplus.gov/genetics/understanding/mutationsanddisorders/structuralchanges/

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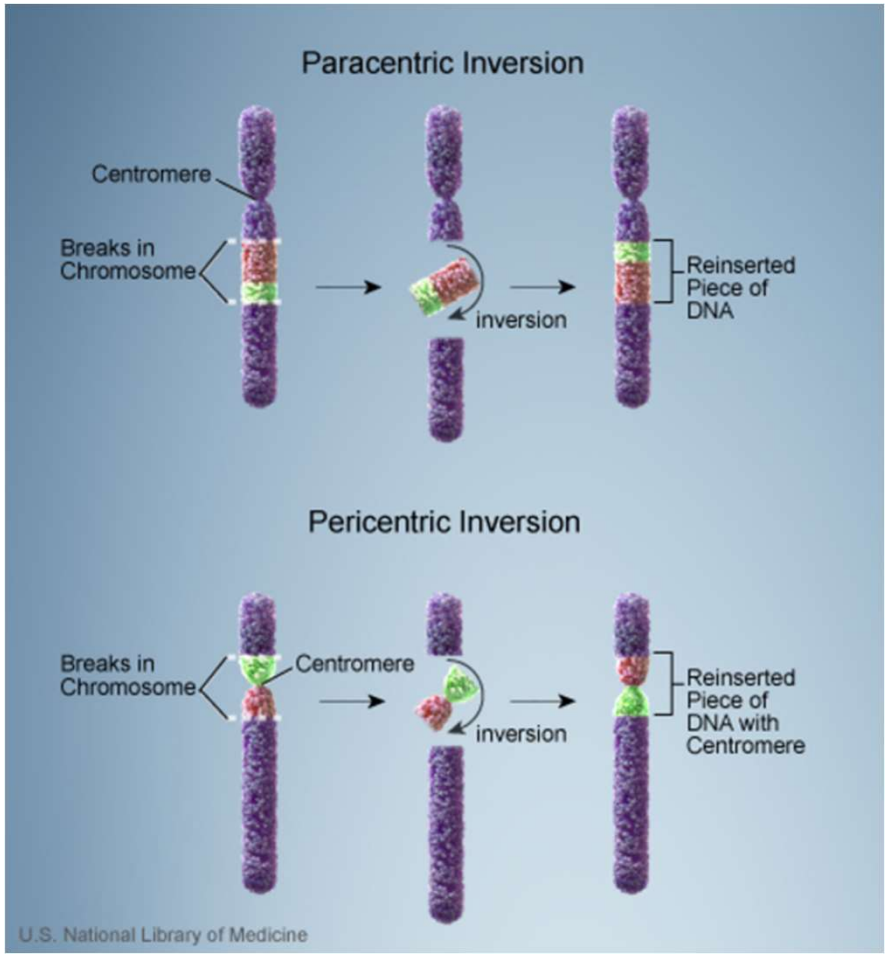
Mutation and structural abberations

- Inversion

- An inversion occurs when a chromosome breaks in two places; the resulting piece of DNA is reversed and re-inserted into the chromosome.

- Genetic material may or may not be lost as a result of the chromosome breaks.

- An inversion that includes
  - the chromosome's constriction point (centromere) is called a pericentric inversion,
  - the long (q) arm or short (p) arm and does not involve the centromere is called a paracentric inversion.



https://medlineplus.gov/genetics/understanding/mutationsanddisorders/structuralchanges/

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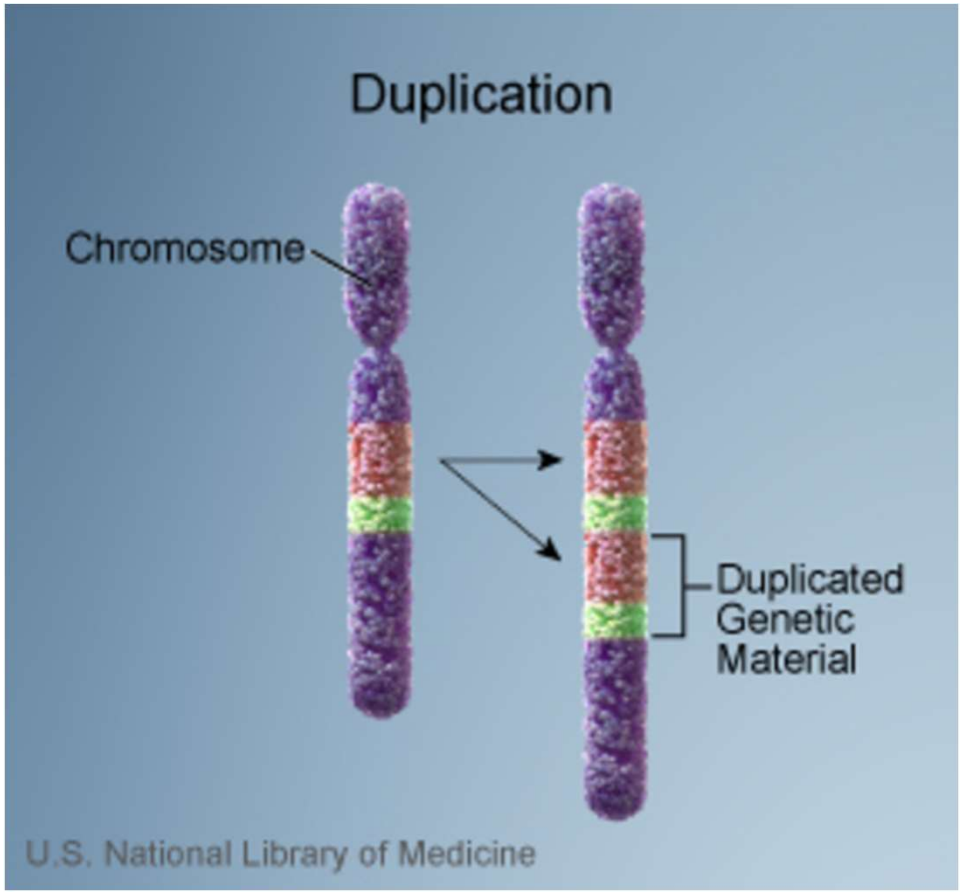
Mutation and structural abberations

- Duplications

- Duplications occur when part of a chromosome is abnormally copied (duplicated). This type of chromosomal change results in extra copies of genetic material from the duplicated segment.



https://medlineplus.gov/genetics/understanding/mutationsanddisorders/structuralchanges/

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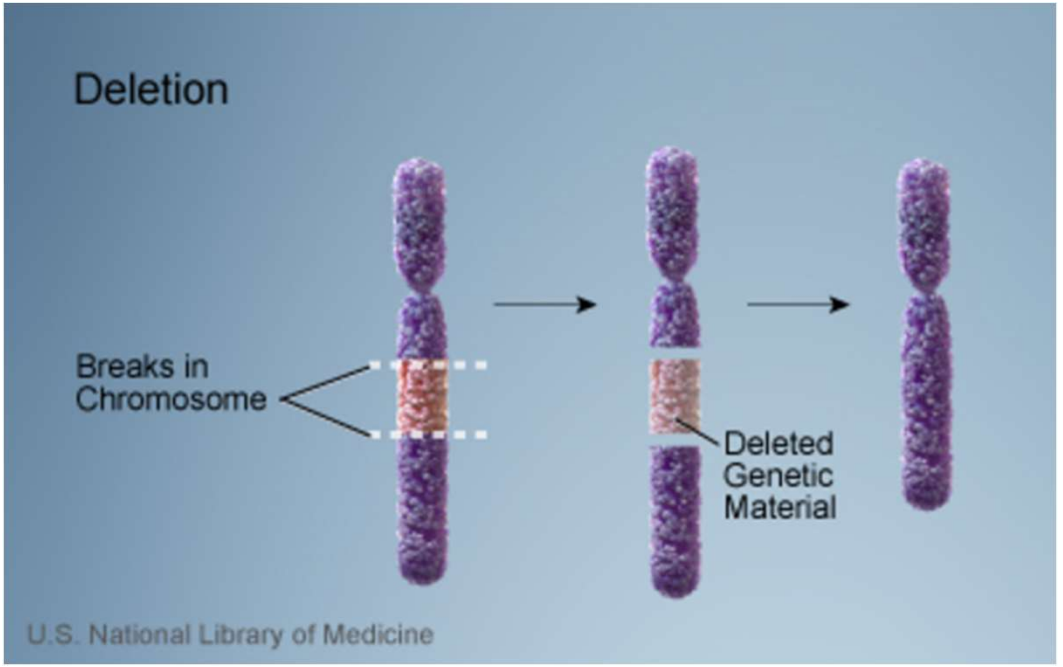
Mutation and structural abberations

- Deletions

- Deletions occur when a chromosome breaks and some genetic material is lost. Deletions can be large or small, and can occur anywhere along a chromosome.

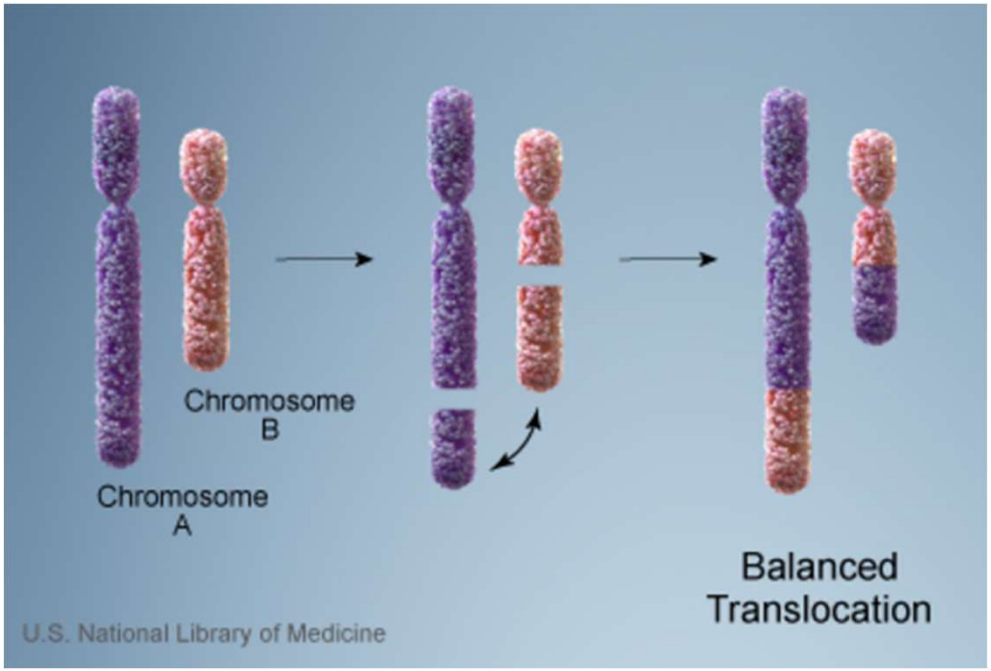


https://medlineplus.gov/genetics/understanding/mutationsanddisorders/structuralchanges/

Mutation and structural abberations

- Translocations

- A translocation occurs when a piece of one chromosome breaks off and attaches to another chromosome. This type of rearrangement is described as balanced if no genetic material is gained or lost in the cell.



https://medlineplus.gov/genetics/understanding/mutationsanddisorders/structuralchanges/

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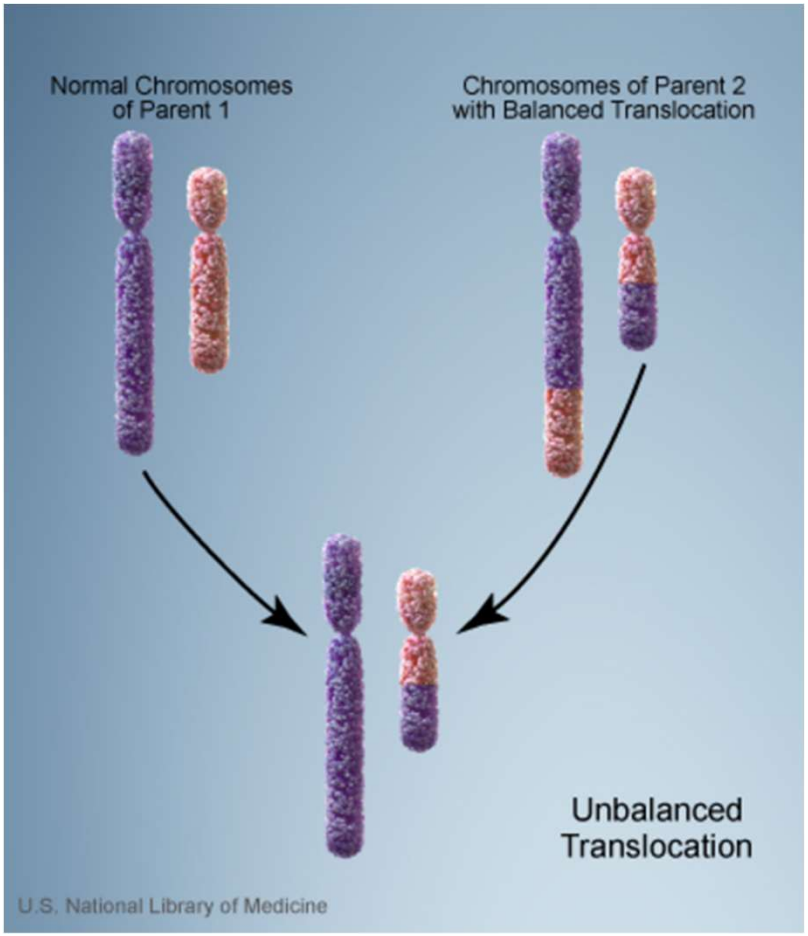
Mutation and structural abberations

- Translocations
- A translocation occurs when a piece of one chromosome breaks off and attaches to another chromosome.
- If there is a gain or loss of genetic material, the translocation is described as unbalanced.



https://medlineplus.gov/genetics/understanding/mutationsanddisorders/structuralchanges/

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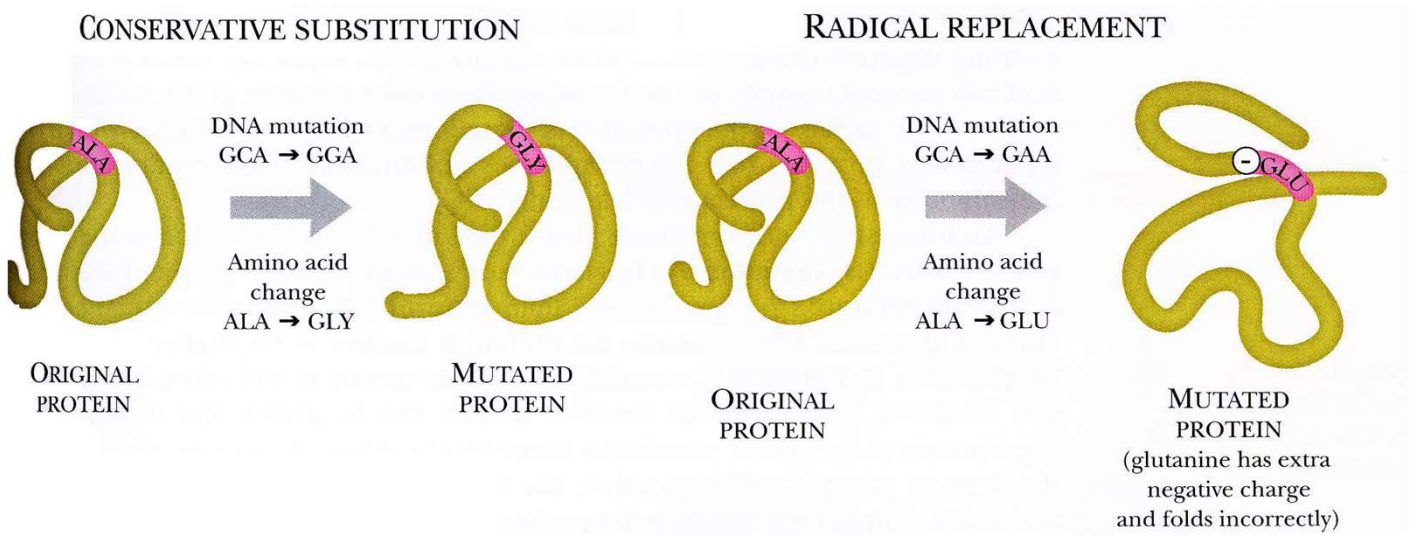
Are mutatins good or bad?

- It depends.
- Mostly harmful with a negative effect on the function of the gene product.
- The damaged gene product may not always be a protein, but also RNA (tRNA, rRNA, atd.).
- Mutations can also damage non-coding but important signal sequences.
- Most mutations do not have a significant effect on the survival of the organism – they are neutral.
- Rarely, the mutation has a positive effect on the survival and reproduction of the organism.
- The accumulation of these beneficial mutations will allow the organism to develop into a changing with the environment.

https://open.lib.umn.edu/evolutionbiology/chapter/5-8-using-the-genetic-code-2/

Impact of mutation depeneds on many aspects

- Mutations that alter the meaning of the codon ("missense mutations") are the most common.
- Lead to the substitution of one amino acid for another in a protein molecule.
- If the mutation replaces the original amino acid with a chemically related amino acid – usually without serious consequences (conservative substitution).
- The consequences are serious if they change the way the protein is composed or the structure active site (non-conservative substitution).



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Classification of Mutations

- According to the method of formation:

Spontaneous – occur in the absence of known mutagen, without apparent external cause.

- Consequence of metabolic disorders in the body.
- Consequence of DNA replication errors.
- Consequence of the presence of an unknown mutagenic substance in the environment.

Induced – occur in the presence of known mutagen

- Physical.
- Chemical.
- Biological factors.

MUTAGENS

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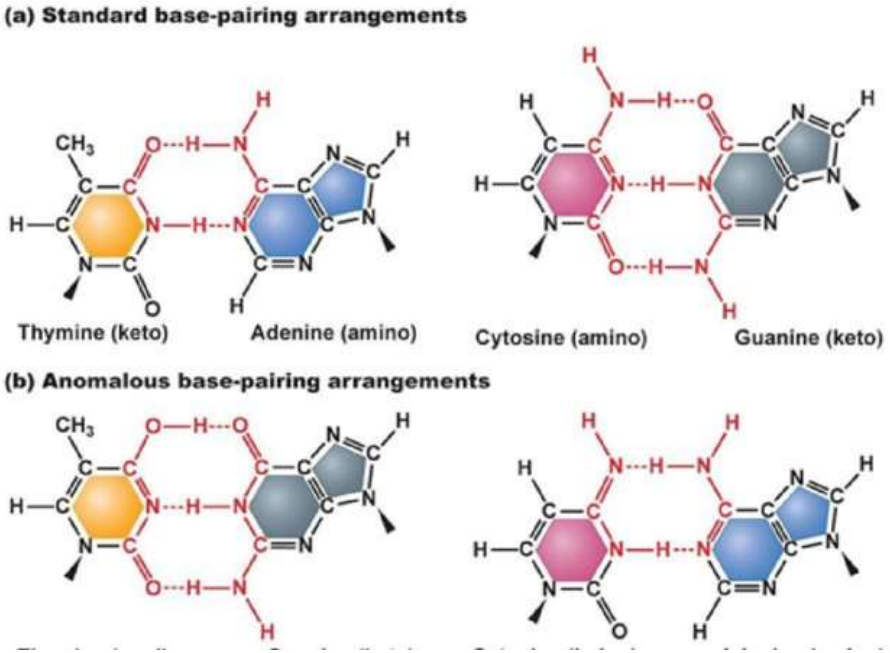
- Spontaneous mutations

Base pairing- tautomerization



- Standard base-pairing arrangements of the canonical nucleotide isomers.
- Anomalous base-pairing arrangements of the tautomers.
- Natural cause of certain genome instability.
- Base transitions to different forms change their pairing specificity.
- When tautomerization occurs during replication, the DNA sequence will be “misread”, and anomalous base-pairing will occur: such as C* with A, or T*.

https://www.researchgate.net/publication/334974633_Unified_Physics_and_the_Entanglement_Nexus_of_Awareness

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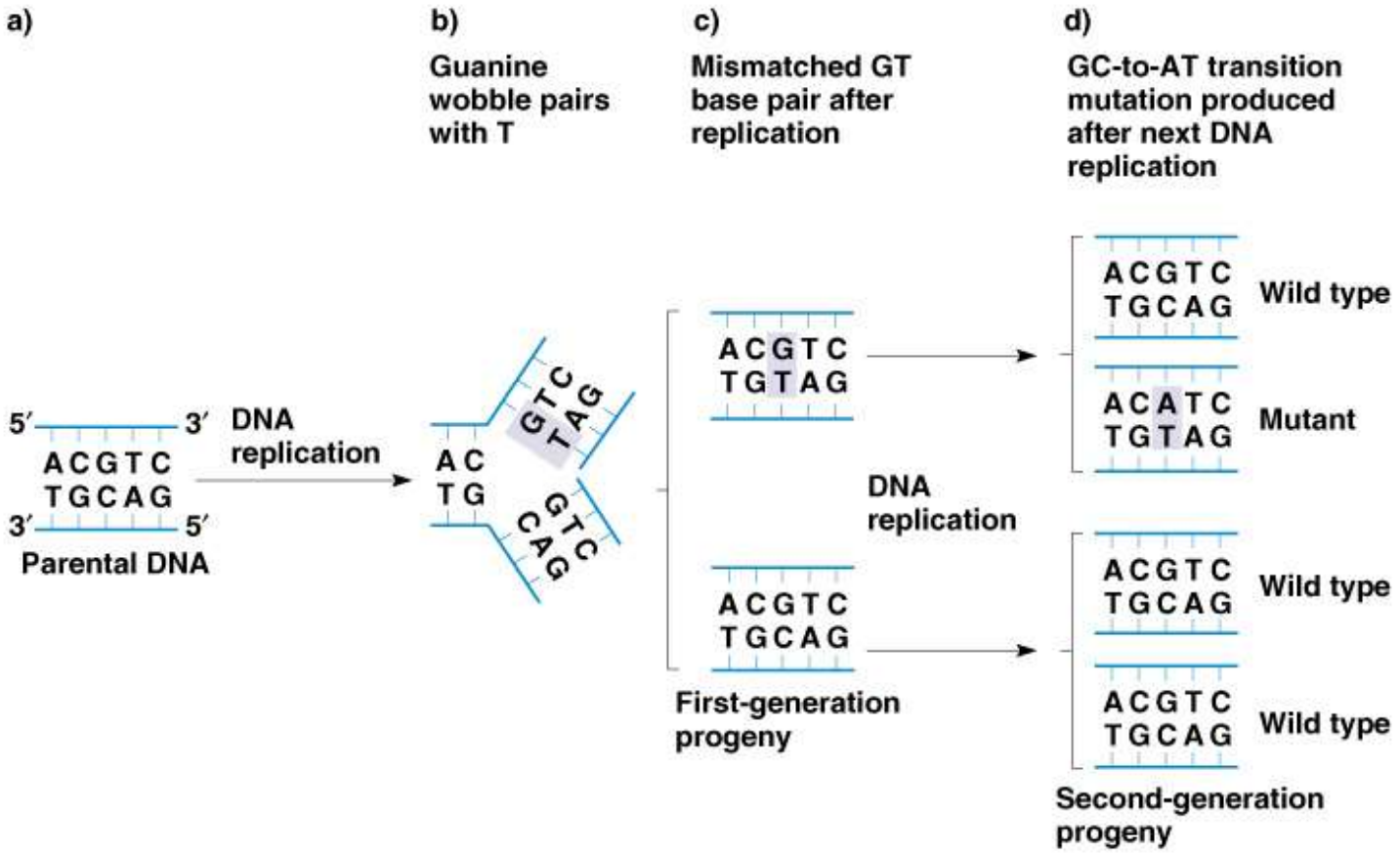
Mutation caused by mismatch wobbl base pairing



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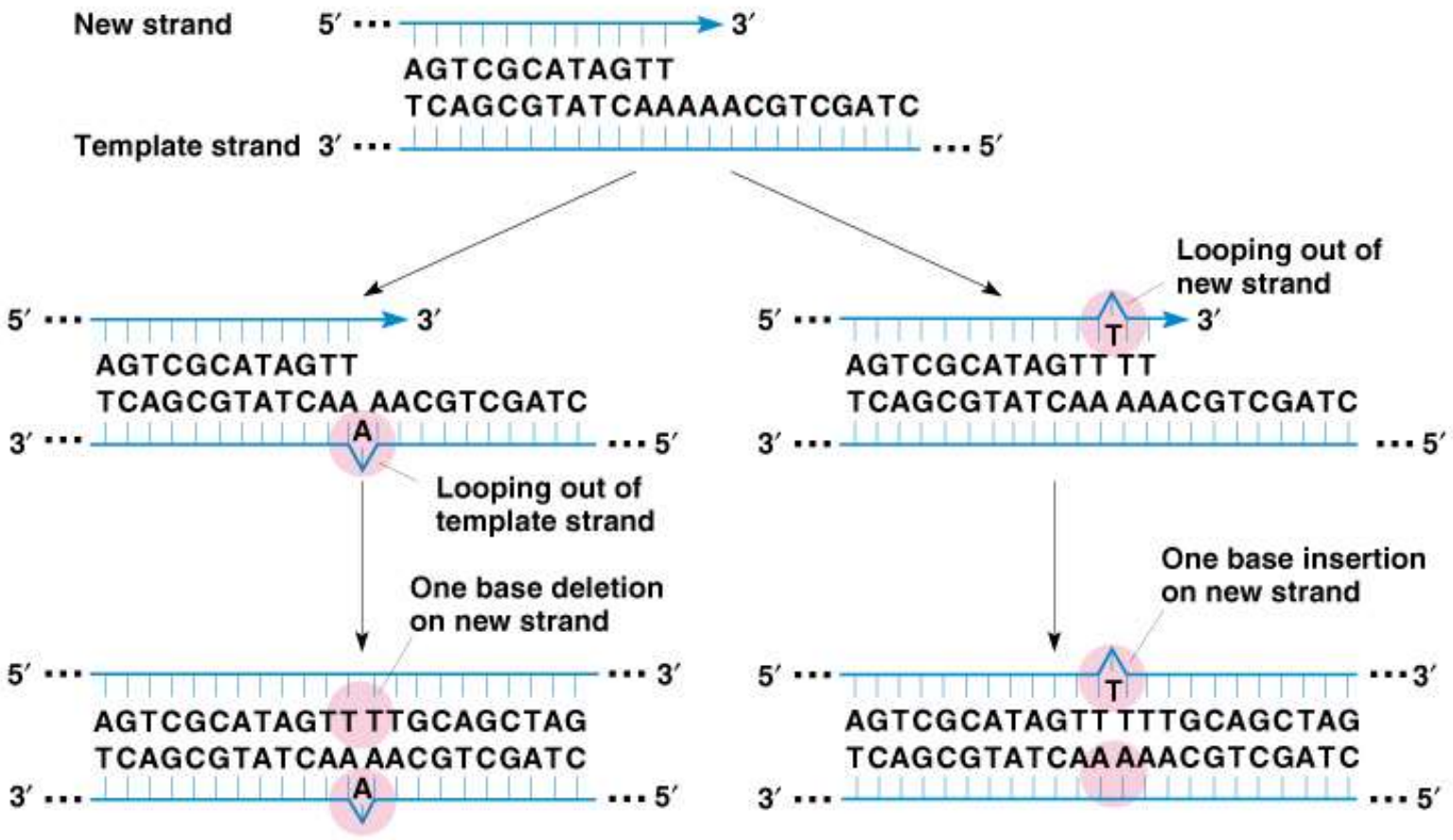
Insertion and deletion by DNA looping-out



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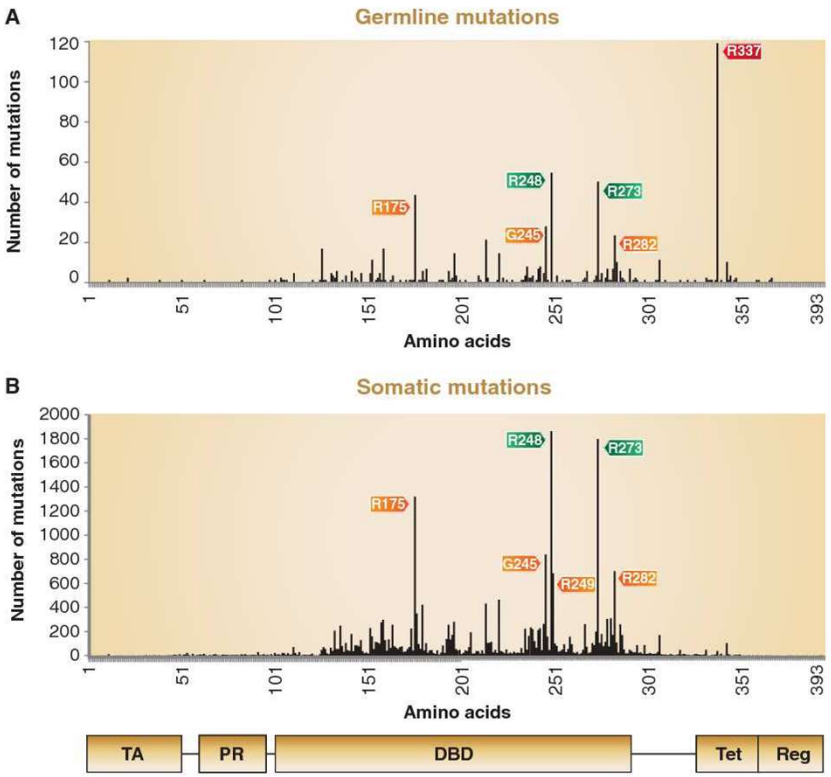
Hot spots in repetitions of DNA



- The distribution of mutations in the gene or genome is not uniform.
- Certain DNA sequences have a higher risk of being affected by mutations „hot spots“.
- Hot-spots - DNA sequences with increased probability of mutation events.
- The replication apparatus has some problems with repetitive sequences.
- Areas of tandem repeats are unstable, often occurence of insertions and deletions.
- Potential clinical relevance (p53).

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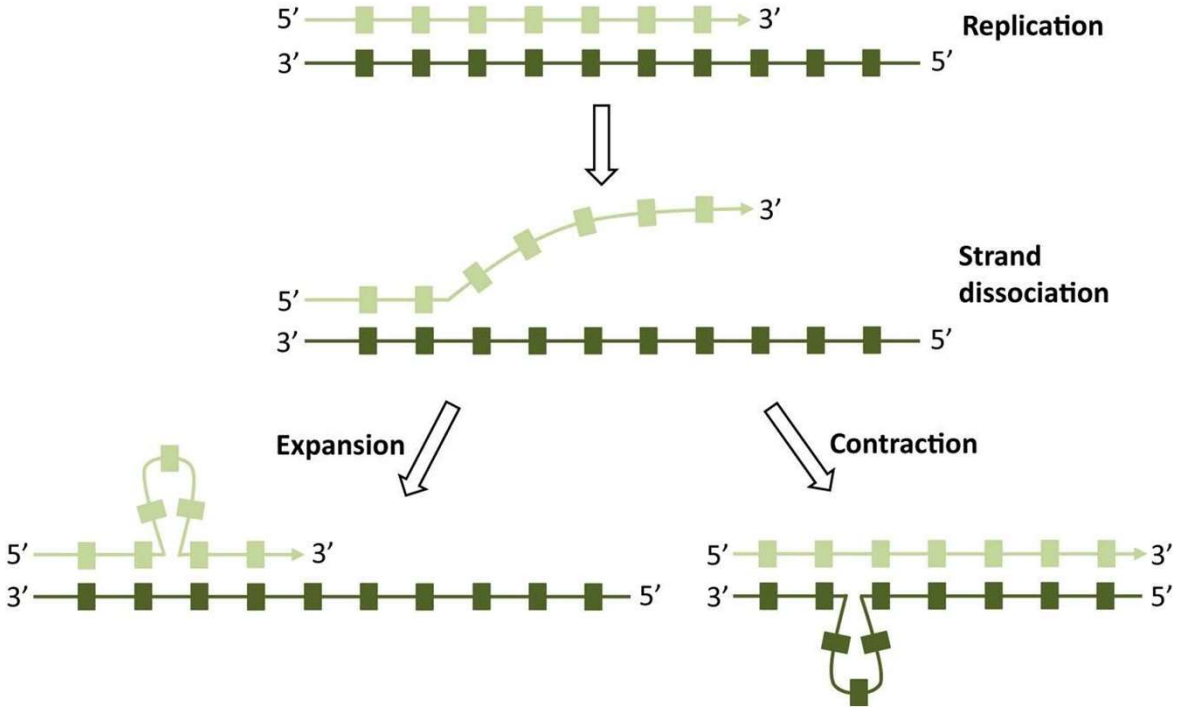
Replication polymerase slippage

- Due to assembly of loops, part of the template can be copied during replication repeatedly or can be omitted „replication slippage“/“polymerase slippage“.
- Trinucleotide expansion: neurological diseases (Huntigton's disease, expansion of trinucleotide CAG).



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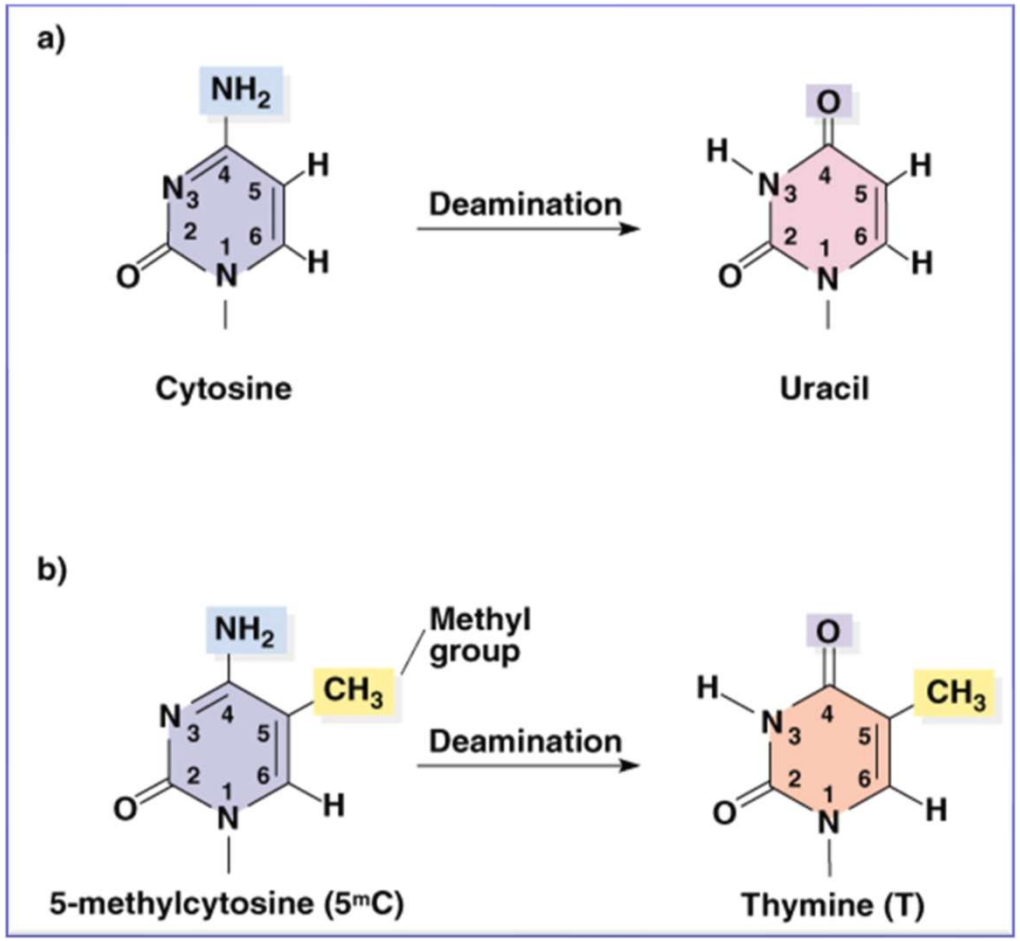
Deamination

- Depurination
- Common; A or G are removed and replaced with a random base.
- Deamination
- Amino group is removed from a base (C → U); if not replaced U pairs with A in next round of replication (CG → TA).
- Prokaryote DNA contains small amounts of $5^{m}C$; deamination of $5^{m}C$ produces T (CG → TA).
- Regions with high levels of $5^{m}C$ are mutation hot spots.

Deamination



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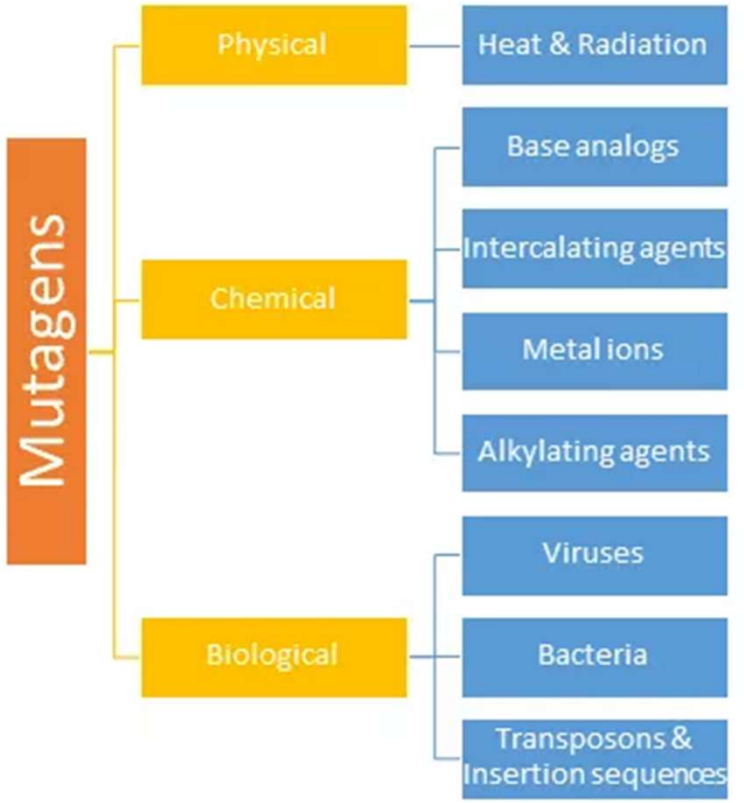
- Induced mutations

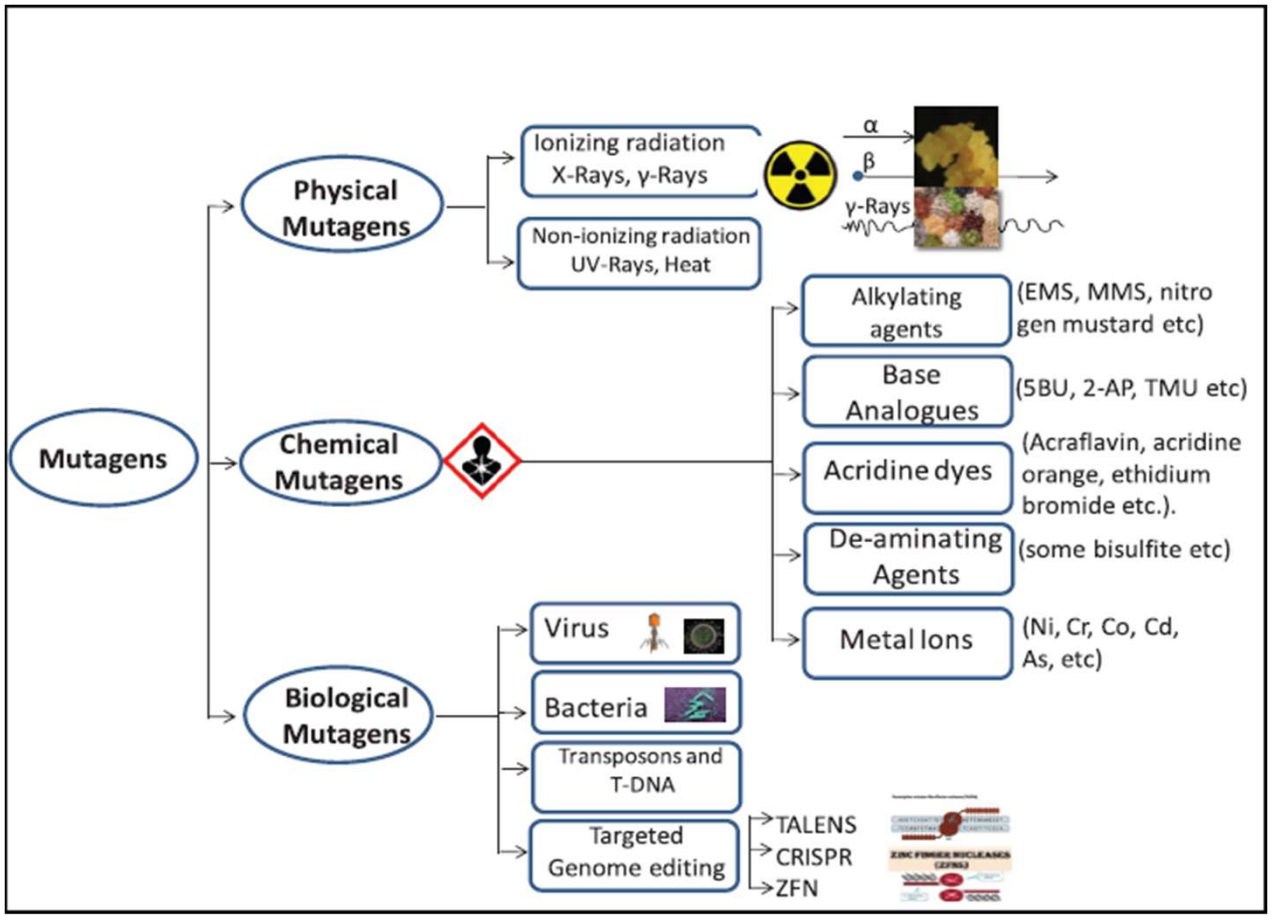
Mutagens

- Mutagen - any agent, physical or environmental, that can induce a genetic mutation or can increase the rate of mutation.
- Mutagens can be physical mutagens, chemical mutagens, or biological mutagens.
- The ability of a substance to induce the alterations in the base pairs of DNA or mutation is known as mutagenicity.



https://www.biologyonline.com/dictionary/mutagen

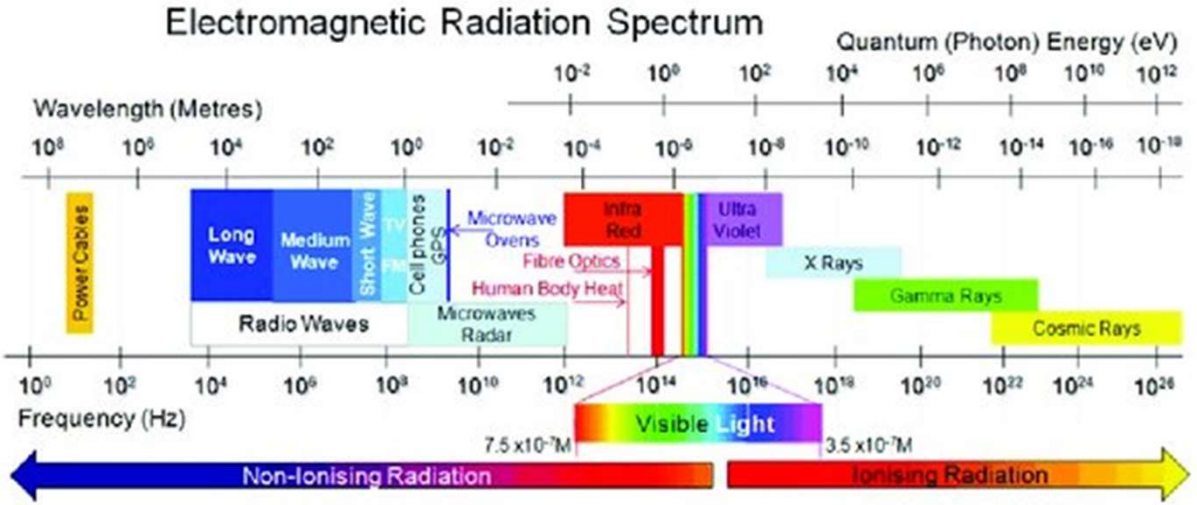
Mutagens



Journal of Applied Biology & Biotechnology Vol. 9(04), pp. 162-169, July, 2021

Physical mutagens

- Ionizing radiation - X-ray, gamma, cosmic, causes DNA breaks.
- Non-ionizing radiation (UV) - specific absorption at wavelength 260 – 280 nm, formation of thymine dimers.
- Heat - the phosphodiester bonds break in DNA when heated above 95°C resulting in breakage of DNA strand.



https://www.biologyonline.com/dictionary/mutagen

IOP Conf. Series: Materials Science and Engineering 374 (2018) 012009

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Ionizing radiation - physical mutagens

- Ionizing radiation – X-ray, Gamma and Cosmic radiations.

- Shorter wavelengths and greater energy than visible light, which penetrates deep into tissues, crashes into atoms, releases electrons, positively charged radicals and ions are formed, which provoke the formation of other ions (ionization process).

- These radiations exert a lethal (i.e., killing the cell) or sub-lethal (i.e., changing the functioning of the cell) effect by directly damaging the DNA or the nucleotides by:
  - inducing-cross-linking of DNA or protein,
  - breaking of chromosomes,
  - breaking of strands or chromosomal loss,
  - molecularly deletion of bases/DNA strand breakages.

https://www.biologyonline.com/dictionary/mutagen

UV radiation - physical mutagens

- Lower energy than ionizing radiation.
- Does not cause ionization.
- Penetrates only into the upper layers.
- Strong mutagen in unicellular organisms.
- The energy of radiation is captured by atoms whose electrons pass to the excited state – increase in the reactivity of atoms and molecules, leading to mutations in DNA.
- Maximum mutagenic effects at 254 nm (maximum absorption).

https://genesiswatertech.com/blog-post/types-of-ultraviolet-uv-lamps/

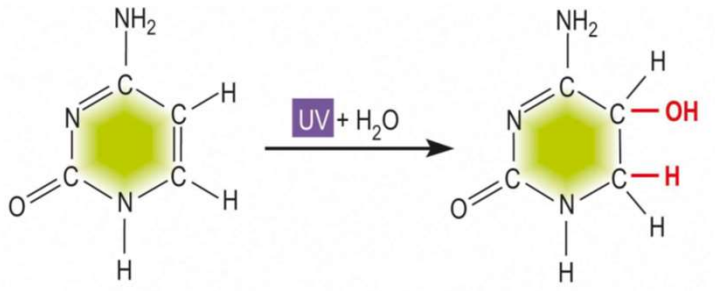
UV radiation - physical mutagens

- UV radiations are of three types - UV A, UV B, and UV C.
- UV-A is the UV rays with a wavelength of 320nm (near-visible range) and is known to result in dimerization of pyrimidines. This kind of pyrimidine dimerization results in alteration of the DNA structure which averts the formation of the replication fork during the process of replication. Such dimerization may lead to health issues.
- UV-B has a wavelength of 290-320nm and highly lethal to DNA.
- UV-C radiations have a wavelength of 180-290nm and are the most lethal as well as carcinogenic. UV-C radiations are majorly absorbed by the ozone layer.

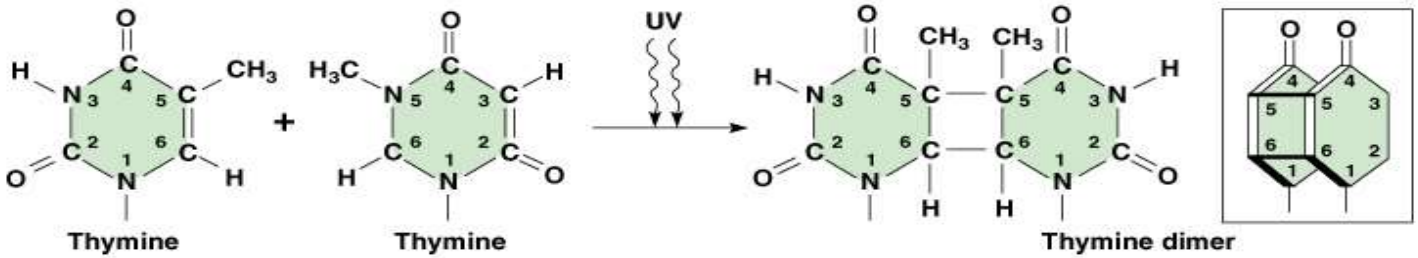
https://www.biologyonline.com/dictionary/mutagen

UV radiation - physical mutagens

- After absorption of UV, Pyrimidines become very reactive and change themself to pyrimidine hydrates and then to the dimers.
- Pyrimidine dimers results in formation of the replication fork and block of replication.



Cytosine Cytosine hydrate



Thymine dimers induced by UV light.

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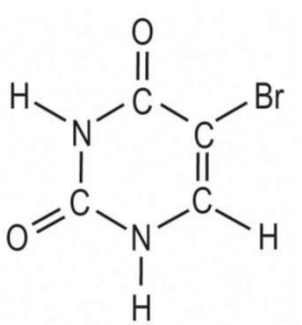
Chemical mutagens

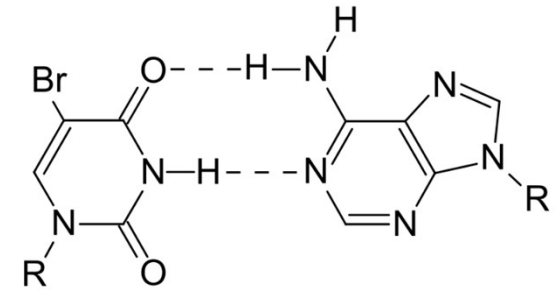
- Chemical mutagens influence the structure of DNA:
  - Oxidizing agents (peroxides, oxygen radicals).
  - Deaminating substances (nitrites).
  - Alkylating agents (mustard gas).
  - Intercalation agents (acridines).
  - Aromatic amines (benzidine, naphthylamine) damaging the cellular apparatus for even distribution genetic information in cell division.
  - Colchicine

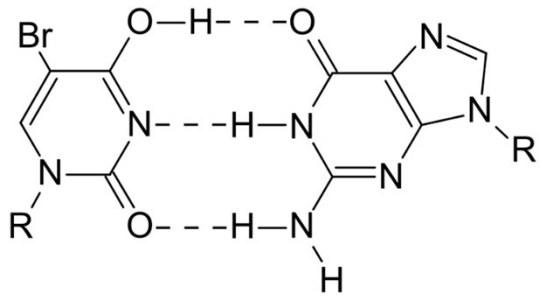
- Chemical mutagens can cause mutations:
  - Regardless of whether DNA replication takes place (e.g. alkylating agents, nitric acid).
  - Only during DNA replication (base analogues, acridine dyes).

Base analogues - chemical mutagens

- 5-bromuracil is a thymine analogue - structurally similar to the normal bases.
- Capable of non-standard pairing (tautomerism).
- Incorporated into DNA during replication.
- 5-bromuracil induces transitions in both directions (different charge distribution increases frequency of tautomeric rearrangement – other pairing).





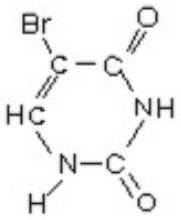


https://pt.wikipedia.org/wiki/5-Bromouracil

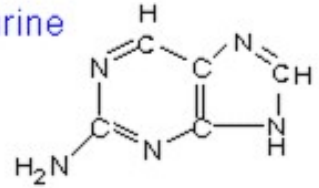
Base analogues - chemical mutagens

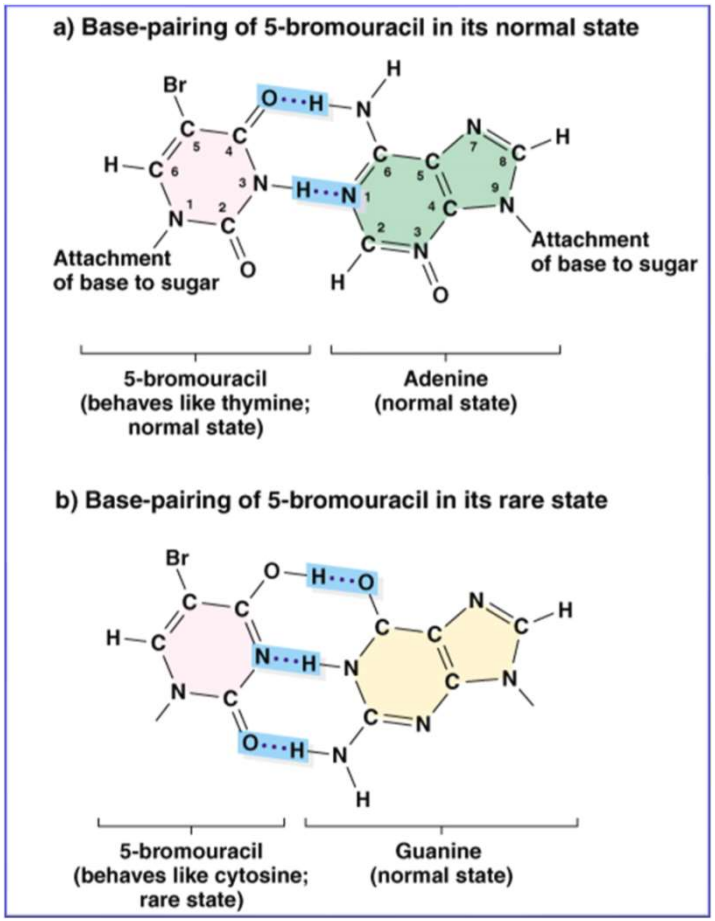
- Due to structural similarities these agents with the DNA bases, base analogs get incorporated in the DNA structure during the process of replication.
- Aminopurine is similar to adenine and can form a base pair with C or T (though base pairing with C is rare).

5-Bromouracil



2-Aminopurine





https://www.biologyonline.com/dictionary/mutagen

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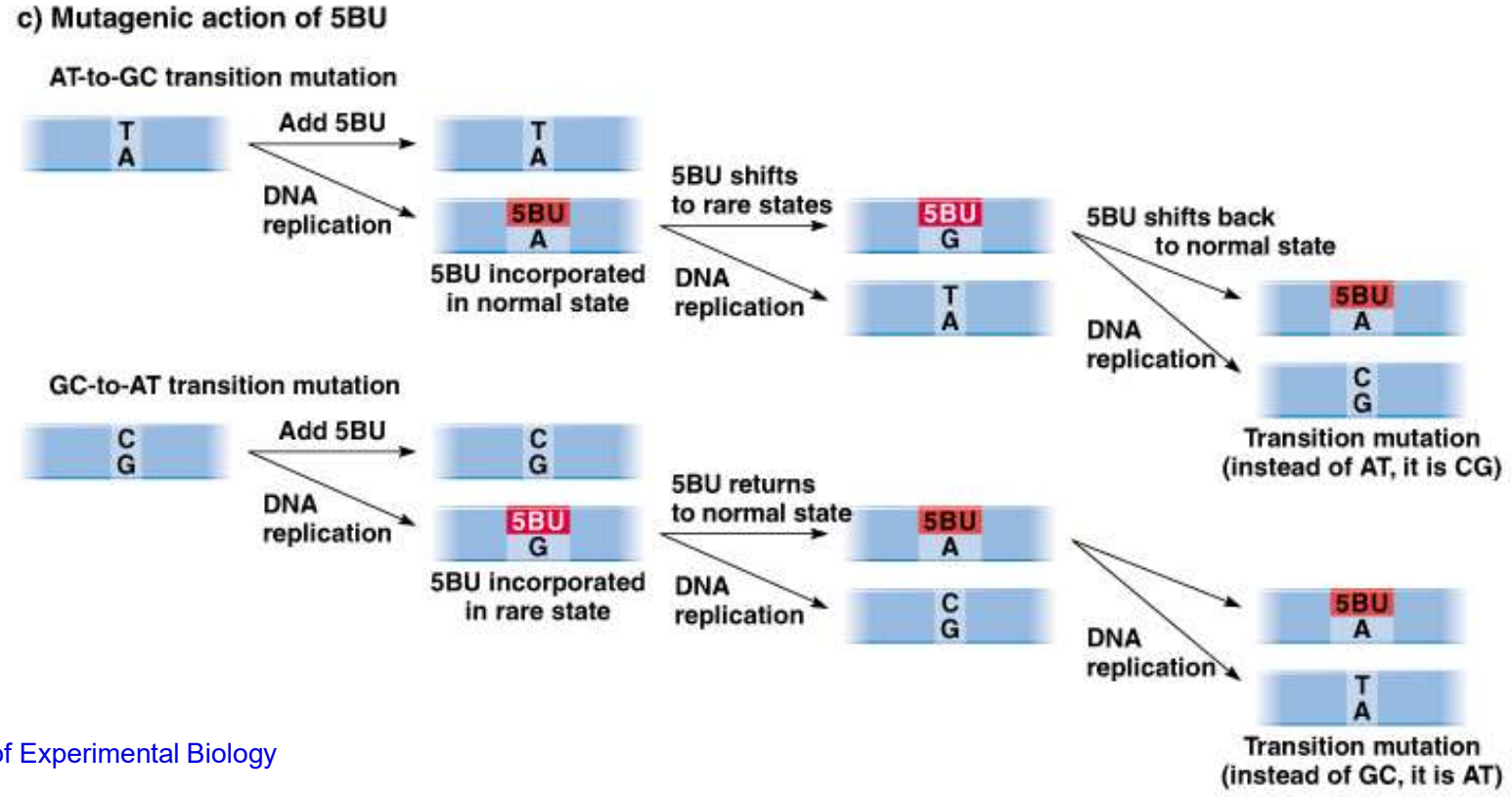
Base analogues - chemical mutagens

- Keto form of the 5-bromouracil replaces thymine, whereas enol tautomeric forms a replaces guanine.

- Upon replication DNA containing 5-Bromouracil changes base pair from A-T to G-C or from a G-C to an A-T.



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Base modifyining agents - chemical mutagens

Base modifying agents affects DNA that is not replicated:

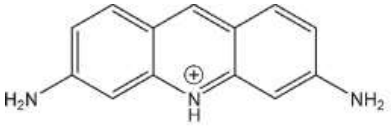
- Intercalating agents
- Nitric acid
- Hydroxylamine
- Sodium bisulfate
- Alkylating agents

DNA intercalating agents - chemical mutagens

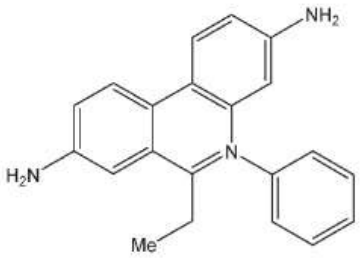
- DNA Intercalating agents are the molecules that have a hydrophobic heterocyclic ring structure and resemble the ring structure of base pairs.
- These agents place themselves in the DNA helix, which eventually interferes with the replication, translation, and transcription resulting in mutation, most commonly frameshift mutation.
- Ethidium bromide, proflavine, acridine orange, actinomycin D, or daunorubicin, etc. are some of the common intercalating agents.
- Daunorubicin along with Epirubicin and Mitoxantrone are some of the common anti-cancer or antineoplastic drugs.

https://www.biologyonline.com/dictionary/mutagen

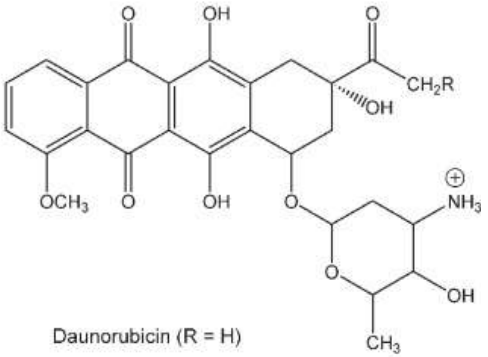
DNA intercalating agents - chemical mutagens



Proflavine

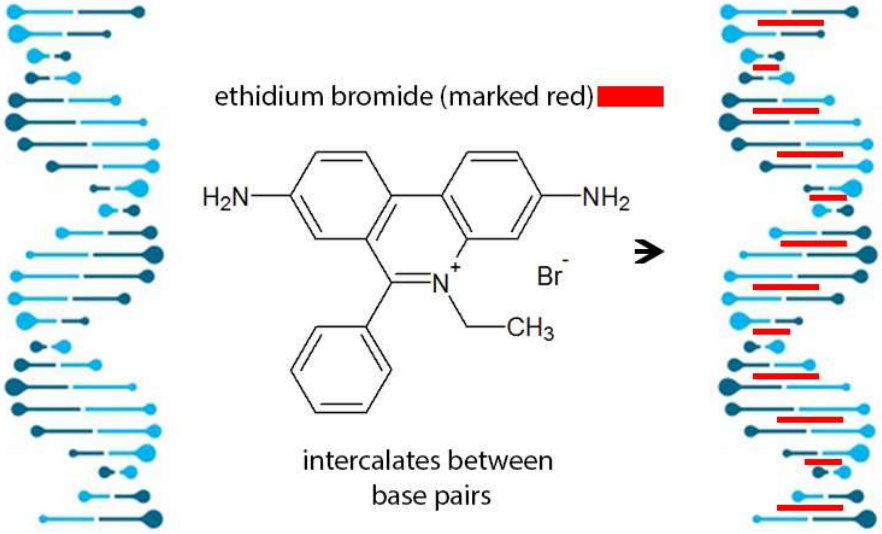


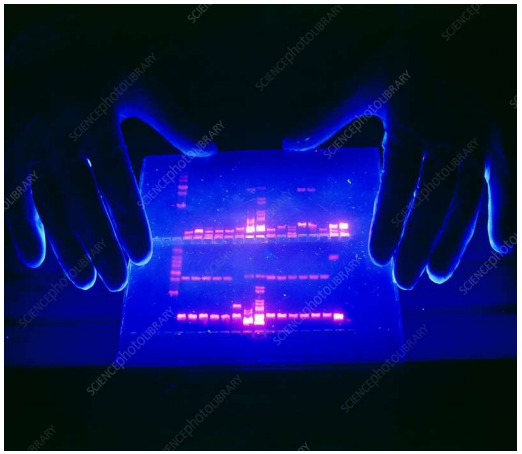
Ethidium



Daunorubicin (R = H)

Adriamycin (R = OH)





https://www.hoelzel-biotech.com/en/infothek/nucleic-acid-detection/

https://www.sciencedirect.com/topics/medicine-and-dentistry/intercalating-agent

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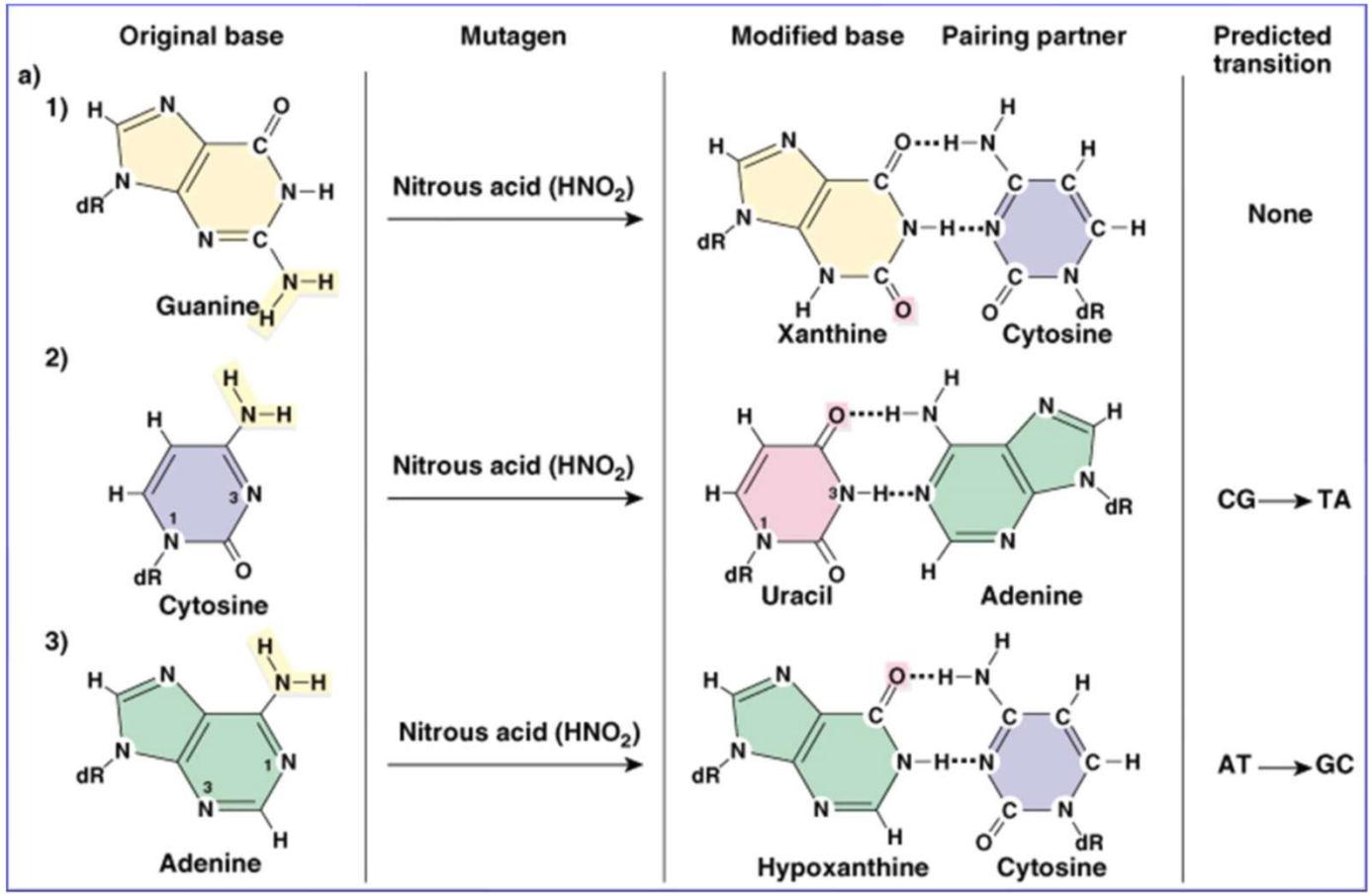
Deaminating agents - chemical mutagens

- Deaminatnig agent causes oxidative deamination of adenine, guanine and cytosine. Amino groups are thus converted into keto groups.
- Adenine → hypoxanthine, which is paired with cytosine.
- Cytosine → uracile, which is paired with cytosine.
- Guanine → xanthine, which pairs with cytosine, thus guanine deamination is not mutagenic.



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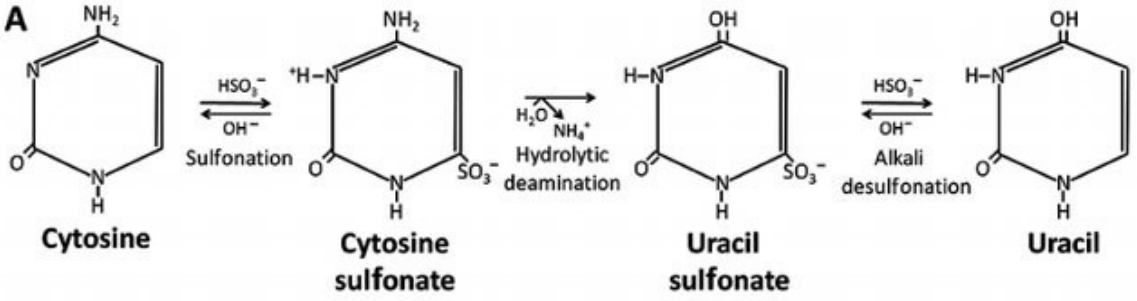
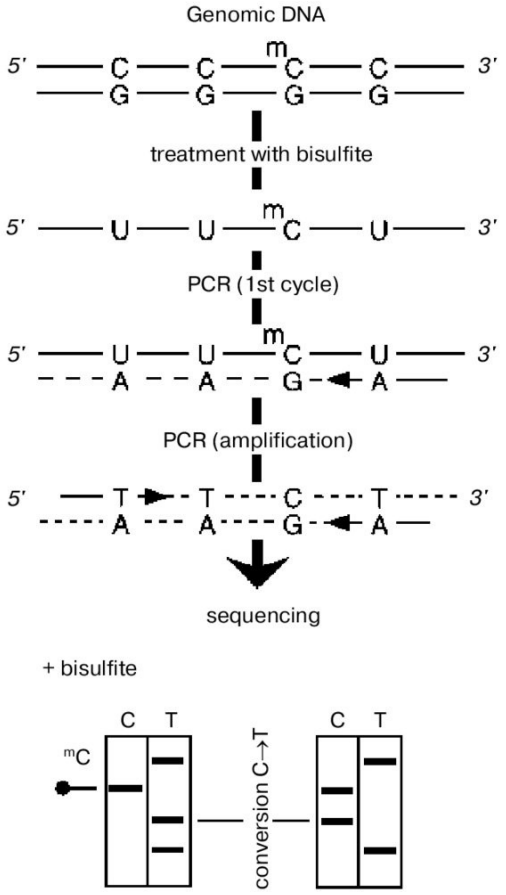
Deaminating agents - chemical mutagens

- Bisulfide has the same effect as nitric acid, deamination of cytosine to uracil, GC → AT.
- 5^{m}C can not be deaminated.



B

	Original sequence	Sequence after bisulfite treatment
Unmethylated DNA	A-T-C-G-G-T-C-A-T-C-G-C-A-T	A-T-U-G-G-T-U-A-T-U-G-U-A-T
Methylated DNA	A-T-C-G-G-T-C-A-T-C-G-C-A-T	A-T-C-G-G-T-U-A-T-C-G-U-A-T



June 2005Biochemistry (Moscow) 70(5):596-603
Clinical Chemistry, 2009, 55(8), 1471-83.

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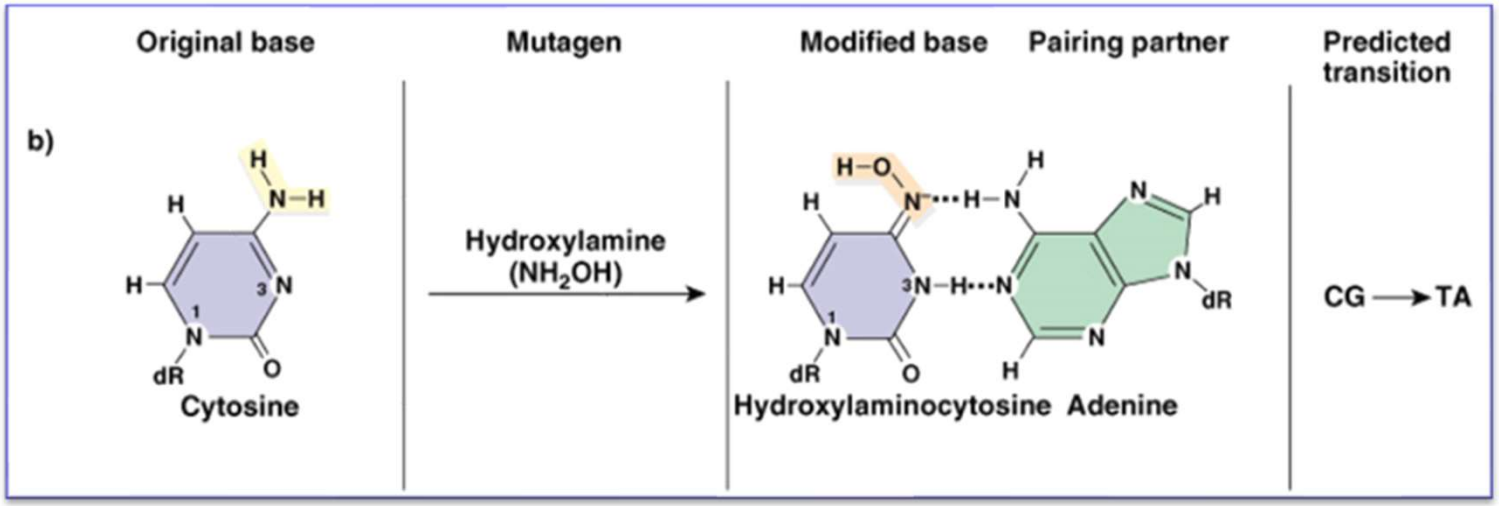
Hydroxylating agents - chemical mutagens

- Hydroxylation of an amino group of cytosine.
- Hydroxyl-amino-cytosine forms pair with adenine.
- Transition CG → TA.



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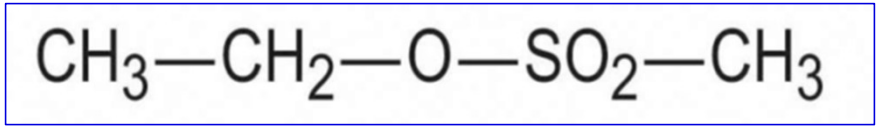
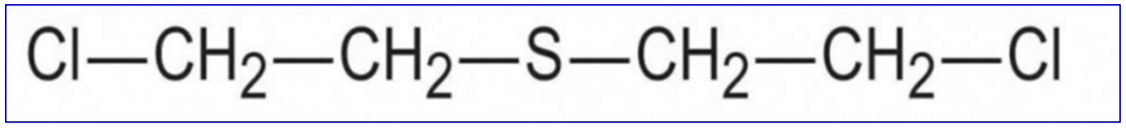
Alkylating agents - chemical mutagens

- Alkylating agents induce alkyl groups (methyl, ethyl) in DNA resulting in altered pairing abilities or create inter-strands cross-links.
- The introduction of the alkyl groups increases ionization that results in base-pairing errors and eventually inducing gaps in the DNA strand.
- Some of the common alkylating agents are ethylnitrosourea, mustard gas, vinyl chloride, Methylhydrazine, Busulfan, Carmustine, lomustine, Dimethyl sulfate, Temozolomide, Dacarbazine, Ethyl ethane sulfate, and Thio-TEPA.
- Induces all types of mutations (transitions, transversions, frameshift mutations and chromosomal aberrations).



Ethylmethansulfonate



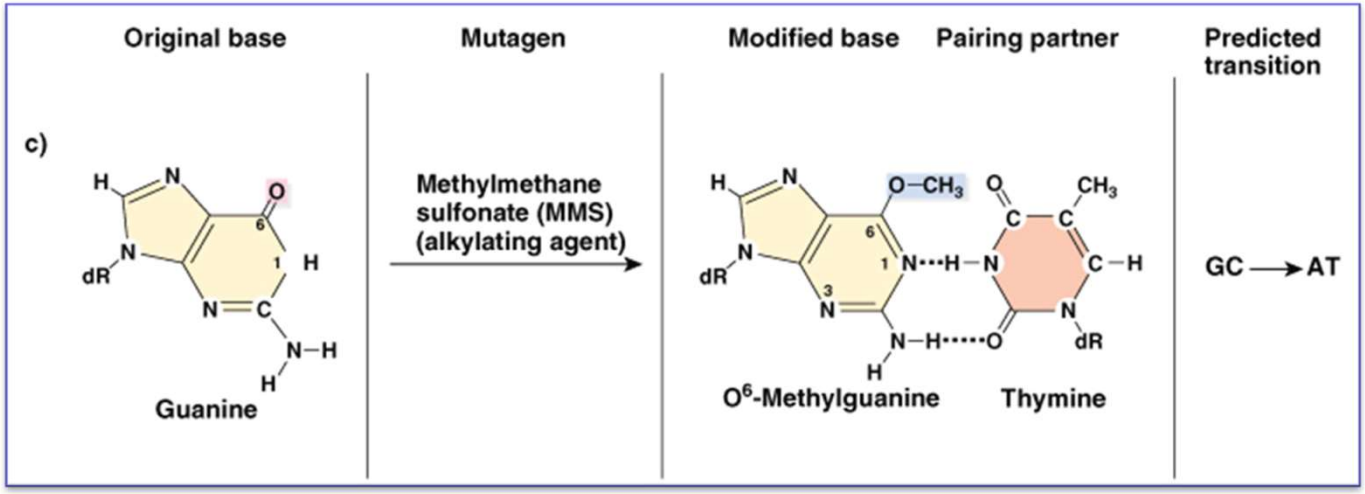
Di-(2-chlorylethyl) sulfide
Mustard gas

MUNI SCI

Alkylating agents - chemical mutagens

- By alkylation of guanine mustard gas causes changes that block replication and cell division (consideration of use in the treatment of cancer).

- Though during the DNA repairing process, alkylated bases can be removed from the DNA by the depurination process. Depurination is a non-mutagenic process.



https://www.biologyonline.com/dictionary/mutagen

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Biological mutagens

- Viruses – can insert its viral DNA into the genome.
- Certain viruses, e.g. *Rous sarcoma virus*, *hepatitis B virus, HIV, Epstein-Barr virus*, directly influence the genetic material in cells, changing the functioning of genes and triggering cancers. Thus, it can be said, that viruses can be mutagenic.
- Bacteria - certain inflammation-inducing bacteria like *Helicobacter pylori* produces reactive oxygen species that results in DNA damage and reduced DNA repair. This raises the likelihood of the mutation.

https://www.biologyonline.com/dictionary/mutagen

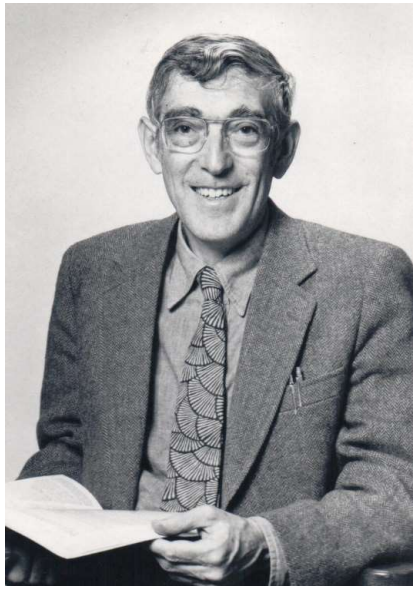
Biological mutagens

- Transposons and Insertion sequences (IS) - are units of DNA that undertake self-directed relocation/multiplication of the DNA fragment.
- Both IS and transposon are also known as jumping genes as they move from throughout the DNA.
- The addition of transposons into chromosomal DNA interrupts the functionality of the genes.
- Three types of transposons are usually found:
  - Replicative transposons - transposons that retain the original locus and translocate its copy.
  - Conservative transposons - the original transposon translocates itself.
  - Retrotransposons transpose - translocate via RNA intermediates.

https://www.biologyonline.com/dictionary/mutagen

Ames test

- American bacteriologist.
- In the late 60s, he is studying mutations in *Salmonella* at Berkeley .
- He observed that mutations in certain genes prevent the growth of bacteria on a Petri dish.
- In this strain there may be another mutation that allows the ability to restore the grow - a bacterial colony is formed on the dish.
- The more colonies, the greater the mutagenic capacity of the substance used, It can be quantified.
- The reversal rate reflects the strength of the mutagen.



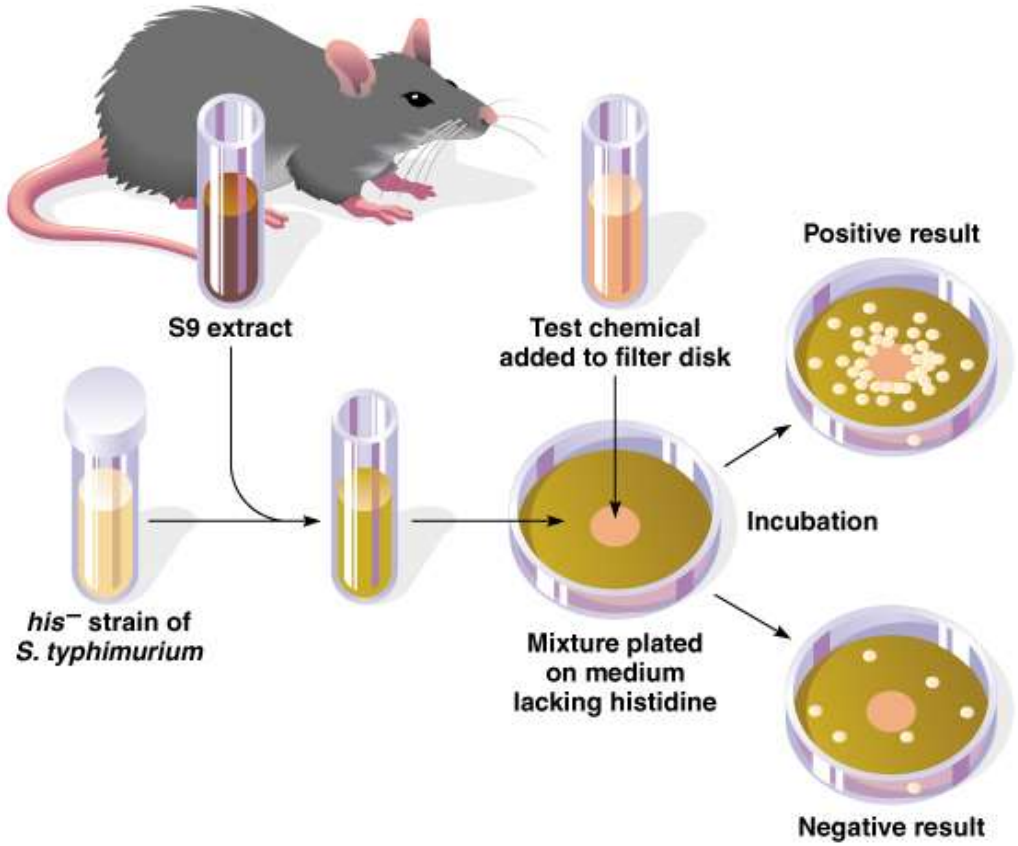
Bruce Ames

https://gairdner.org/award_winners/bruce-n-ames/

Ames test

- Ames Test is an inexpensive method used to screen possible carcinogens and mutagens.
- Histidine auxotroph *Salmonella typhimurium* (requires Histidine to grow) are mixed with rat liver enzymes and plated on media lacking histidine.
- Liver enzymes are required to detect mutagens that are converted to carcinogenic forms by the liver (e.g., procarcinogens).
- Test chemical is then added to medium.
- Control plates show only a small of revertants (bacteria cells growing without histidine).
- Plates inoculated with mutagens or procarcinogens show a larger of revertants.
- Auxotroph will not grow without Histidine unless a mutation has occurred.

Ames test



https://slidetodoc.com/chapter-7-a-dna-mutation-and-repair-mutation/

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