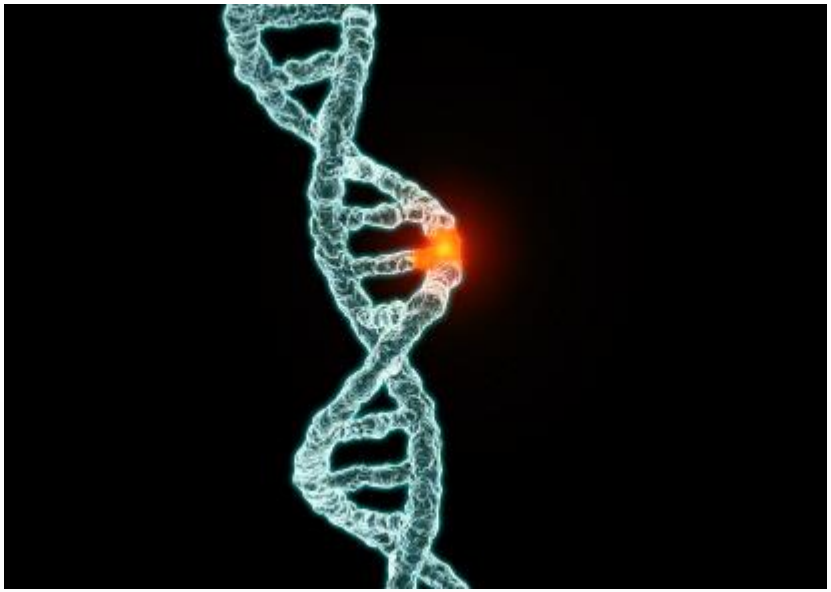
Molecular mechanisms of mutagenesis, spontaneous and induced mutations, and reversions

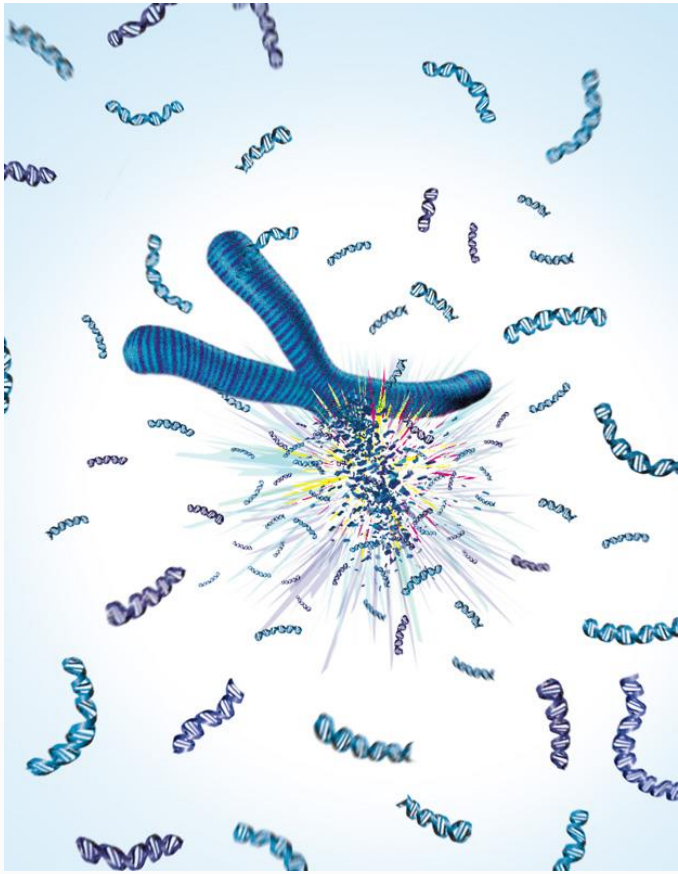


Mutation - is a change of the nucleotide sequence of the genome of an organism, virus, or extra chromosomal genetic element.

- process

Mutations = the source of genetic variability

- **heritable changes in the genetic material:**
  - changes at the level of individual **genes** (**substitutions, insertions, deletions**)
  - changes in the **structure or number** of **chromosomes**



- not to be mistaken with changes in genotype (and hence the phenotype), which are the result of new combinations of existing genetic variants (**recombination**)

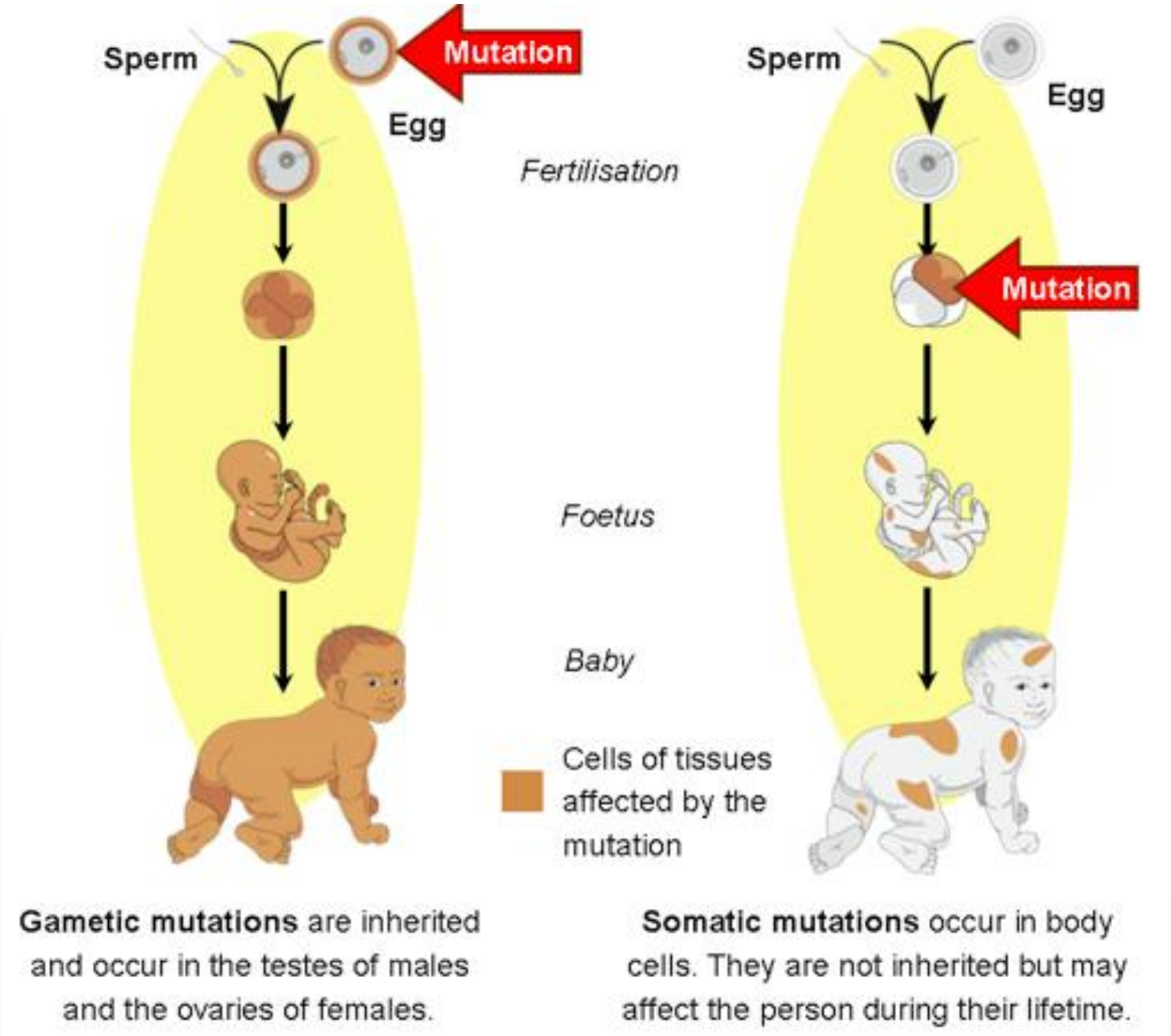
- **source of genetic variability** - providing new genetic variants that are a prerequisite for the evolution of organisms

Occurrence and distribution of mutations

- in unicellular organisms, each mutation duplicates in replication and passes to the next cell generation

- in multicellular organisms, mutations are transmitted to offspring only when they appear in the genome of cells of the **germ line**
- mutations in the DNA of **somatic** cells occur only in the progeny of these cells (population of cells that are genetically different from the rest of the body)

- **Classification of mutations:**
- **gametic**: only in cells the germ line, can cause hereditary diseases
- **somatic**: only in somatic cells, may cause cancer



Gametic mutations are inherited and occur in the testes of males and the ovaries of females.

Somatic mutations occur in body cells. They are not inherited but may affect the person during their lifetime.

Diploid state protects the cells against the harmful effects of mutations

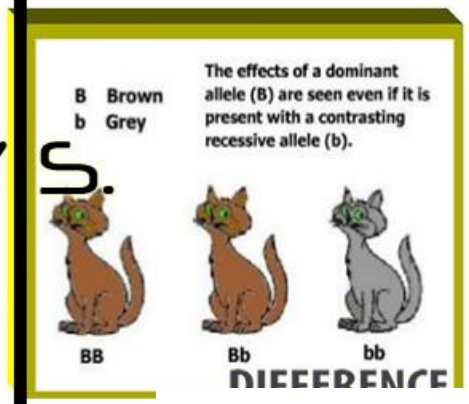
- **diploid organisms**: each gene has **two copies**
- when one becomes damaged, the other to provides the correct gene / protein - **recessive** mutations
- preventing the defect (if the mutation is not **dominant**)
- estimation: each person carries many harmful mutations that would in haploid configuration mean eightfold lethality
- due to the accumulation of mutations over the centuries, today's people are genetically different from their predecessors

Dominant

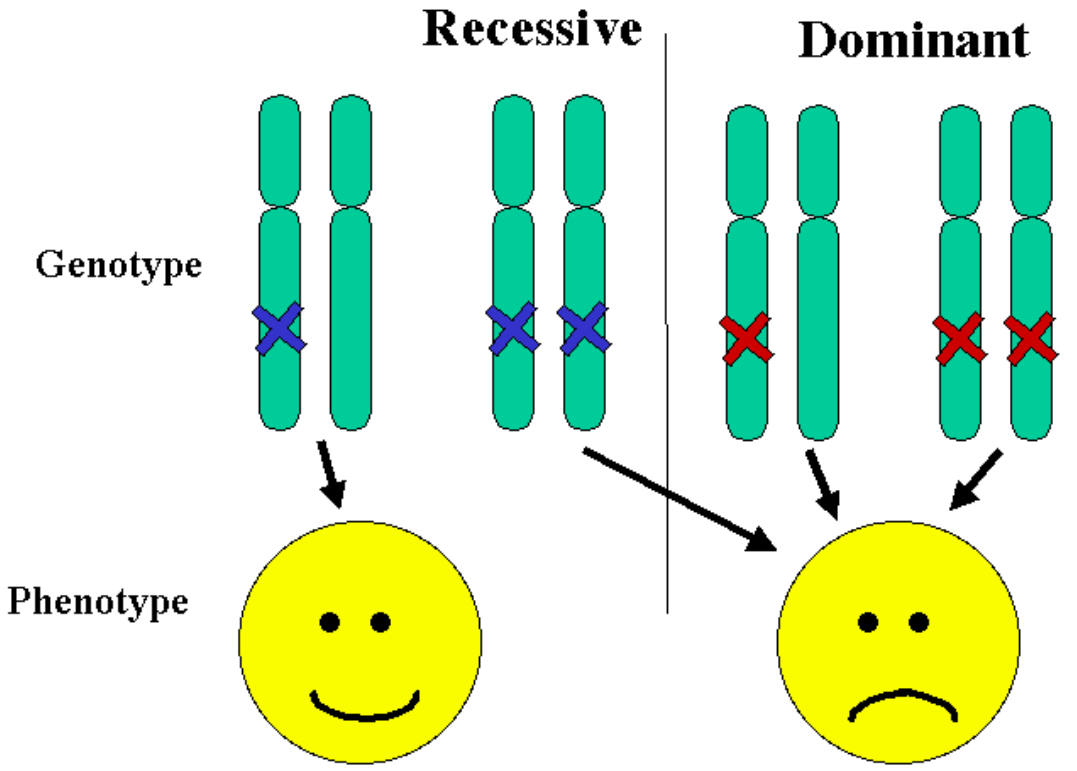
- A *dominant* allele is expressed even if it is paired with a recessive allele.

- A *recessive* allele is only visible when paired with another recessive allele.

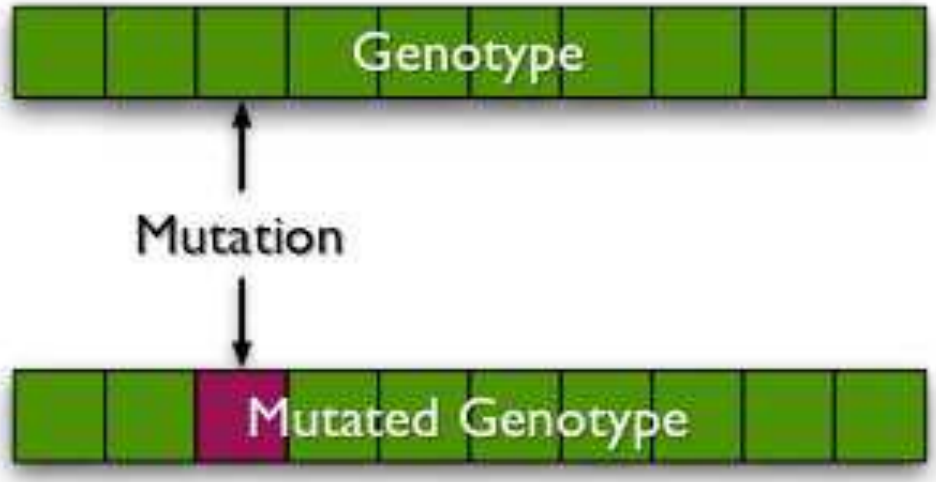
Recessive



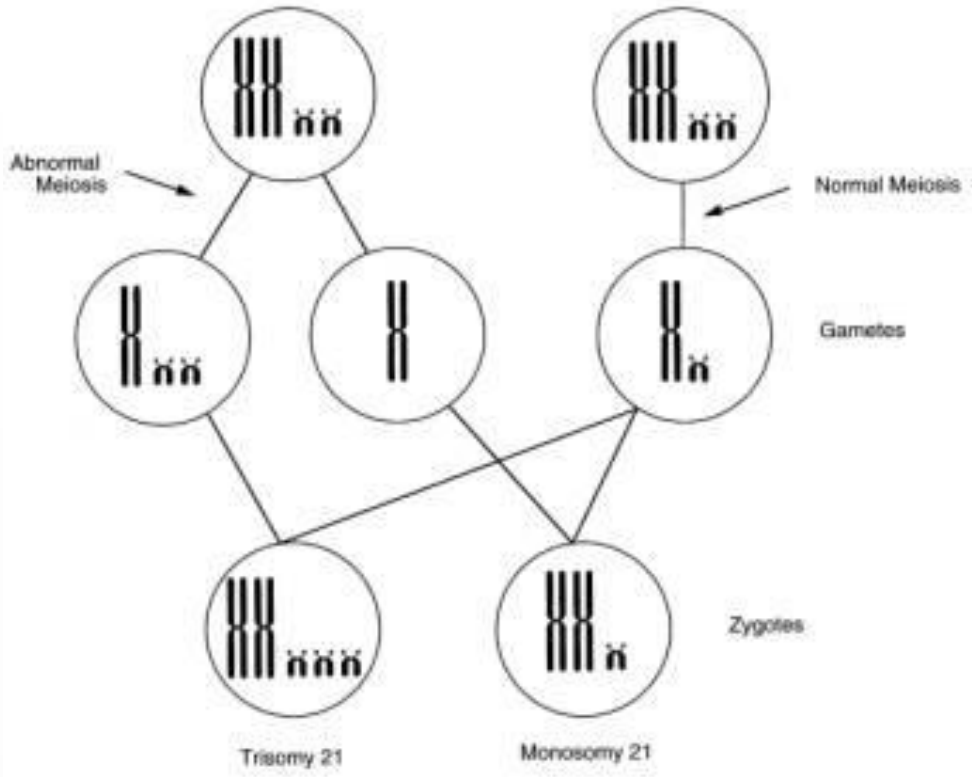
Dominant and Recessive mutations



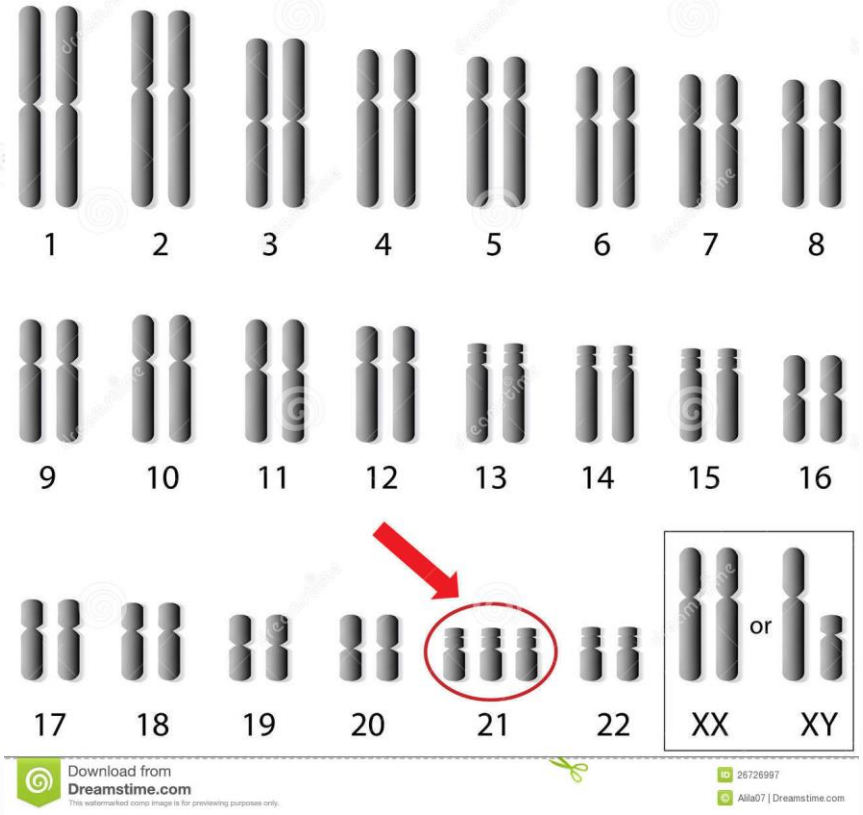
Range of mutations? What do the mutations influence?

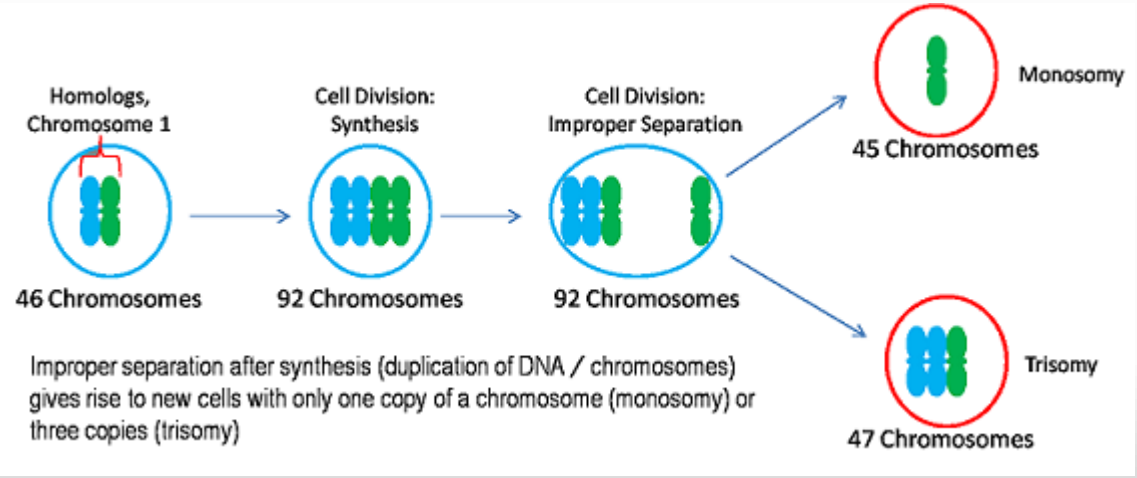


- affect the structure of **genes (and their products) or regulatory regions of DNA**
- changing the structure of **chromosomes** (cause chromosome aberrations: duplications, deletions, inversions, translocations)
- the structure of the genome (chromosome numbers vary:
- aneuploidy - change in the number of certain chromosomes - monosomy, trisomy - eg. **Down syndrome**
- euploidy - change in chromosome set number - haploidy, polyploidy)



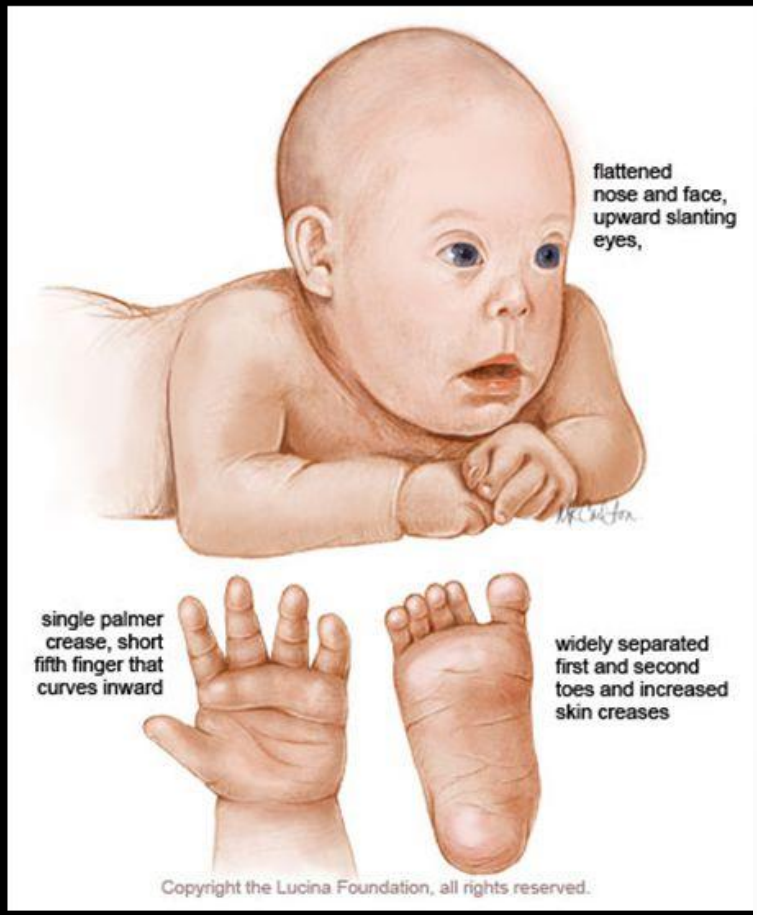
Down Syndrome - Trisomy 21





Down Syndrome

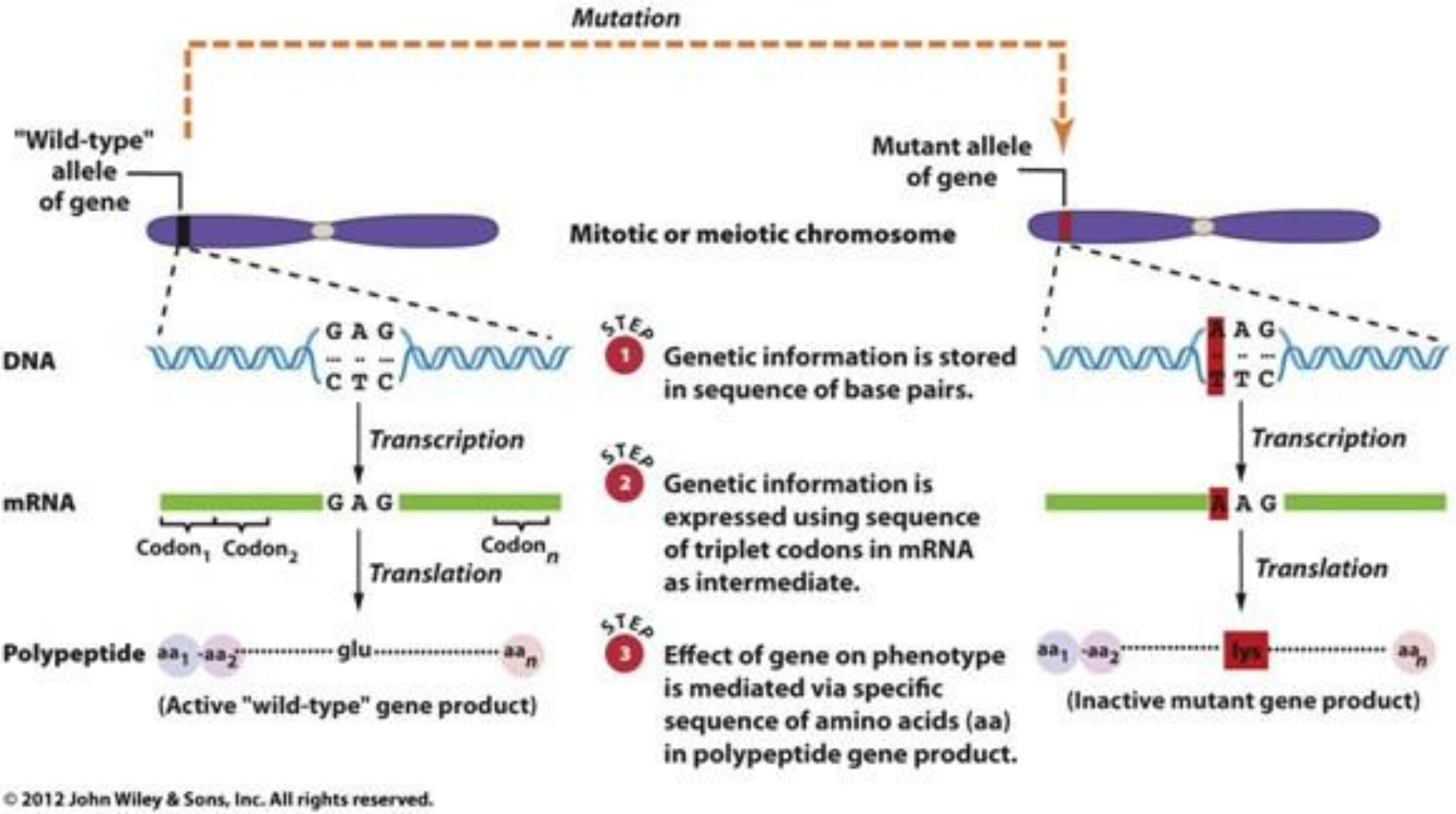
- Trisomy 21
- Often Down syndrome is associated with poor physical development and mental retardation, people with Down syndrome have features characteristic feature of the disease has been nimita and mongolism.

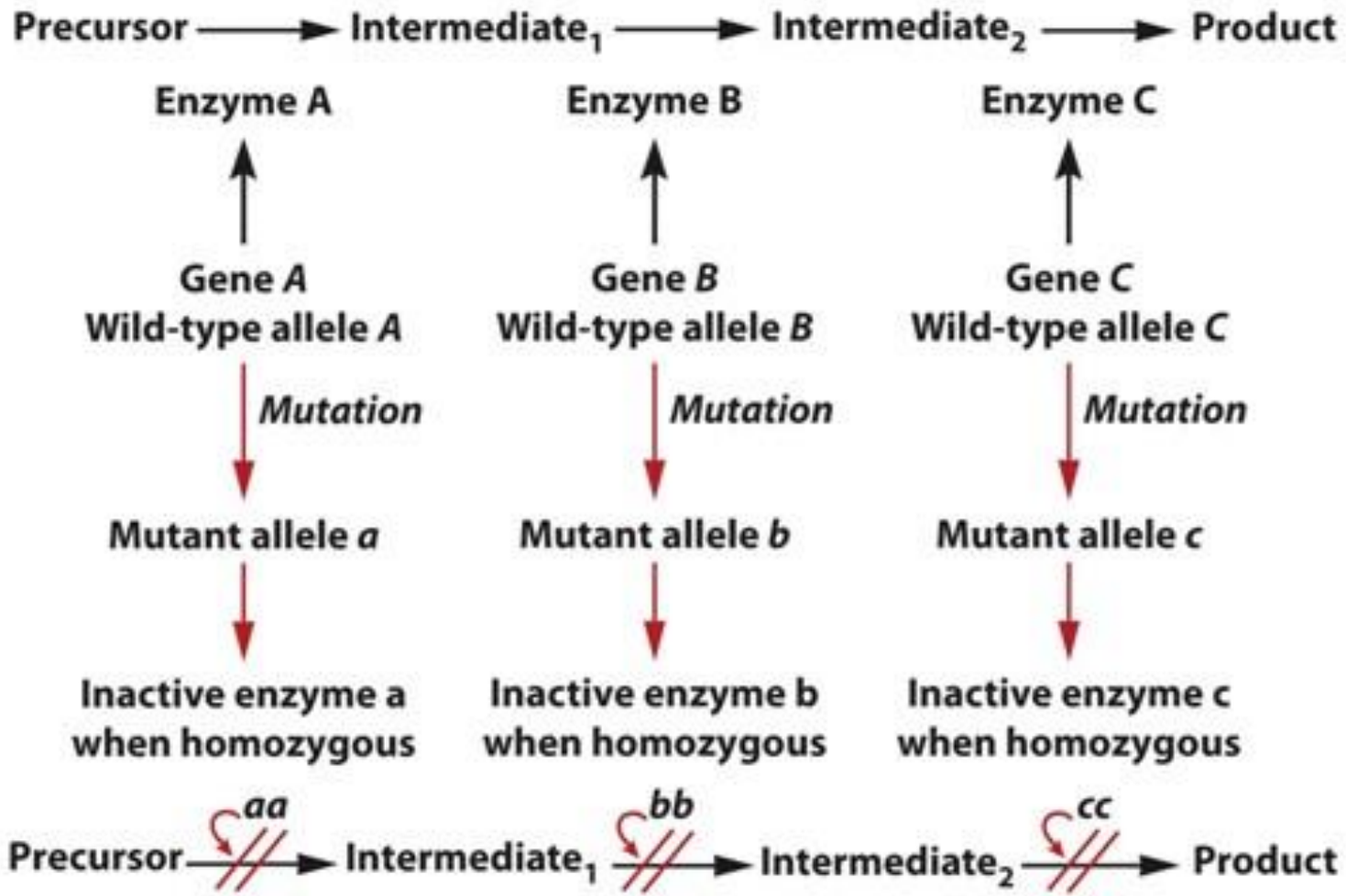


Are mutations are useful or harmful?

- often **harmful** because it adversely affects the function of the **gene product** and thereby damaging the cells
- damaged new product may not be only **protein but also RNA (tRNA, rRNA, etc.)**
- mutations can also damage the **non-coding, but important signal sequences**
- most mutations have no significant impact on the survival of the organism - they are **neutral**
- rarely mutation has a positive effect on the survival and reproduction of the organism
- accumulation of these beneficial mutations will enable the development of an organism in a changing environment

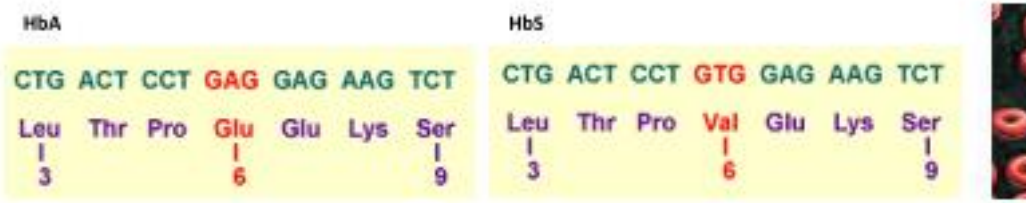
Expression of Wild-type and Mutant Alleles





• Sickle-cell anemia - hemoglobin S

- Mutation in alleles HBBA change that led to the emergence of **alleles HBSS, the substitution of nucleotide pair T: A to A: T,** with thymine in rewriting the chain in the first case and the second case adenine



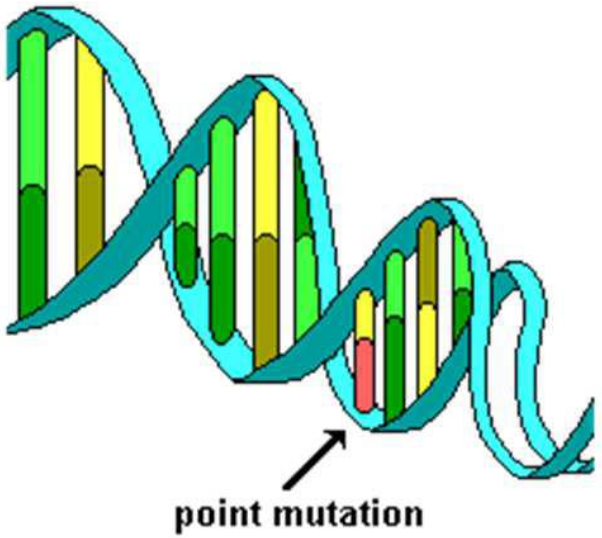
• Phenylketonuria

The best studied hereditary disease in phenylalanine-tyrosine metabolism is **phenylketonuria**, autosomal recessive disease which is caused by the **lack of phenylalanine hydroxylase enzyme which converts phenylalanine to tyrosine.** Newborns affected by phenylketonuria should have a strict diet minimal in phenylalanine, otherwise they may develop severe mental retardation

• Albinism

Albinism, a disorder caused by a lack of pigmentation of the skin, hair and eyes, is the result of mutations blocking the conversion of tyrosine to dark pigment melanin

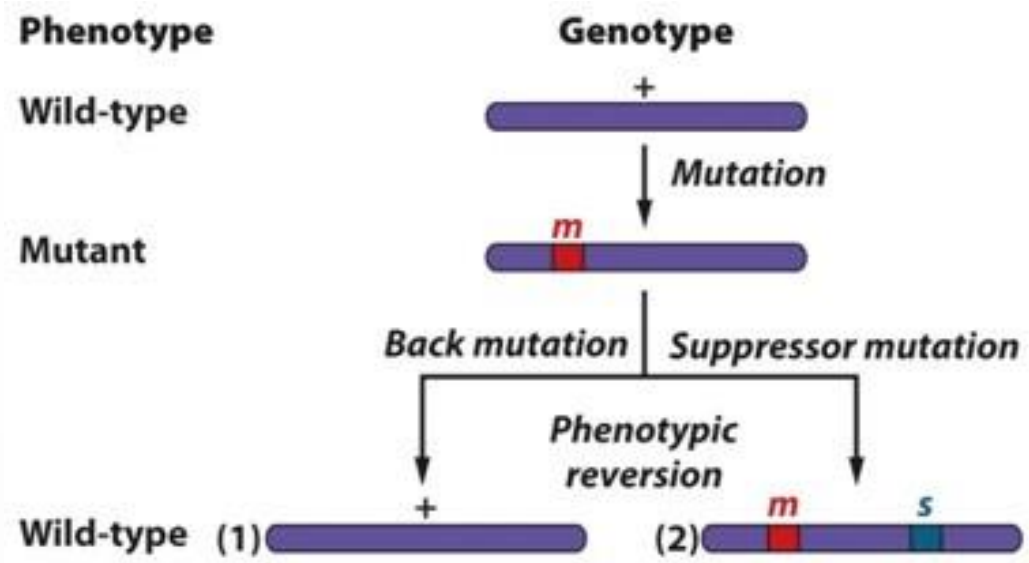
Types of mutations



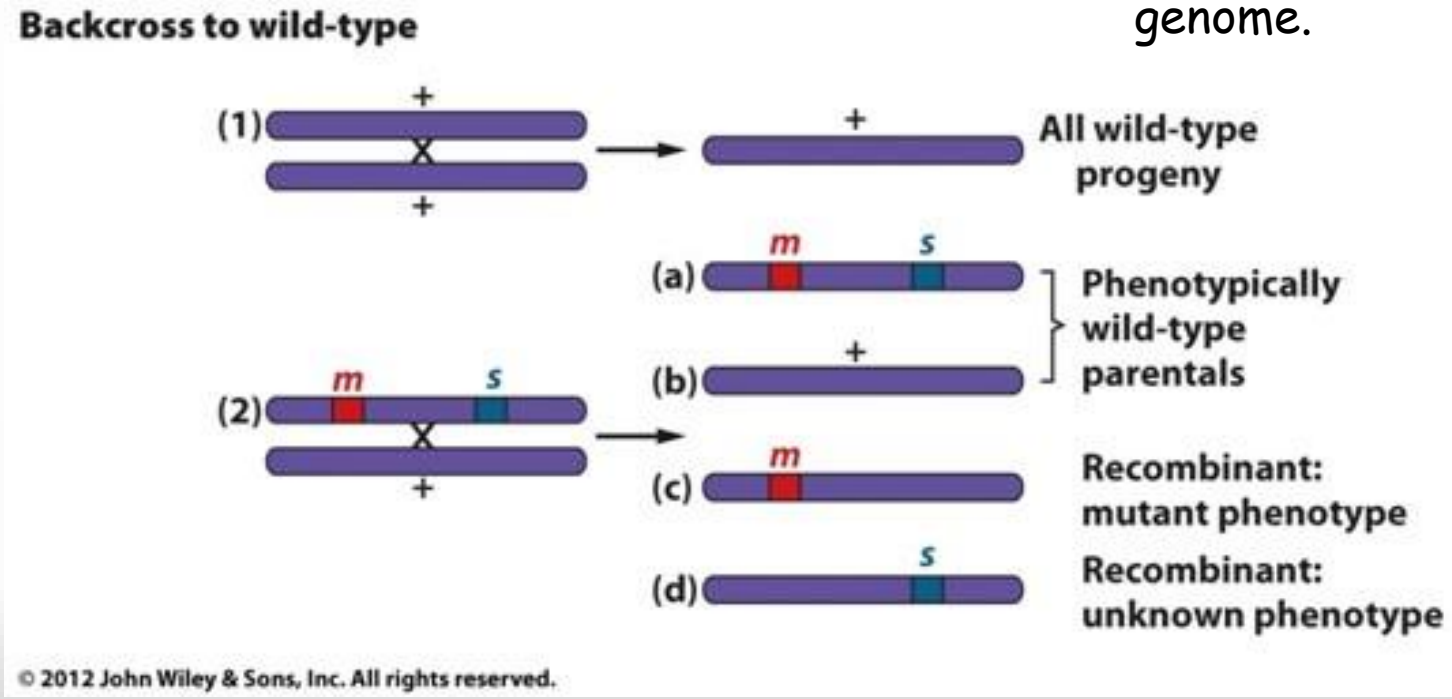
- point mutation: substitution of a single base
- null mutation: complete loss of gene function

- tight mutation has a clear phenotype (eg. full loss of ability to grow under certain conditions or prevent formation of product of the biochemical pathway, if the mutation knocks out an enzyme)
- leaky mutation: partial activity of the gene product is maintained (eg. the residual activity of the enzyme allows at least slow growth under certain circumstances)

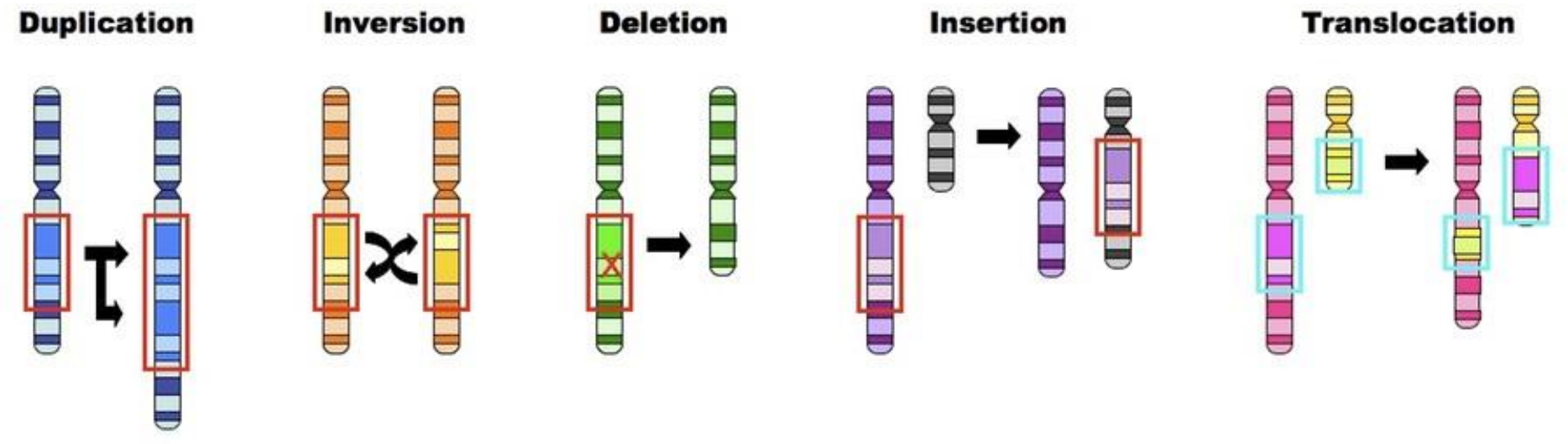
- direct mutation - standard allele changes in mutant
- back mutation (reversion) - mutant allele changes into standard
- suppressor mutation - a second mutation, which offsets the effect of the first direct mutation



- **Mutation:** A Reversible Process Forward mutation—mutation of a wild- type allele to a mutant allele. Reverse mutation (reversion)—a second mutation that restores the original phenotype. -**Back mutation**—a second mutation at the same site. -**Suppressor mutation**—a second mutation at a different location in the genome.



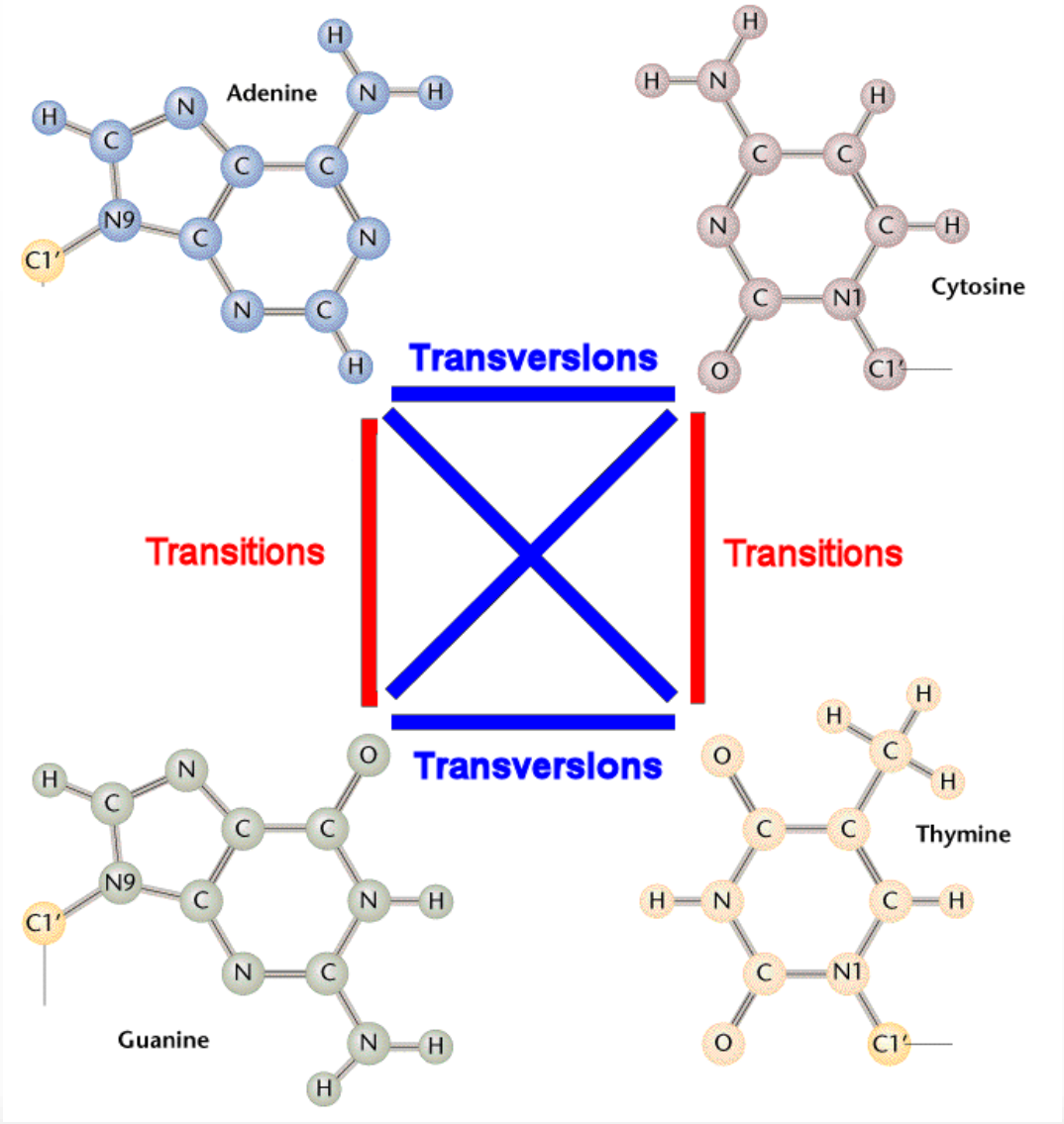
Types of mutations



- substitution: change in the sequence of bases
- inversion: a piece of DNA is inverted, remains in the same place
- duplication: a piece of DNA is duplicated, the second copy usually remains in the same place as the original
- deletion: one or more bases are removed
- insertion: one or more bases are added
- translocation: stretch of DNA is transferred from its original location to another location - either the same or different DNA molecule

Base substitution

- transition: pyrimidine is replaced by another pyrimidine (T for C and vice versa) or the purine is replaced by another purine (A for G and vice versa)
- transversion: pyrimidine is replaced by a purine or vice versa



Consequences of substitution

- **missense mutation**:

  TGG (Trp) -> CGG (Arg)

- **nonsense mutation – causes formation of stop codon:**

  TGG (Trp) -> TAG (Term.)

- **neutral mutations - changes and are not reflected in the function**:

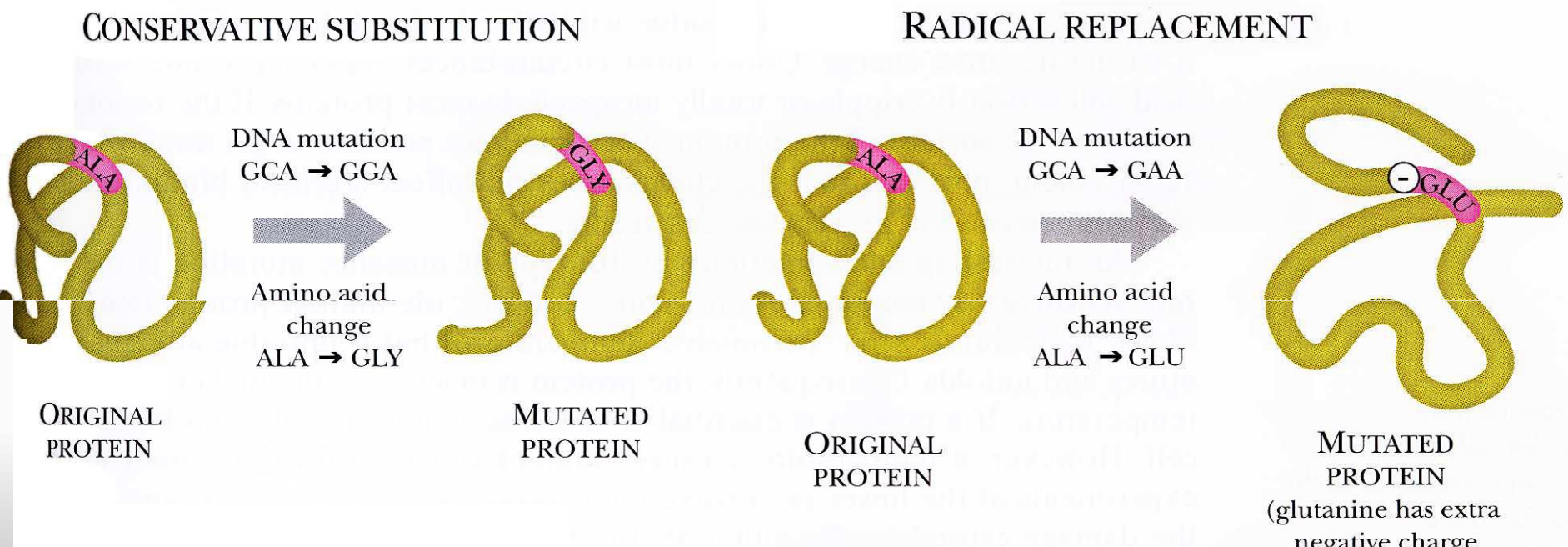
  AAA (Lys) -> AGA (Arg)

- **silent mutation - a different codon, but the same AA**:

  AAA (Lys) -> AAG (Lys)

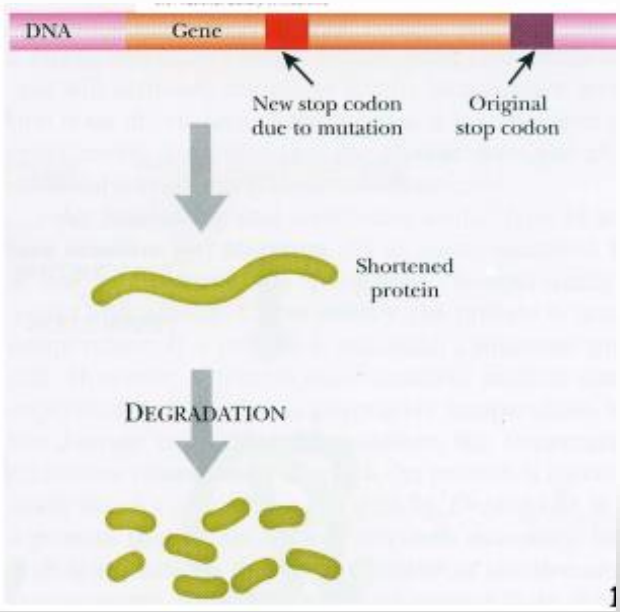
Severity of mutation depends on their type and location

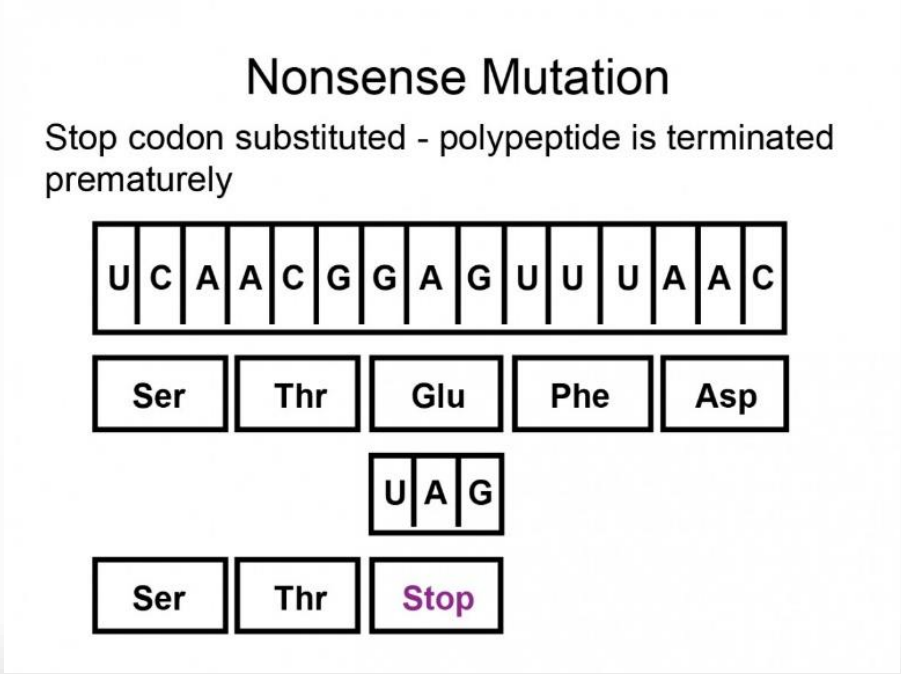
- missense mutations are the most common - lead to the replacement of one amino acid in a protein for another
- when the original amino acid mutations is replaced by chemically related - usually not serious consequences (conservative substitution)
- the consequences are serious when changes folding or structure of the protein active site (radical replacement)



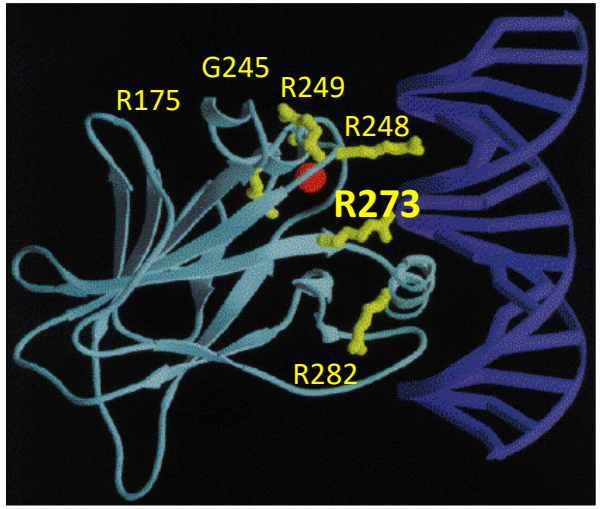
Nonsense mutations

- mutations, when the codons encoding amino acids arechanged to meaningless codons - terminator (UAA, UAG, UGA in RNA)
- cause premature termination of the synthesis of polypeptide chain
- truncated polypeptide is not folded correctly
- usually undergoes degradation





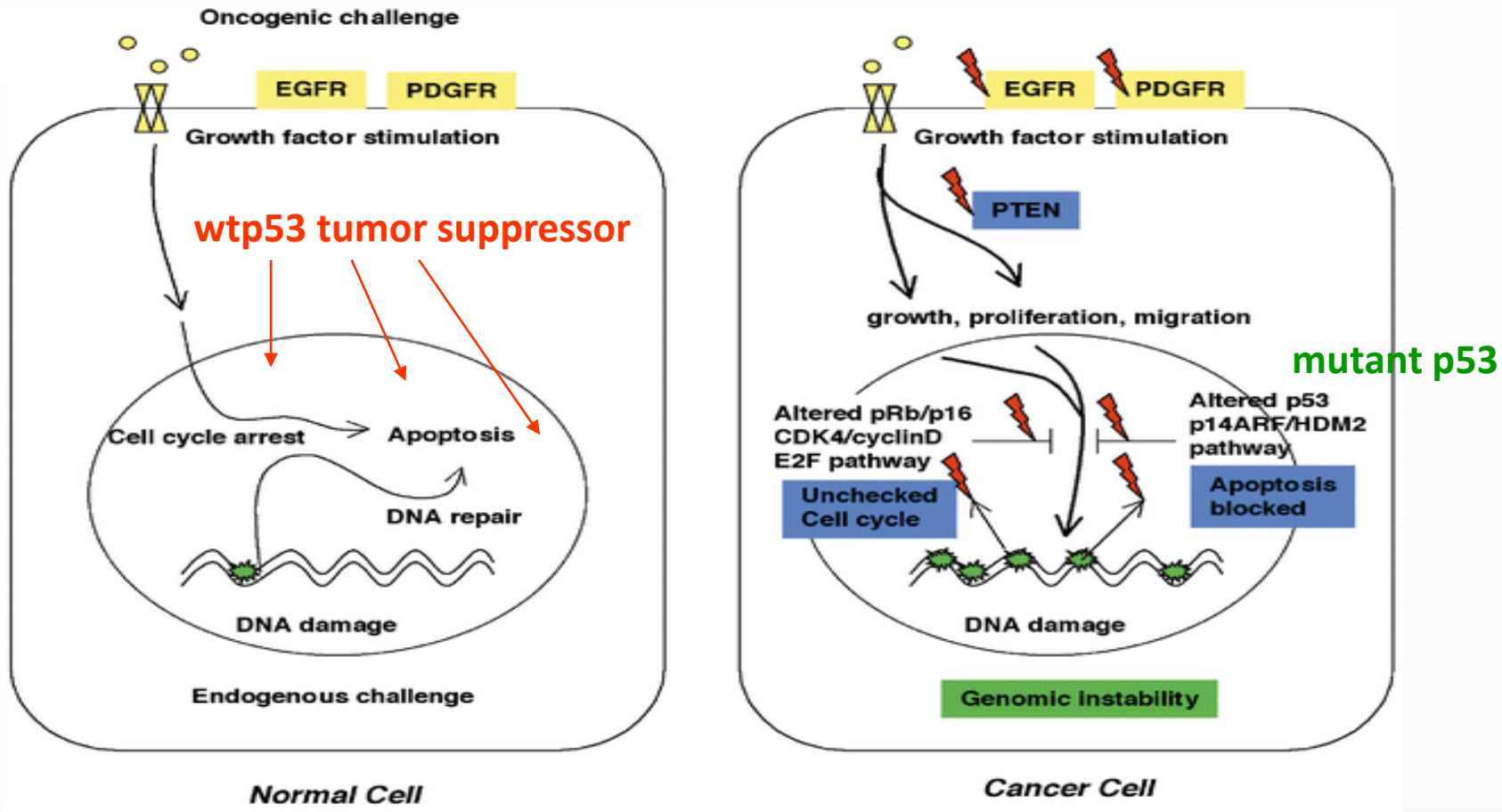
TP53 point mutations will cause loss of p53 function as a tumor suppressor



Cho et al. 1994

PuPuPuC(A/T)(T/A)GPyPyPy

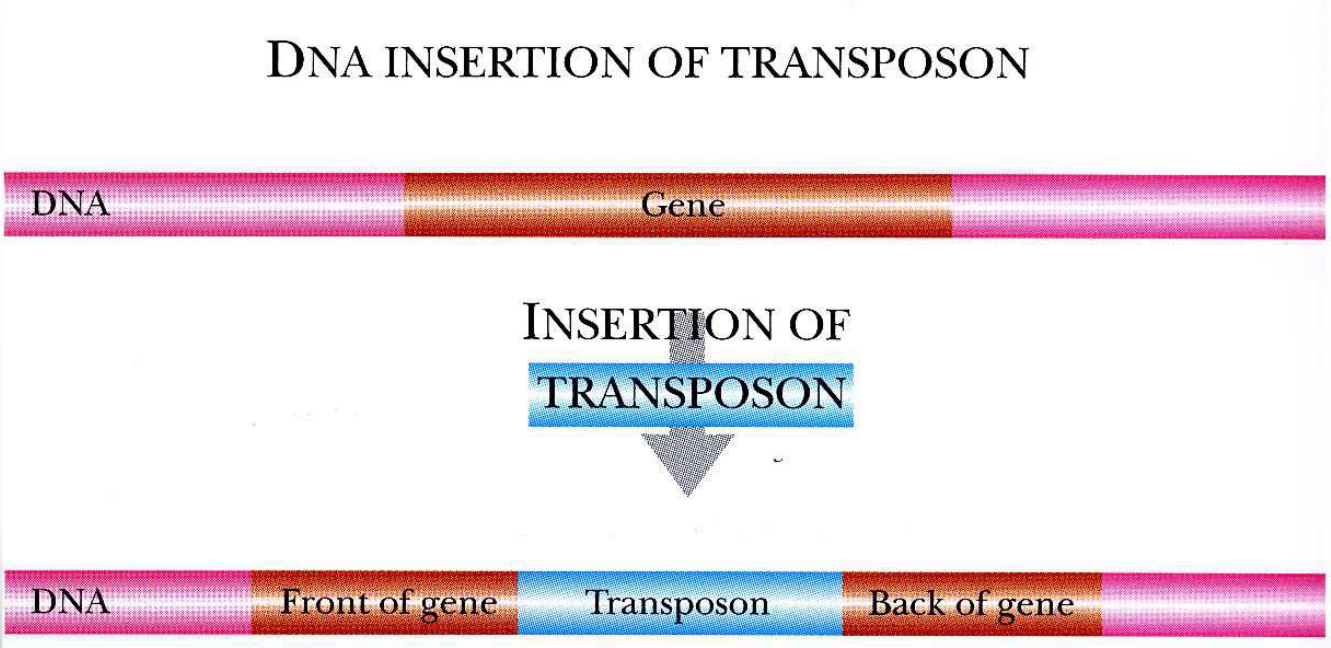
Different reactions of normal and tumor cells to the oncogenic stress



A.Merlo, 2004

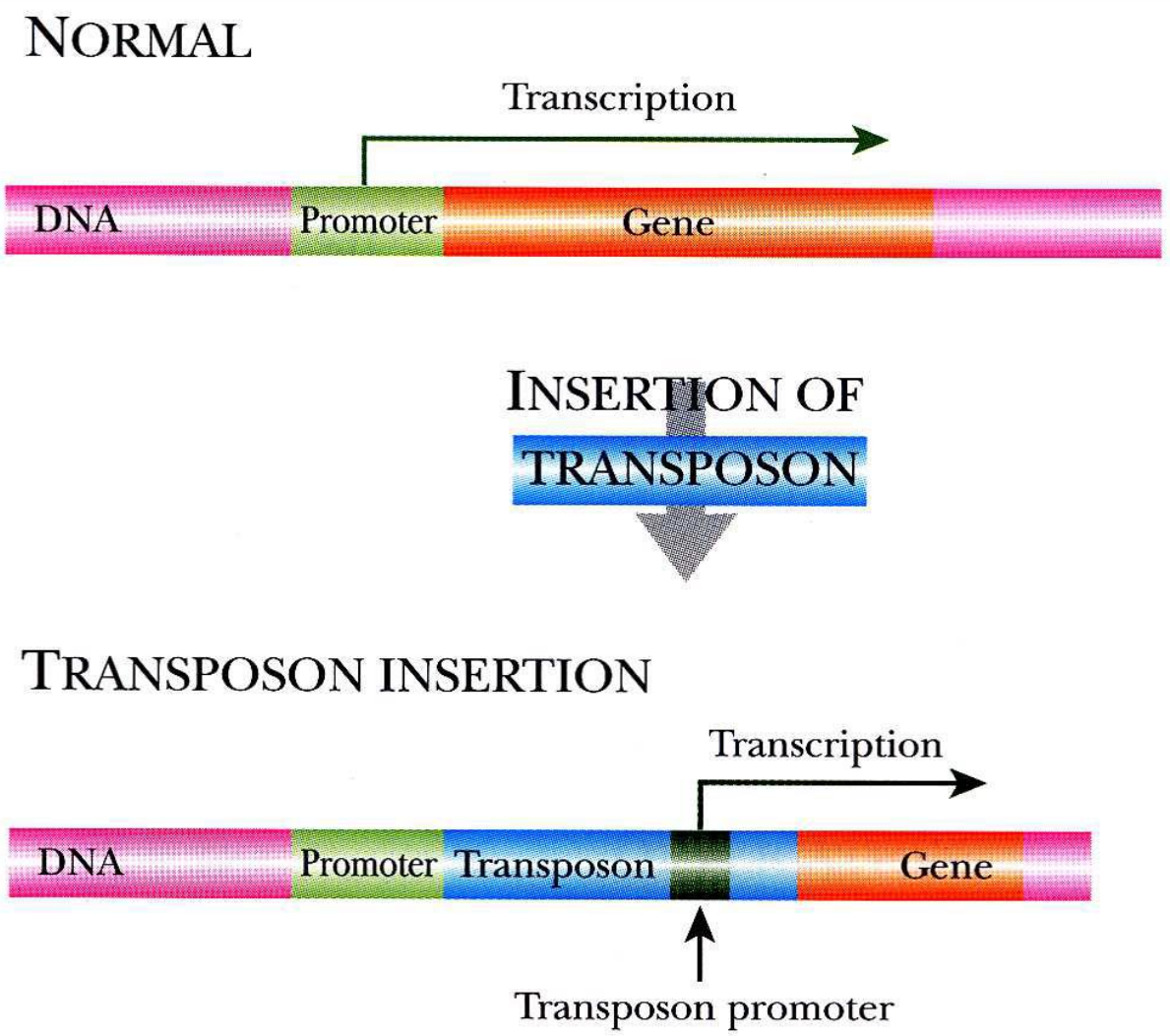
Insertions and deletions

- Insertion or loss of DNA section
- incorporation into the DNA segment into coding sequence will usually cause its **inactivation**
- effect depends on the extent and location (shorter insertion may allow at least partial activity of the original protein)
- mobile genetic elements (**transposons**) can be as long as several thousand bp



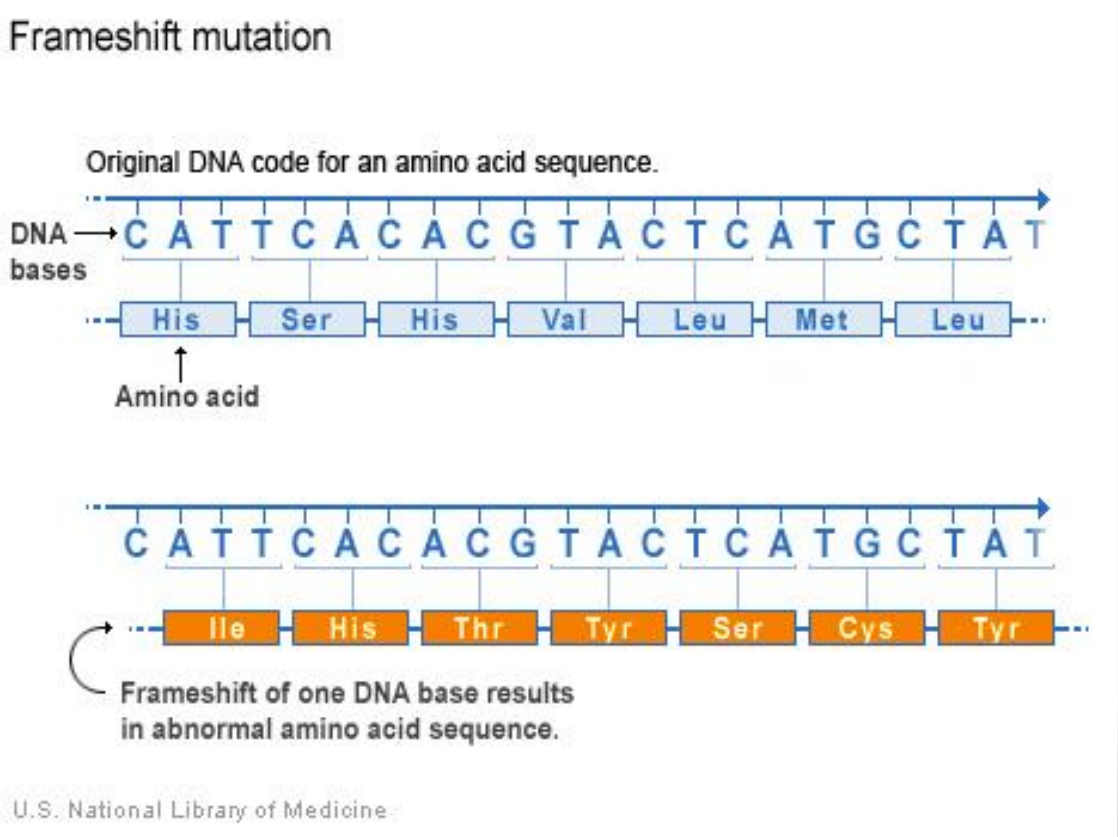
Insertion can activate gene expression

- change of repressor binding site
- change in promoter - e.g. conversion of gene regulation under the control of the transposon promoter



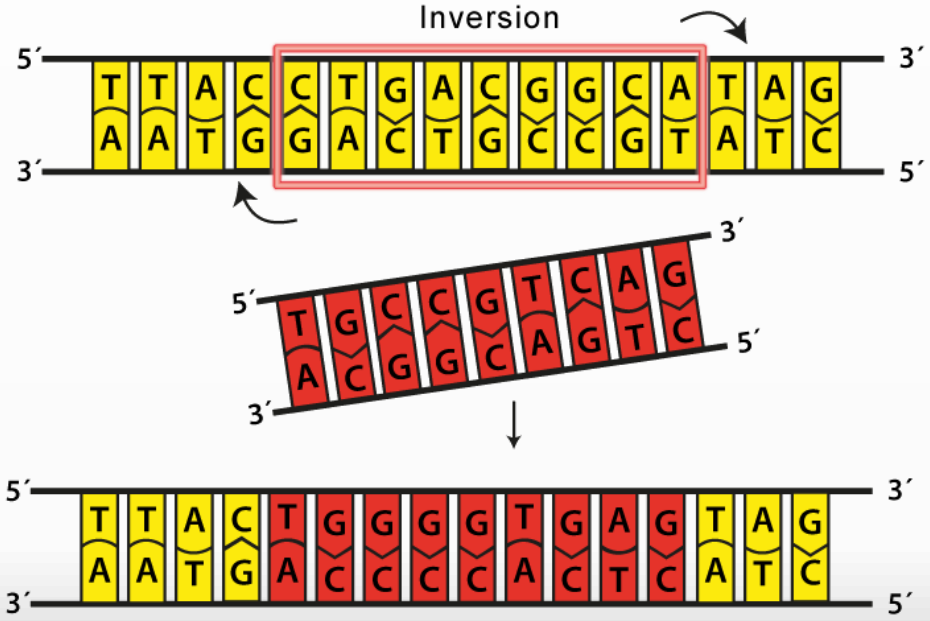
Mutations changing the reading frame

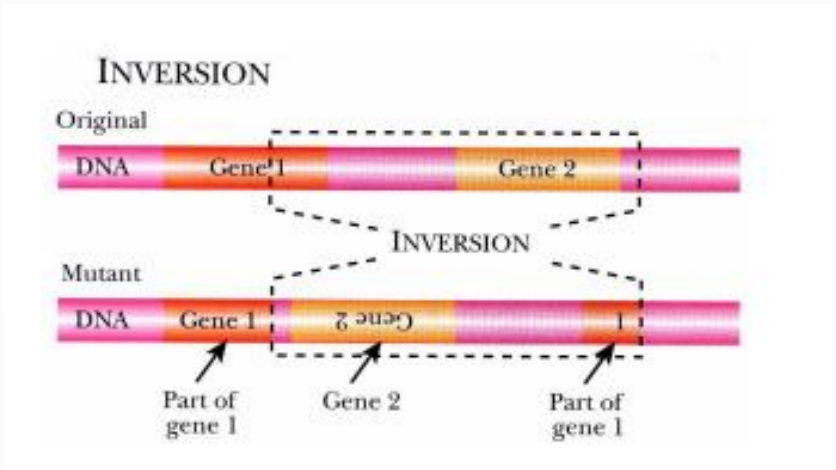
- bases are read as codons (3 bases)
- the inclusion or removal of one or two bases fundamentally changes the genetic information - leading to loss of protein function
- insertion or deletion of three bases - reading frame does not change - the protein has 1 AA more or less, its function is usually not significantly altered



Inversion

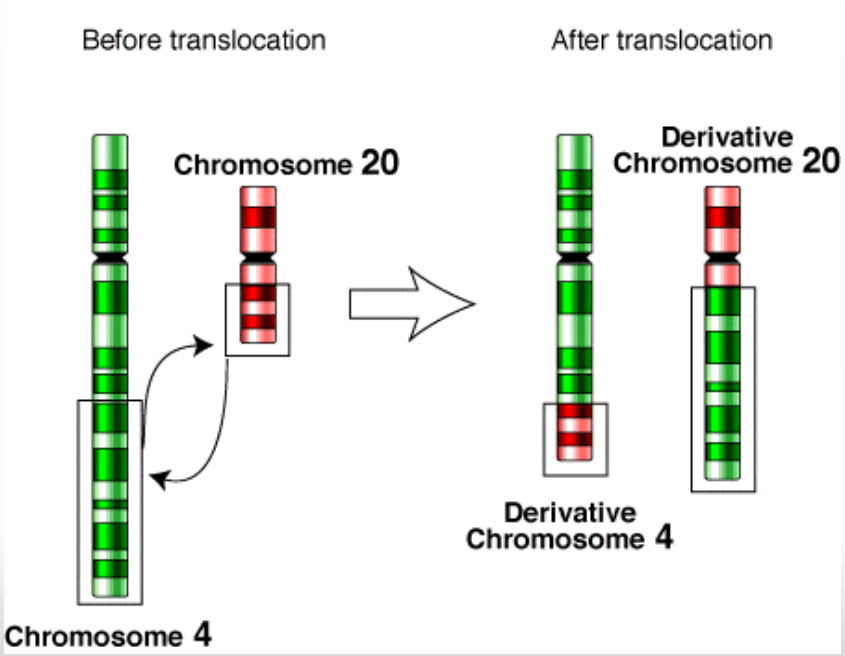
- reversal of a DNA segment
- interruption of the coding sequence - loss of gene function
- when terminal sequences of the inverted portion are in intergenic regions (inversion includes promoter) - gene remains intact, even if it is transcribed in the reverse orientation - does not lose its function





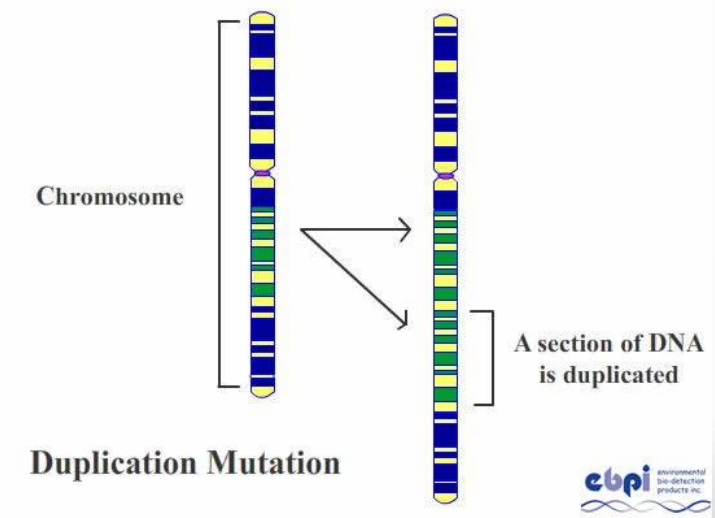
Translocation

- exclusion of a DNA segment from its original site and its insertion at the same or another chromosome
- if the coding sequence is intact - protein function may not be lost
- inclusion of one gene to another gene - loss or change in function



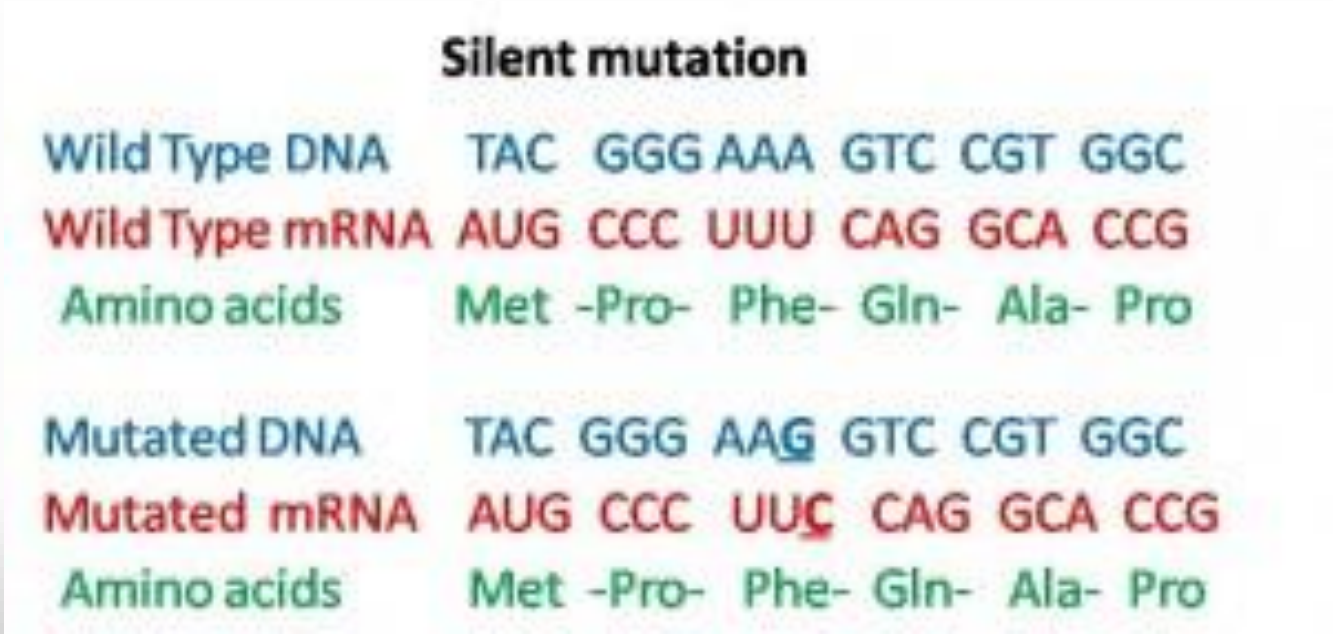
Duplication

- stretch of DNA is duplicated and both copies remain on the chromosome
- usually in the location of the original and the copy adjacent to it (tandem duplication)
- can generate two copies of gene - subsequent divergence allows the creation of new genes during evolution
- multiple duplication (amplification) may significantly increase the gene copy number and thus the level of product



Silent mutations

- does not alter the phenotype
- in the noncoding intergenic regions
- introns (can not be in critical splicing sites)
- mutations changing codon sense (e.g. glutamic acid codons : GAA, GAG, for alanine 4 codons exist: GCU, GCC, GCA, GCG)



Reversion = back mutation

- reversion = complete recovery of sequence
- pseudoreversion = codon restoring the original function of the polypeptide
- intragenic suppressor mutations = restores the original phenotype after suppressor-sensitive mutation in the same gene occurs
- intergenic suppressor mutations = arises in another gene. Suppressor is a mutant allele of the gene - suppressing phenotype of suppressor-sensitive mutation

Alleles and phenotype

- Allele = specific form of a gene
- Standard allele = prevalent in the population
- Mutant allele = changed by mutation

- standard phenotype = standard allele in the phenotype
- mutant phenotype = expression of the mutant allele in phenotype

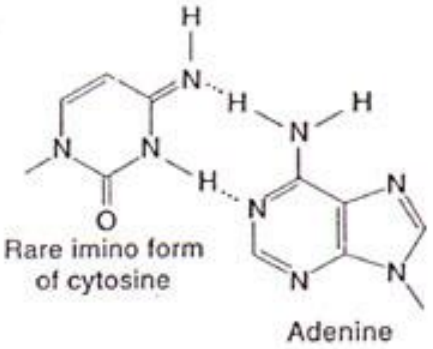
KEY POINTS

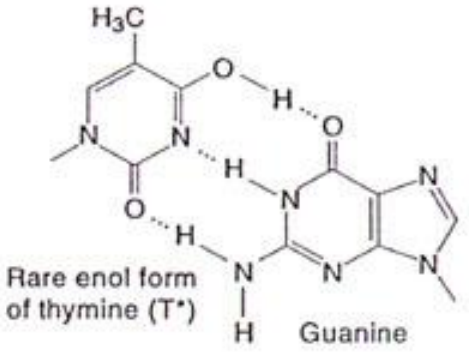
- Mutations occur in both embryonic and the somatic cells but to the progeny are transmitted only mutations in the germ cells.
- Mutations can arise spontaneously or may be induced by mutagenic substances in the environment.
- The mutation is usually a non-adaptive process in which the individulals with preexisting randomly arising mutations are selected in given conditions.
- Restoring the standard phenotype in the mutant organism is the result of back or suppressor mutations.

Spontaneous and induced mutations

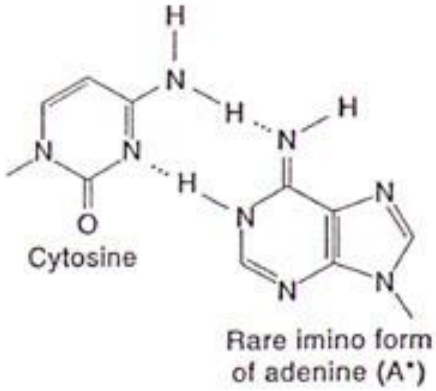
- **Spontaneous mutations** occur without apparent external cause – result of metabolic disorders in the body, mistakes in DNA replication or the presence of unknown substances in the environment
- **induced mutations** are formed by the known physical, chemical or biological factors capable of inducing changes in DNA - mutagens
- Mutagens are also causes of neoplastic transformation - **carcinogens**
  1. mutagenic directly
  2. promutagens, metabolic activation

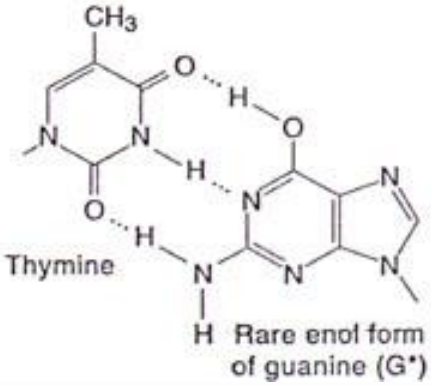
Spontaneous mutations





- **Incorrect base pairing**

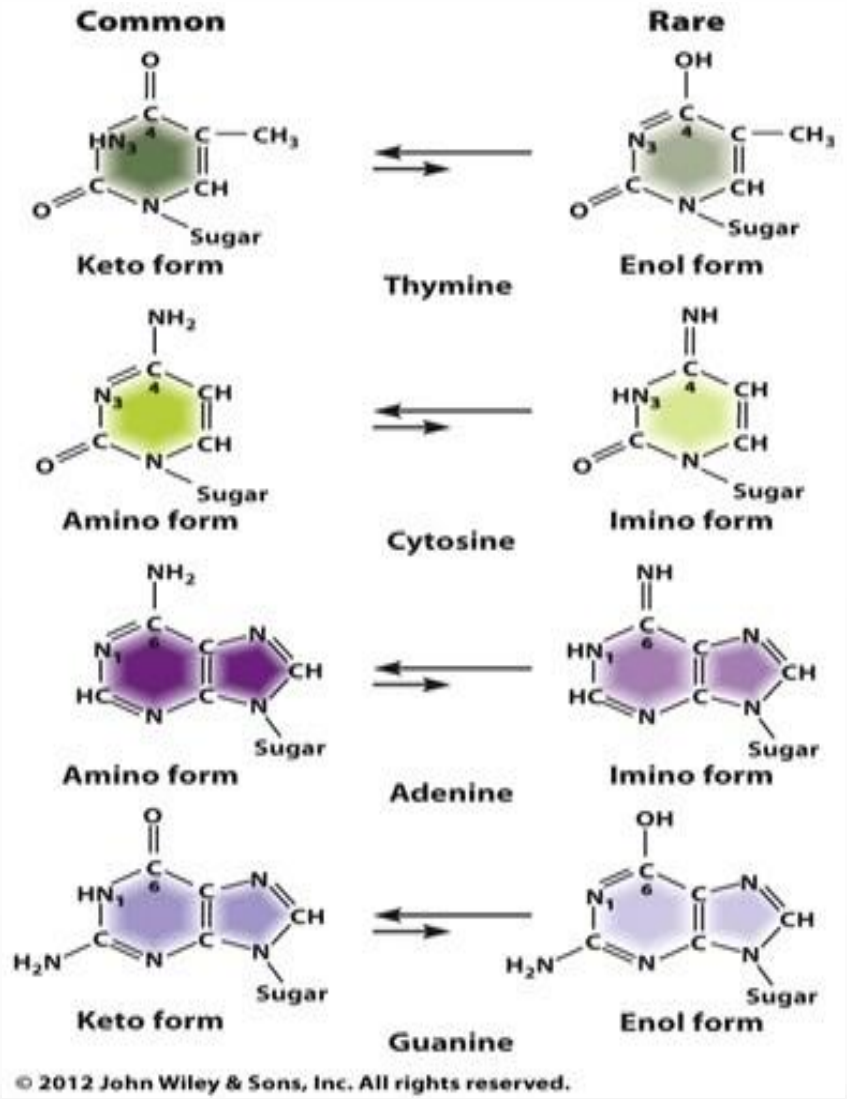




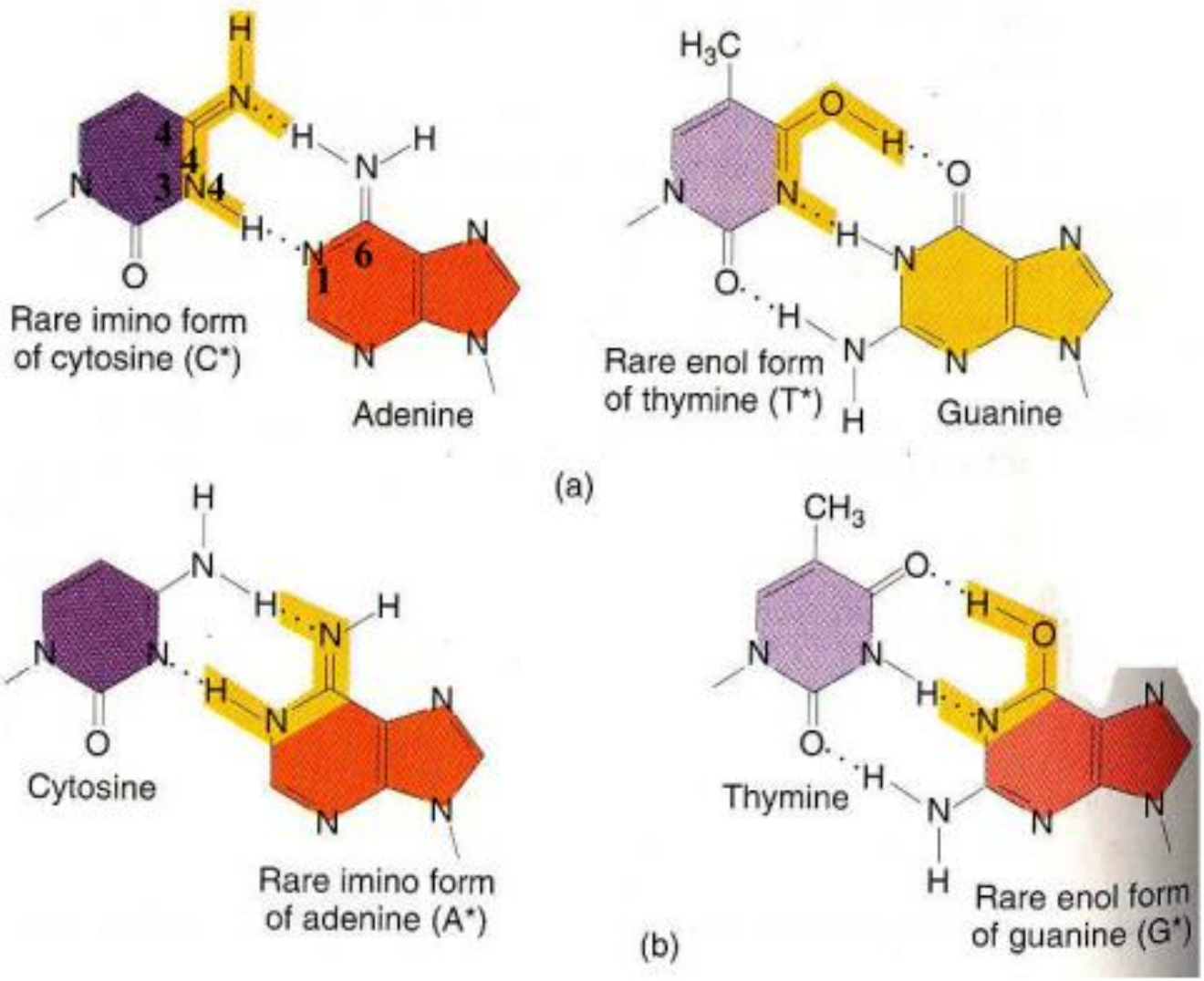
- tautomeric base changes
- wobble base pairing
- depurination and depyrimidination of bases
- deamination of bases
- uracil incorporation into DNA during replication
- oxidative DNA damage

Molecular basis of mutation

- structures of bases in DNA are not static. Hydrogen atoms can move from one position in purine or pyrimidine to another position – for example, from the amino group to the ring.
- Such chemical modifications are referred to as **tautomerism**. Although tautomeric rearrangements are rare, they can have an important role in metabolism of DNA, because some of them are changing the base pairing

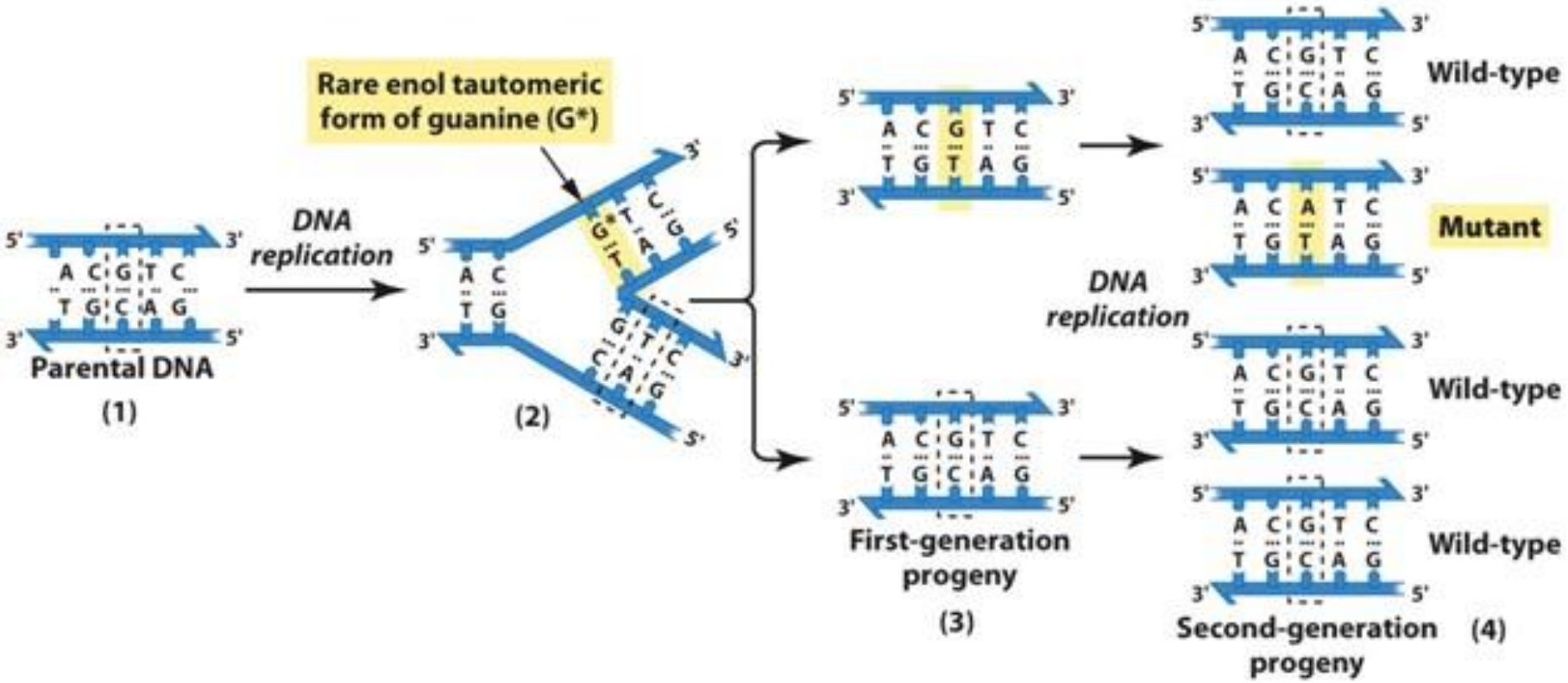


Tautomeric Shifts Affect Base-Pairing



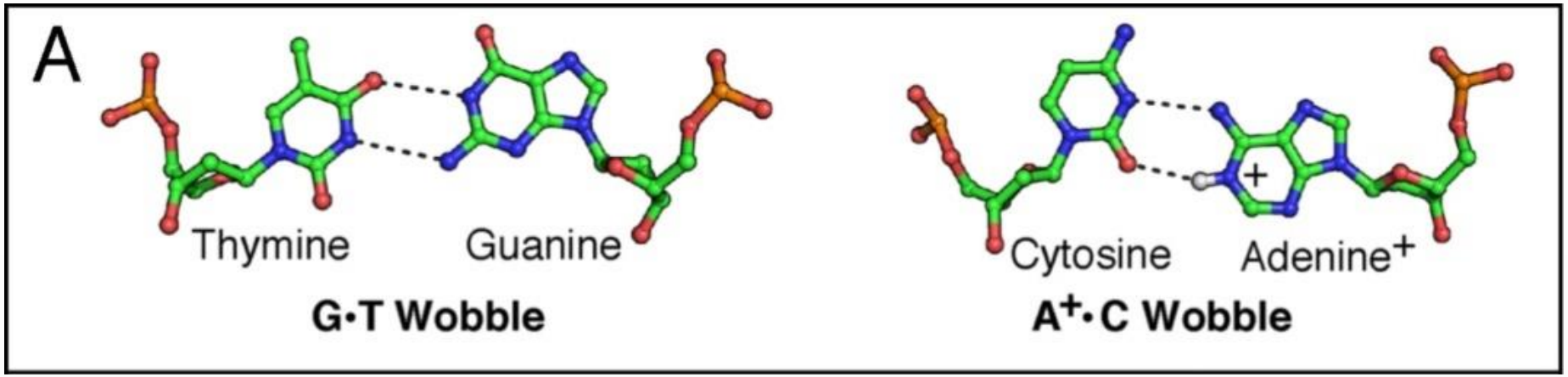
Mutation Caused by Tautomeric Shifts

Mechanism by which tautomeric shifts in the bases in DNA cause mutations.



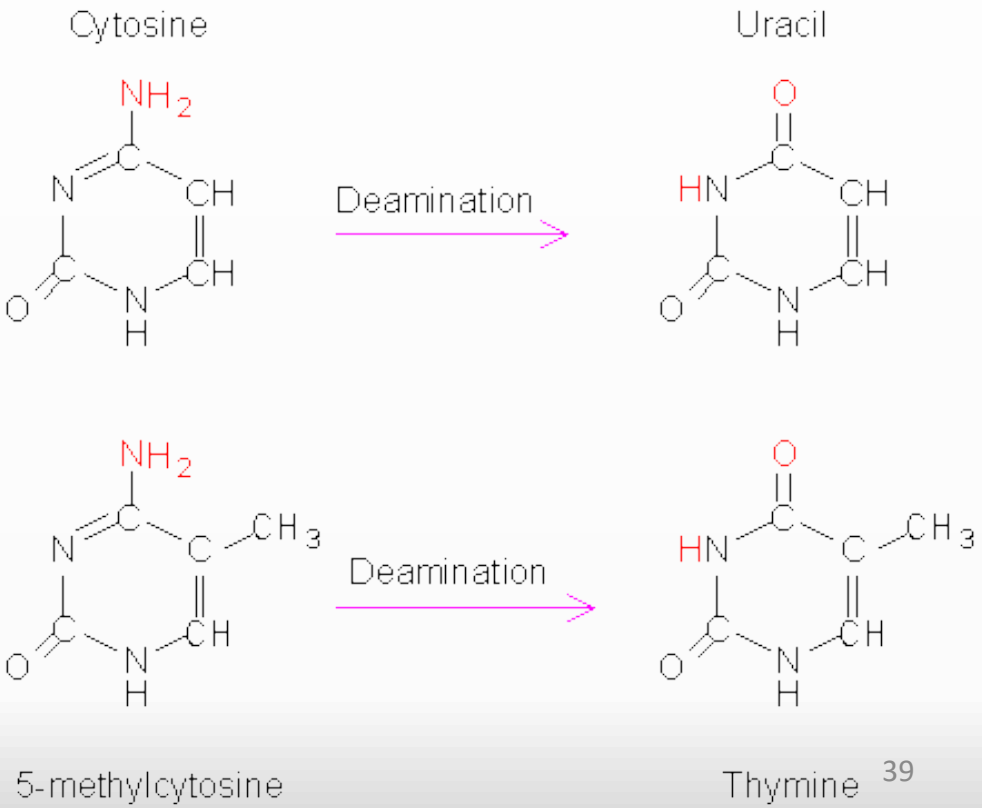
Wobble base pairing

- between tRNA anticodon and the mRNA codon
- during replication
- correction mechanism of DNA polymerase III



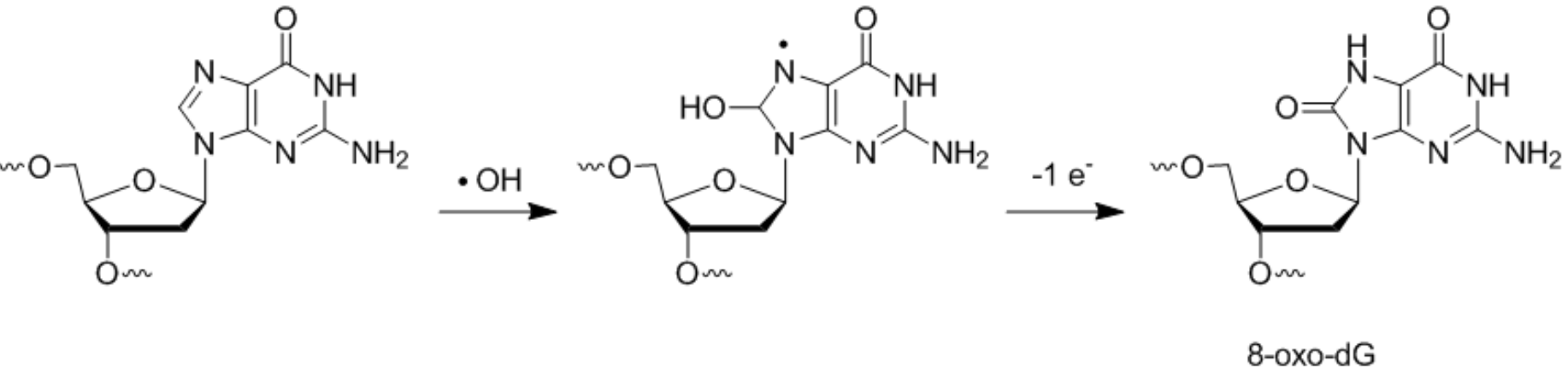
Incorporation of uracil into DNA

- associated with spontaneous deamination of cytosine and producing uracil
- occasionally incorporated into the DNA - removed by uracil-DNA-glycosylase
- a frequent phenomenon in human lymphocytes
- transversion causes *GC* to *AT*



Oxidative DNA damage

- produces hydroxyl radical •OH
- formed from the hydrogen peroxide in respiratory chain
- causes transversion of GC to AT



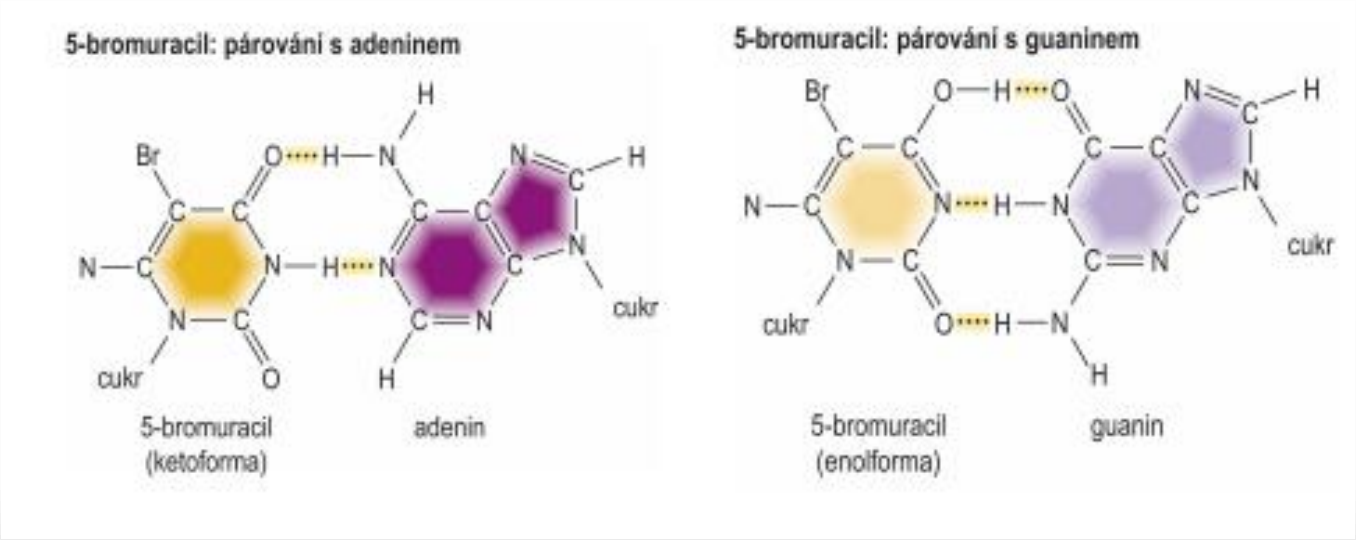
Induced mutations:chemical mutagens

- **influencing the structure of DNA**
- **oxidizing agents (peroxides, oxygen radicals)**
- **deaminating substances (nitrites)**
- **alkylating agents (ethyl methanesulfonate, yperite)**
- **intercalating agents (acridines)**
- **aromatic amines (benzidine, naphthylamine)**

- substances damaging **cellular machinery** for the equitable division of genetic information during cell division – colchicine
- substances inducing mutation, regardless of the **ongoing replication of DNA** (e.g., alkylating agents, nitrous acid)
- substances causing **mutations only in DNA replication** (base analogues, acridine dye)

Mutagenic base analogues

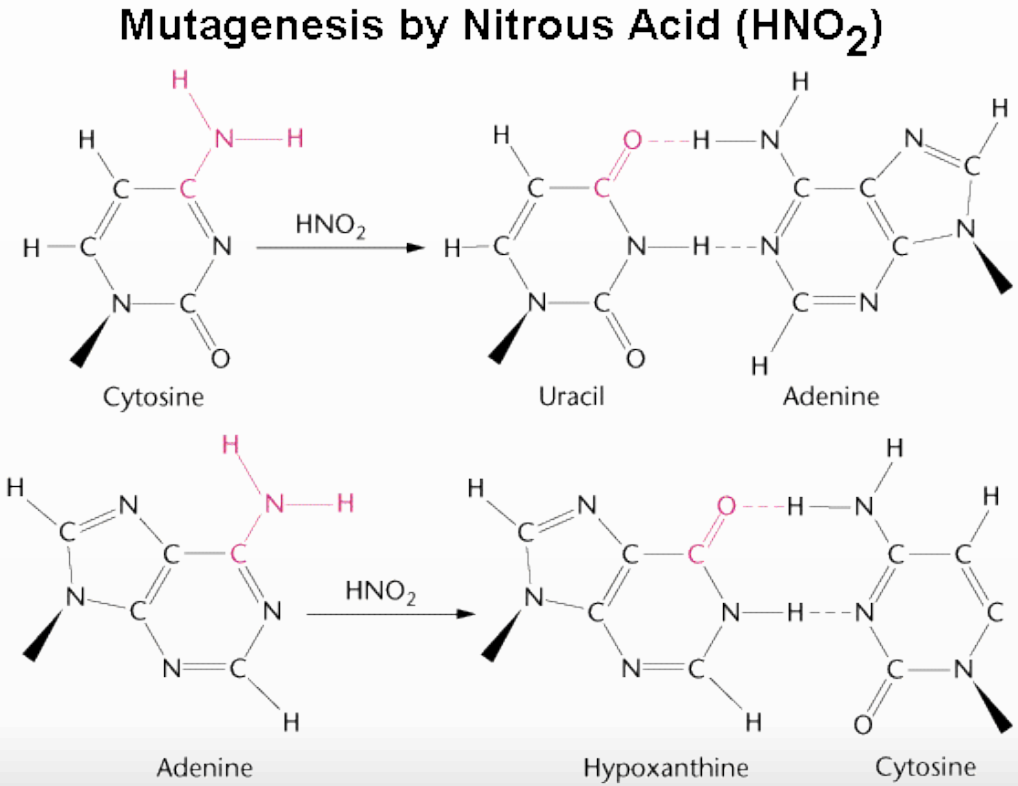
- structurally similar to the normal bases
- incorporated into the DNA during replication
- structural differences from the normal bases, increase the rate of mismatch thereby creating mutations
- e.g. 5-bromouracil: thymine analogue - induces a transition from AT to GC (different charge distribution - increased frequency of tautomerization to enol form, which is paired with guanine)



Nitrous acid

HNO_2

- causing the oxidative deamination of amino groups of adenine, guanine and cytosine: amino groups thereby changing to keto group
- adenine deamination - changed to hypoxanthine, which is paired with cytosine
- cytosine is deaminated to uracil, which pairs with adenine

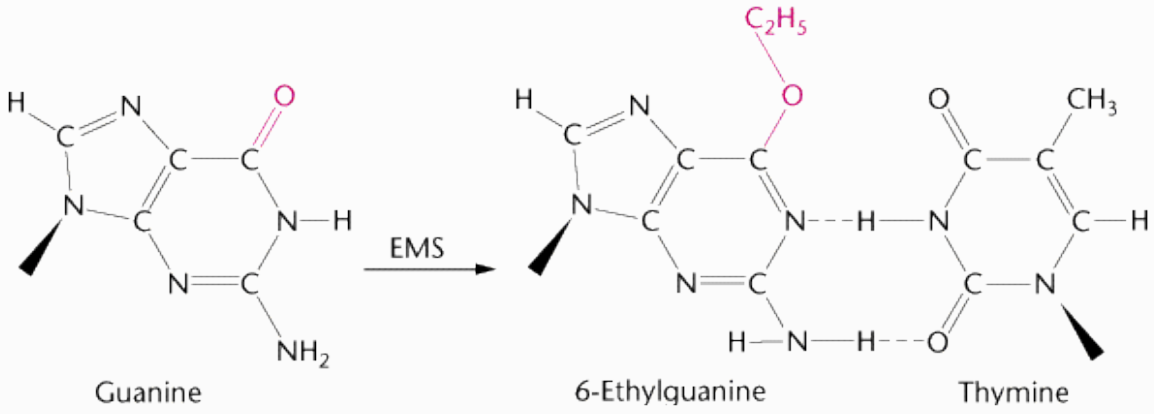


(Klug & Cummings 1997)

Alkylating agents

- Ethyl methanesulfonate, aziridine, yperite
- transfer of a methyl or ethyl groups to DNA bases, which causes a change in base-pairing
- induces all types of mutations (transitions, transversions, frameshift mutations and chromosomal aberrations)

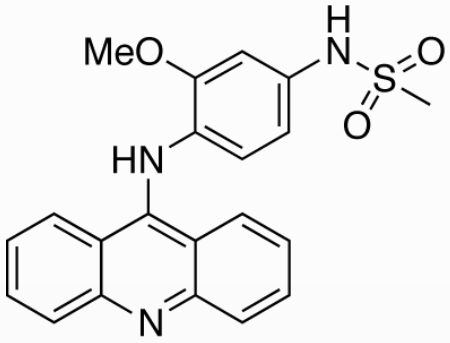
Mutagenesis by Ethyl Methane Sulfonate (EMS)



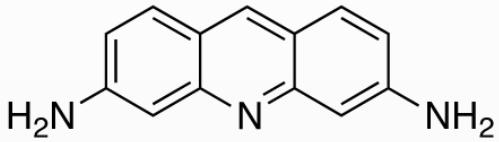
(Klug & Cummings 1997)

Acridine dyes

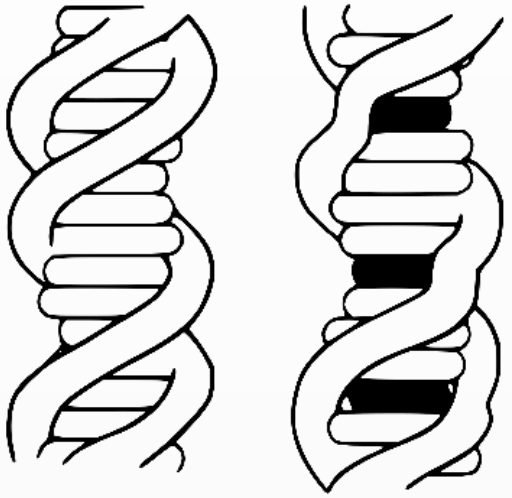
- e.g. proflavine, acridine orange, acridine blue
- intercalators – incorporation to DNA groove
- tighten and alter the conformation of the DNA double helix
- during replication occur deletions or insertions of one or more base pairs - often frameshift mutation (change of reading frame)



amsacrine

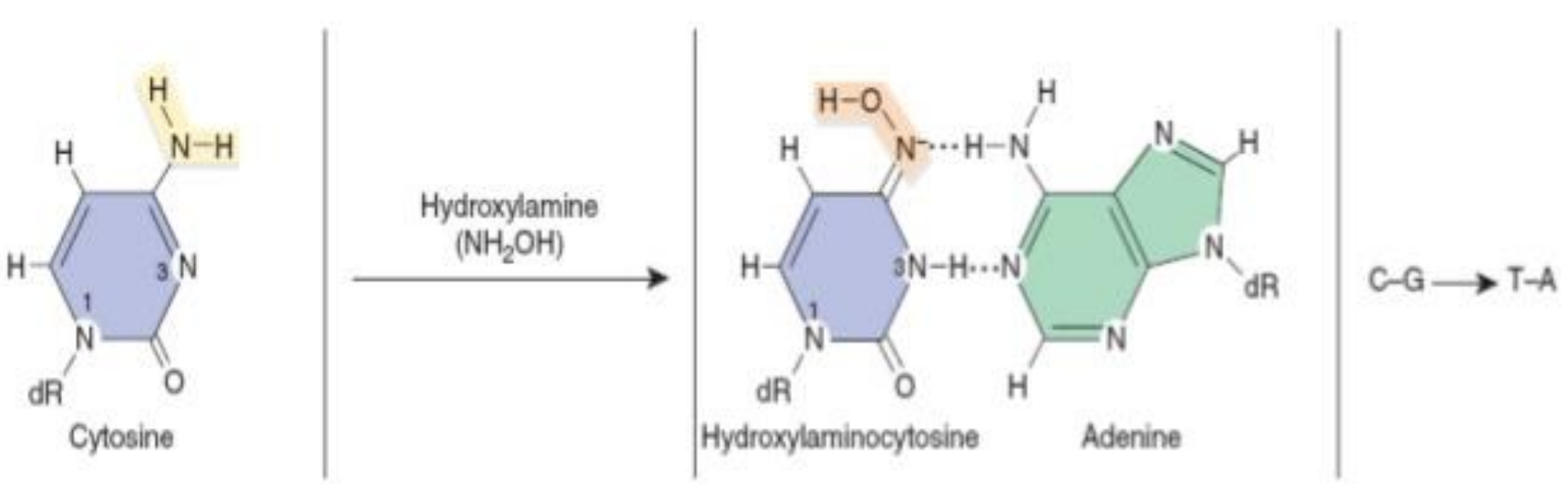


proflavine



Hydroxylamine

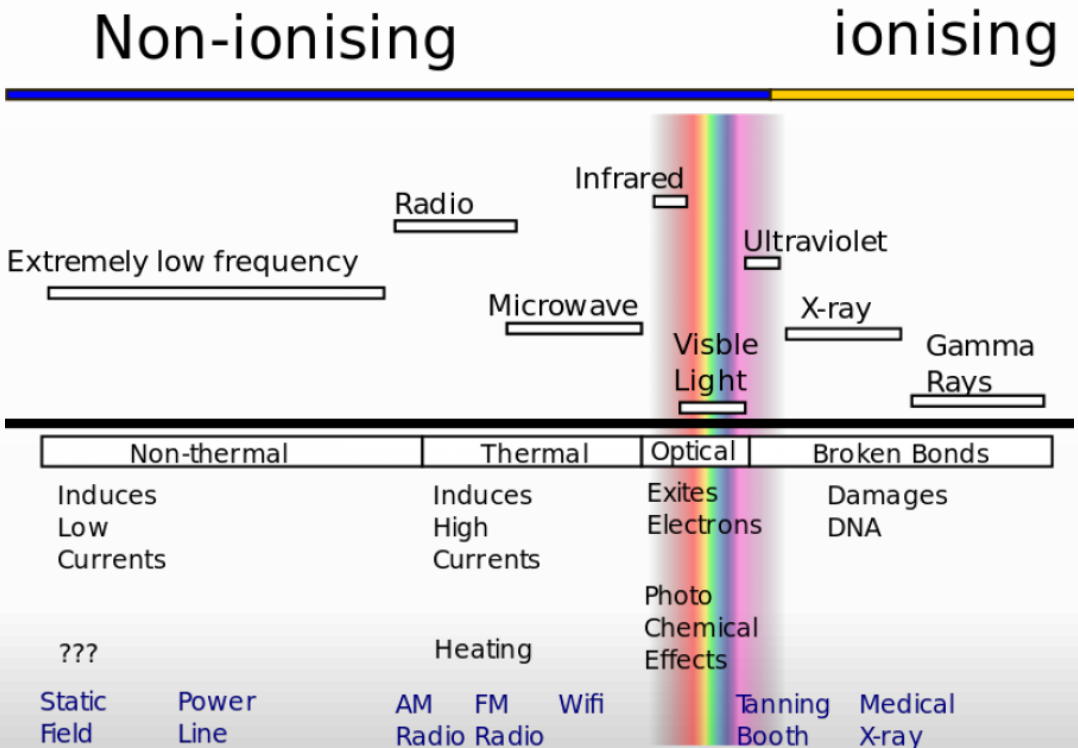
- NH_2OH
- causes hydroxylation of amino group of cytosine
- resulting hydroxylaminecytosine pairs with adenine (transition of *GC* to *AT*)



(Russell PJ, 2010)

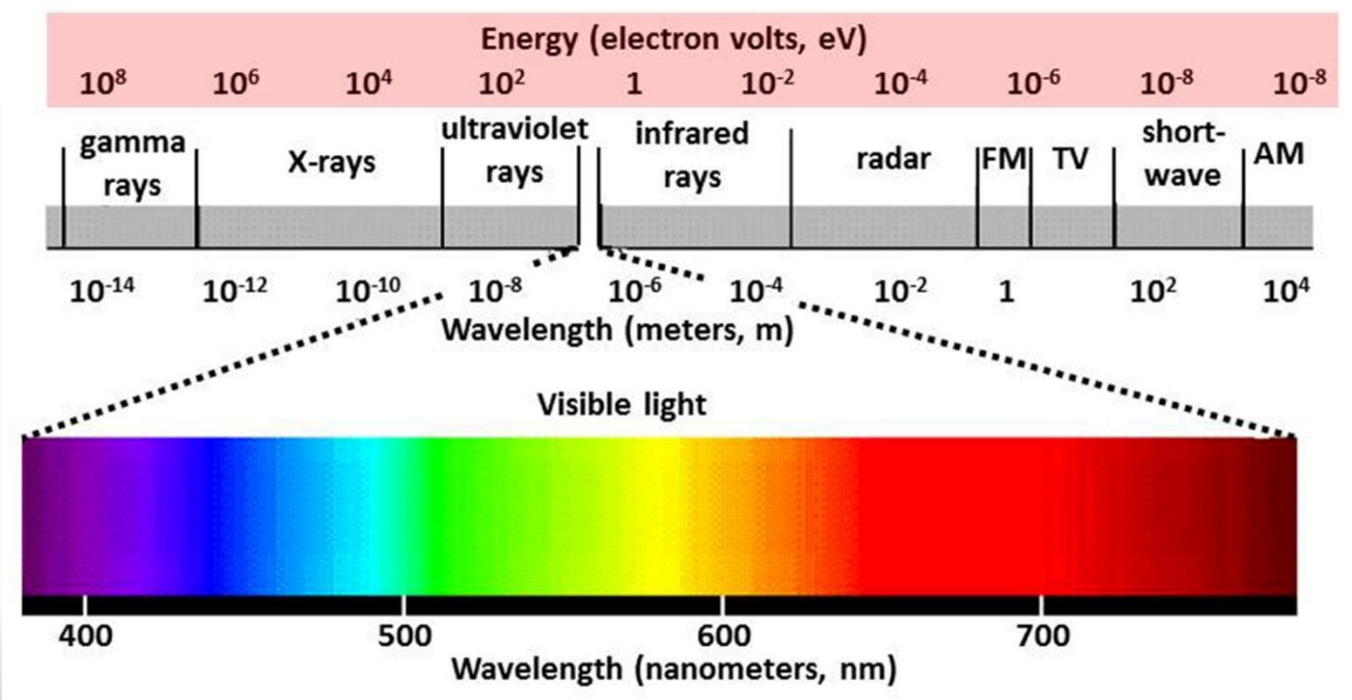
Physical mutagens

- ionizing radiation (X-ray, gamma, cosmic) induces breaks in DNA
- non-ionizing radiation (UV) absorption at a specific wavelength of 260-280 nm, formation of thymine dimers
- the degree of DNA damage is equivalent to the type and dose of radiation absorbed



Mutations induced by ionizing radiation

- shorter wavelength and higher energy than the visible light
- penetrates deep into the tissue, strikes the atoms, releases electrons to form positively charged ions and radicals which give rise to other ions (ionization process)

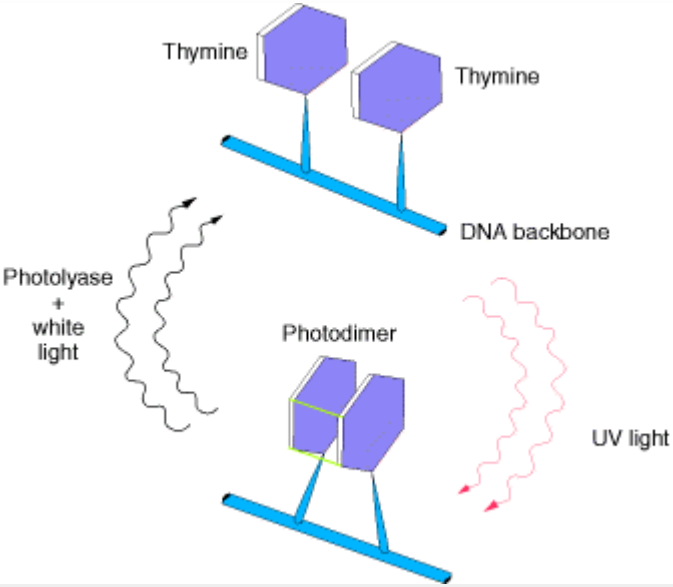


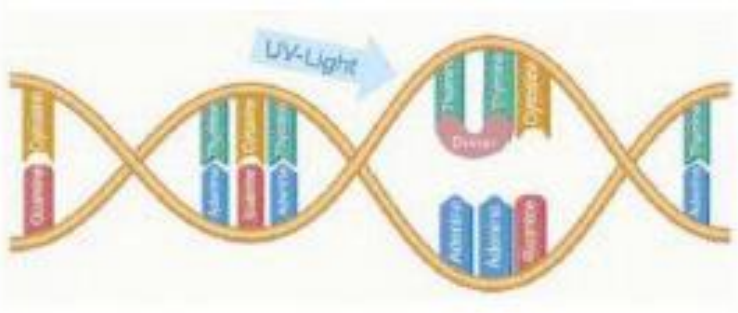
UV radiation

- less energy than ionizing radiation
- only penetrates into the upper layers of cells, potent mutagen in unicellular organisms
- not causing ionization
- radiant energy is captured by atoms, the electrons pass into the excited state - to increase the reactivity of atoms and molecules in DNA that leads to creation of mutations
- most mutagenic effects at 254 nm (absorption maximum of the bases at this wavelength)

UV and pyrimidines

- After UV absorption pyrimidines react to form pyrimidine dimers and hydrates
- **thymine dimers** disrupt the structure of DNA and **disrupt the replication**

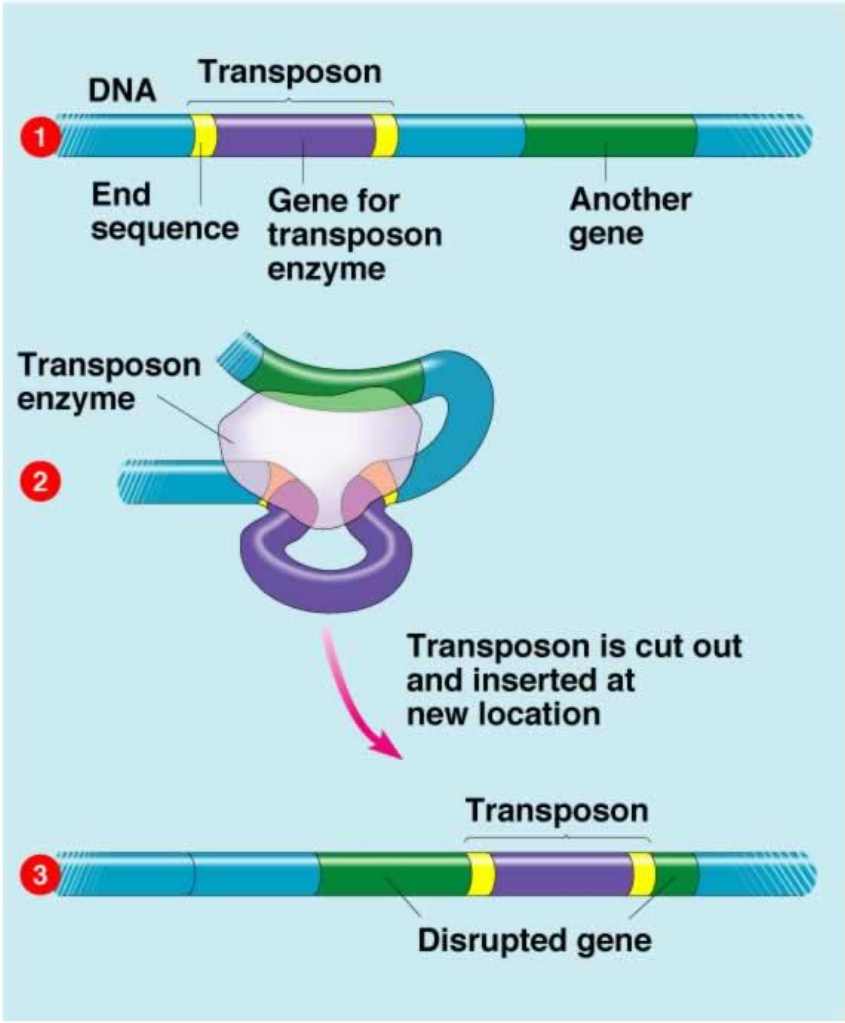




Biological mutagens

- **Viruses** - incorporation into the DNA of of the host
- transposable elements- **transposons**: DNA regions which can be be moved from one place to another in the genome

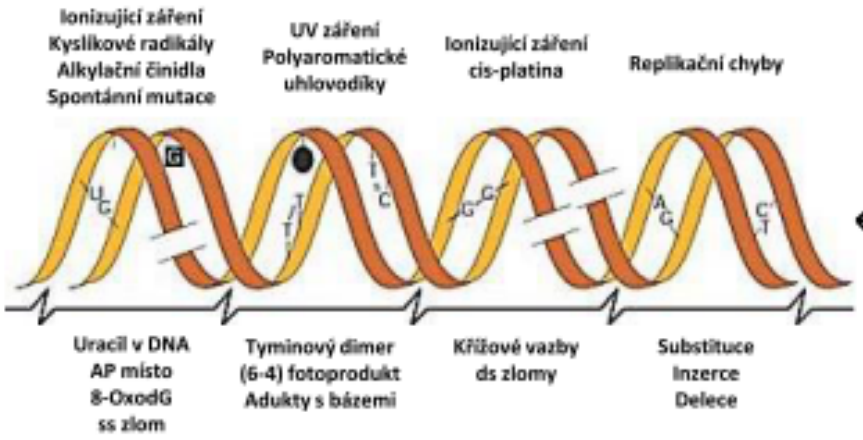
- Insertion of transposon may inactivate the gene (mutagenic)



2. DNA repair mechanisms

Living organisms contain many **proteins that scan their DNA for damage** and initiate repair processes when damage is detected.

DNA repair



Cell cycle arrest senescence

Inhibition of transcription, replication, segregation of chromosomes apoptosis

Mutation, chromosome aberration cancer, heriditary diseases

- In cells, there are mechanisms by which cell recognizes and completely or to some extent removes DNA damage. These repair mechanisms are catalyzed by different sets of enzymes.
- Ability to repair damaged DNA is essential for maintaining the integrity of the genome of the cell and for the normal functioning of a multicellular organism.
- Tomas R. Lindahl, Paul L. Modrich, Aziz Sancar won the 2015 Nobel Prize in Chemistry for his research on the molecular mechanisms of DNA repair.

Types of DNA repair:

- **complete repair** - repairs to the original state without DNA synthesis
- **excision repair** - excision of damaged sites, synthesis of DNA
- **tolerant repair** - restoration of function without complete DNA damage repair

DNA repair mechanisms

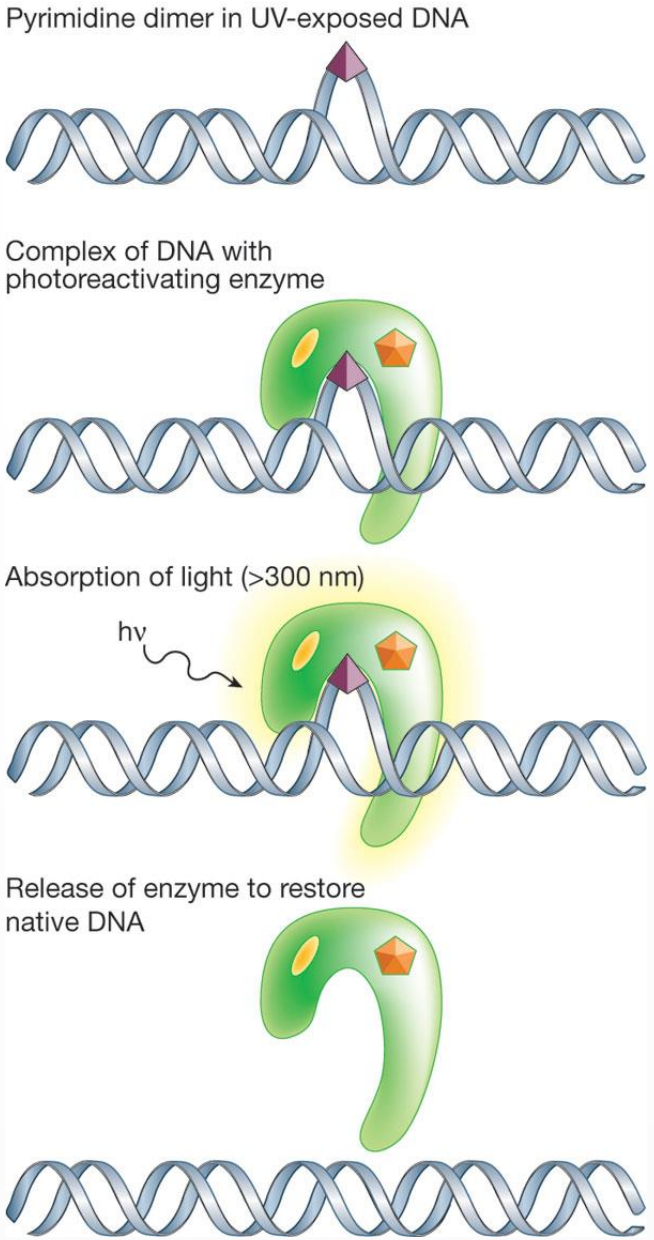
- *enzymes seek DNA damage and when it is found, they activate any of the repair processes existing from bacteria to humans*

- **mismatch repair** controlled by methylation
- **excision repair** (base and nucleotide)
- **photoreactivation-**correction dependent on the light - only in bacteria
- **postreplication repair**
- **error-prone repair** (SOS response)
- Mutation frequencies: 10^{-10} mutations / bp / replication

E.coli: 5 mechanisms (photoreactivation, excision repair, mismatch repair, postreplication, error-prone)
mammals: all except for photoreactivation

1. Photoreactivation

- only in **bacteria, eukaryotes**, but **not in mammals**
- complete repair
- correction dependent on light
- removes **thymine dimers** mediated by the enzyme **DNA photolyase**, which is activated by visible light (especially blue 340-400nm)
- DNA photolyase recognizes dimers, binds to them and cleaves covalent cross-linking using light energy
- binding to dimers occurs in the dark, cleavage only after activation by light energy
- No endonuclease, no polymerase, no DNA ligase



2. Excision repair

(Excision of the damaged sites, synthesis of new DNA)

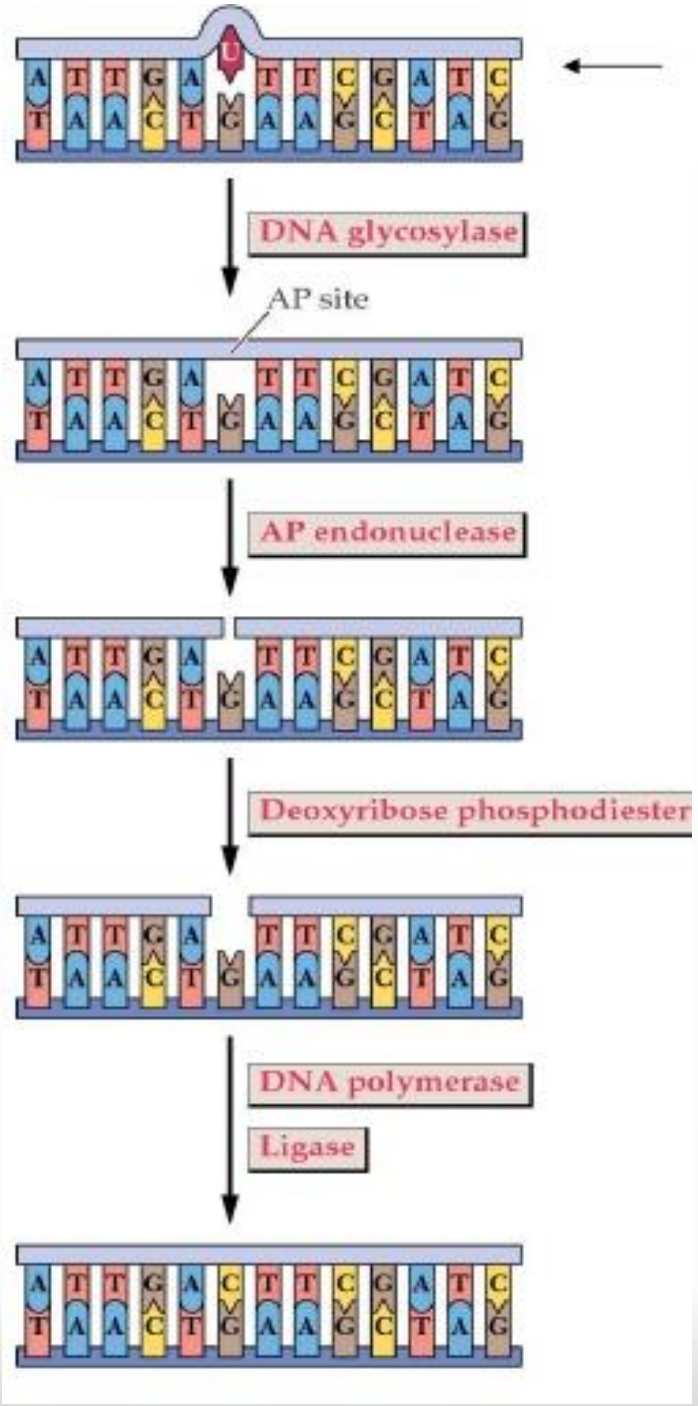
- performed in three steps:

- **DNA endonuclease** recognizes damaged base in DNA, it binds to it and cleaves it
- **DNA polymerase** fills the gap using the intact complementary strand as a template
- **DNA ligase** connects strands retained by DNA polymerase

- **Base excision repair (BER)** removes **abnormal or chemically modified bases from DNA**
- **Nucleotide excision repair (NER)** removes larger defects in DNA

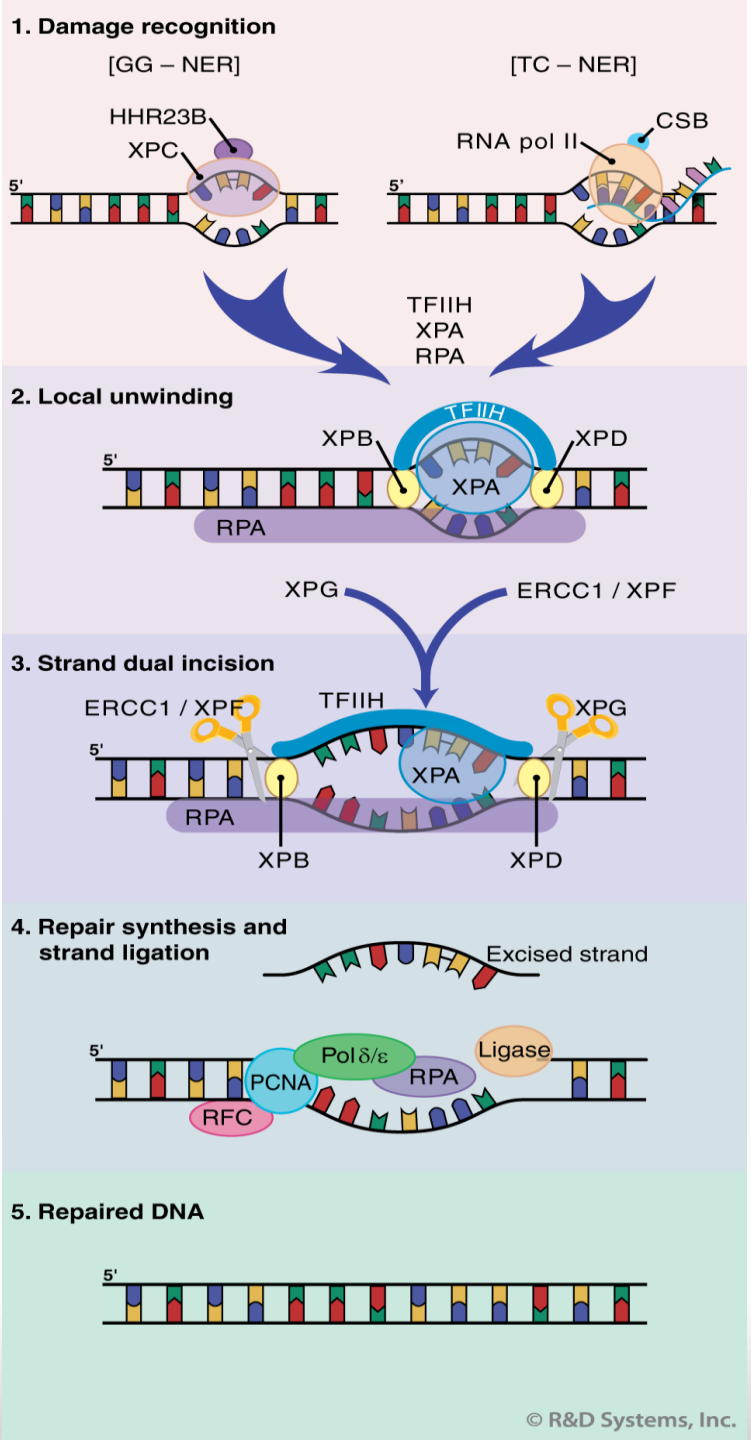
2A Base excision repair

- **repair of damaged bases** (oxidation, alkylation, deamination), removal of U
- **DNA glycosylase** creates **apurine or apyrimidine sites (AP sites)**
- **AP endonucleases** recognize AP-space, which together with **phosphodiesterases** cleave - interrupt the sugar-phosphate backbone, creating 3 OH
- in humans **APEX1, APEX2**
- **DNA polymerase** replaces a missing nucleotide by complementary strand
- **Polβ in eukaryotes, Pol1 in prokaryotes**
- **DNA ligase** restores the sugar-phosphate backbone
- Increased risk of colorectal tumors with mutations in the **Polβ, DNA glycosylase**



2B Nucleotide excision repair (NER)

- removes DNA from more damaged sites that distort the double helix, DNA adducts, UV photoproducts
- specific endonucleases (**excision nuclease**) cleave on both sides of the section of damaged nucleotides
- cleavage of the oligonucleotide fragment containing the damaged bases
- gap is filled by **DNA polymerase and DNA ligase**



The stability of genes depends on DNA repair

- repair mechanisms based on the existence of two copies of the genetic information in the DNA double helix
- corrupted strand is corrected according to undamaged
- corrupted strand is identified by abnormal DNA structures that are generated by errors
- **mutations in genes** encoding repair proteins increases the frequency of mutations which frequently lead to a **predisposition to cancer**

Inherited DNA repair gene mutations that increase cancer risk			
DNA repair gene	**Protein**	**Repair pathways affected**	**Cancers with increased risk**
breast cancer 1 & 2	BRCA1 BRCA2	HRR of double strand breaks and daughter strand gaps[16]	breast, ovarian [17]
ataxia telangiectasia mutated	ATM	Different mutations in *ATM* reduce HRR, SSA or NHEJ [18]	leukemia, lymphoma, breast [18][19]
Nijmegen breakage syndrome	NBS (NBN)	NHEJ [20]	lymphoid cancers [20]
MRE11A	MRE11	HRR and NHEJ [21]	breast [22]
Bloom syndrome	BLM (helicase)	HRR [23]	leukemia, lymphoma, colon, breast, skin, lung, auditory canal, tongue, esophagus, stomach, tonsil, larynx, uterus [24]
WRN	WRN	HRR, NHEJ, long patch BER [25]	soft tissue sarcoma, colorectal, skin, thyroid, pancreas [26]
RECQL4	RECQ4	Helicase likely active in HRR [27]	basal cell carcinoma, squamous cell carcinoma, intraepidermal carcinoma [28]
Fanconi anemia genes FANCA,B,C,D1,D2,E,F,G,I,J,L,M,N	FANCA etc.	HRR and TLS [29]	leukemia, liver tumors, solid tumors many areas [30]
XPC, XPE (DDB2)	XPC, XPE	Global genomic NER, repairs damage in both transcribed and untranscribed DNA [31][32]	skin cancer (melanoma and non-melanoma) [31][32]
XPA, XPB, XPD, XPF, XPG	XPA XPB XPD XPF XPG	Transcription coupled NER repairs the transcribed strands of transcriptionally active genes [33]	skin cancer (melanoma and non-melanoma) [33]
XPV (also called polymerase H)	XPV (POLH)	Translesion synthesis (TLS) [34]	skin cancers (basal cell, squamous cell, melanoma) [34]
mutS H2, mutS homolog 6, mutL (*E. coli*) homolog 1, postmeiotic segregation increased 2 (*S. cerevisiae*)	MSH2 MSH6 MLH1 PMS2	MMR [35]	colorectal, endometrial [35]
mutY homolog (*E. coli*)	MUTYH	BER of A paired with 8-oxo-dG [36]	colon [36]
TP53	P53	Direct role in HRR, BER, NER and acts in DNA damage response[37] for those pathways and for NHEJ and MMR [38]	sarcomas, breast cancers, brain tumors, and adrenocortical carcinomas [39]
NTHL1	NTHL1	BER for Tg, FapyG, 5-hC, 5-hU in dsDNA[40]	Colon cancer, endometrial cancer, duodenal cancer, basal-cell carcinoma[41]

The similarity of proteins in different species

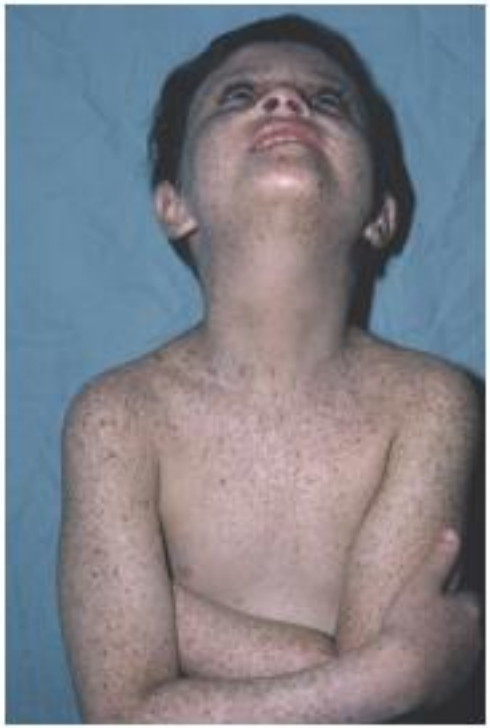
- thanks to the repair mechanisms (and selection pressure) changes in the DNA structure during evolution accumulates slowly
- between man and chimpanzee is about 5 million years divergence, but their nucleotide sequences in DNA are 98% identical
- Thanks to the precision replication processes and reparations occurred over millions of years, only minimal changes in the genetic information

Hereditary diseases in humans caused by disorders in DNA repair

- xeroderma pigmentosum
- **Ataxia-telangiectasia**
- **Fanconi anemia**
- **Bloom syndrome**
- **Werner syndrome**
- **Rothmund-Thompson syndrome**
- **Nijmegen breakage syndrome**

- Errors in DNA repair (damaged excision repair) proteins, helicases
- high frequency of chromosomal aberrations
- high risk of malignancy

Hereditary diseases in humans caused by disorders in DNA repair



- **xeroderma pigmentosum** – extreme sensitivity to sunlight, skin cancer formation
- It affects about 1 in 250,000 newborns
- mutations in genes encoding proteins involved in **nucleotide excision repair** – excision nuclease activity

Cells in individuals with XP have a malfunction in the repair of DNA damage induced by UV radiation, as thymine dimers. XP disease can arise as a result of defects in any one of at least eight different genes. Products of seven of these genes, **XPA, XPB, XPC, XPD, XPE, XPG and XPF** are required for **nucleoside are nucleotide excision repair**

Hereditary diseases in humans caused by disorders in DNA repair

- **Cockayne syndrome** – short stature, impaired mental ability
- **Trichothiodystrophy** - short limbs, brittle hair, scaly skin, psychomotoric retardation
- also a consequence of disturbances in **nucleotide excision repair**

DNA repair defects cause disease

TABLE 23-1 Some Human Hereditary Diseases and Cancers Associated with DNA-Repair Defects

Disease	DNA-Repair System Affected	Sensitivity	Cancer Susceptibility	Symptoms
PREVENTION OF POINT MUTATIONS, INSERTIONS, AND DELETIONS				
Hereditary nonpolyposis colorectal cancer	DNA mismatch repair	UV irradiation, chemical mutagens	Colon, ovary	Early development of tumors
Xeroderma pigmentosum	Nucleotide excision repair	UV irradiation, point mutations	Skin carcinomas, melanomas	Skin and eye photosensitivity, keratoses
REPAIR OF DOUBLE-STRAND BREAKS				
Bloom's syndrome	Repair of double-strand breaks by homologous recombination	Mild alkylating agents	Carcinomas, leukemias, lymphomas	Photosensitivity, facial telangiectases, chromosome alterations
Fanconi anemia	Repair of double-strand breaks by homologous recombination	DNA cross-linking agents, reactive oxidant chemicals	Acute myeloid leukemia, squamous-cell carcinomas	Developmental abnormalities including infertility and deformities of the skeleton; anemia
Hereditary breast cancer, BRCA-1 and BRCA-2 deficiency	Repair of double-strand breaks by homologous recombination		Breast and ovarian cancer	Breast and ovarian cancer

SOURCES: Modified from A. Kornberg and T. Baker, 1992, *DNA Replication*, 2d ed., W. H. Freeman and Company, p. 788; J. Hoeijmakers, 2001, *Nature* **411**:366; and L. Thompson and D. Schild, 2002, *Mutation Res.* **509**:49.