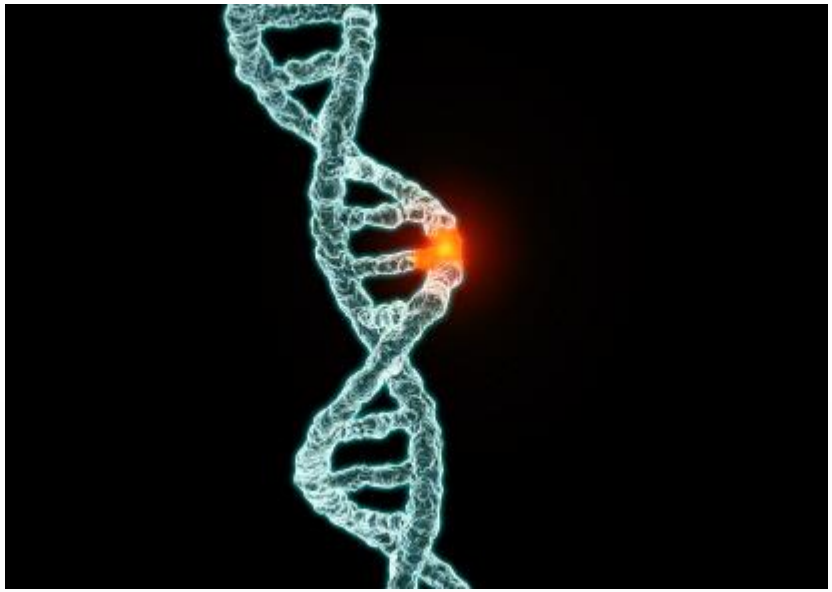
Molecular mechanisms of mutagenesis, spontaneous and induced mutations and reversions

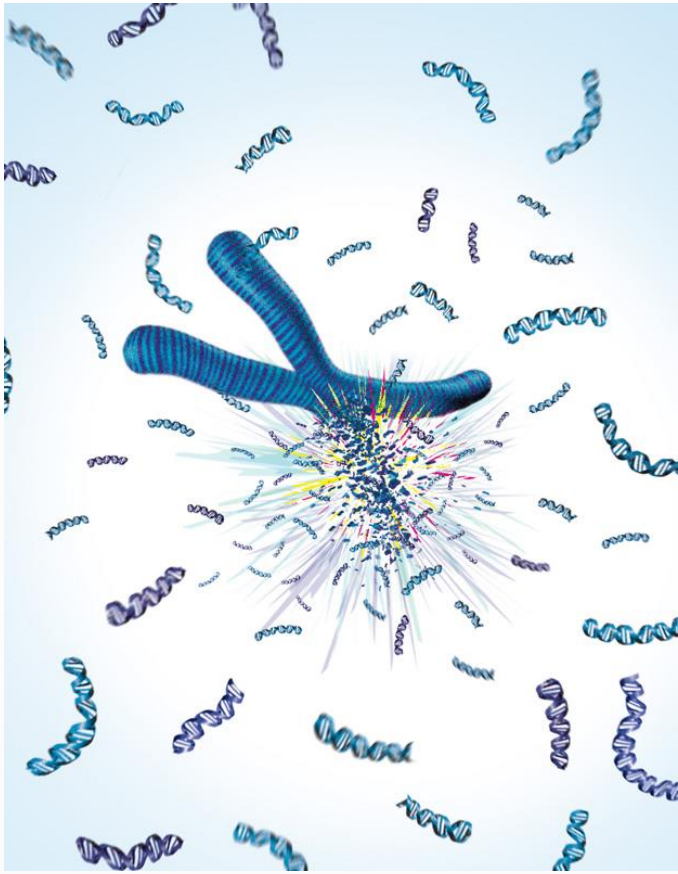


Mutation - is a permanent change of the nucleotide sequence in the genome of an organism, virus, or extra chromosomal genetic element.

- a process

Mutations = 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) that are the result of new combinations of existing genetic variants (**recombination**)

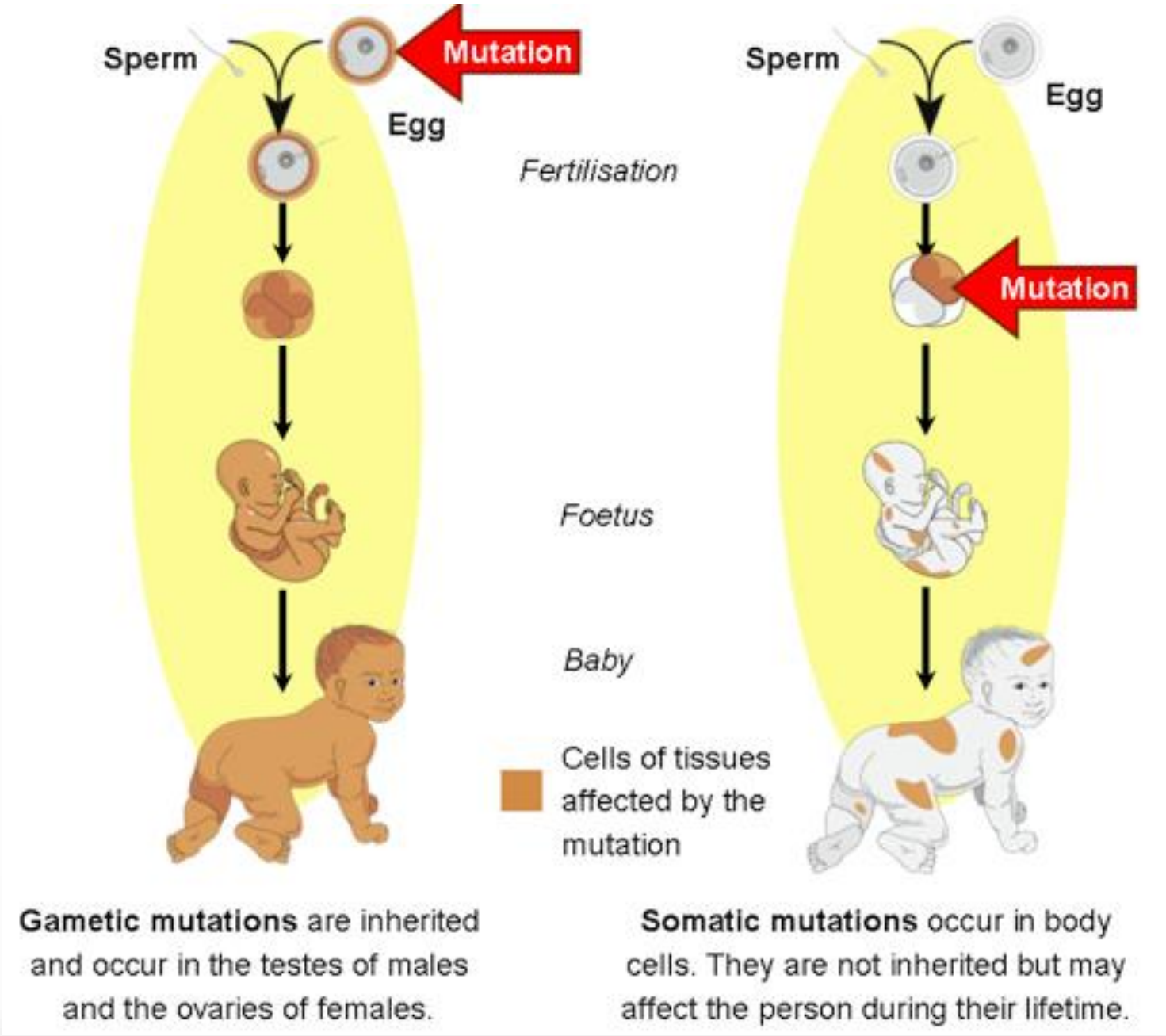
- **source of genetic variability** - provide new genetic variants that are a prerequisite for the evolution of organisms

Occurrence and distribution of mutations

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

- in multicellular organisms, mutations can be 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**: in germ line cells, may cause hereditary diseases
- **somatic**: 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 cells against the harmful effects of mutations

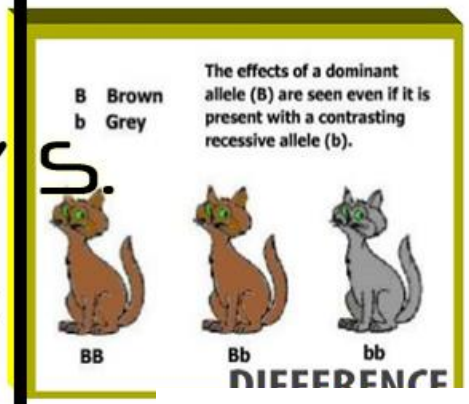
- **diploid organism**: each gene has **two copies**
- when one becomes damaged, the other can still provide the correct gene / protein - **recessive** mutation, preventing the defect (that is if the mutation is not **dominant**)
- estimate: each person carries so many harmful mutations that would in haploid configuration cause lethality about eight times
- people today are genetically different from their predecessors, due to the accumulation of mutations over the centuries

Dominant

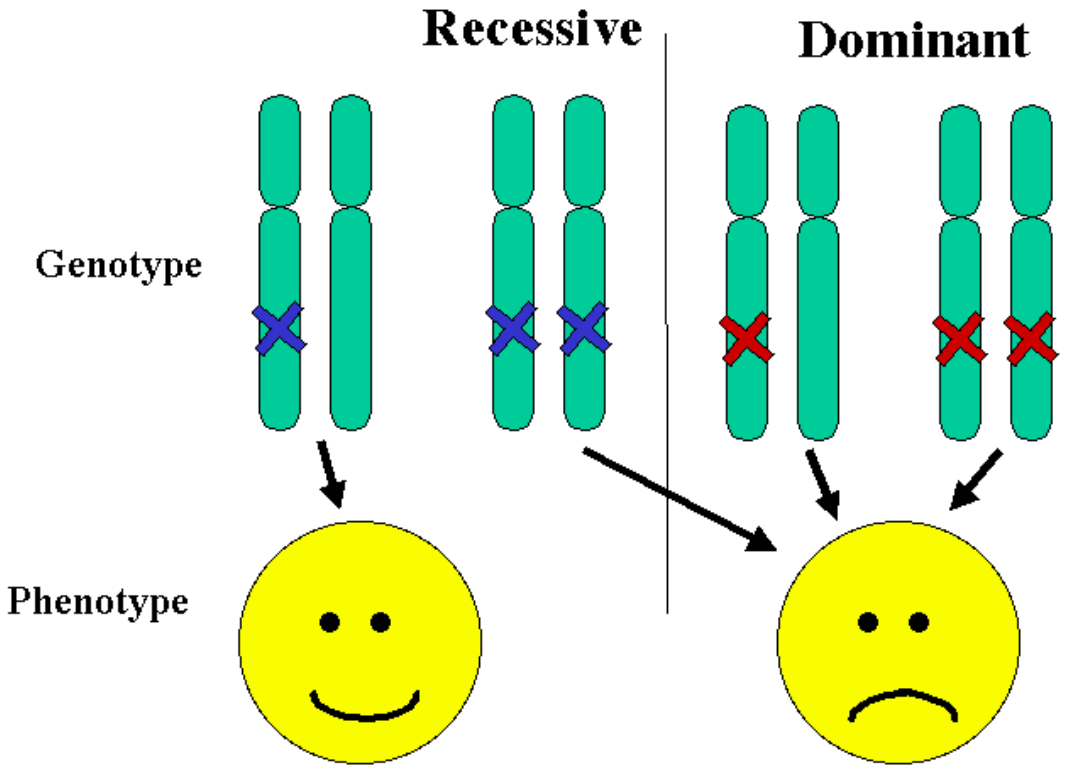
- A *dominant* allele is expressed even if it is paired with a recessive allele.

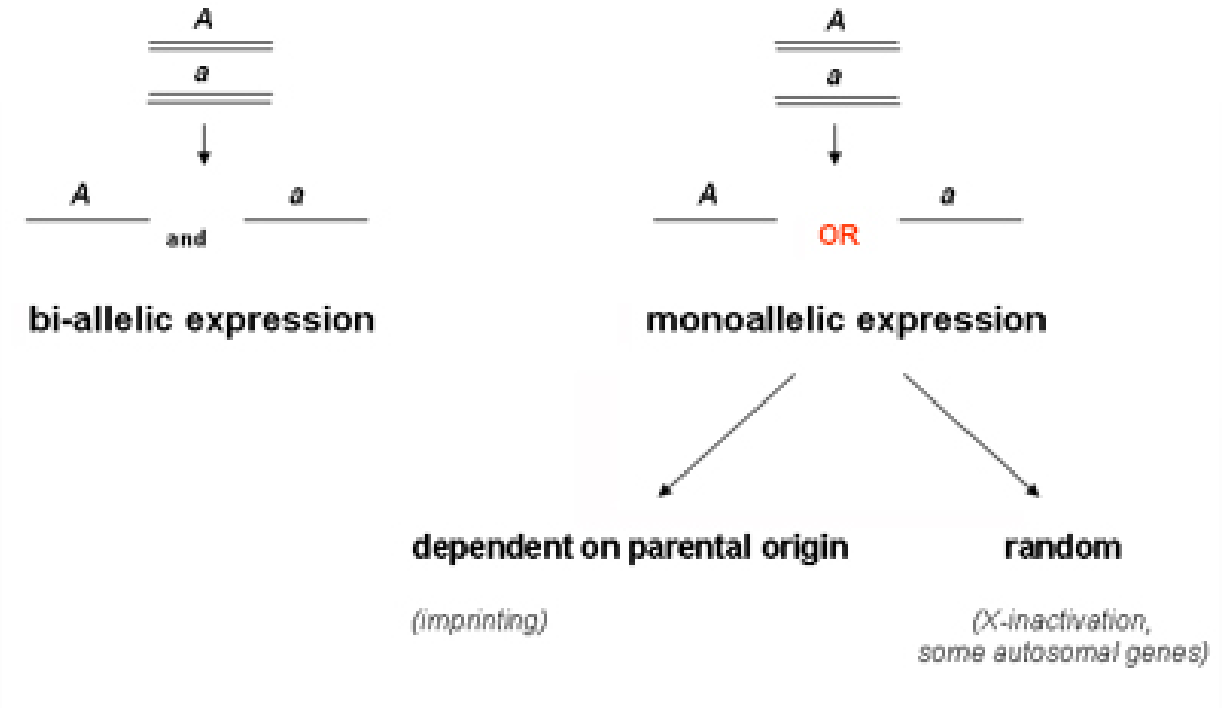
- A *recessive* allele is only visible when paired with another recessive allele.

Recessive



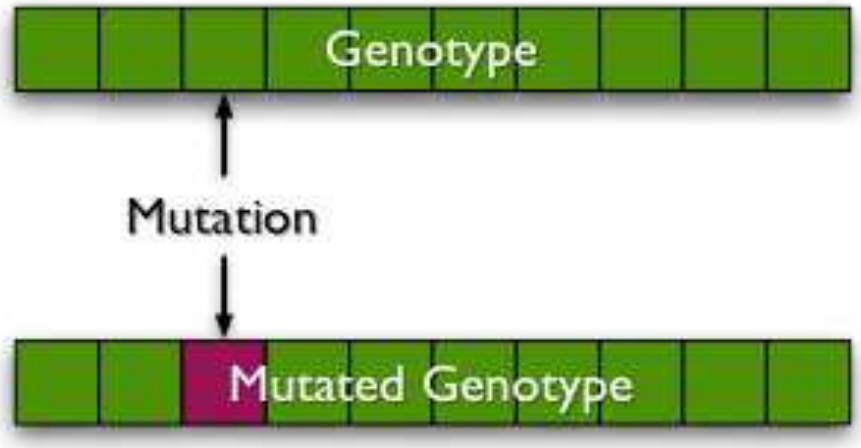
Dominant and Recessive mutations





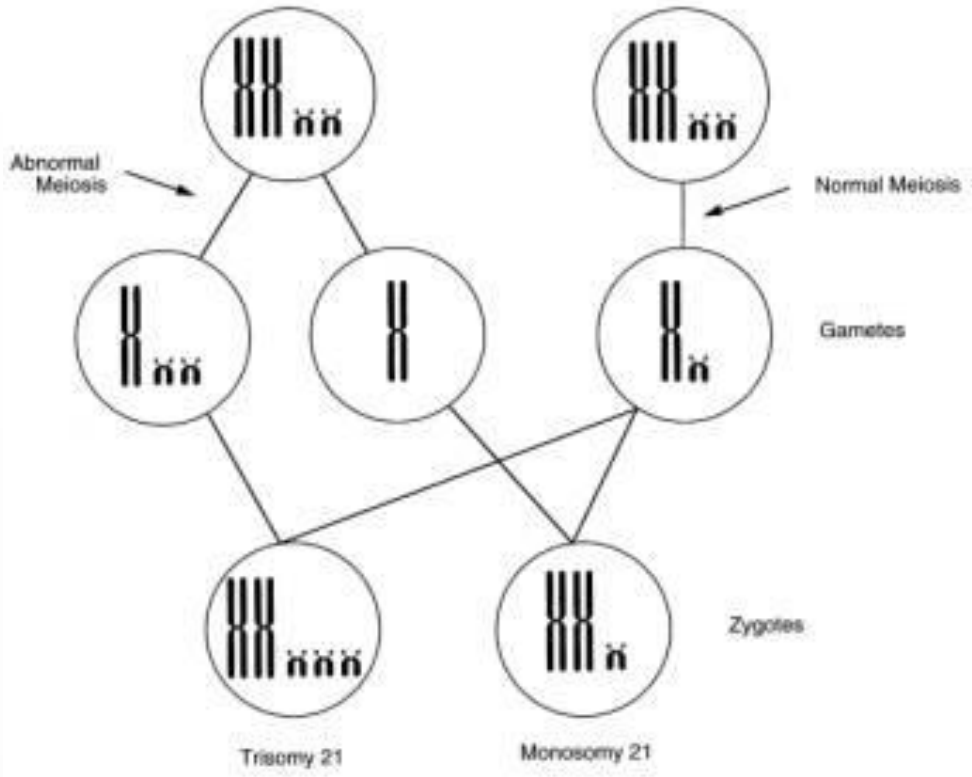
- Monoallelic expression can be best understood in comparison to bi-allelic expression, the more prevalent form of gene expression (Figure 1). In most cases, both alleles of a gene are transcribed; this is known as bi-allelic expression. However, a minority of genes show monoallelic expression. In these cases, only one allele of a gene is expressed. Which one of the two alleles is expressed may be determined by the parental origin of the allele (such as in imprinting), or the choice may be random.

Mutations – from genes to chromosomes

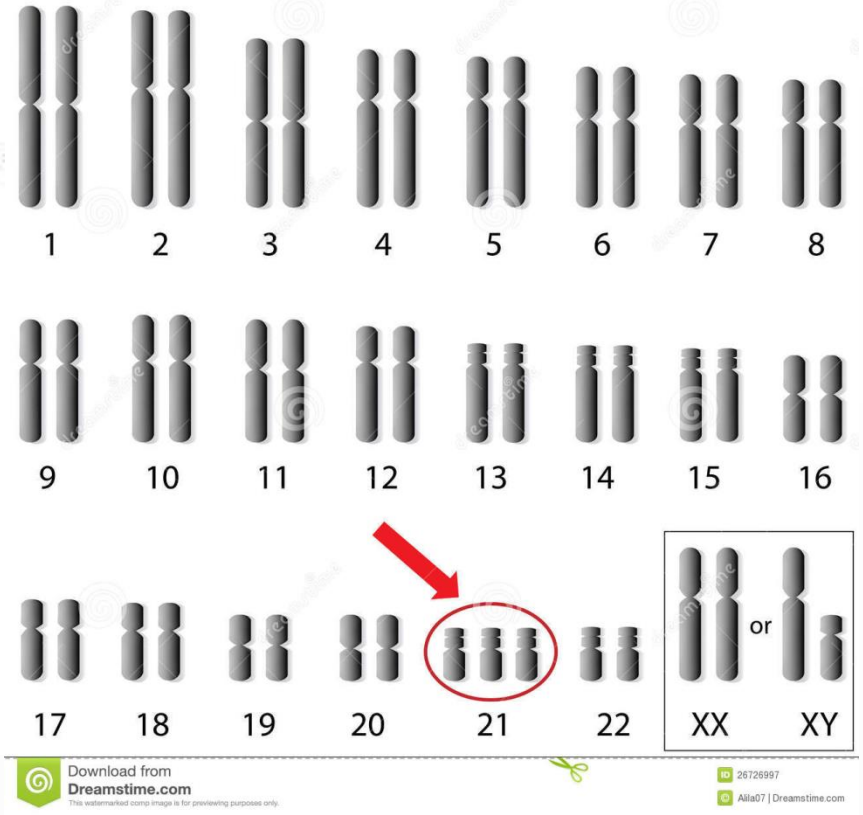


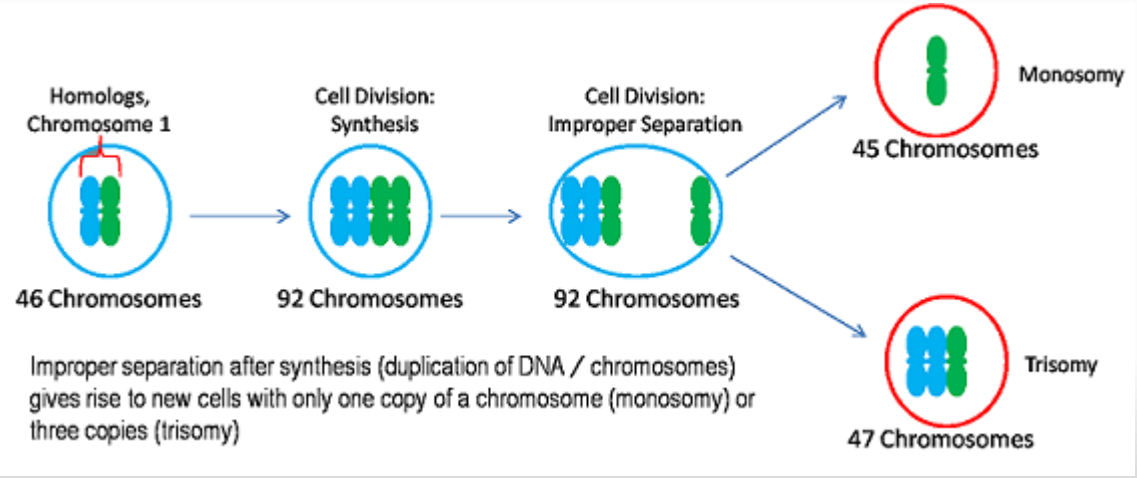
- affect the structure of genes **(and their products) or regulatory regions of DNA**
- change the structure of chromosomes (chromosome aberrations: duplications, deletions, inversions, translocations)

- variations in chromosome numbers:
- aneuploidy – a particular chromosome undergoes a change in its number- monosomy, trisomy - eg. **Down syndrome**
- euploidy – a variation in which the chromosomes are present in a abnormal whole-number multiple of a haploid genome - haploidy, polyploidy)



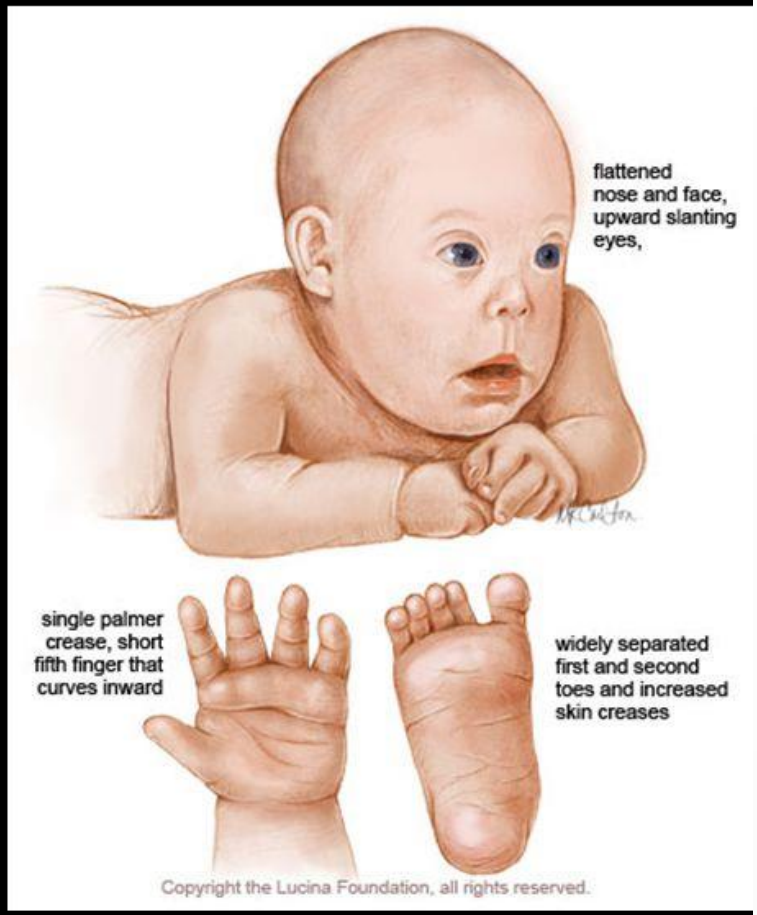
Down Syndrome - Trisomy 21





Down Syndrome

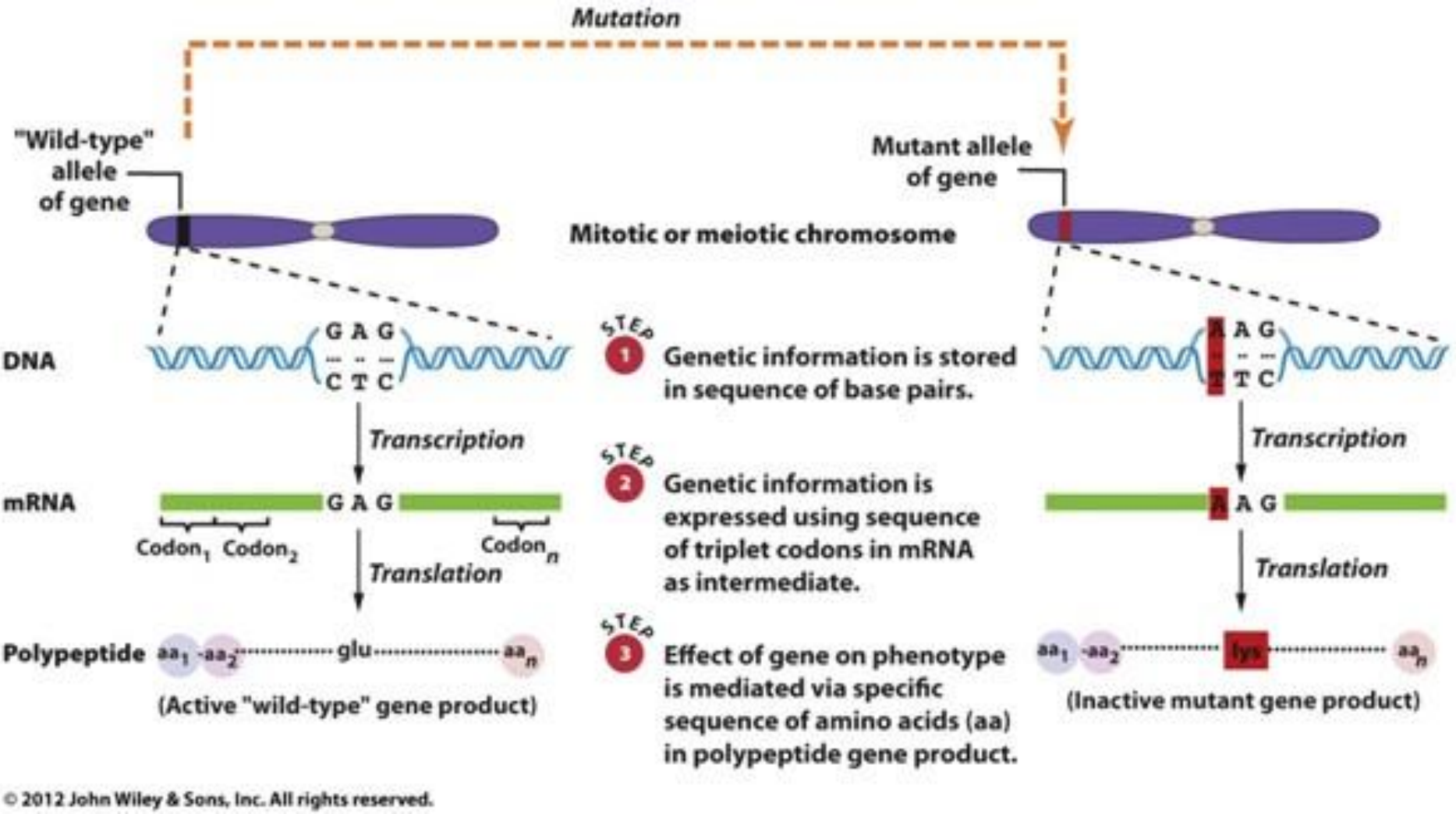
- Trisomy 21
- Often Down syndrome is associated with poor physical development and mental retardation, people with Down syndrome have features characteristic feature of the disease has been nimita and mongolism.

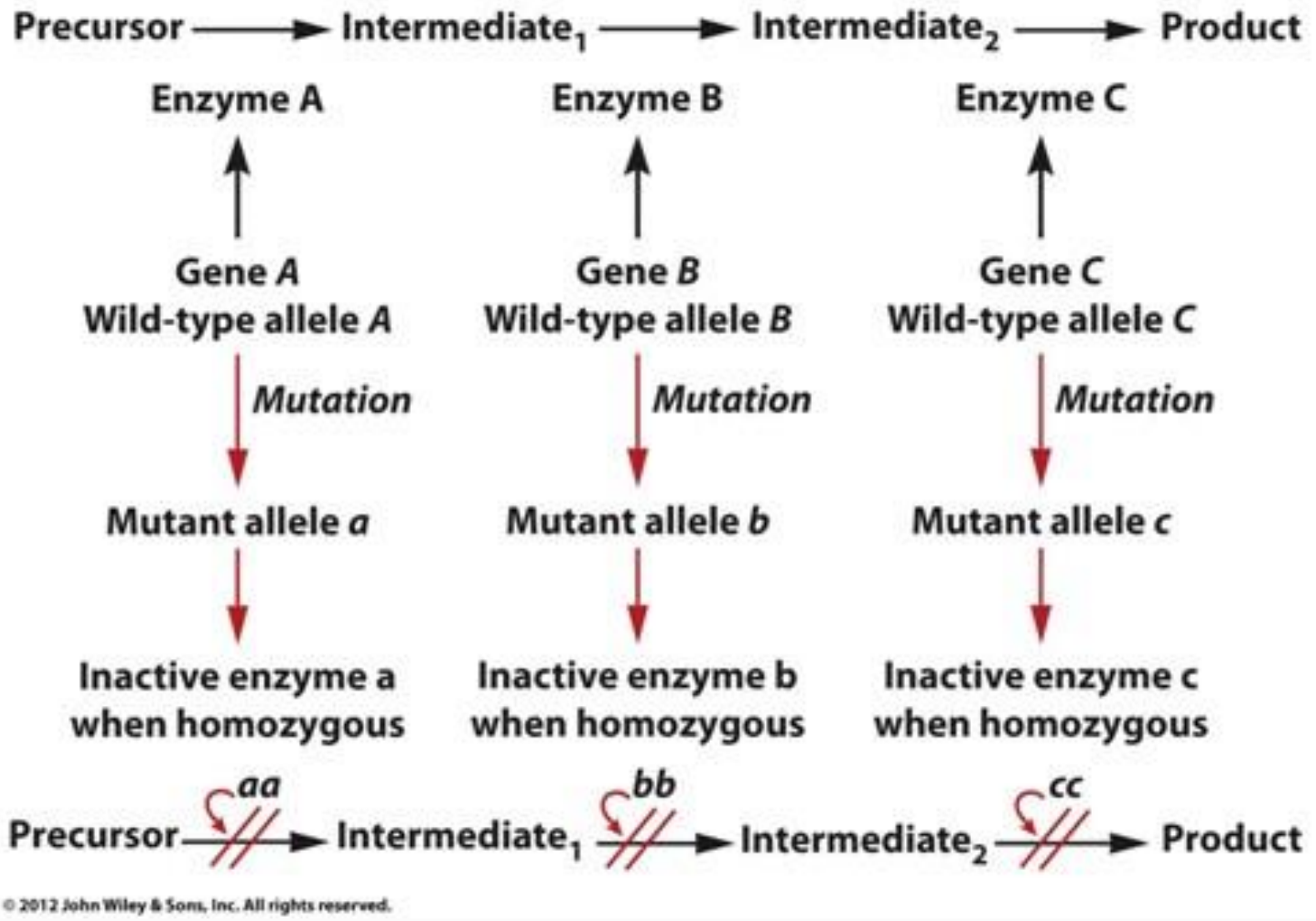


Are mutations are useful or harmful?

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

Expression of Wild-type and Mutant Alleles





• Sickle-cell anemia - haemoglobin S

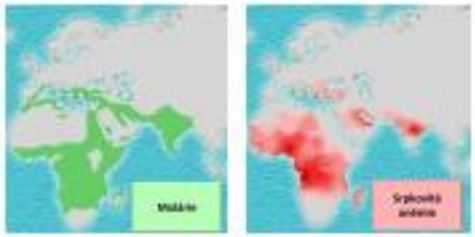
- Mutation in allele HBBA led to the emergence of **allele HBSS, the substitution of one T: A nucleotide pair to A: T**

HbA

CTG	ACT	CCT	GAG	GAG	AAG	TCT
Leu	Thr	Pro	Glu	Glu	Lys	Ser
3			6			9

HbS

CTG	ACT	CCT	GTG	GAG	AAG	TCT
Leu	Thr	Pro	Val	Glu	Lys	Ser
3			6			9



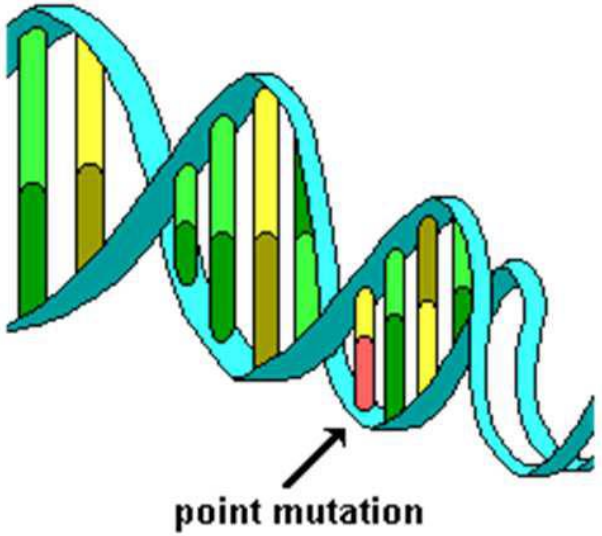
• Phenylketonuria

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

• Albinism

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

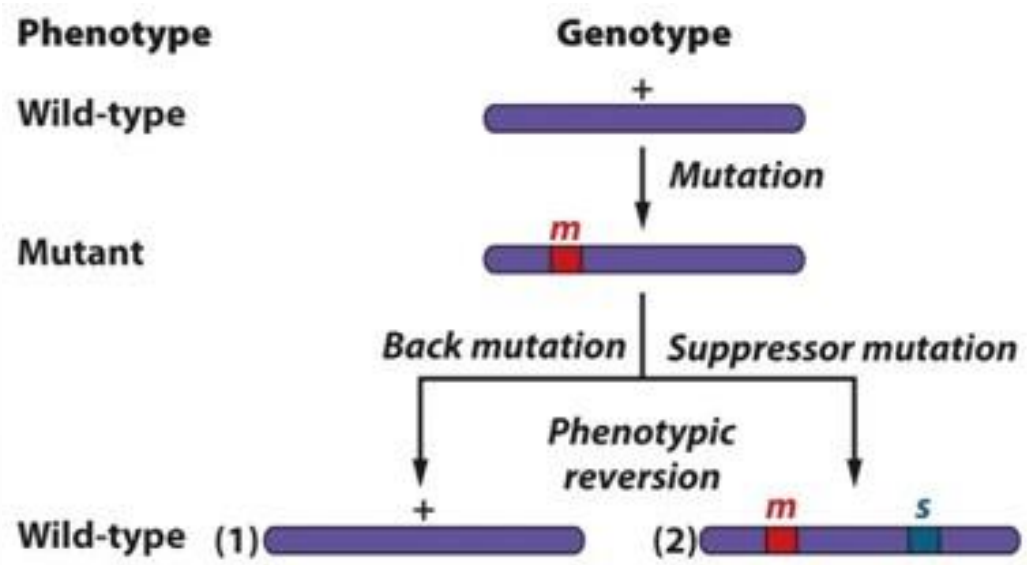
Types of mutations



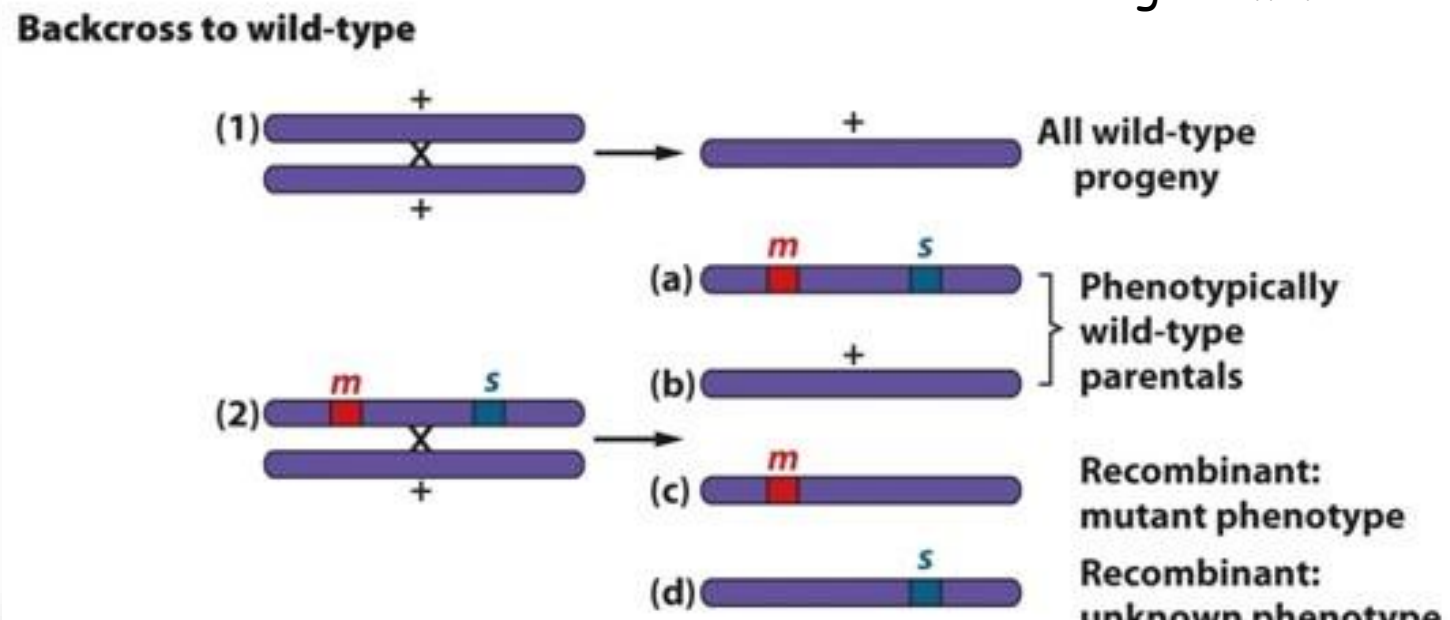
- point mutation: substitution of a single base
- null mutation: complete loss of gene function

- 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)

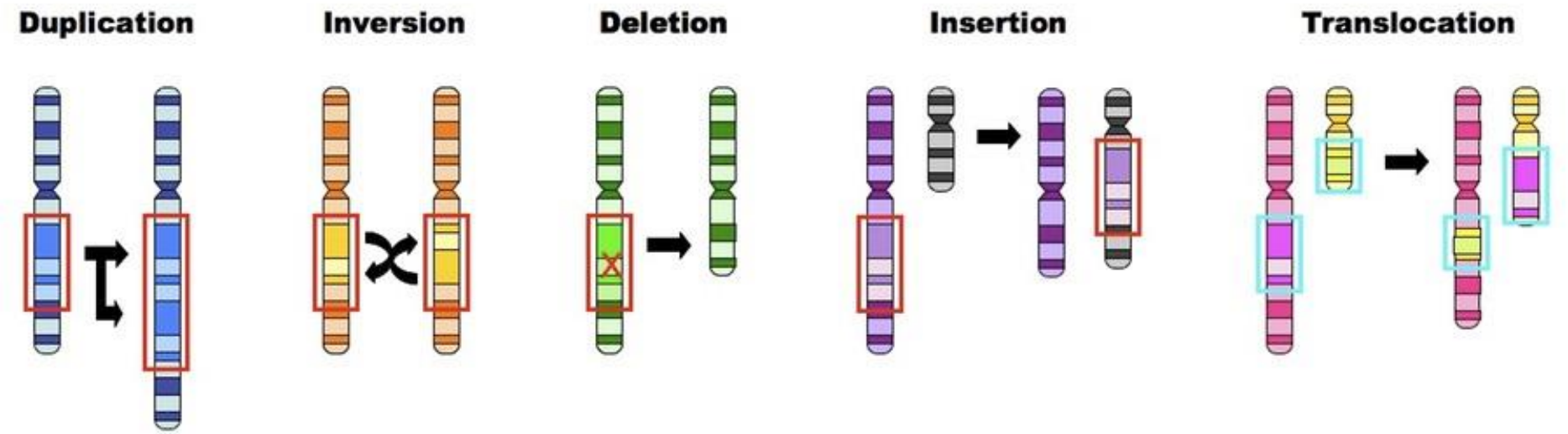
- direct/forward mutation - standard allele turns into mutant allele
- reverse mutation (reversion) - mutant allele changes back into standard
- suppressor mutation - a second mutation, which offsets the effects of the first direct mutation



- **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.



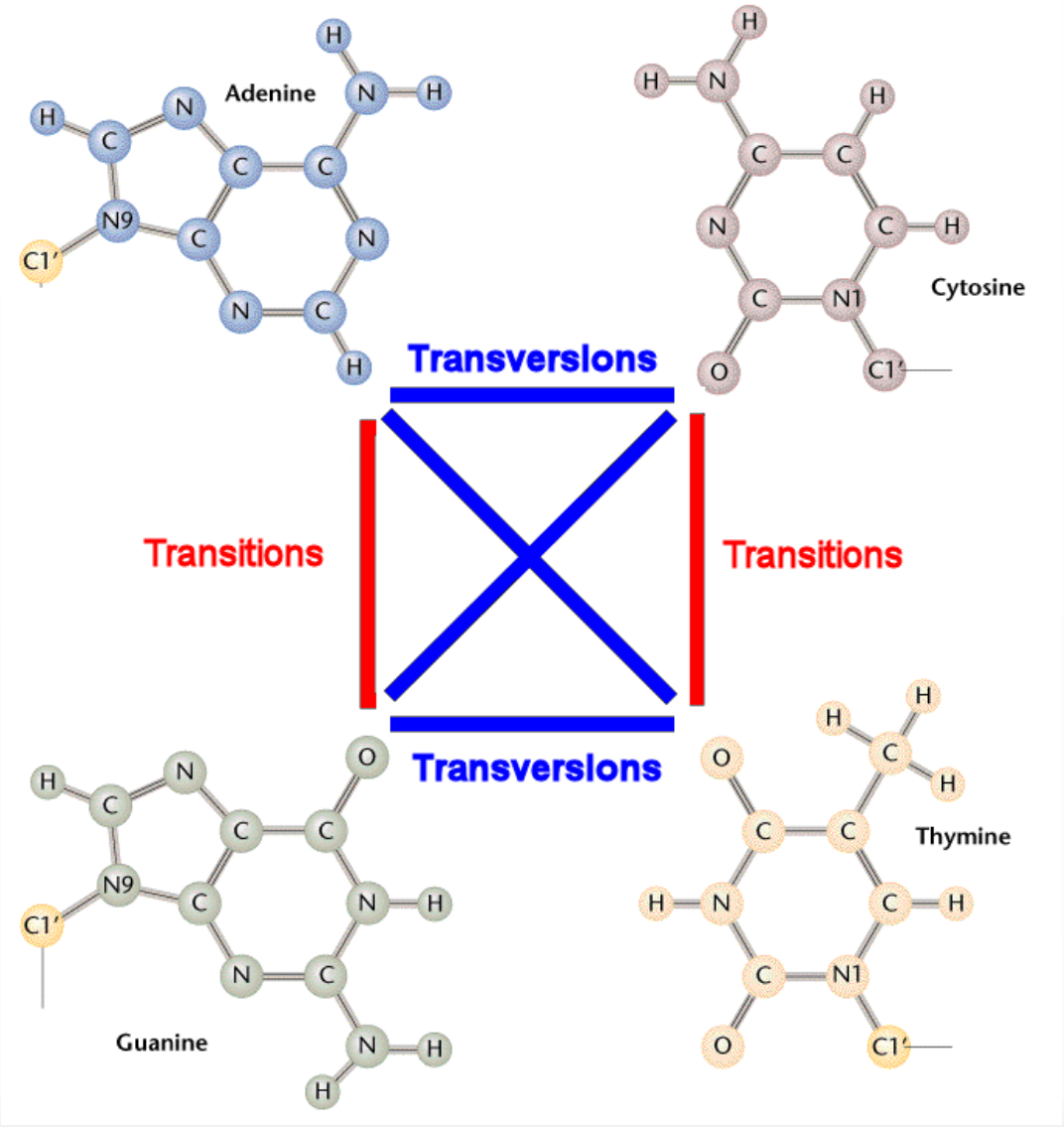
mutations - classification



- substitution: substitution 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 just next to 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



Substitution mutation outcomes

- **missense mutation**:

  TGG (Trp) -> CGG (Arg)

- **nonsense mutation – causes premature termination by a stop codon:**

  TGG (Trp) -> TAG (Term.)

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

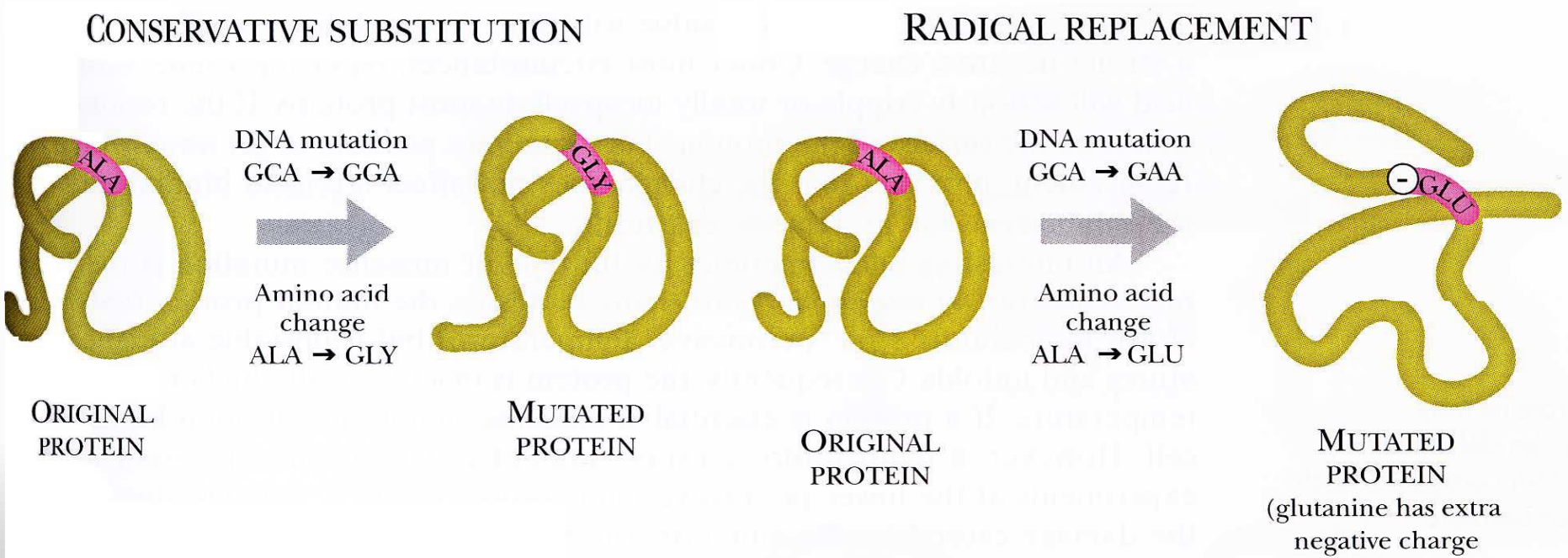
  AAA (Lys) -> AGA (Arg)

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

  AAA (Lys) -> AAG (Lys)

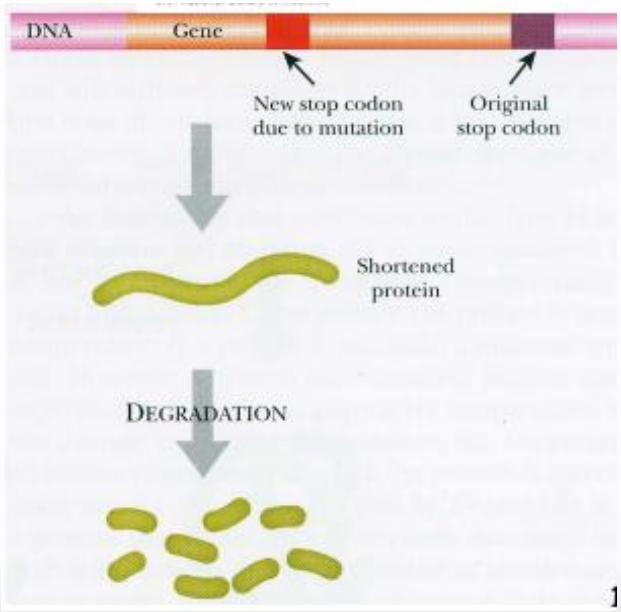
Severity of mutation depends on their type and location

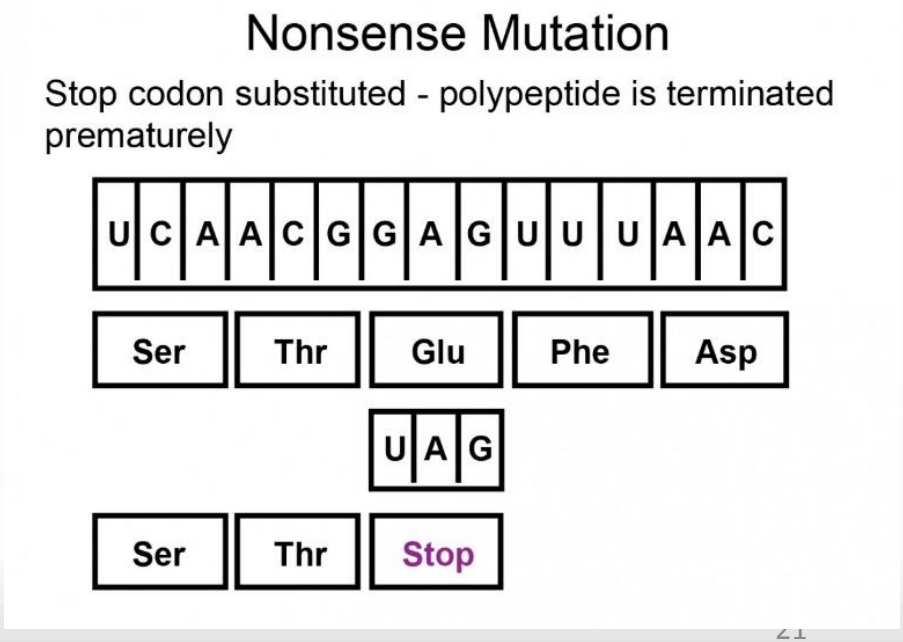
- missense mutations are the most common - lead to the replacement of one amino acid in a protein for another
- when the original amino acid is replaced by a chemically related - usually not serious consequences (conservative substitution)
- the consequences are serious when the protein folding or the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



Nonsense mutations

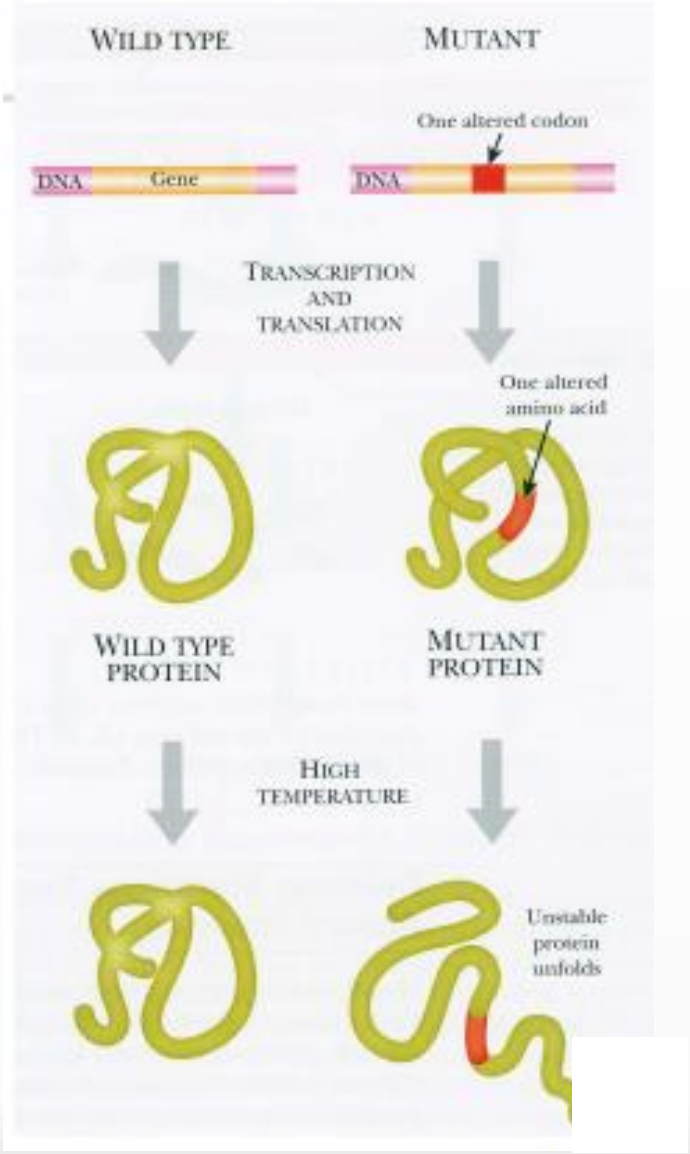
- mutations, in which the codons encoding amino acids are changed to meaningless codons - terminator (UAA, UAG, UGA in RNA)
- premature termination of the polypeptide chain synthesis
- truncated polypeptide is not folded correctly
- usually undergoes degradation





Some mutations are lethal only under specific conditions

- eg. temperature-sensitive mutants will grow at one temperature but not at another.
- Suppressor-sensitive mutants are viable only when a second genetic factor, a suppressor, is present.

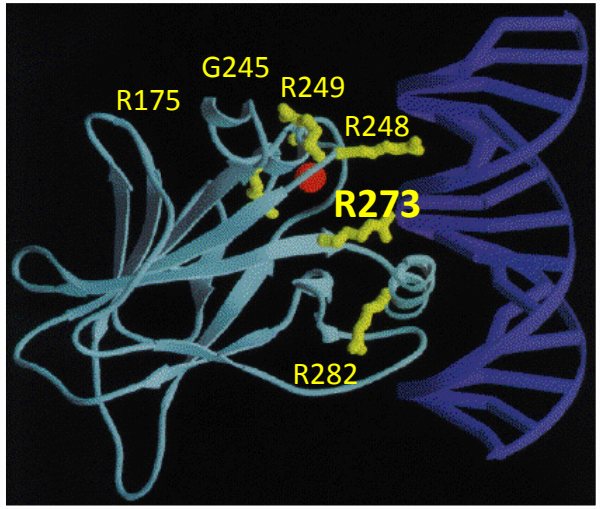


Example of a temperature sensitive mutation

Pointed cats are partial albinos. Albinism is a mutation in the TYR gene, responsible for the enzyme tyrosinase. Tyrosinase, in turn, is necessary for the production of melanin, or dark pigmentation. Albinism is not a black or white trait; it has shades of gray—literally. In cats, various mutated alleles in TYR can result in phenotypes with different degrees of pigmentation. Each of these phenotypes falls in a specific place in an allelic series with a hierarchical inheritance pattern from dominant to recessive—in other words, some of these traits are dominant over others. The Siamese phenotype falls in the middle of the series, recessive to normal coloration but dominant over a completely white coat.

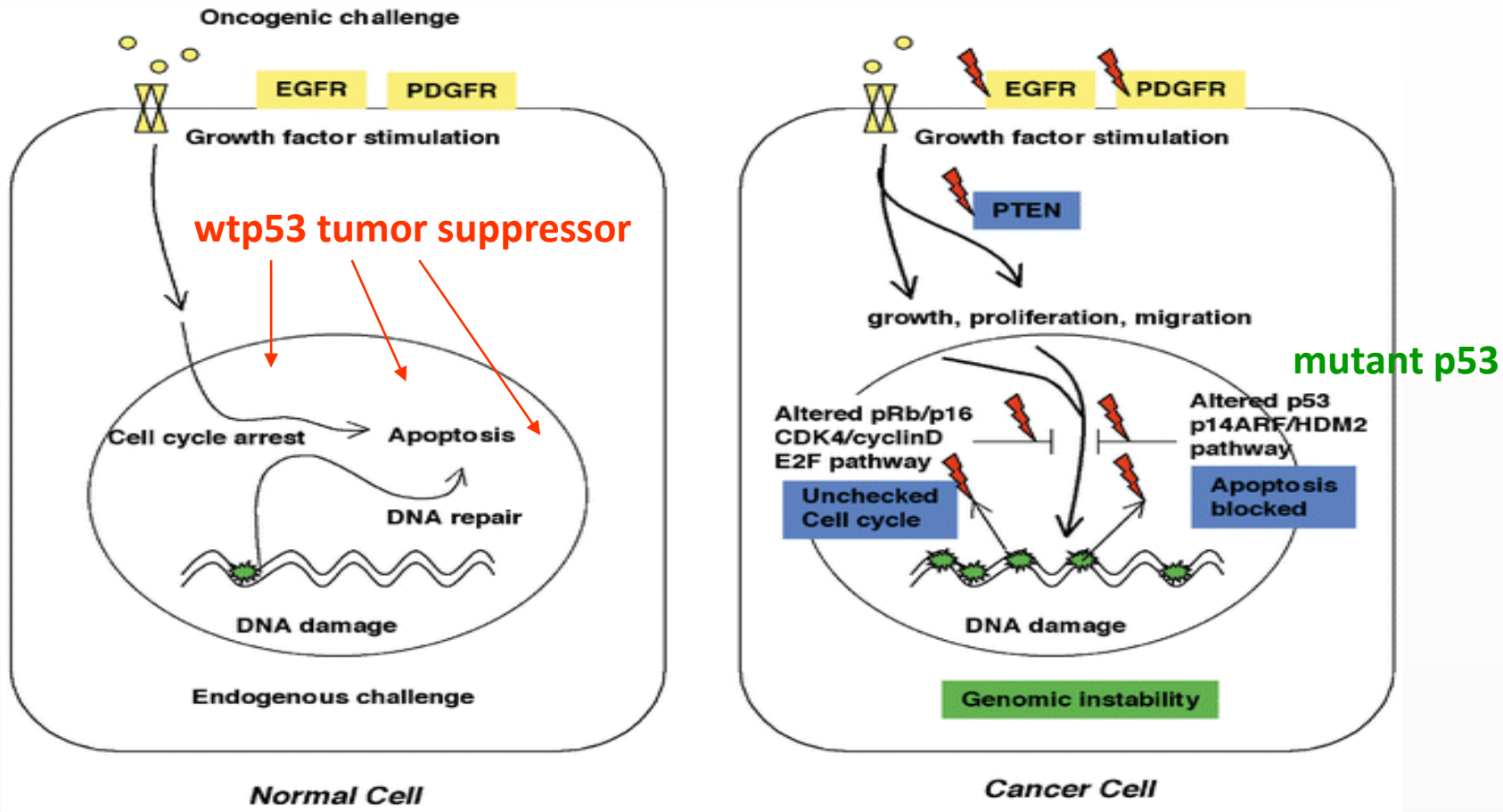
The albino mutation in Siamese cats results in a defective form of tyrosinase which does not function at normal body temperature. Therefore, dark coloration can only appear in parts of the body that are cooler than the core body temperature. The extremities are always the coolest parts of the body. The face is also cooler because of air passing through the sinuses. The back is warmer than the extremities, being closer to the body core, but it is also exposed. The result is a medium degree of tyrosinase function, resulting in a medium degree of shading

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



Cho et al. 1994

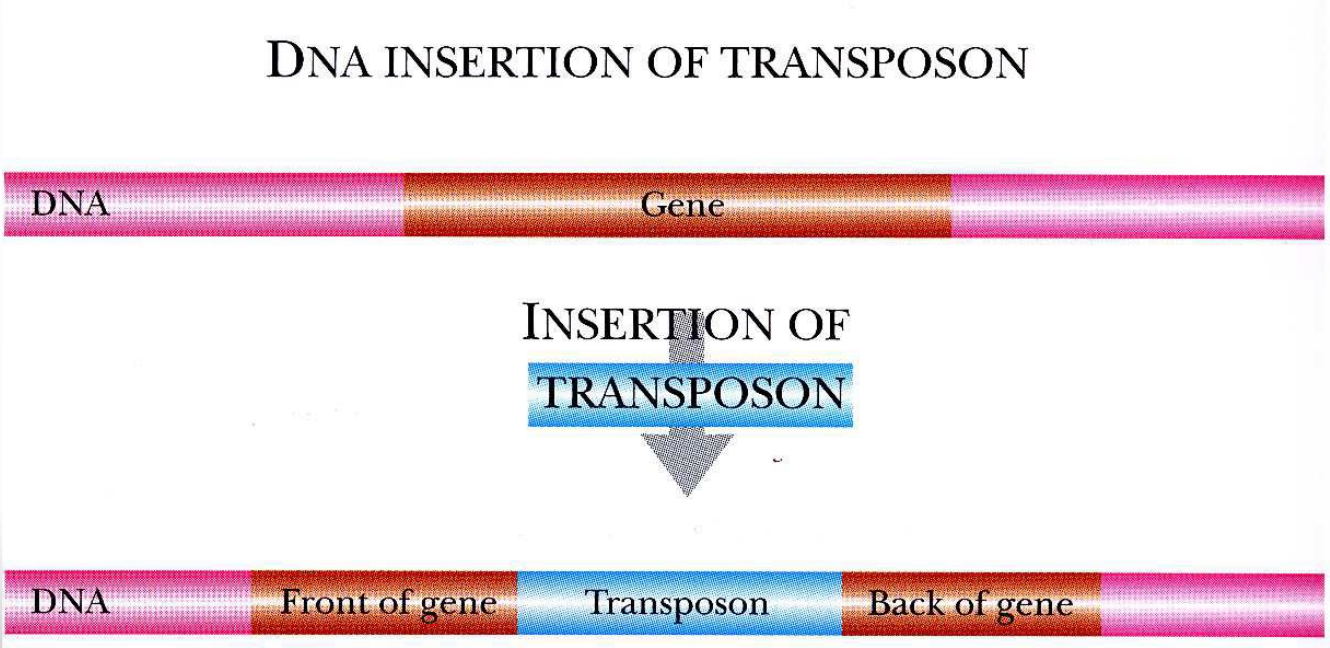
Reactions of normal and tumor cells to the oncogenic stress



A.Merlo, 2004

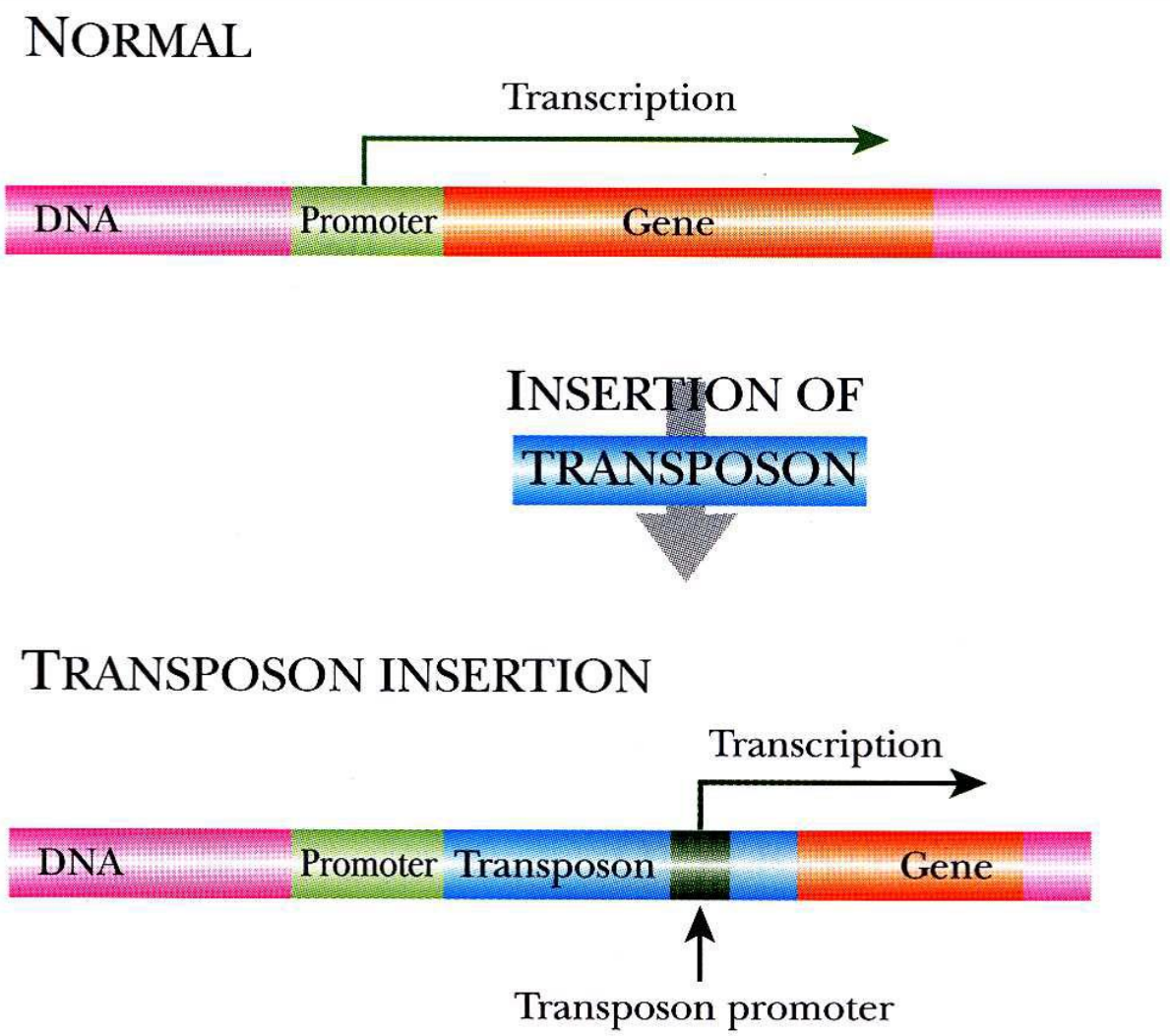
Insertions and deletions

- Gain or loss of a DNA section
- random DNA segment incorporation into coding sequence will usually **inactivate** the original sequence
- phenotypic response depends on the extent and location (shorter insertion may allow for at least partial activity of the original protein)
- mobile genetic elements (**transposons**) can be as long as several thousand bp



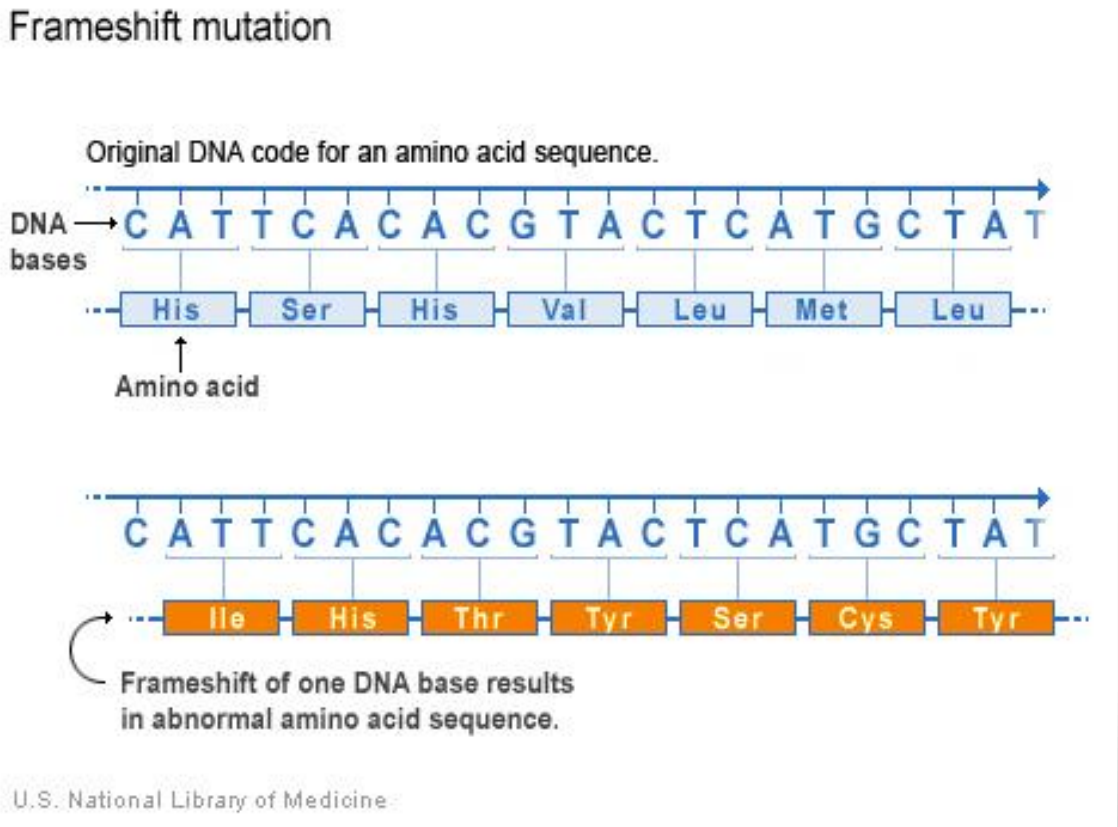
Insertion can activate gene expression

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



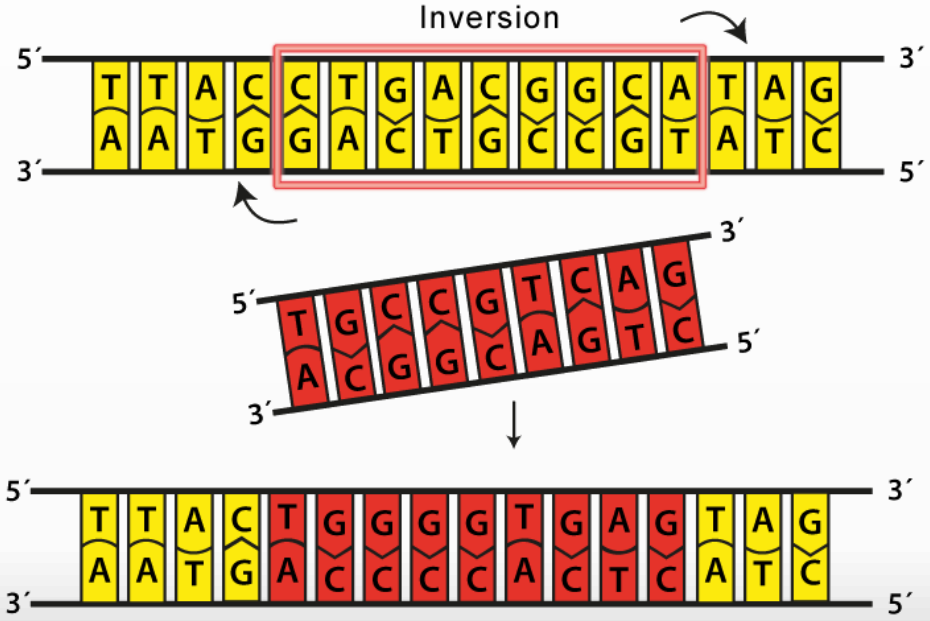
Mutations altering the reading frame

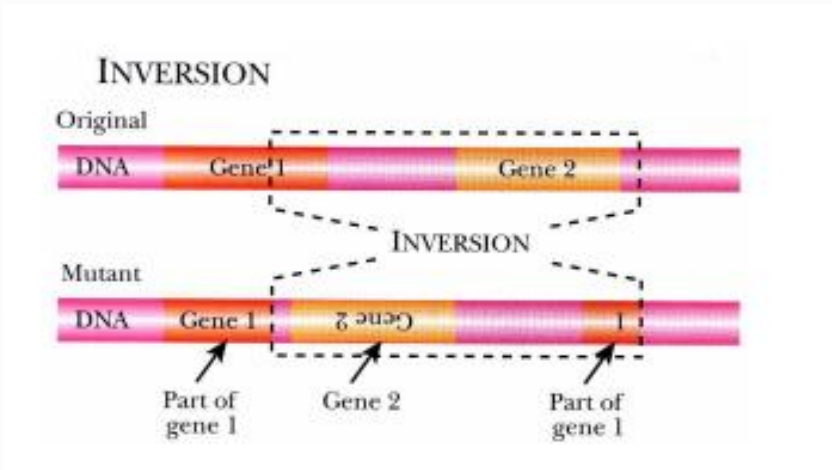
- bases are read as codons (3 bases)
- the inclusion or removal of one or two bases inside the reading frame fundamentally changes the genetic information – protein function is often lost
- three base insertion or deletion inside reading frame - reading frame does not change drastically- the protein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



Inversion

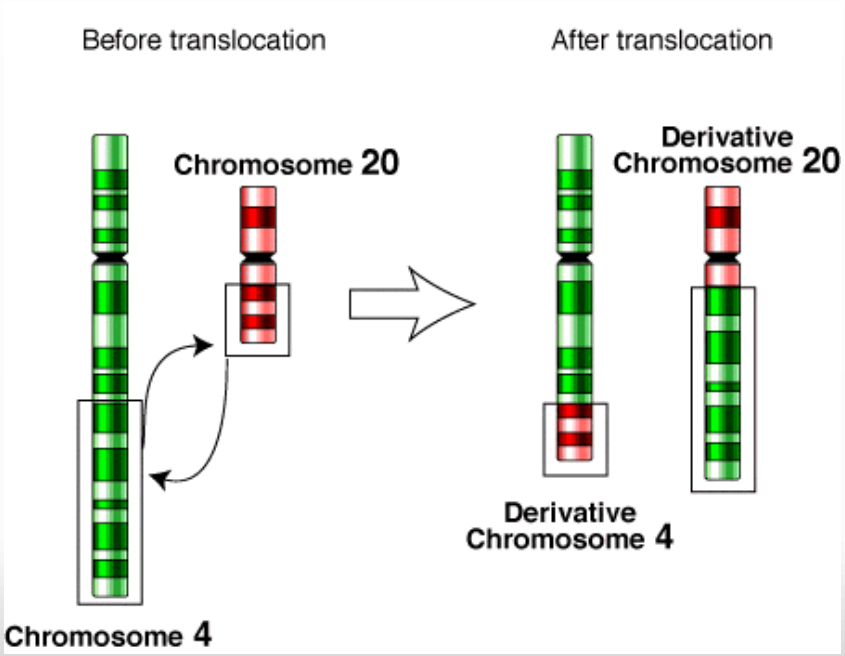
- DNA segment reversal
- coding sequence gets interrupted- loss of gene function
- when terminal sequences of the inverted portion are in intergenic regions - gene can remain intact, if the inversion includes promoter with the whole gene it can even be transcribed in the reverse orientation - does not lose its function





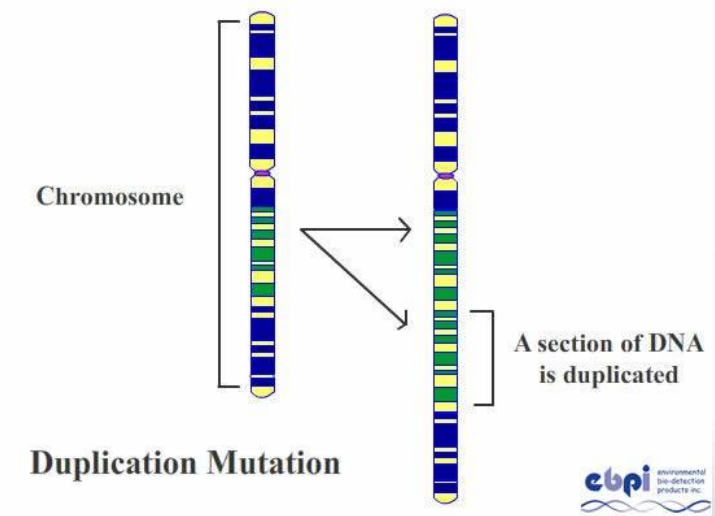
Translocation

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



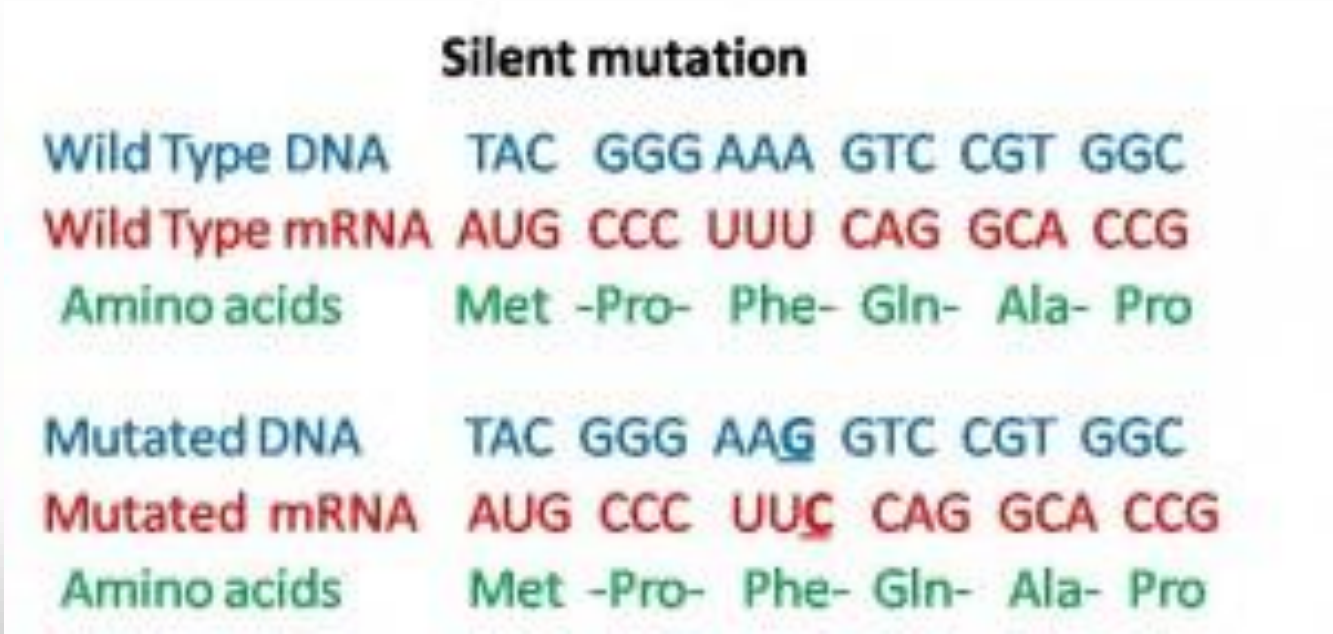
Duplication

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



Silent mutations

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



Reversion = back mutation

- reversion = original sequence is completely recovered
- pseudoreversion = original function of the polypeptide is restored, by a degenerate codon
- 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 = gene variant
- Standard allele = prevalent in the population
- Mutant allele = changed by mutation

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

Spontaneous and induced mutations

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

How often do mutations occur?

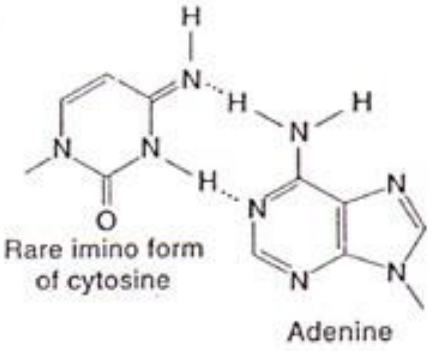
- Spontaneous mutations occur sporadically, the actual observed rates vary from gene to gene and between different organisms.
- **phages and bacteria**: 10^{-8} to 10^{-10} mutations per base pair and generation
- **eukaryotes**: 10^{-7} - 10^{-9} mutations per base pair and generation
- The action of mutagenic substances may increase the mutation rates by several orders.

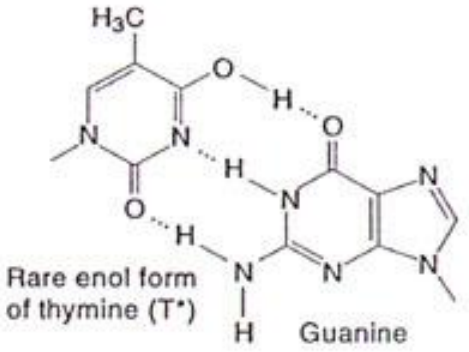
KEY POINTS

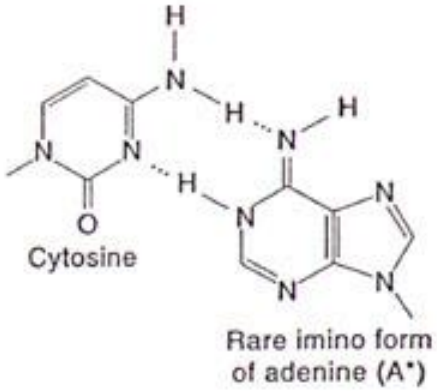
- Mutations occur in both embryonic and somatic cells but only mutations in the germ cells are transmitted to progeny.
- Mutations can arise spontaneously or may be induced by mutagenic substances in the environment.
- Standard phenotype can be restored in the mutant organism by reversions or suppressor mutations.

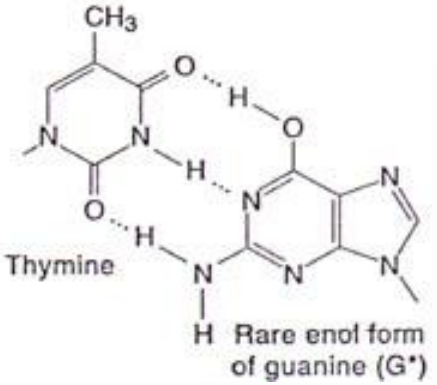
Spontaneous mutations

- **Incorrect base pairing arises in these processes and from the intrinsic base properties**





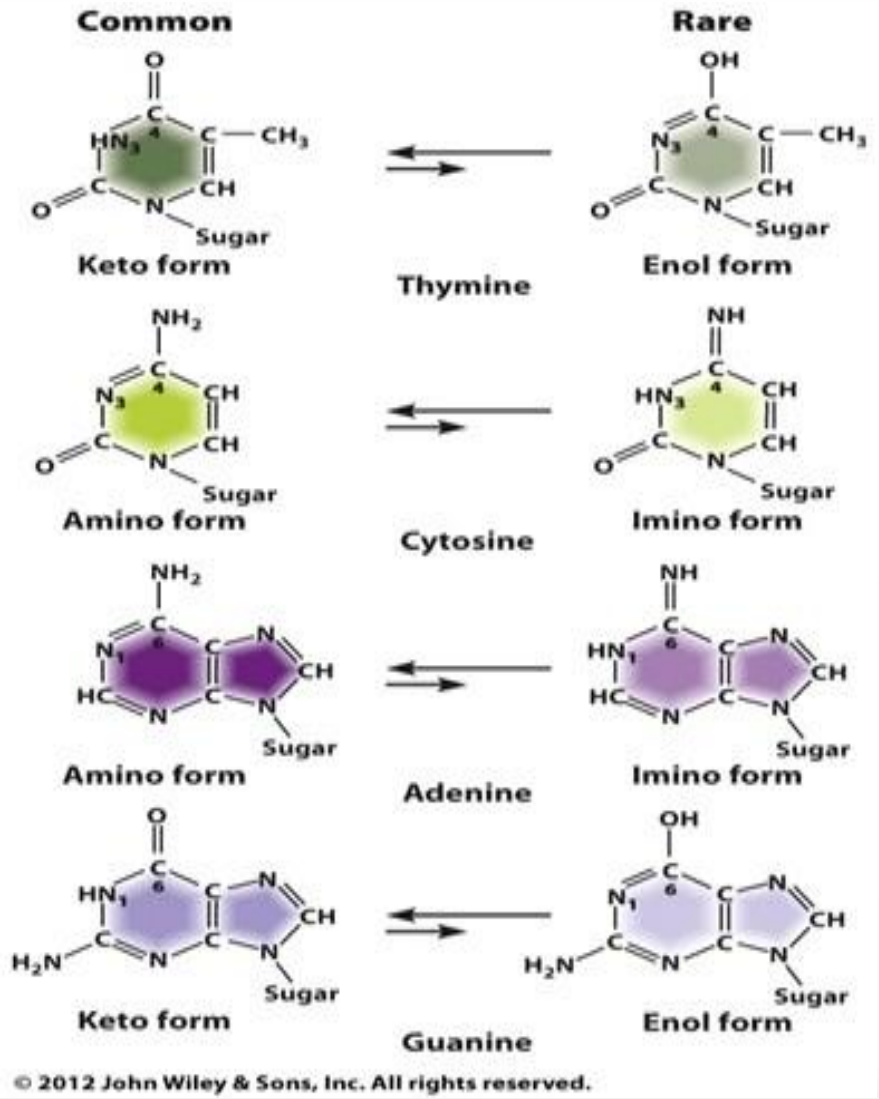




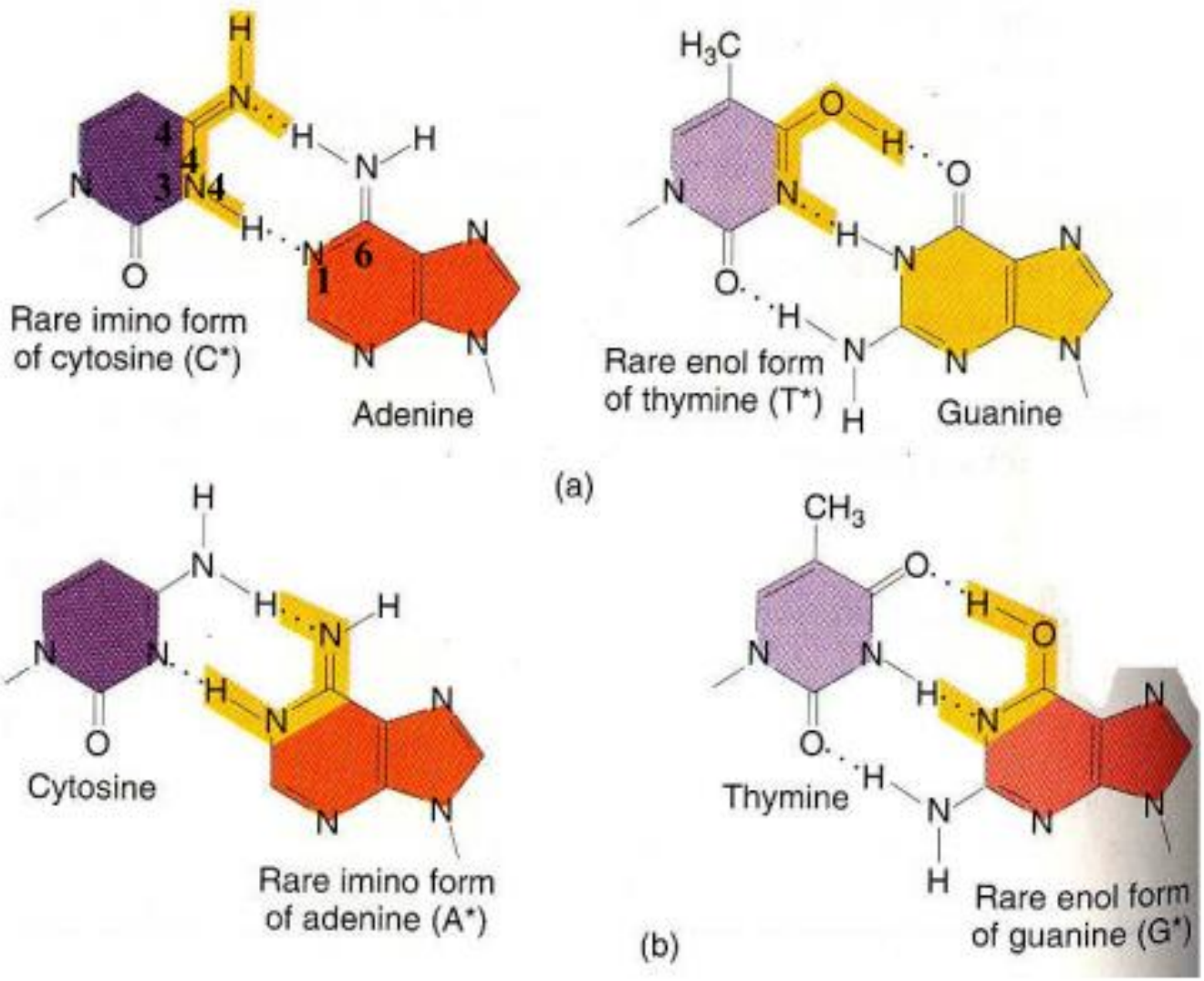
- tautomeric base changes
- wobble base pairing
- depurination and depyrimidination of bases
- deamination of bases
- uracil incorporation into DNA during replication
- oxidative DNA damage

Tautomeric base changes

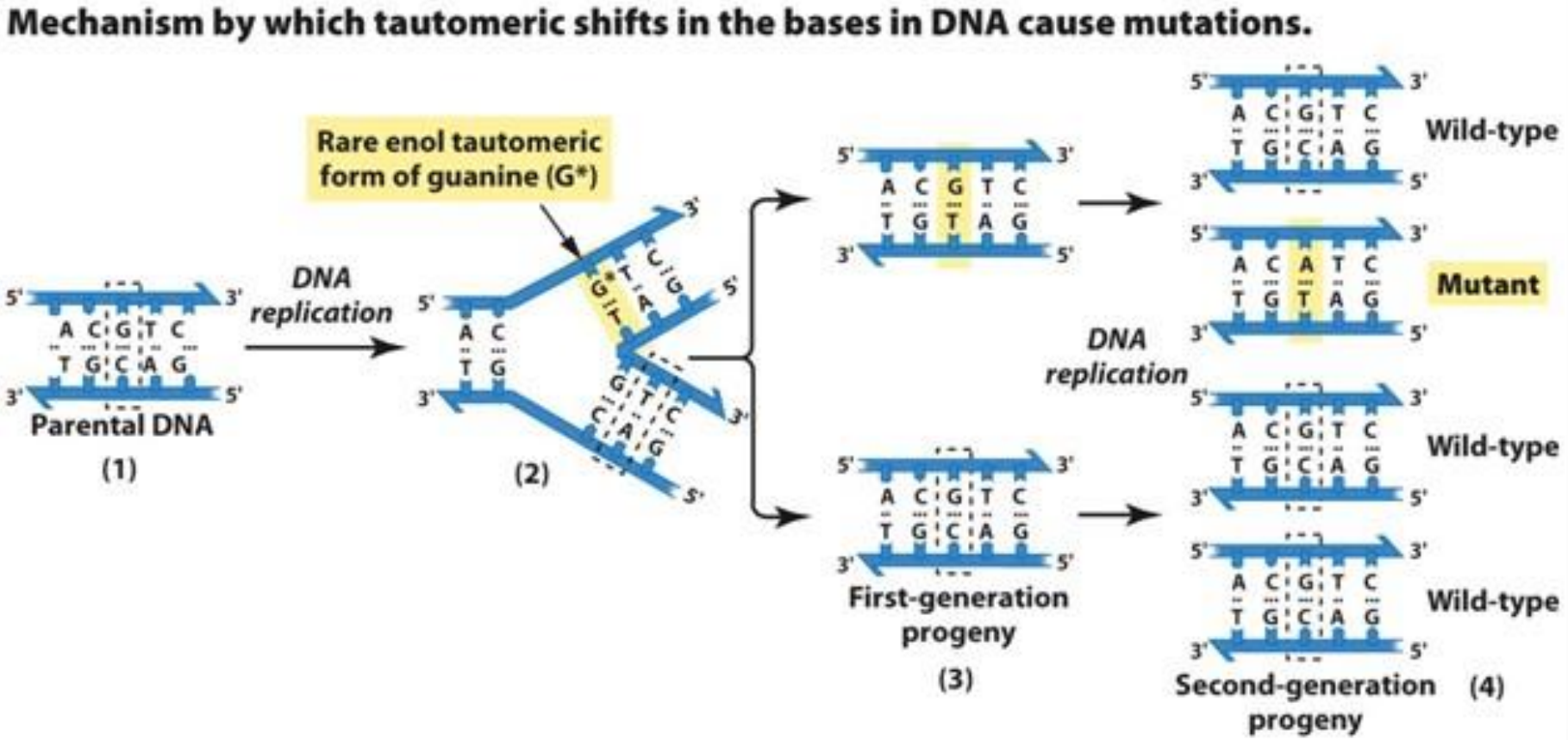
- Bases in DNA are not static. Hydrogen atoms can move from one position on purine or pyrimidine to another - for example, from the amino group to the ring.
- Such chemical isomorphism is referred to as **tautomerism**. Although tautomeric rearrangements are rare, they can have an important role in DNA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



Tautomeric Shifts Affect Base-Pairing

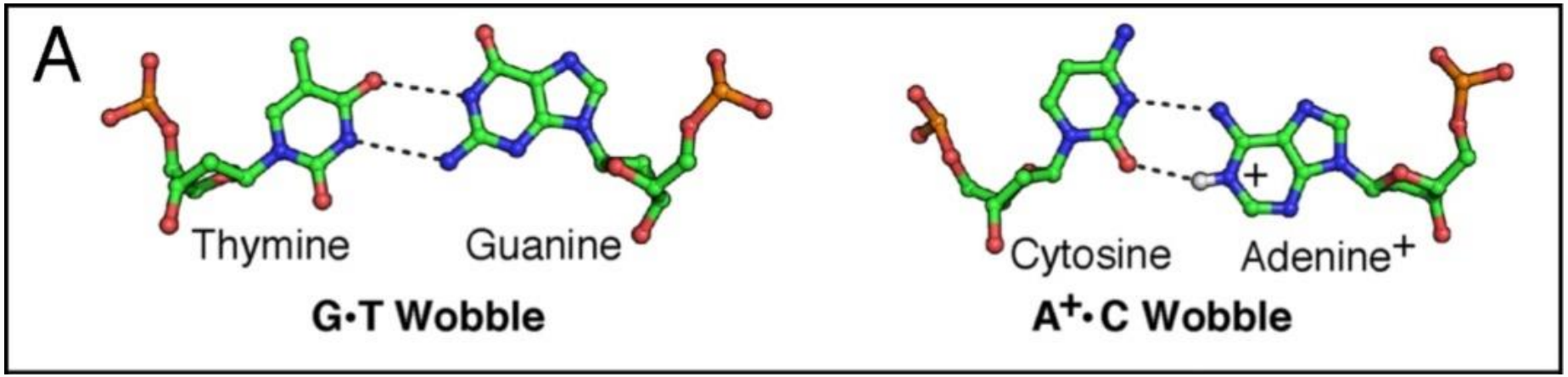


Mutation caused by tautomeric shift



Wobble base pairing

- CT,GA,TG – wobble base pairs possible
- during replication
- also between tRNA anticodon and the mRNA codon
- correction mechanism - DNA polymerase III

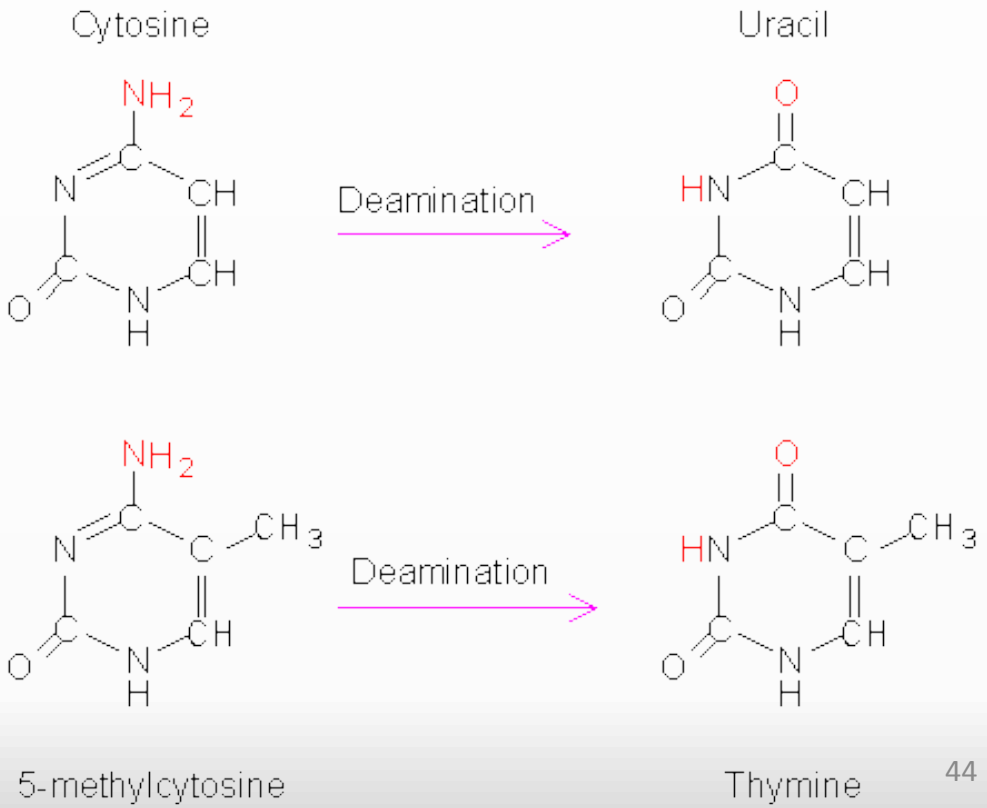


Depurination and depyrimidination

- glycosidic bond between the base and the sugar can eventually be abolished and the base then lost (phenomenon used also to repair mispaired bases, as they can be excised and then replaced)
- under physiological conditions in mammals - daily several thousand nucleotide bases are lost
- during replication any nucleotide can be incorporated to the vacant slot, usually dAMP (GC -> AT)

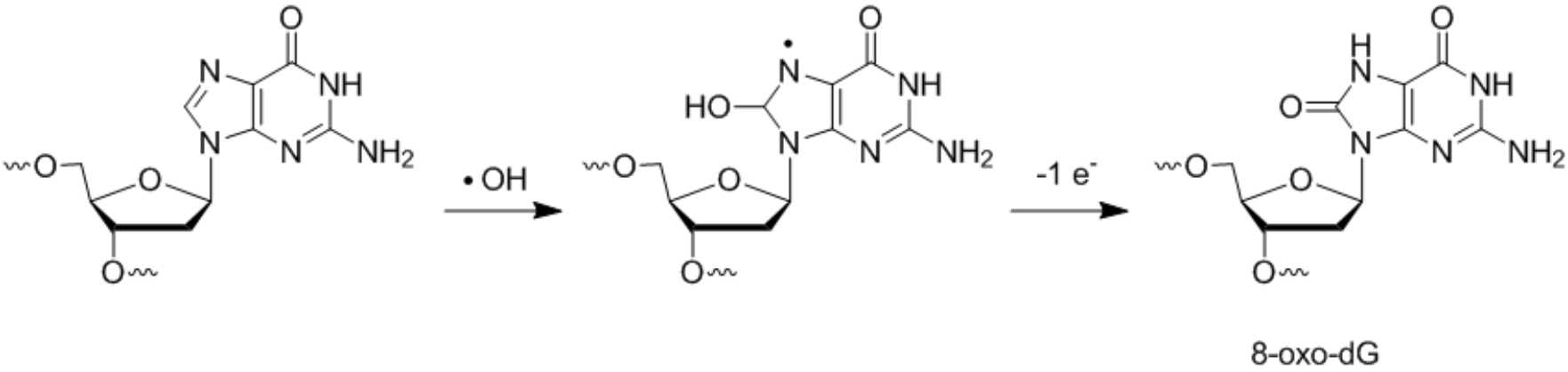
Uracil incorporation into DNA

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



Oxidative DNA damage

- hydroxyl radical •OH, acts on bases (mostly guanine)
- formed from hydrogen peroxide in respiratory chain
- causes transversion from GC to AT



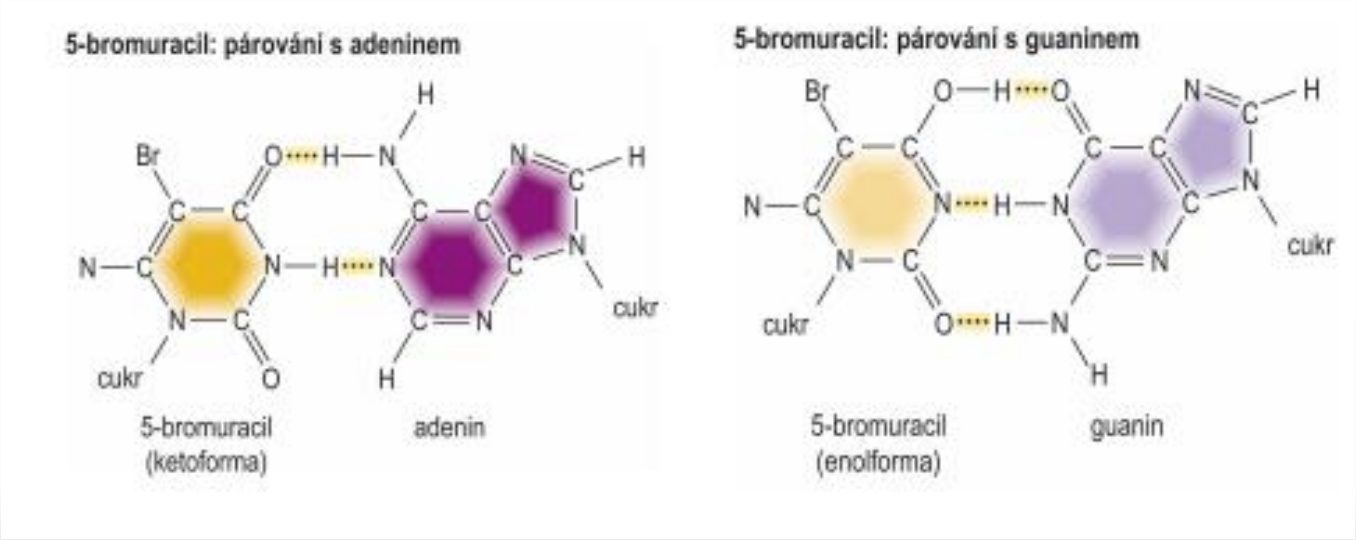
Induced mutations: chemical mutagens

- **influence DNA structure**
- **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 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 over DNA replication** (base analogues, acridine dye)

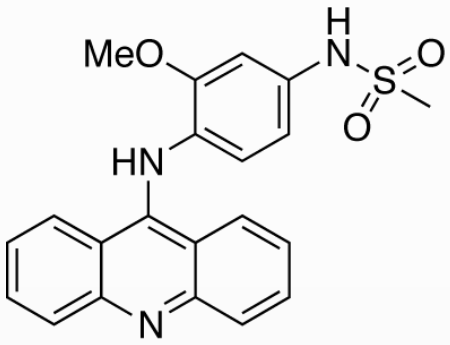
Mutagenic base analogues

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

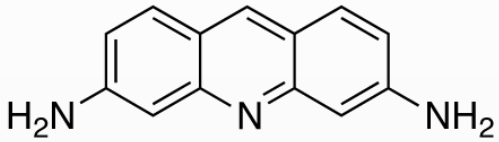


Acridine dyes

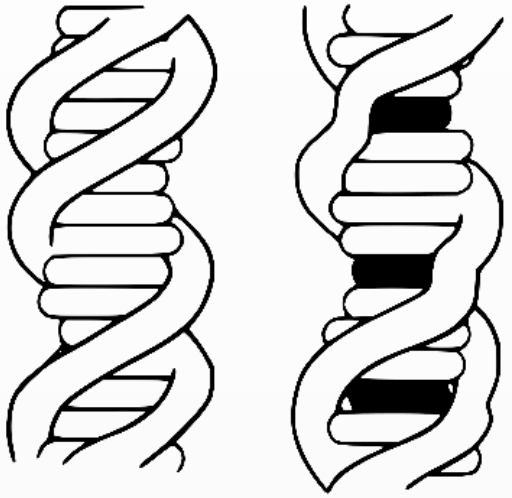
- e.g. proflavine, acridine orange, acridine blue
- intercalators – incorporation to DNA groove
- tighten and alter the conformation of the DNA double helix
- during replication deletions or insertions of one or more base pairs occur- often frameshift mutations



amsacrine



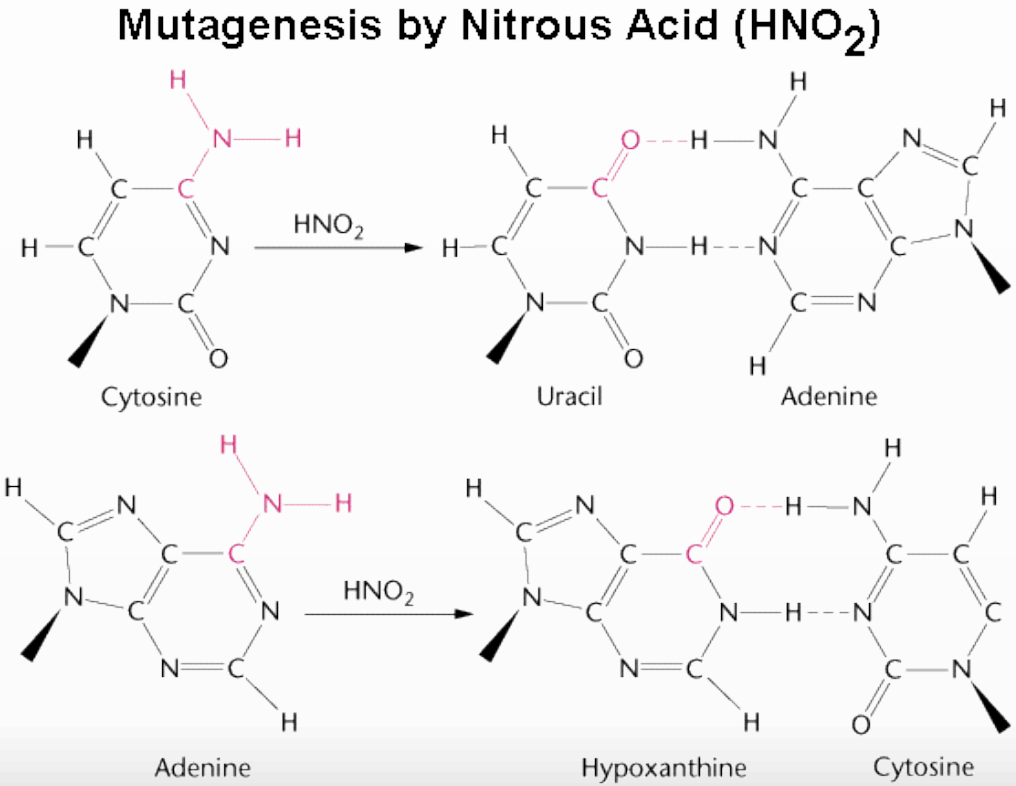
proflavine



Nitrous acid

HNO_2

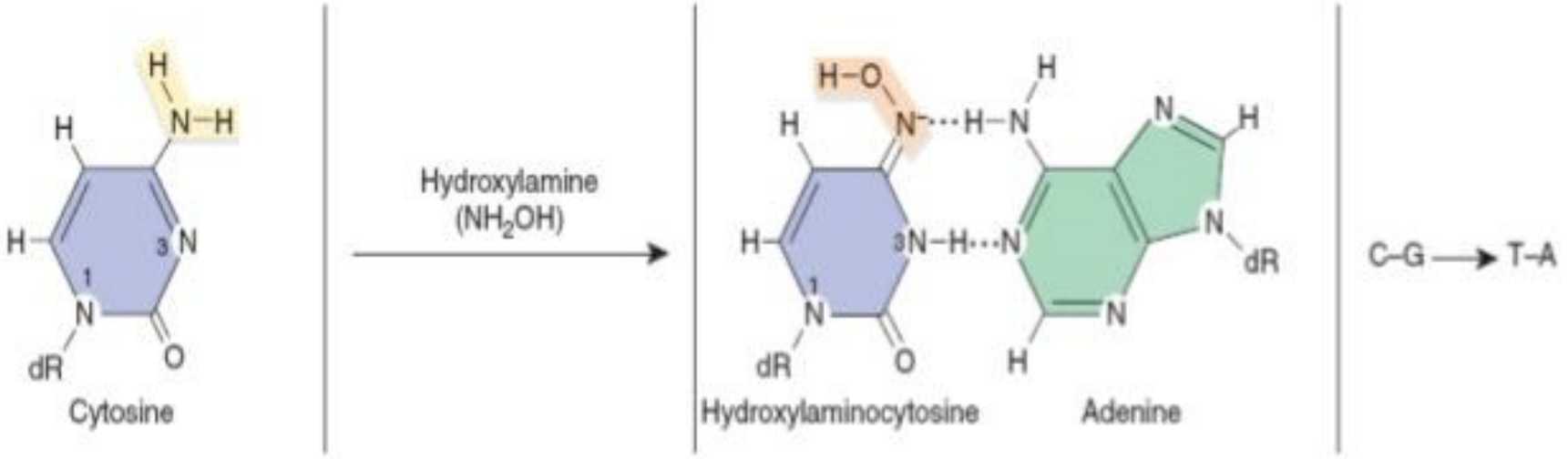
- causes oxidative deamination of amino groups of adenine, guanine and cytosine: amino groups thereby change to keto groups
- adenine deamination - change to hypoxanthine, which is paired with cytosine
- cytosine is deaminated to uracil, which pairs with adenine



(Klug & Cummings 1997)

Hydroxylamine

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

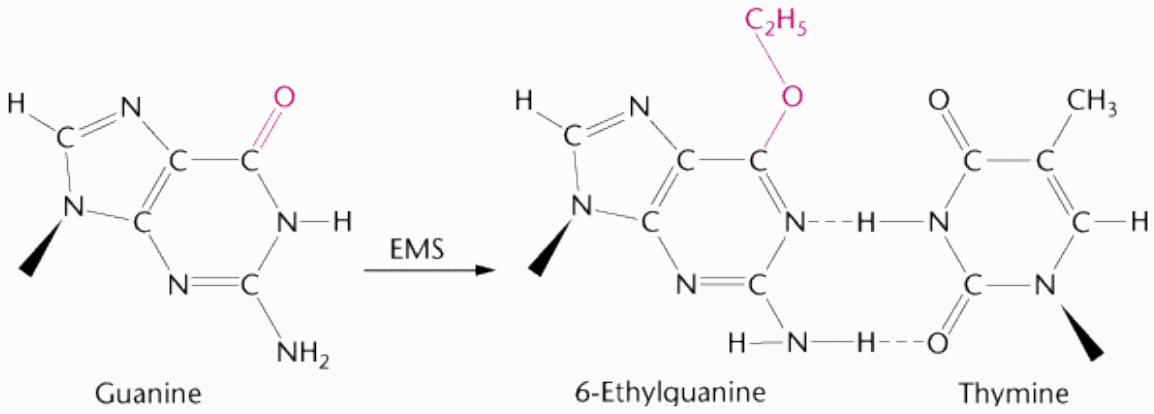


(Russell PJ, 2010)

Alkylating agents

- Ethyl methanesulfonate, aziridine, yperite
- DNA bases methylation or ethylation, causes a change in base-pairing
- induces all types of mutations (transitions, transversions, frameshift mutations and chromosomal aberrations)

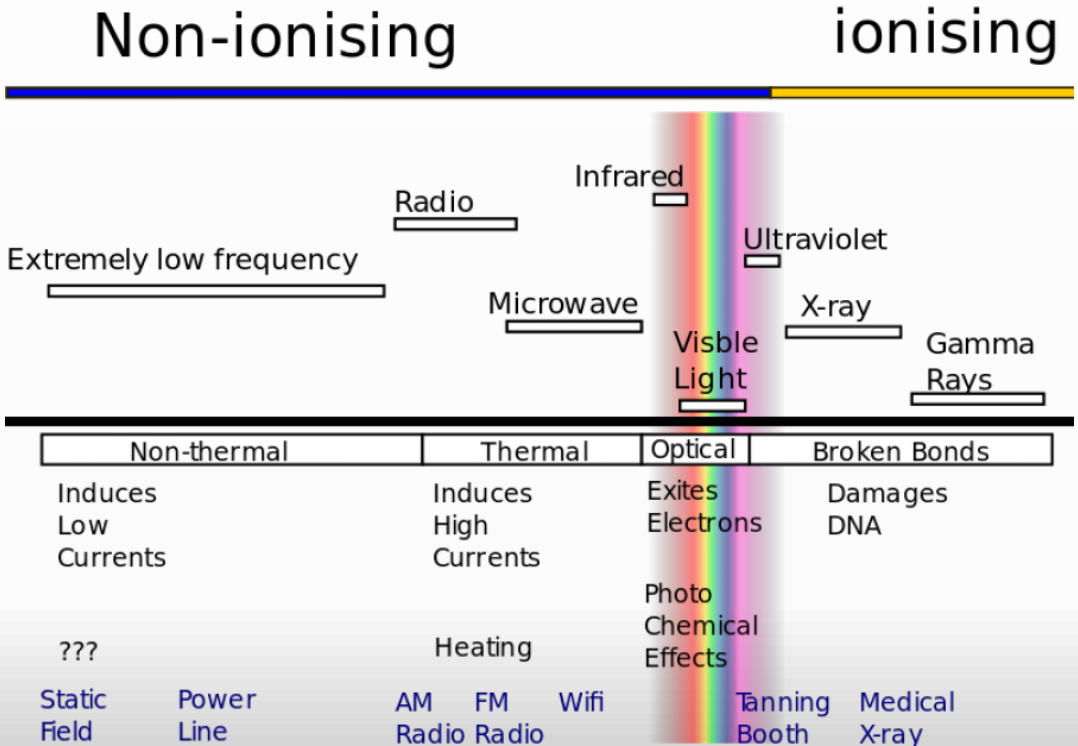
Mutagenesis by Ethyl Methane Sulfonate (EMS)



(Klug & Cummings 1997)

Physical mutagens

- ionizing radiation (X-ray, gamma, cosmic) induces breaks in DNA
- non-ionizing radiation (UV) absorbed 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

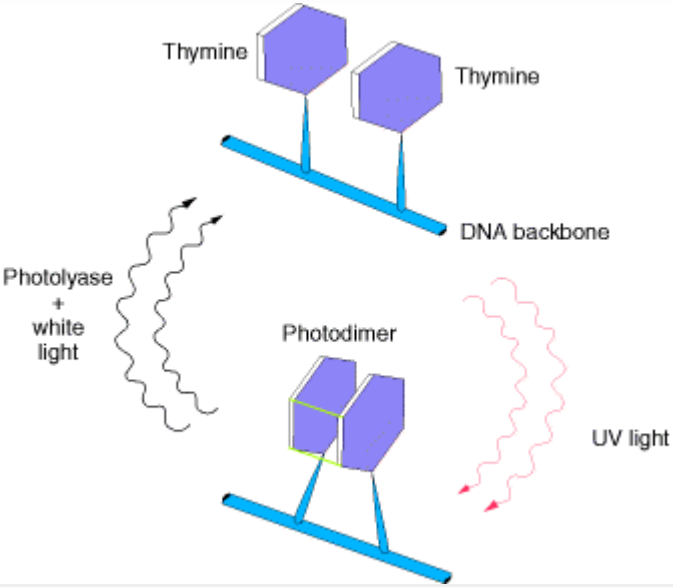
- shorter wavelength ~ higher energy than the visible light
- penetrates deep into the tissue, strikes the atoms, releases electrons to form positively charged ions and radicals which give rise to other ions (ionization process)

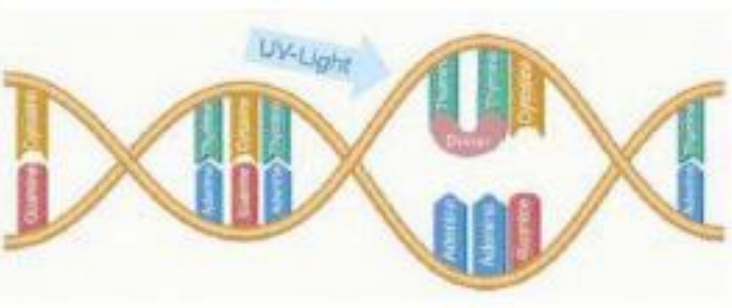
UV radiation

- lower energy than ionizing radiation
- only penetrates into the upper layers of cells, potent mutagen in unicellular organisms
- does not cause ionization
- radiant energy is captured by atoms, electrons pass into the excited state – increasing 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 that are close react to form pyrimidine dimers and hydrates
- **thymine dimers** disrupt the DNA structure and **disrupt the replication**

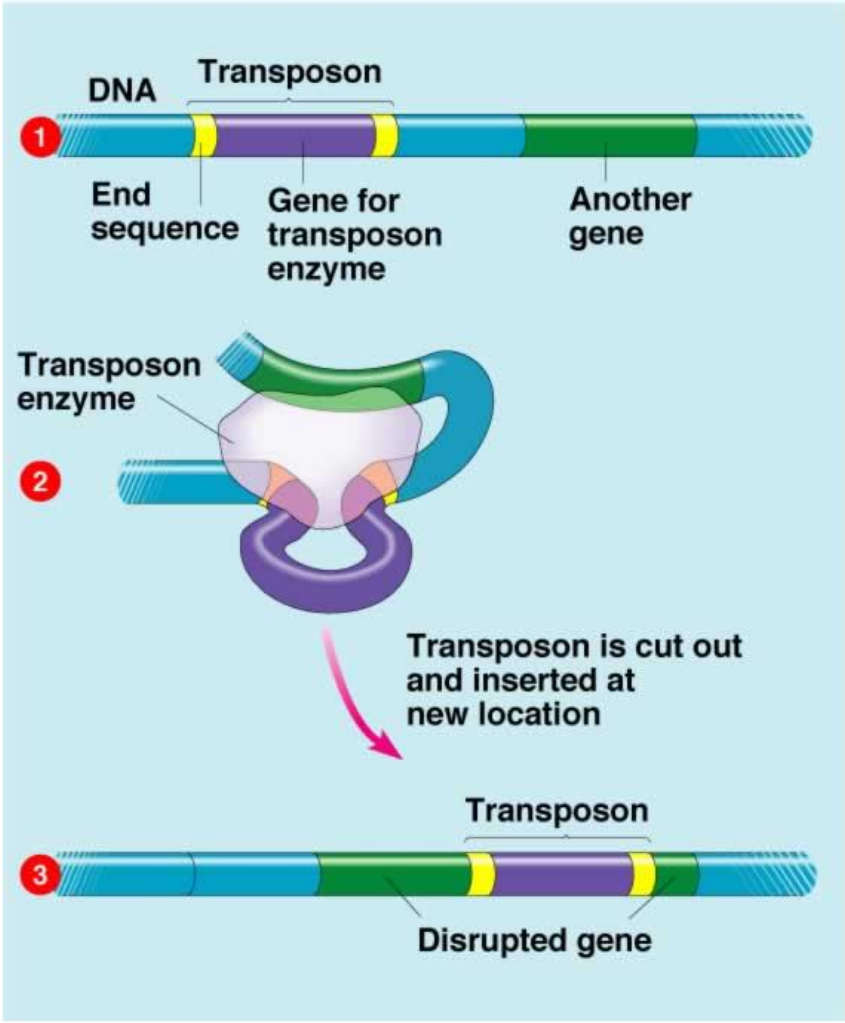




Biological mutagens

- **Viruses** - incorporate into the host DNA
- transposable elements- **transposons**: DNA regions which can move from one place to another in the genome

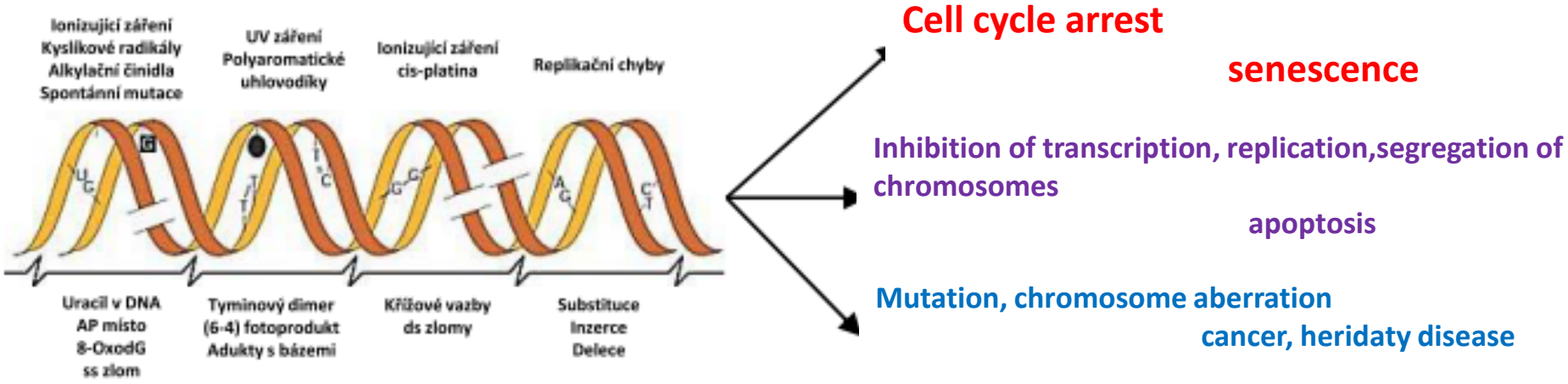
- Insertion of transposon may inactivate a gene (mutagenic)



DNA Repair mechanisms

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

DNA repair



- 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 genome integrity of the cell and for normal functioning of a multicellular organism.
- Tomas R. Lindahl, Paul L. Modrich, Aziz Sancar won the 2015 Nobel Prize in Chemistry for their research on the molecular mechanisms of DNA repair.

Types of DNA repair:

- **complete repair** - repairs to the original state without DNA synthesis
  - photoreactivation
  - direct repair of alkylated bases
- **excision repair** - excision of damaged sites, synthesis of DNA
  - NER – nucleotide excision repair
  - BER – base excision repair
  - mismatch repair
- **tolerant repair** – function restoration without DNA damage repair
  - SOS response
- **double strand break 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, DIVERSITY*

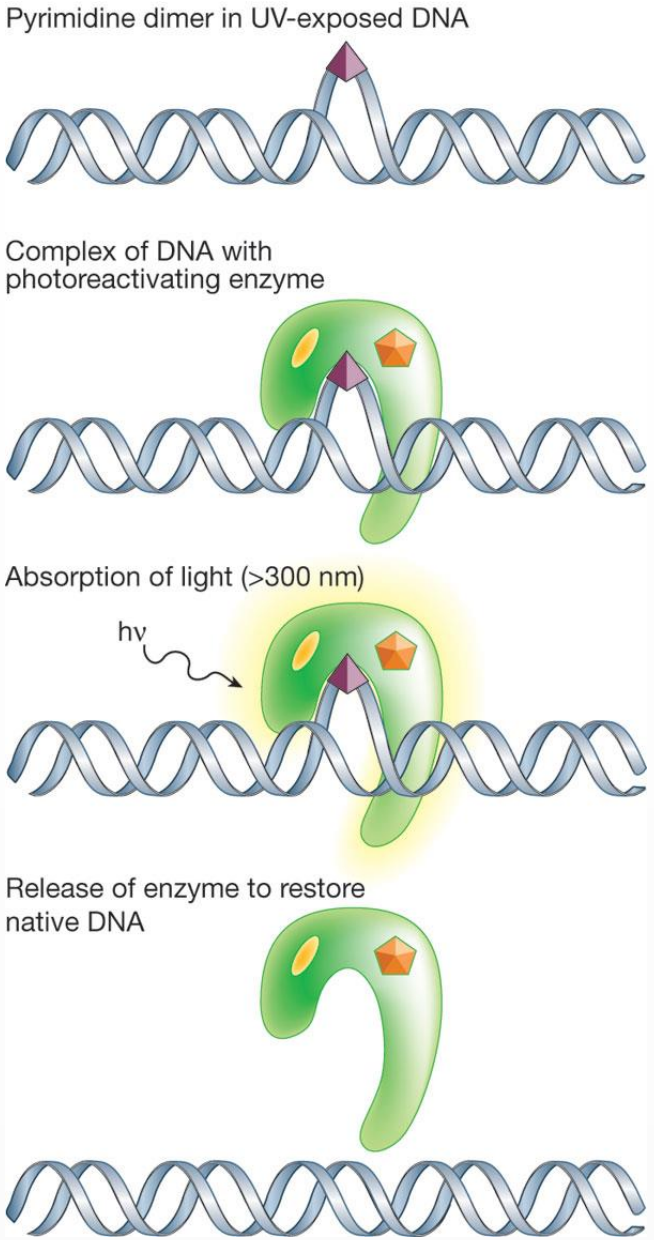
- **mismatch repair** controlled by methylation
- **excision repair** (base and nucleotide)
- **photoreactivation-**correction dependent on the light - only in bacteria
- **postreplication repair**
- **error-prone repair** (SOS response)
- Mutation frequencies: 10^{-10} mutations / bp / replication

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

1) complete repair

1A. Photoreactivation

- only in **bacteria, eukaryotes**, but **not in mammals**
- total 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



1B. Direct repair of alkylated bases

- Another type of damage, methylation of guanine bases, is directly reversed by the **protein methyl guanine methyl transferase (MGMT),** the bacterial equivalent of which is called ogt.
- This is an expensive process because each MGMT molecule can be used only once; that is, the reaction is stoichiometric rather than catalytic.
- A generalized response to methylating agents in bacteria is known as the adaptive response and confers a level of resistance to alkylating agents upon sustained exposure by upregulation of alkylation repair enzymes.
- The third type of DNA damage reversed by cells is certain methylation of the bases cytosine and adenine.

2. Excision repair

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

- performed in three steps:

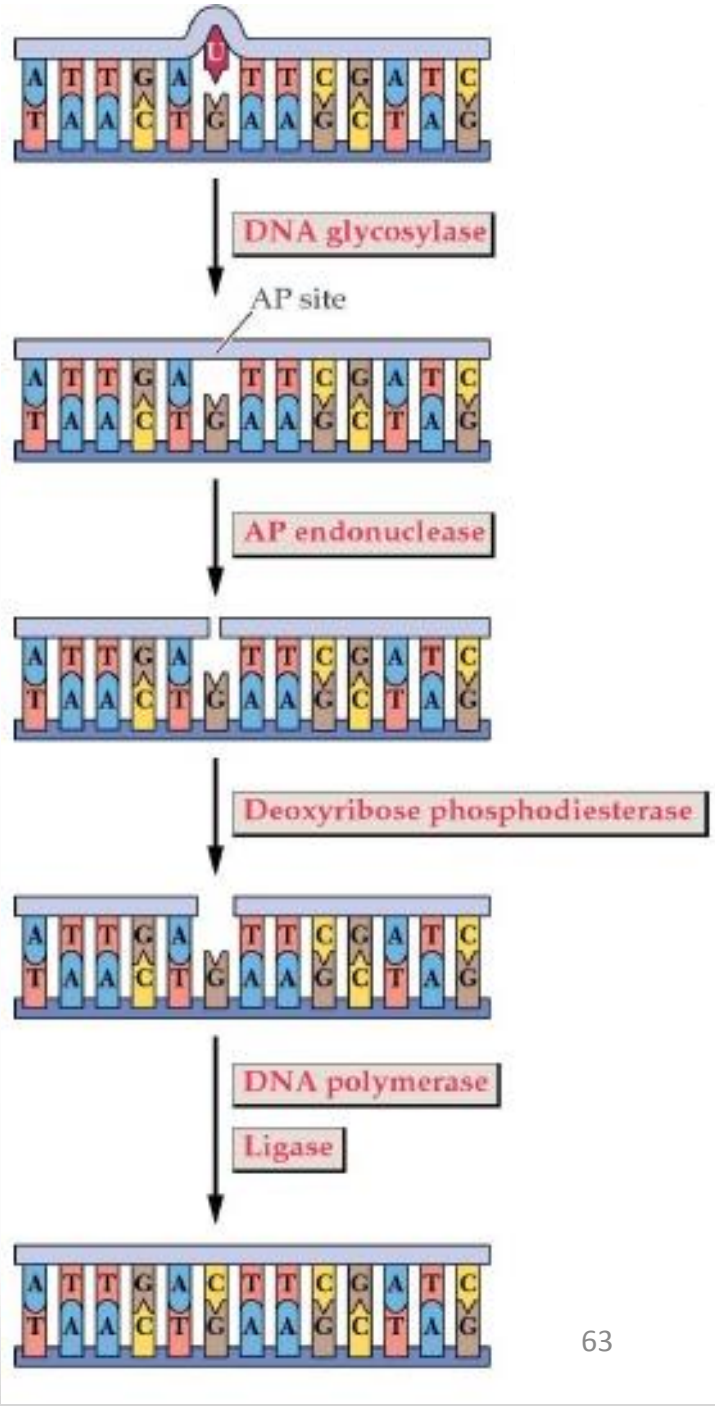
- **DNA endonuclease** recognizes damaged base in DNA, it binds to it and cleaves it
- **DNA polymerase** fills the gap using the intact complementary strand as a template
- **DNA ligase** connects strands retained by DNA polymerase

- **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

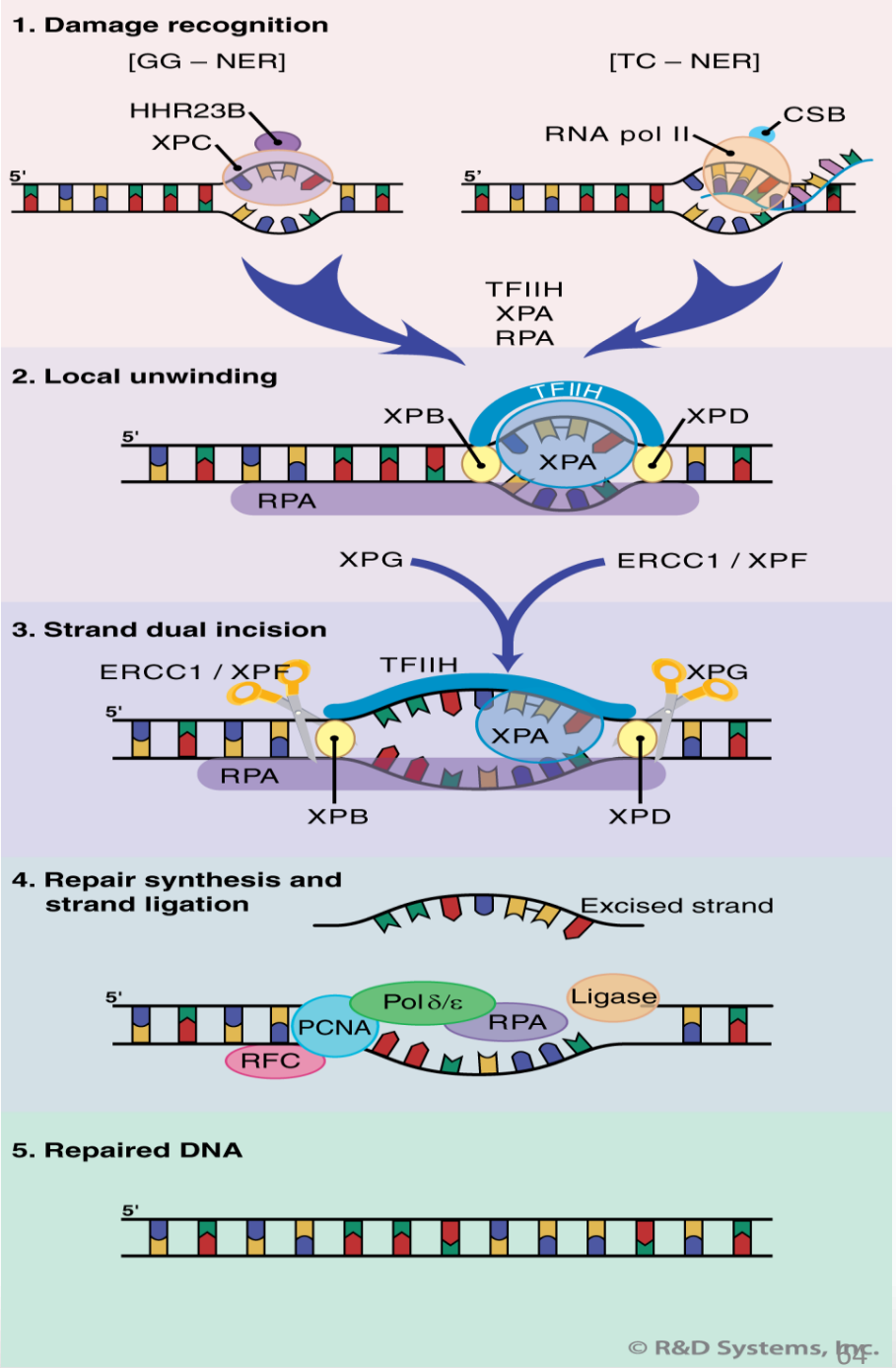
repair of damaged bases (oxidation, alkylation, deamination, removing U)

- creates **apurinic or apyrimidinic sites (AP sites)**
- **AP endonucleases** recognize AP-space, which they cleave - interrupt the sugar-phosphate backbone, creating 3 OH
- sugar is cleaved off later with **phosphodiesterases** (in humans **APEX1, APEX2)**
- **DNA polymerase** replaces a missing nucleotide by complementarity to the opposite strand (has no proofreading activity, makes mistakes → checked by APE1)
- **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**

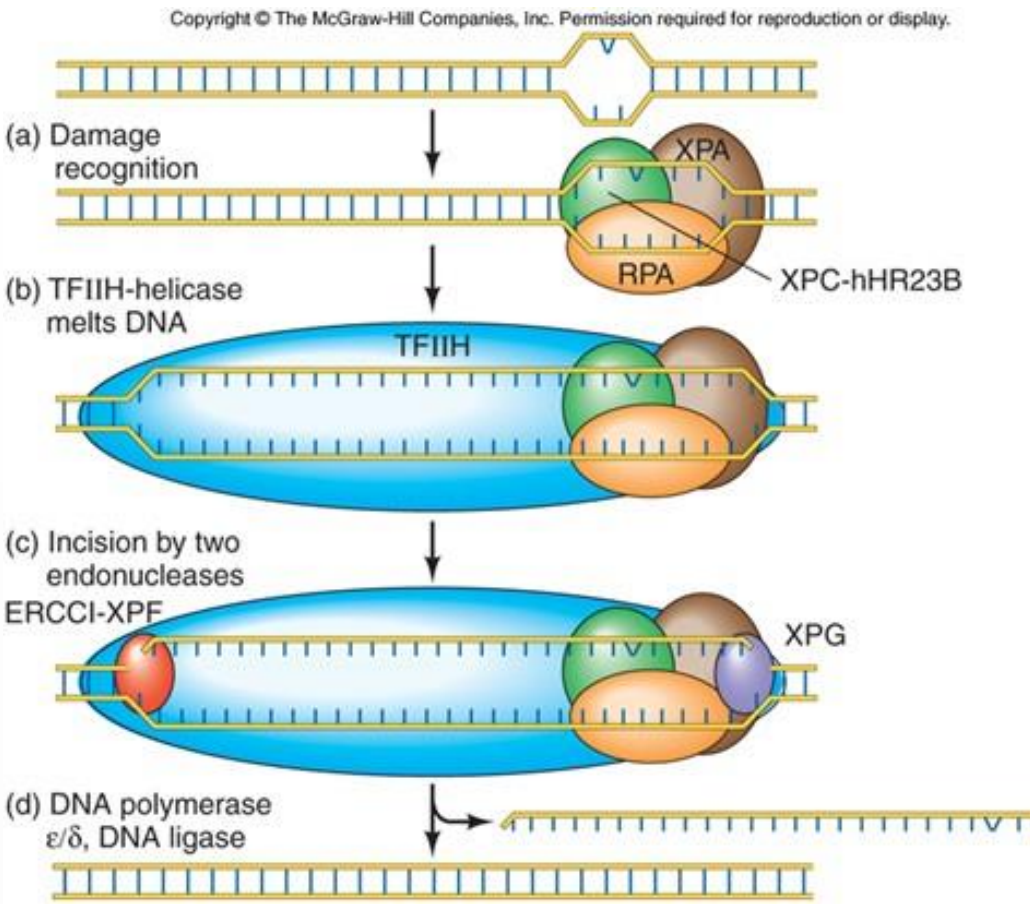


2B Nucleotide excision repair (NER)

- 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 damaged section
- oligonucleotide fragment containing the damaged bases is cleaved off
- gap is then filled by **DNA polymerase and DNA ligase**



- **Nucleotide excision repair (NER)** repairs damaged DNA which commonly consists of bulky, helix-distorting damage, such as pyrimidine dimerization caused by UV light.
- Damaged regions are removed in 12-24 nucleotide-long strands in a three-step process which consists of recognition of damage, excision of damaged DNA both upstream and downstream of damage by endonucleases, and resynthesis of removed DNA region.[19]
- **NER is a highly evolutionarily conserved repair mechanism and is used in nearly all eukaryotic and prokaryotic cells**.[19] In prokaryotes, NER is mediated by Uvr proteins.[19] In eukaryotes, many more proteins are involved, although the general strategy is the same.[19]

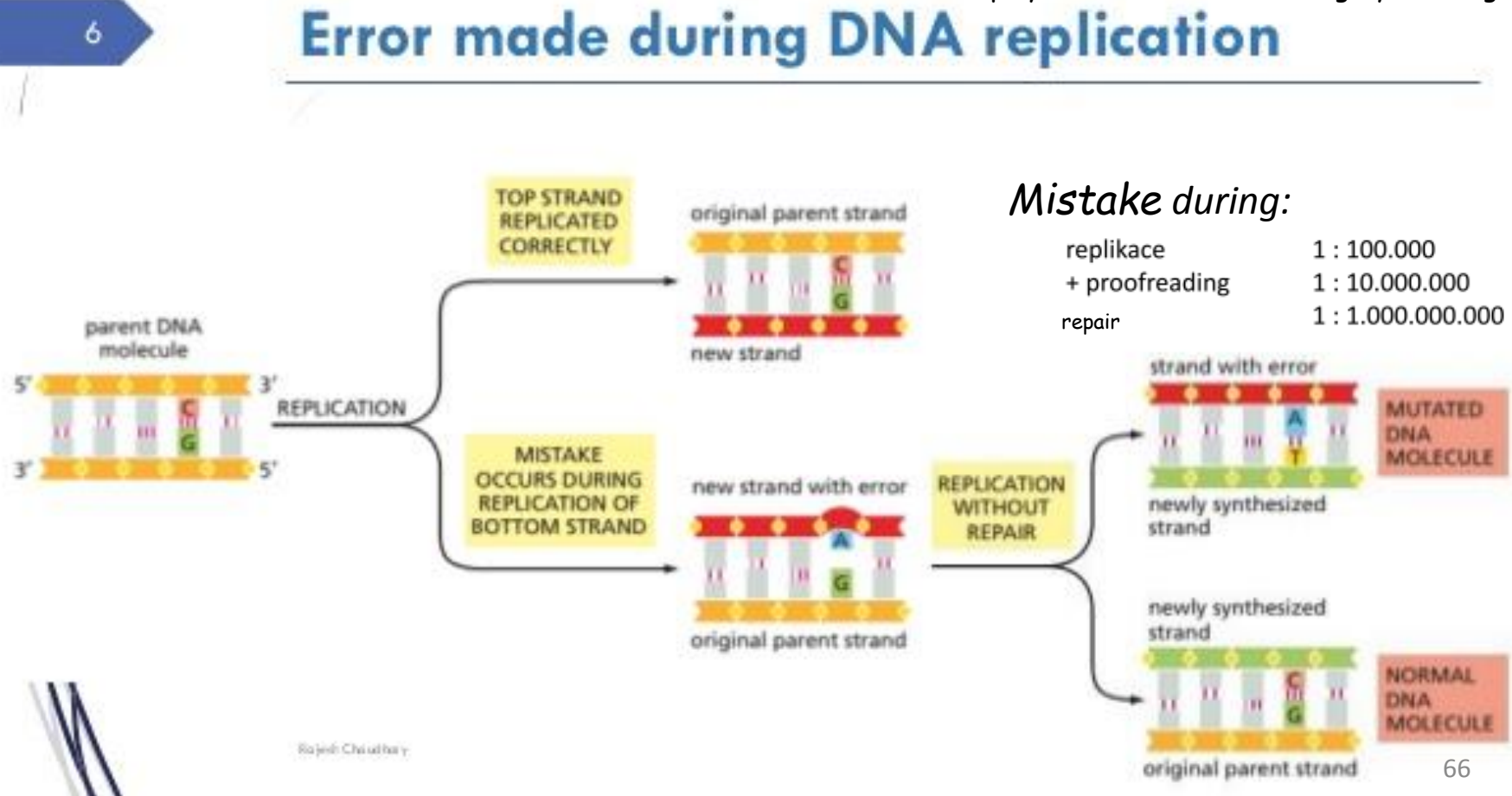


3 Correction of errors in DNA replication

mismatch repair

- repair mechanisms which repair 99% of mismatched bases created during replication
- repair proteins find mismatches and bind to them, ensuring removal of part of the newly synthesized strand and its resynthesis

Mismatch repair systems are present in essentially all cells to correct errors that are not corrected by proofreading. These systems consist of at least two proteins. One detects the mismatch, and the other recruits an endonuclease that cleaves the newly synthesized DNA strand close to the region of damage. In *E. coli* , the proteins involved are the Mut class proteins. This is followed by removal of damaged region by an exonuclease, resynthesis by DNA polymerase, and nick sealing by DNA ligase.[20]

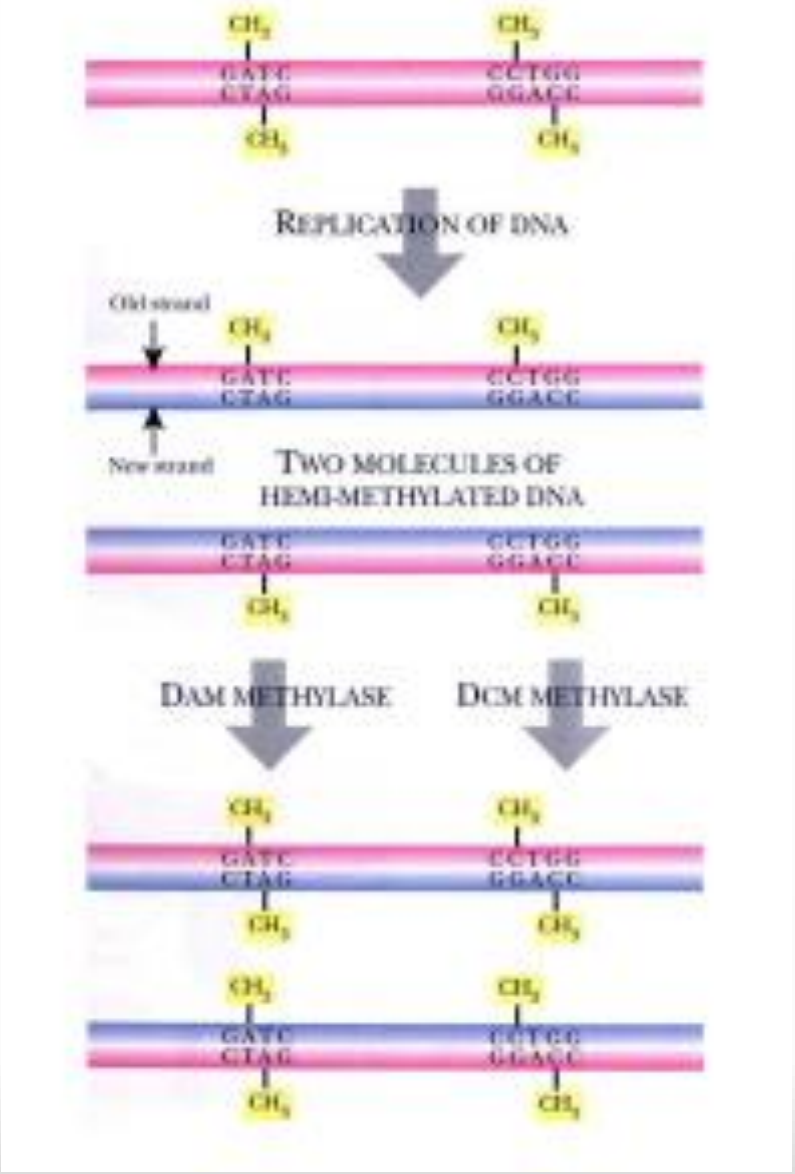


Mismatch Repair in E. coli

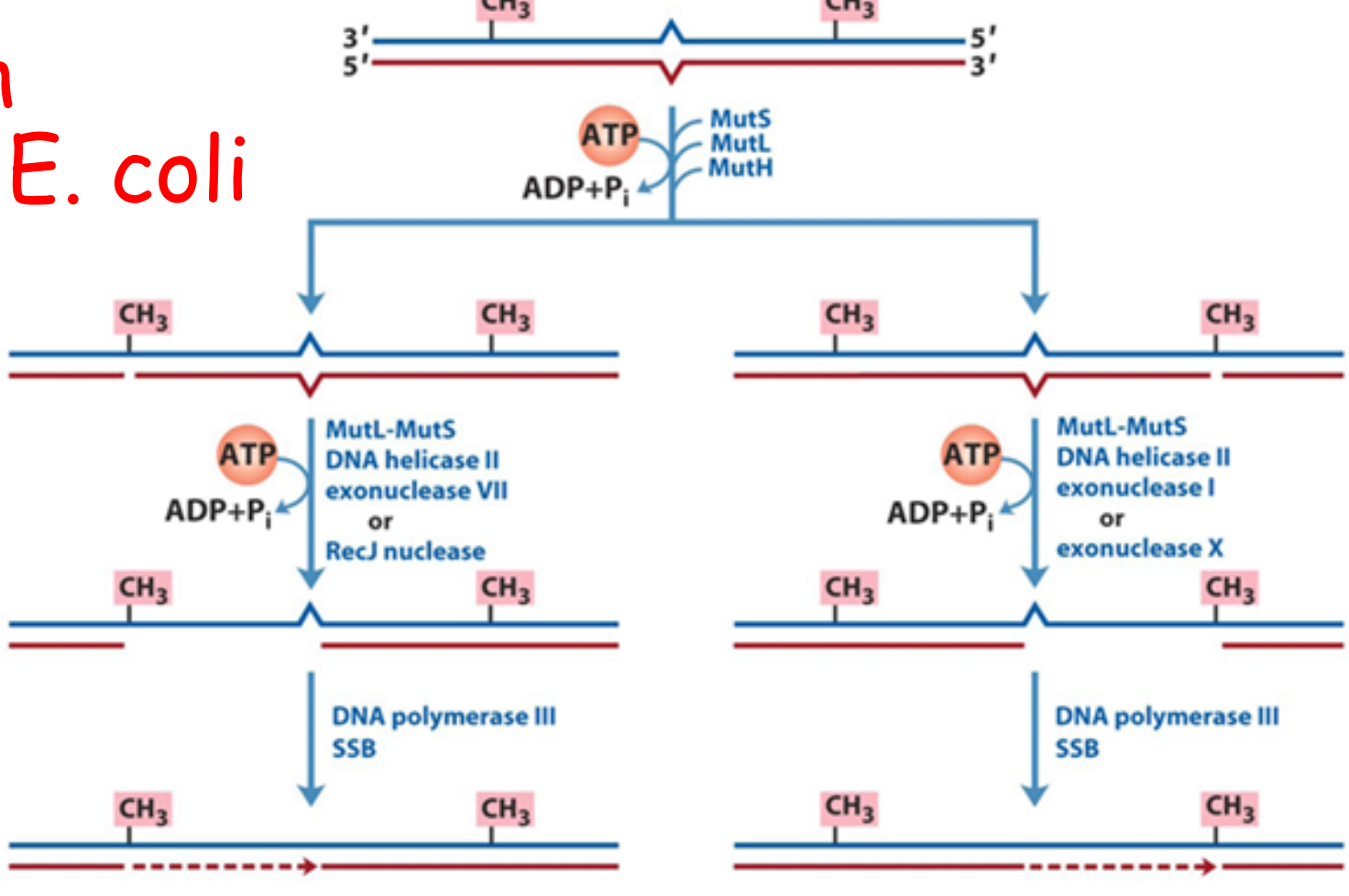
- Mismatching or mispairing of **G and T (DNA polymerase/exonuclease proofreading activity)**
- The A in GATC sequences is methylated subsequent to DNA replication.
- In newly replicated DNA, the parental strand is methylated, but the new strand is not. This difference allows the mismatch repair system to distinguish the new strand from the old strand.
- The mismatched nucleotide is excised from the new strand and replaced with the correct nucleotide, using the methylated parental strand as a template.

distinguishment of a new strand with an error from the original - *E. coli* Dam **methylase** that methylates A sequence GATC, just after the replication hemimethylated status

Correction system recognizes this fact based on the identification of strand of the matrix that contains the original nucleotide sequence, and a newly synthesized strand which contains the erroneously inserted bases (bug)

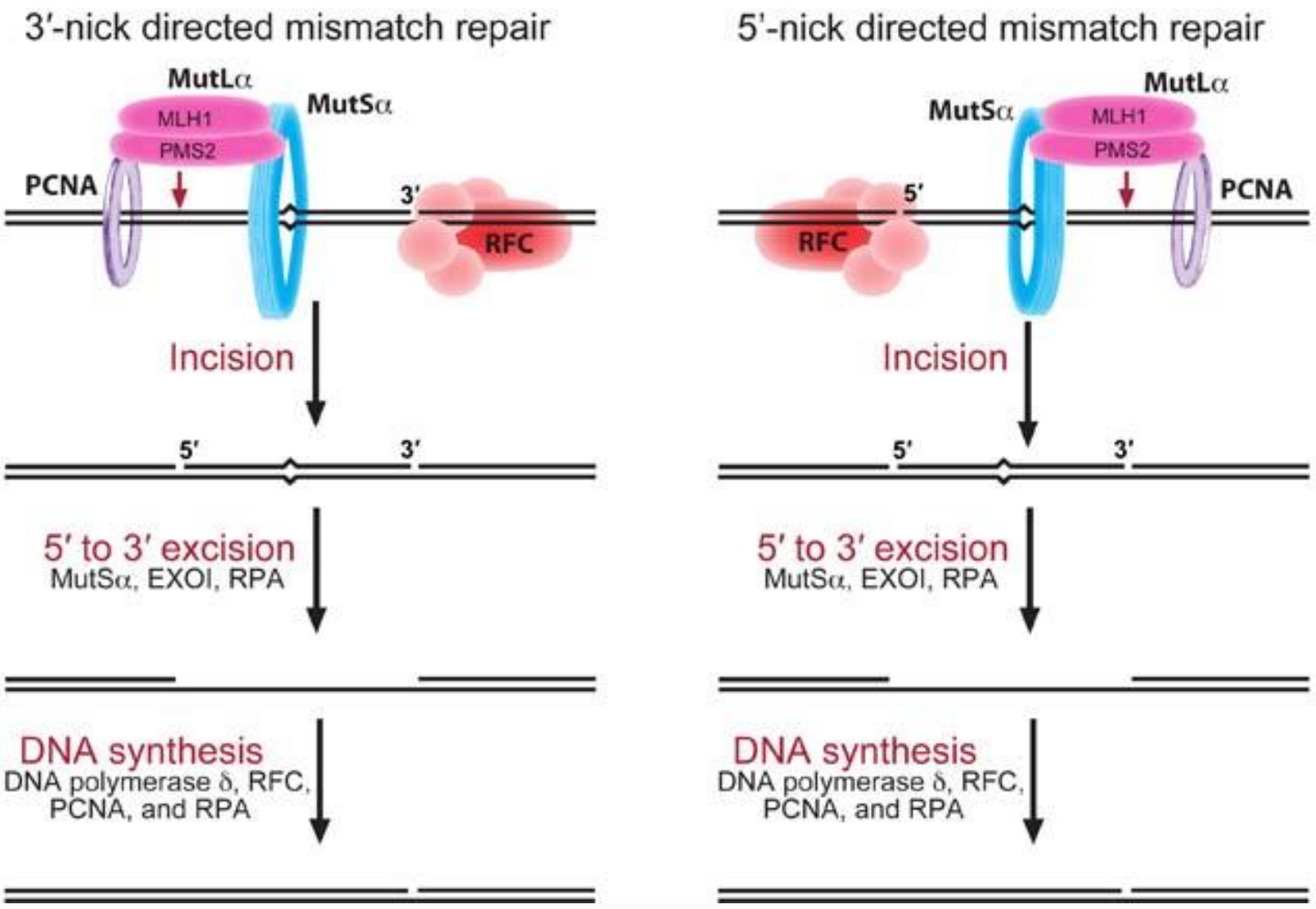


Mismatch Repair in E. coli



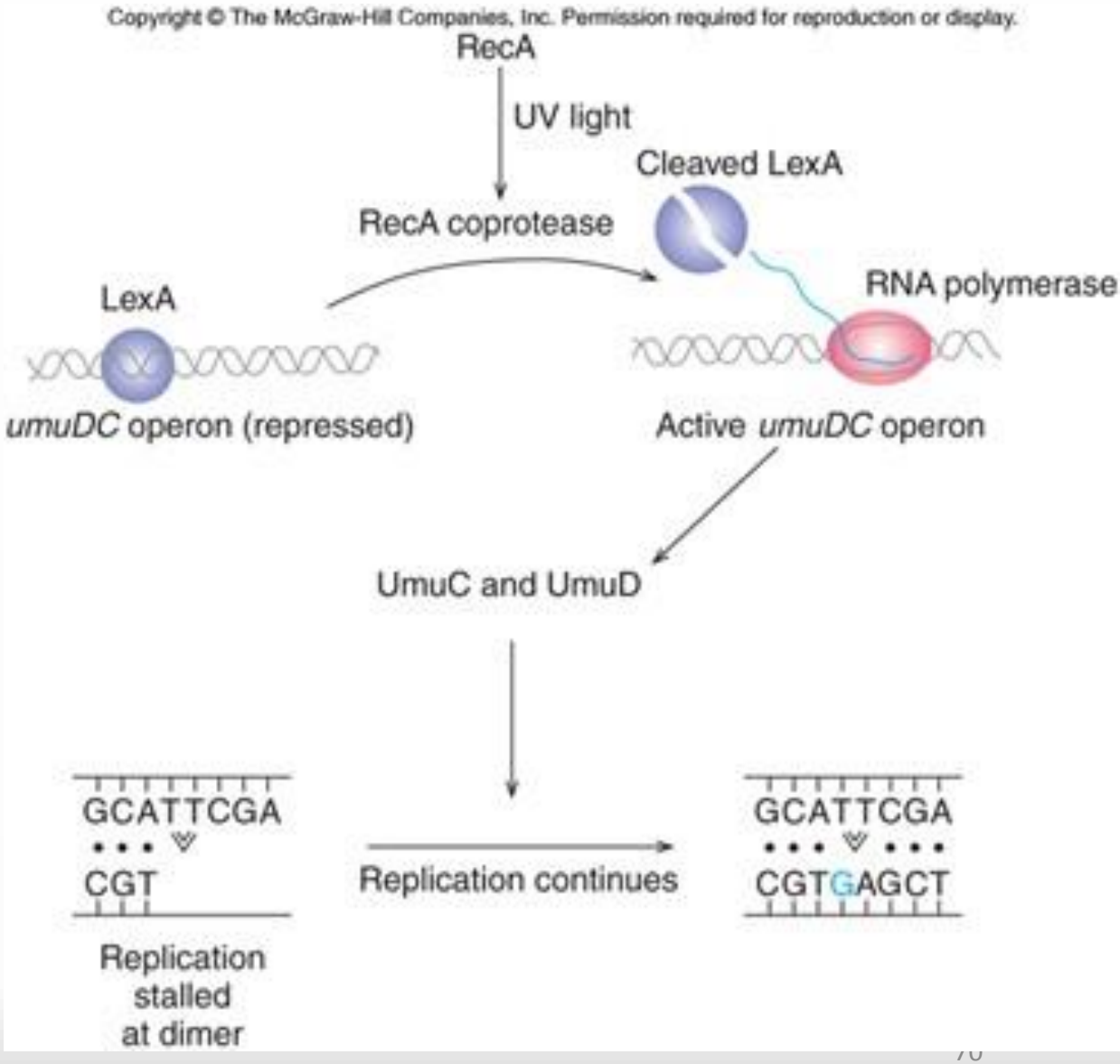
- MutS recognizes mismatches and binds to them to initiate the repair process.
- MutH and MutL join the complex.
- MutH cleaves the unmethylated strand at hemimethylated GATC sequences on either side of the mismatch.
- Excision requires MutS, MutL, MutU (DNA helicase II), and an exonuclease.
- DNA polymerase III fills in the gap, and DNA ligase seals the nick.

Human mismatch repair



Tolerant repair- error prone bipass

Error-Prone Bypass
Induce the **SOS response**
This causes DNA to replicate even though the damaged region cannot be read correctly
Result is errors in the newly made DNA



Genetic stability depends on DNA repair

- repair mechanisms are based on the existence of two copies of the genetic information in the DNA double helix
- corrupted strand is repaired according to the undamaged strand
- the corrupted strand is identified by abnormal DNA structures generated by errors (bulges, modified bases, mispaired..)
- **mutations in genes encoding repair proteins increase the frequency of further mutations, creating 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**	breast, ovarian
ataxia telangiectasia mutated	**ATM**	**Different mutations in *ATM* reduce HRR, SSA or NHEJ**	leukemia, lymphoma, breast
Nijmegen breakage syndrome	**NBS (NBN)**	**NHEJ**	lymphoid cancers
MRE11A	**MRE11**	**HRR and NHEJ**	breast
Bloom syndrome	**BLM (helicase)**	**HRR**	leukemia, lymphoma, colon, breast, skin, lung, auditory canal, tongue, esophagus, stomach, tonsil, larynx, uterus
WRN	**WRN**	**HRR, NHEJ, long patch BER**	soft tissue sarcoma, colorectal, skin, thyroid, pancreas
RECQL4	**RECQ4**	**Helicase likely active in HRR**	basal cell carcinoma, squamous cell carcinoma, intraepidermal carcinoma
Fanconi anemia genes FANCA,B,C,D1,D2,E,F,G,I,J,L,M,N	**FANCA etc.**	**HRR and TLS**	leukemia, liver tumors, solid tumors many areas
XPC, XPE (DDB2)	**XPC, XPE**	**Global genomic NER, repairs damage in both transcribed and untranscribed DNA**	skin cancer (melanoma and non-melanoma)
XPA, XPB, XPD, XPF, XPG	**XPA XPB XPD XPF XPG**	**Transcription coupled NER repairs the transcribed strands of transcriptionally active genes**	skin cancer (melanoma and non-melanoma)
XPV (also called polymerase H)	**XPV (POLH)**	**Translesion synthesis (TLS)**	skin cancers (basal cell, squamous cell, melanoma)
mutS H2, mutS homolog 6, mutL (*E. coli*) homolog 1, postmeiotic segregation increased 2 (*S. cerevisiae*)	**MSH2 MSH6 MLH1 PMS2**	**MMR**	colorectal, endometrial
mutY homolog (*E. coli*)	**MUTYH**	**BER of A paired with 8-oxo-dG**	colon
TP53	**P53**	**Direct role in HRR, BER, NER and acts in DNA damage response for those pathways and for NHEJ and MMR**	sarcomas, breast cancers, brain tumors, and adrenocortical carcinomas
NTHL1	**NTHL1**	**BER for Tg, FapyG, 5-hC, 5-hU in dsDNA**	Colon cancer, endometrial cancer, duodenal cancer, basal-cell carcinoma

DNA repair defects cause disease

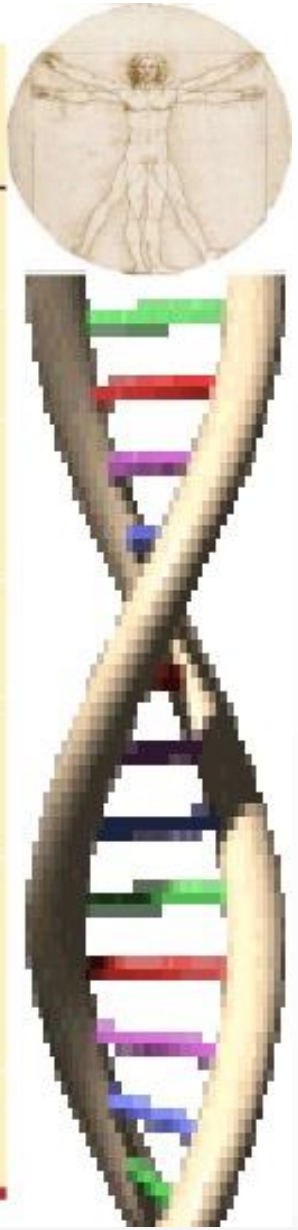
TABLE 23-1 Some Human Hereditary Diseases and Cancers Associated with DNA-Repair Defects

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.

Genetic diseases associated with defects in DNA repair systems

Disease	Symptoms	Genetic defect
Xeroderma pigmentosum	Frecklelike spots on skin, sensitivity to sunlight, predisposition to skin cancer	Defects in nucleotide-excision repair
Cockayne syndrome	Dwarfism, sensitivity to sunlight, premature aging, deafness, mental retardation	Defects in nucleotide-excision repair
Trichothiodystrophy	Brittle hair, skin abnormalities, short stature, immature sexual development, characteristic facial features	Defects in nucleotide-excision repair
Hereditary nonpolyposis colon cancer	Predisposition to colon cancer	Defects in mismatch repair
Fanconi anemia	Increased skin pigmentation, abnormalities of skeleton, heart, and kidneys, predisposition to leukemia	Possibly defects in the repair of interstrand cross-links
Ataxia telangiectasia	Defective muscle coordination, dilation of blood vessels in skin and eyes, immune deficiencies, sensitivity to ionizing radiation, predisposition to cancer	Defects in DNA damage detection and response
Li-Fraumeni syndrome	Predisposition to cancer in many different tissues	Defects in DNA damage response



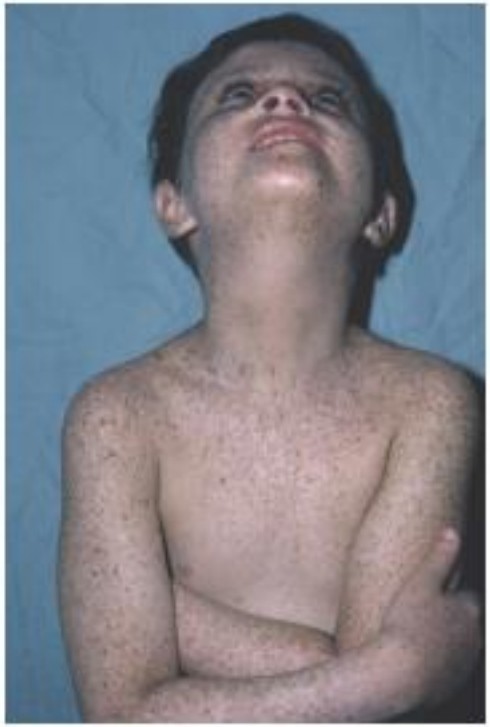
Hereditary diseases caused by disorders in DNA repair

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

- Errors in DNA repair (defective enzymes – helicases, nucleases, regulatory proteins…)
- 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 predisposition
- affects about 1 in 250,000 newborns
- defective DNA repair after UV radiation (most often aberration - 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 **nucleotide excision repair**



Hereditary diseases caused by DNA repair disorders

- **Cocayne syndrome** - stunted growth, impaired mental ability
- **trichothiodystrophy** - short limbs, brittle hair, scaly skin, psychomotoric retardation

Protein homology in between different species

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