

DNA damage, Mutations and DNA repair

part A

Biol 3005 Genome maintenance

Fall 2011

DNA Damage and Mutation

- DNA can be damaged in several different ways
 - modification of the bases
 - modification of the sugar-phosphate backbone
 - breaking of the sugar-phosphate backbone
- **DNA damage is not the same as mutation but if unrepaired it may lead to mutation**
- DNA damage involves an alteration to the chemical structure of DNA whereas mutation is a change in the sequence of the DNA

Why study DNA repair?

1. Sources of DNA damage

- ✓ Exogenous: sun, radon decay, tobacco, chemotherapy, various industrial chemicals etc.
- ✓ Endogenous: oxidative damage, spontaneous alterations, V(D)J recombination

2. DNA repair mechanisms

- ✓ Repair occurs by many different mechanisms
- ✓ Repair genes account for a significant fractions of the genome

3. DNA repair defects lead to genetic instability, which is a hallmark of cancer

- ✓ Genetic instability allows enough mutations to accumulate to cause cancer
- ✓ Genetic instability in cancer cells allows them to become resistant to treatment

The importance of DNA repair

DNA is:

- the repository of hereditary information
- the blueprint for operation of individual cells.

Nearly all DNA damage is harmful. Therefore, it is essential to reduce this damage to a tolerable level.

The importance of repair can be seen from the facts that

- DNA is the only biomolecule that is specifically ***repaired***. All others are ***replaced***.
- >100 genes participate in various aspects of DNA repair, even in organisms with very small genomes
- Cancer is caused by mutations. In most cases, "genetic instability" (elevated mutation rate) is required to permit accumulation of sufficient mutations to generate cancer during a human lifetime. DNA repair mechanisms promote genomic stability and prevent cancer. Many, perhaps most, cancers are at least partially attributable to defects in DNA repair.

Mutations

Mutation – an alteration in the nucleotide sequence of a DNA molecule

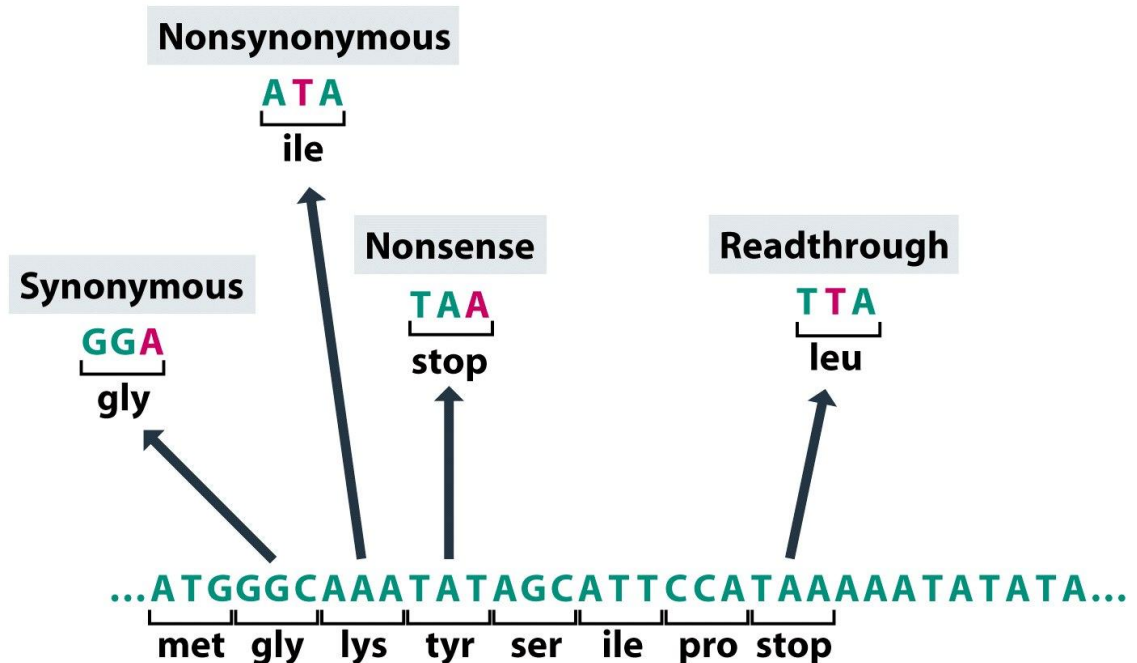
Types of mutations

- **Point mutations** (simple mutations or single-site mutations):
 - **categories:**
 - **Transitions** are purine-to-purine or pyrimidine-to-pyrimidine changes:
 - **A → G, G → A**
 - **C → T, T → C**
 - **Transversions** are purine-to-pyrimidine or pyrimidine-to-purine changes:
 - **A → C, A → T, G → C, G → T**
 - **C → A, C → G, T → A, T → G**
- **Large mutations:**
 - **deletions**
 - **Insertions**
 - **duplications**
 - **re-arrangements**

Types of point mutations

(based on their effects)

- **Synonymous** = **silent** – the new codon specifying the same amino acid as the unmutated codon.
- **Non-synonymous** = **missense** – mutation altering codons – and a protein will have an amino acid change.
- **Nonsense** – mutation converting a sense codon (coding for an amino acid) to a stop-codon.
- **Readthrough** – mutation converting a termination codon into one specifying an amino acid.



Frameshift mutations

Wild-type sequences

Amino acid	N-Phe	Arg	Trp	Ile	Ala	Asn-C
mRNA	5`-UUU	CGA	UGG	AUA	GCC	AAU-3`
DNA	3`-AAA	GCT	ACC	TAT	CGG	TTA-5`
	5`-TTT	CGA	TGG	ATA	GCC	AAT-5`

Frameshift by addition

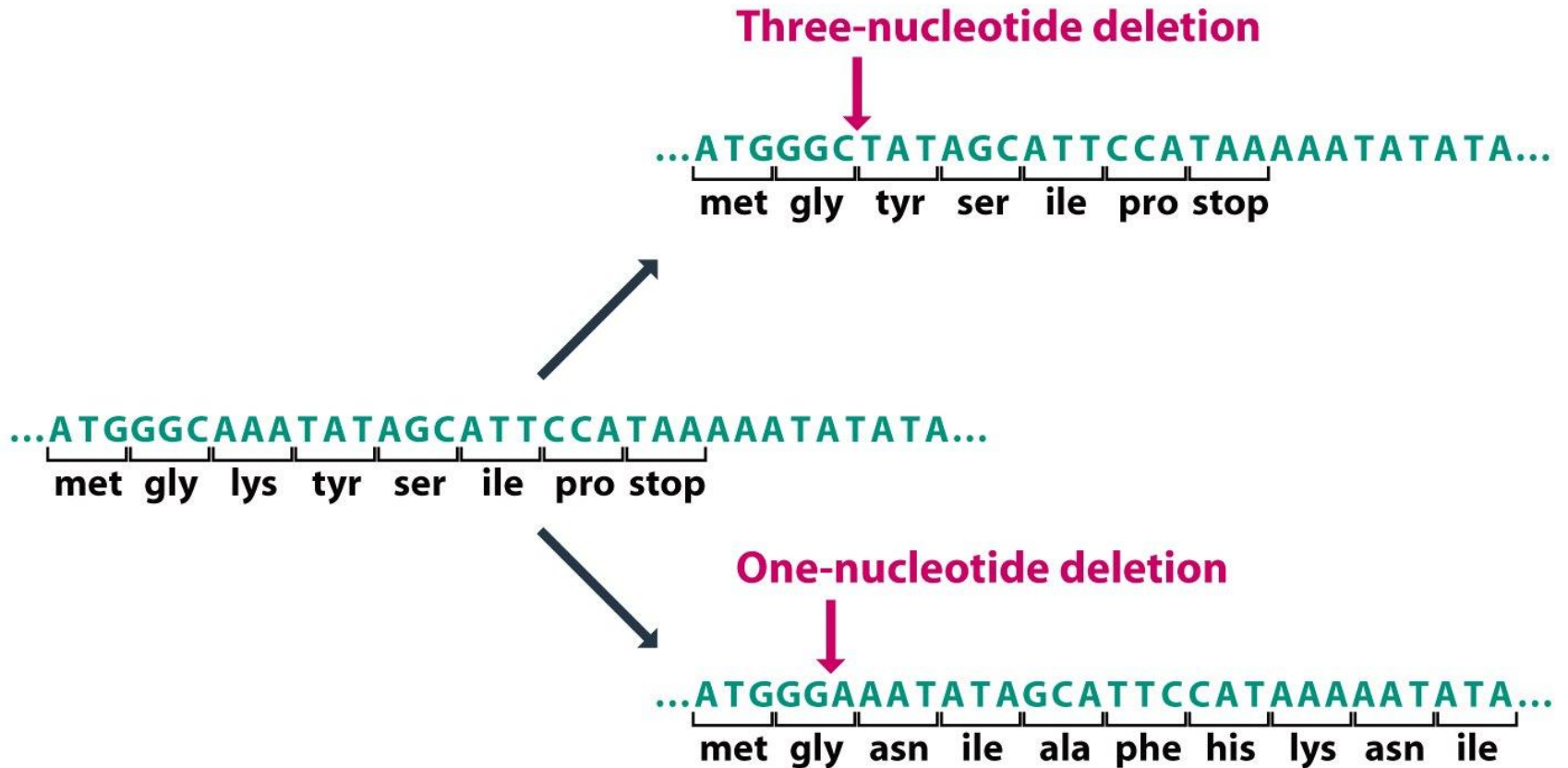
Amino acid	N-Phe	Arg	Trp	Tyr	Ser	Gln-C
mRNA	5`-UUU	CGA	UGG	TUT	AGC	CAU U-3`
DNA	3`-AAA	GCT	ACC	ATA	TCG	GTT A-5`
	5`-TTT	CGA	TGG	TAT	AGC	CAT T-5`

Frameshift by deletion

Amino acid	N-Phe	Gly	Stop
mRNA	5`-UUU	GGA	UAG-3`
DNA	3`-AAA	CCT	ATC GGT TA-5`
	5`-TTT	▲GGA	TAG CCA AT-5`
		[GCTA]	
		[CGAT]	

Deletion and insertion mutations have distinct capabilities on coding capacities of the genes. May result in **frameshift**.

Deletion mutations

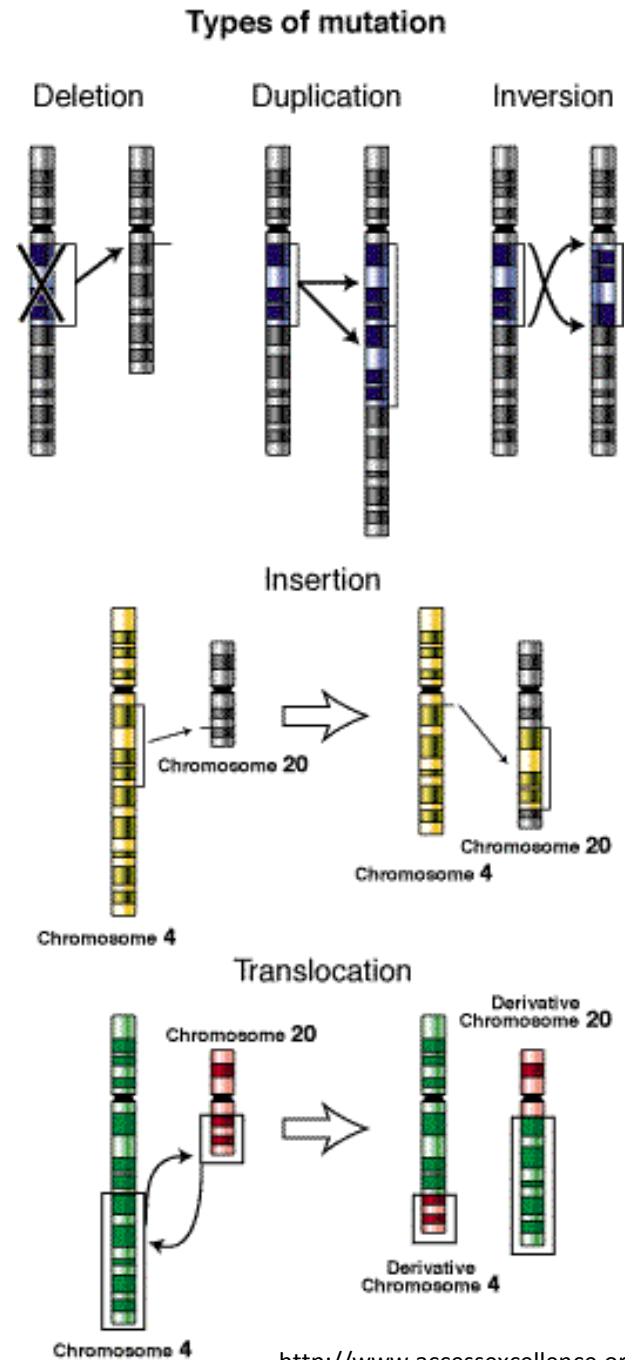


Insertion or deletion of 3 nucleotides (or its multiple) does not result in frameshift.

Large mutations

- **Chromosomal mutation:**

- Deletions
- Insertions
- Duplications
- Re-arrangements:
 - inversions
 - translocations



The effects of mutations

Silent mutations – have no effect on the organism.

Mutations in coding regions are much more important.

Loss-of-function mutation:

Diploid organisms (eukaryotes) - **mutation is usually recessive, since a functional copy of the gene is present in the homologous chromosome.**

Haploid organisms (prokaryotes) – **mutation is usually conditional-lethal.**

Gain-of-function mutations are less common and occur in regulatory sequences rather than in coding regions.

Mutations in introns and intron-exon boundaries – effects on splicing.

Origins of replication can be made non-functional by mutations, but these possibilities are not well documented.

Mutations: random or programmed?

The causes of mutations

Mutation can arise in two ways:

Some mutations are **spontaneous**

Other mutations arise because a **mutagen** has reacted with the parent DNA, causing a structural change that affects the base-pairing capability of the altered nucleotide.

- **Errors in replication**
- **Mutagens**

Replication errors

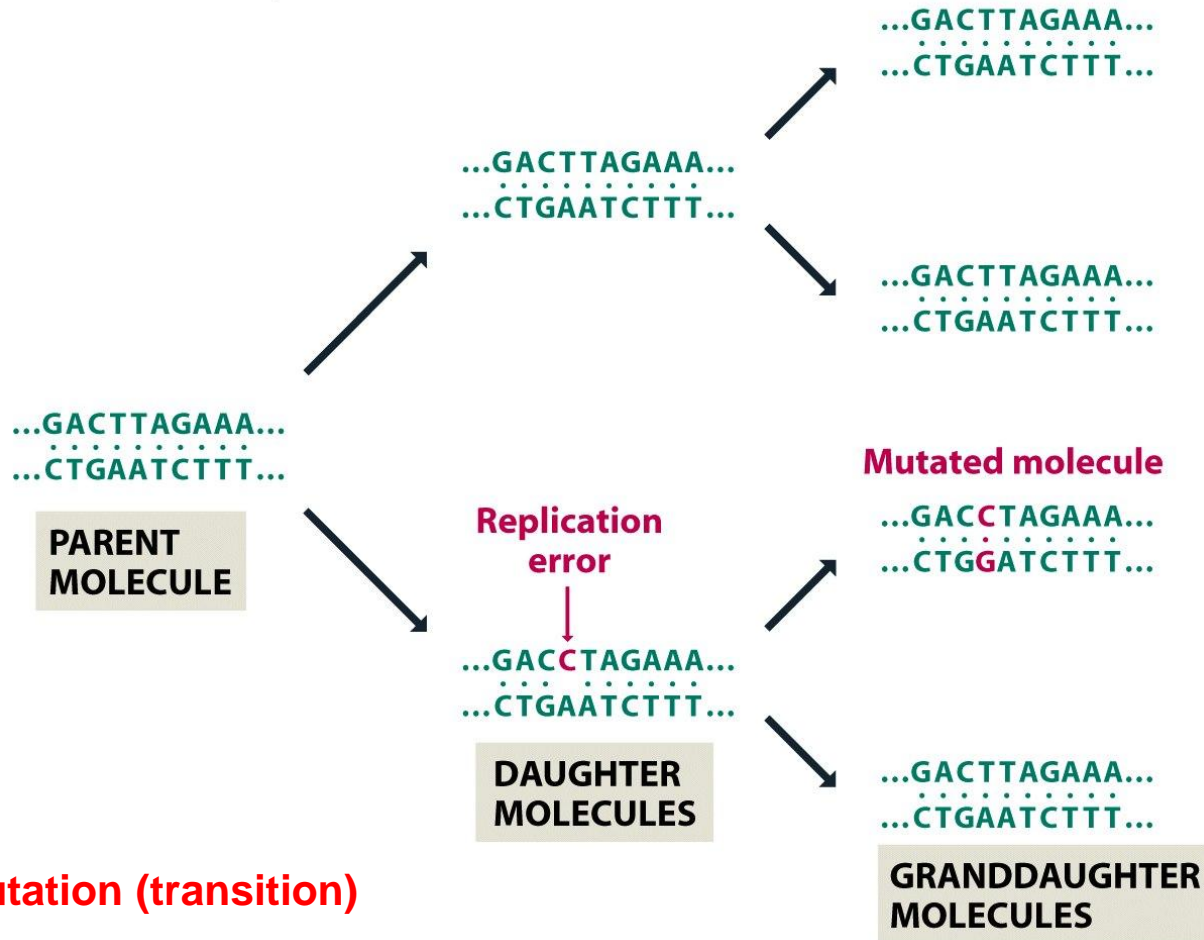
Errors in replication are sources of point mutations. Replication errors can also lead to insertion and deletion mutations resulting in frameshift.

- **Misincorporation**
- **Tautomers**
- **Replication slippage**

Misincorporation - an error in replication

Misincorporation leads to a mismatch in one of the daughter double helices

An error in replication



TA → CG mutation (transition)

Mechanisms for ensuring the accuracy of DNA replication

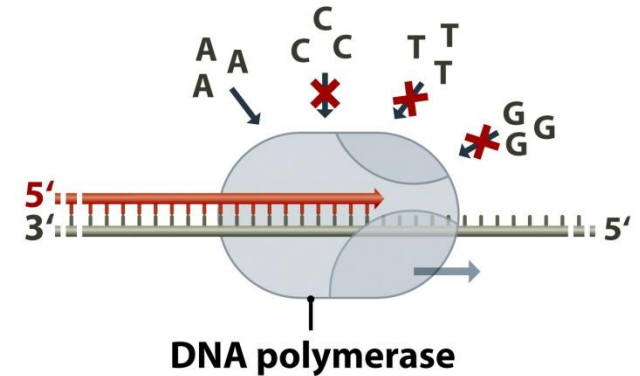
Nucleotide selection process (discrimination against incorrect nucleotide) probably acts at three different stages during the polymerization reaction:

- when the nucleotide is first bound to DNA polymerase,
- when it is shifted to the active site of the enzyme,
- and when it is attached to the 3' end of the polynucleotide that is being synthesized.

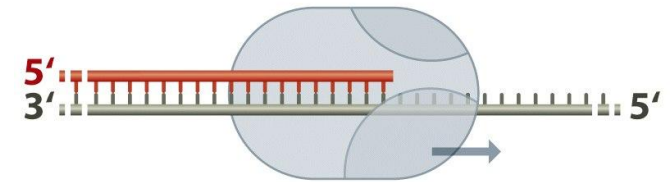
Proofreading: removal of an incorrect nucleotide via a 3'→5' exonuclease activity of DNA polymerase:

- active checking process?
- competition between DNA synthesis and exonuclease activities

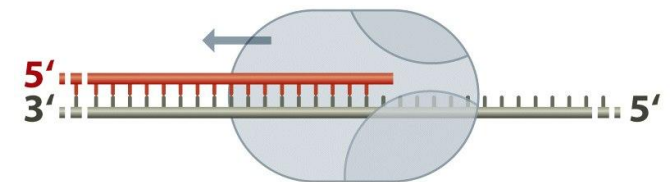
Nucleotide selection



"Proofreading"

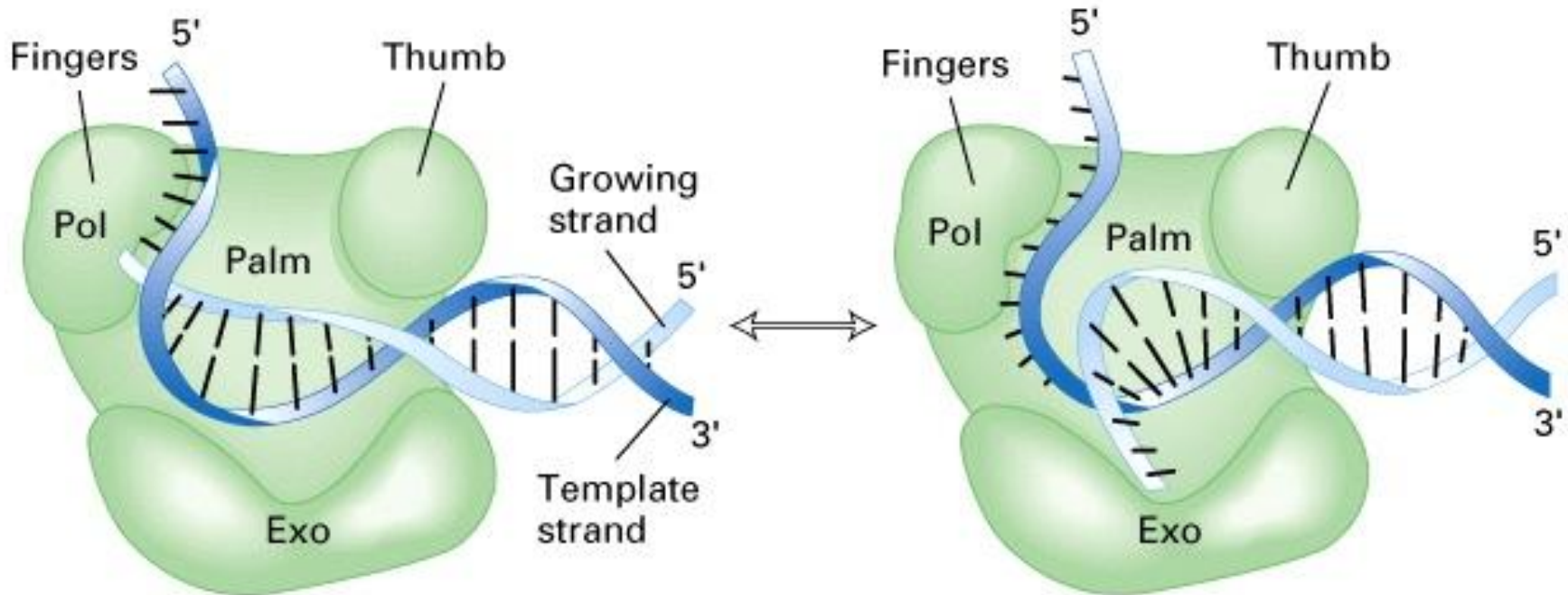


Last nucleotide is base-paired
POLYMERASE WINS



Last nucleotide is not base-paired
EXONUCLEASE WINS

Schematic model of the proofreading function of DNA polymerase



The polymerase is usually winning because it is more active than exonuclease, at least when the 3' terminal nucleotide is base-paired to the template.

But the polymerase activity is less efficient if the terminal nucleotide is not base-paired, the resulting pause in polymerization allowing the exonuclease activity to predominate so the incorrect nucleotide is removed.

Tautomers

structural isomers of bases

Some nitrogen bases exists as two tautomers:

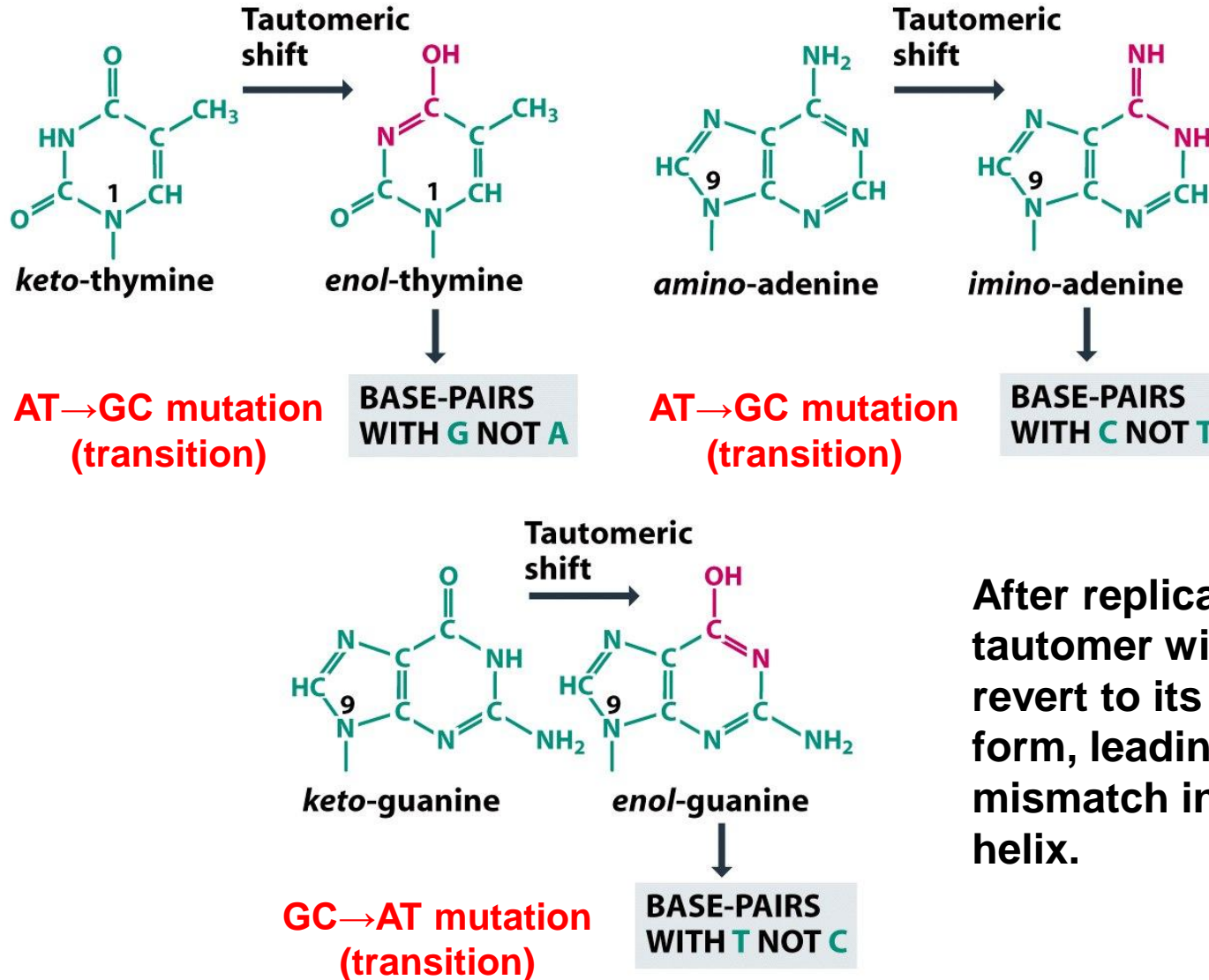
- *keto* and *enol* forms for thymine and guanine
- *amino* and *imino* for adenine

Individual molecules occasionally undergo a shift from one tautomer to another.

The equilibrium is biased very much towards *keto/amino* forms.

The two tautomeric forms of the base have different pairing properties.

The effects of tautomerism on base pairing



After replication rare tautomer will inevitably revert to its more common form, leading to a mismatch in the double helix.

Replication slippage

Normal DNA replication

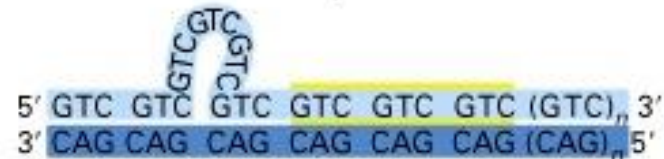


Single-stranded loop

Error in DNA replication

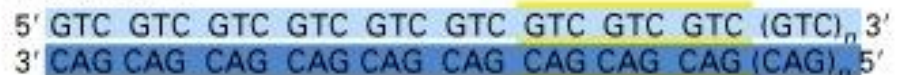


Synthesis completed



Second round of replication

Mutant DNA



+

Wild-type DNA



Trinucleotide repeat expansion diseases

Table 16.1 Examples of human trinucleotide repeat expansions

Locus	Repeat sequence		Associated disease
	Normal	Mutated	
Polyglutamine expansions (all in coding regions of genes)			
<i>HD</i>	(CAG) _{6–35}	(CAG) _{36–121}	Huntington's disease
<i>AR</i>	(CAG) _{9–36}	(CAG) _{38–62}	Spinal and bulbar muscular atrophy
<i>DRPLA</i>	(CAG) _{6–35}	(CAG) _{49–88}	Dentatorubral-pallidoluysian atrophy
<i>SCA1</i>	(CAG) _{6–39}	(CAG) _{39–82}	Spinocerebellar ataxia type 1
<i>SCA3</i>	(CAG) _{12–40}	(CAG) _{55–84}	Machado–Joseph disease
Fragile site expansions (both in the untranslated leader regions of genes)			
<i>FRM1</i>	(CGG) _{6–53}	(CGG) _{60–over 230}	Fragile X syndrome
<i>FRM2</i>	(GCC) _{6–35}	(GCC) _{61–over 200}	Fragile XE mental retardation
Other expansions (positions described below)			
<i>DMPK</i>	(CTG) _{5–37}	(CTG) _{50–3000}	Myotonic dystrophy
<i>X25</i>	(GAA) _{7–34}	(GAA) _{34–over 200}	Friedreich's ataxia

The *DMPK* and *X25* expansions are in the trailer and intron regions of their genes, respectively, and are thought to affect RNA processing. There are also a few disease-causing mutations that involve expansions of longer sequences, for example progressive myoclonus epilepsy caused by a (CCCCGCCCGCG)_{2–3} to (CCCCGCCCGCG)_{over 12} expansion in the promoter region of the *EPM1* locus.

Studies in yeast : mutations in *Rad27* gene (coding for FEN1). This might indicate that a trinucleotide repeat expansion is caused by an aberration in lagging strand synthesis.

The causes of mutations

- Errors in replication
- **Mutagens:**
 - **Chemical mutagens**
 - **Physical mutagens**

Chemical mutagens

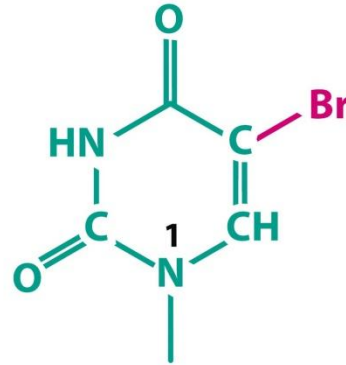
- **Base analogs**
- **Intercalating agents**
- **Factors causing chemical modifications of bases**
- **Reactive oxygen species**

Base analogs

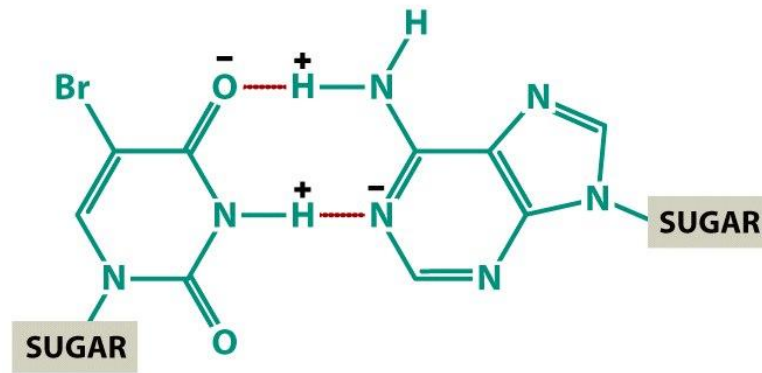
- **Base analogs** are purine and pyrimidine bases that are similar enough to the standard bases to be incorporated into nucleotides when these are synthesized by cells. The resulting unusual nucleotides can then be used as substrates for DNA synthesis during replication.
- E.g – **5-bromouracil** and **2-Aminopurine**

5-Bromouracil and its tautomerism

5-Bromouracil

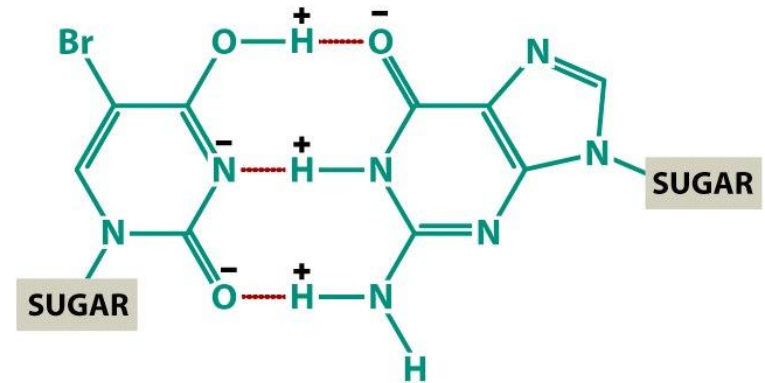


Base pairing with 5-bromouracil



5-Bromouracil
keto form

Adenine

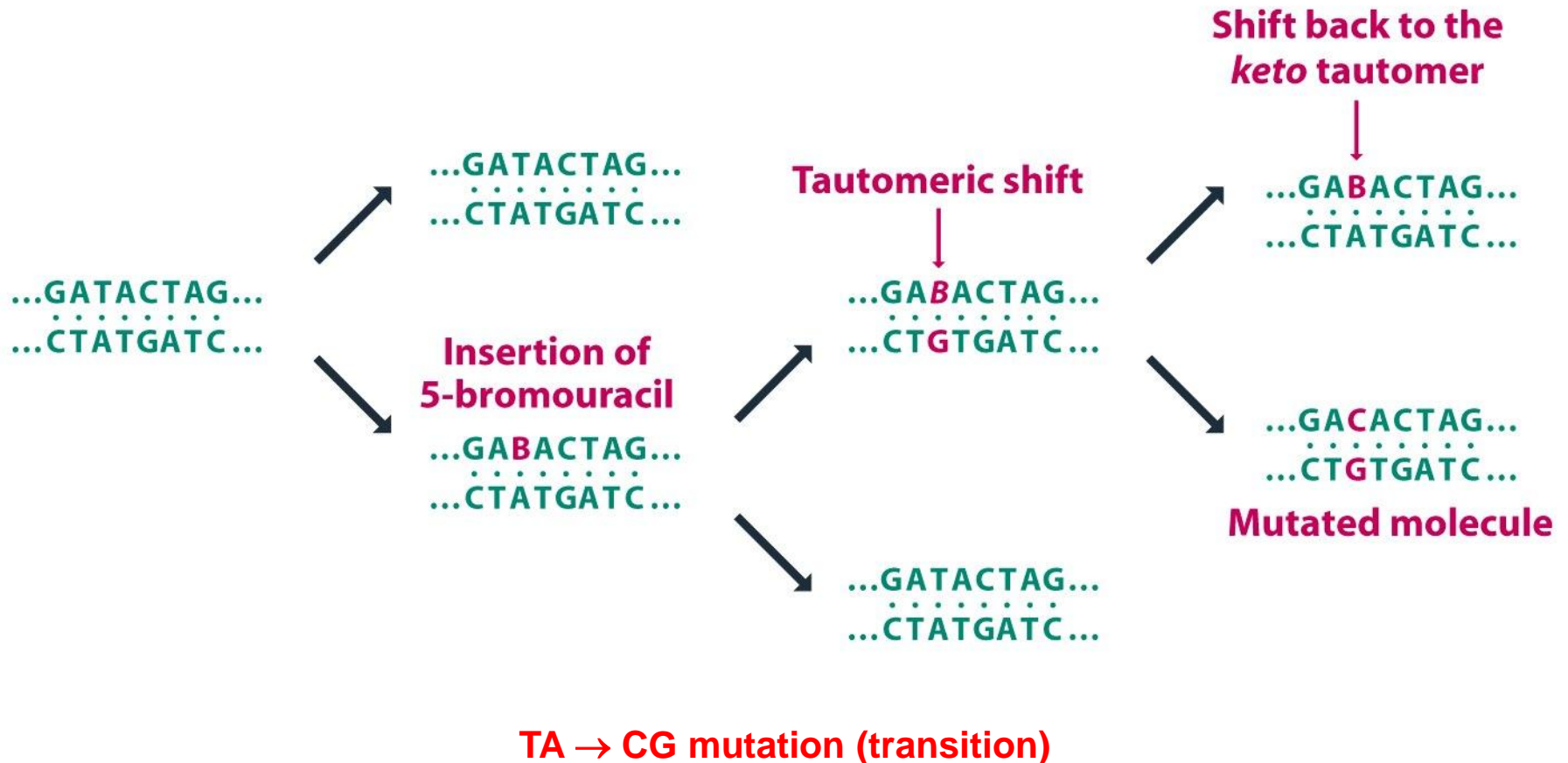


5-Bromouracil
enol form

Guanine

5-bromouracil and its mutagenic effect

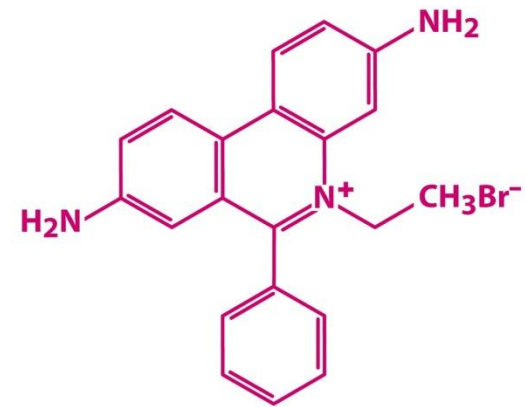
The mutagenic effect of 5-bromouracil



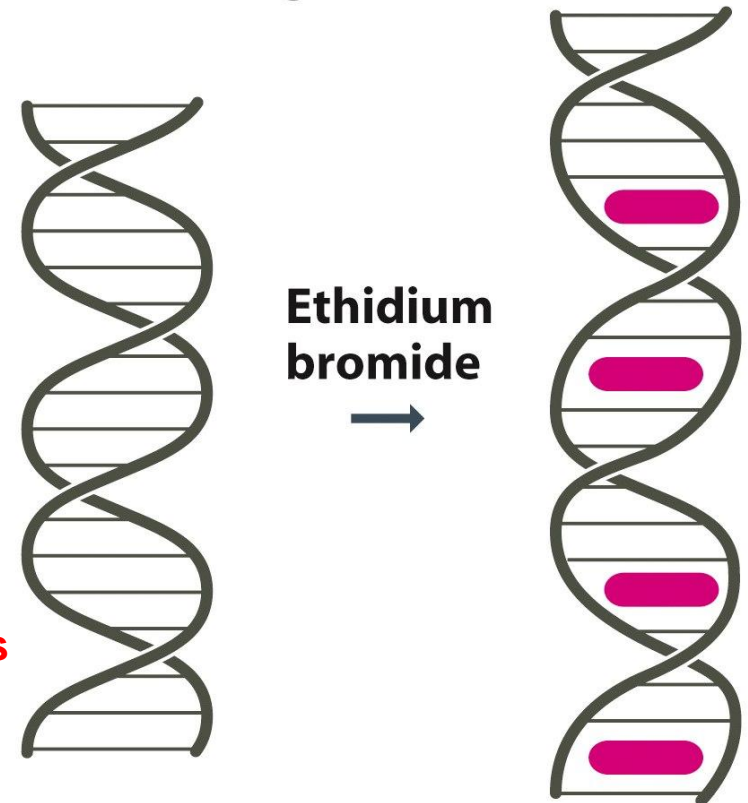
Intercalating agents

- **Ethidium bromide** – causes insertion mutations.
- It can slip between the base pairs in the double helix, slightly unwinding the helix and increasing the distance between adjacent base pairs.

Ethidium bromide



The mutagenic effect

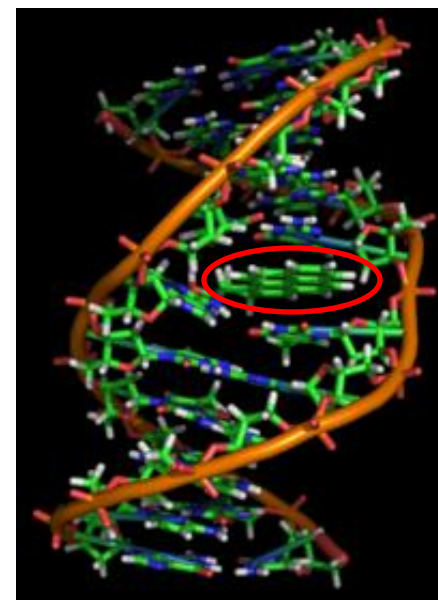


Small insertions or deletions → frameshift mutations

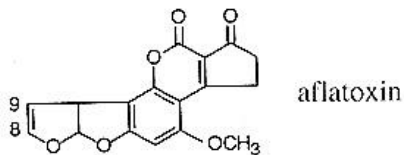
DNA adducts formation

Aflatoxin and **Benzo(a)pyrene** are incorporated into DNA during DNA synthesis by formation of covalent bonds with purines (chemical modification of bases). Due to their large size they are also considered as intercalating agents.

A DNA adduct of benzo[a]pyrene is the major mutagen in **tobacco smoke**.

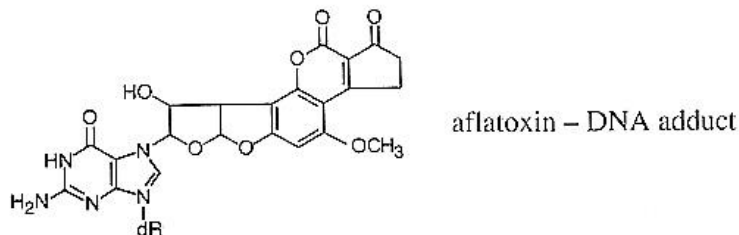


Base adduct: aflatoxin

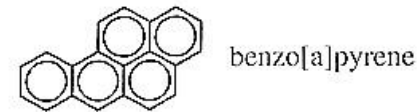


⇓ liver activation

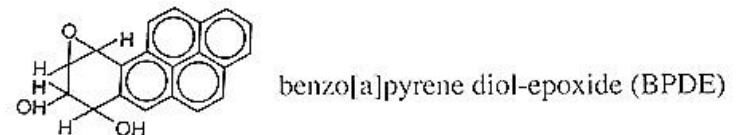
⇓ DNA incorporation



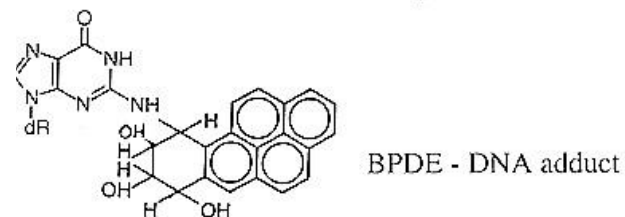
Base adduct: benzo[a]pyrene



⇓ metabolic activation



⇓ DNA incorporation



Factors causing chemical modifications of bases

- **Alkylating agents**
- **Deaminating agents**
- **DNA depurination (base loss)**

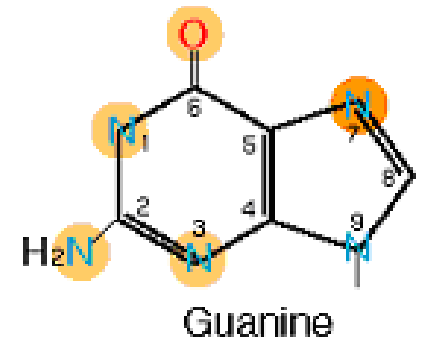
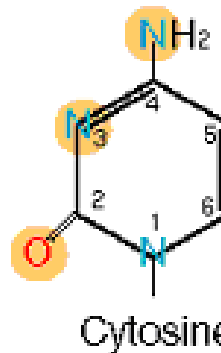
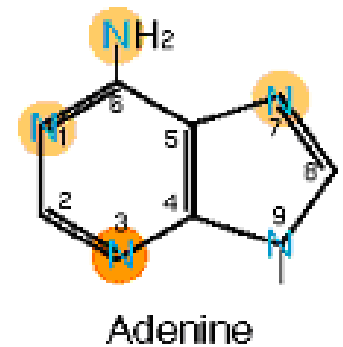
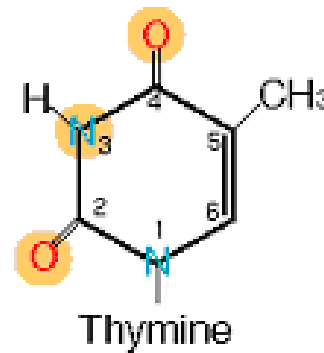
Alkylation of bases

Many environmental chemicals, including "natural" ones (frequently in the food we eat) can also modify DNA bases, frequently by addition of **a methyl (methylation)** or other **alkyl group (alkylation)**.

In addition, normal metabolism frequently leads to alkylation.

S-adenosylmethionine (SAM), the normal biological methyl group donor, reacts accidentally with DNA to produce alkylated bases like 3-methyladenine at a rate of several hundred per day per mammalian haploid genome.

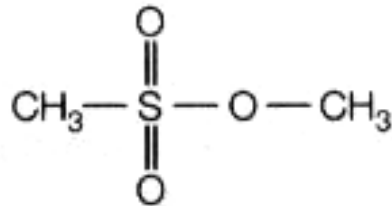
Alkylation occurs most readily at the nucleophilic positions shown in this diagram (the darker orange circles denote the most reactive positions).



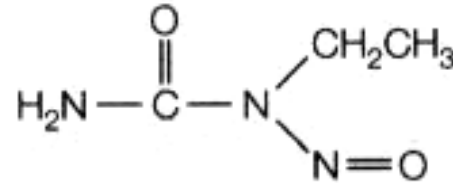
Based on Fig. 1-32 in Friedberg, Walker and Siede

Examples of alkylating agents and alkylated bases

Alkylating agents

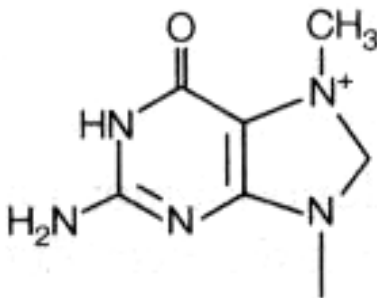


(MMS) methylmethane sulfonate

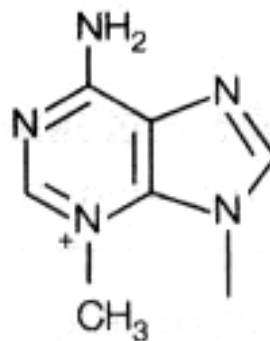


ethylnitrosamine

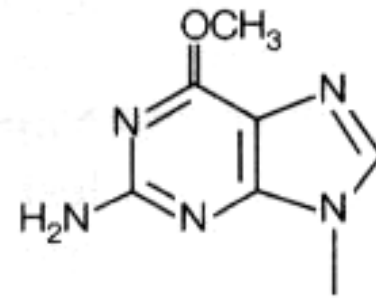
Methylated bases



7-methylguanine



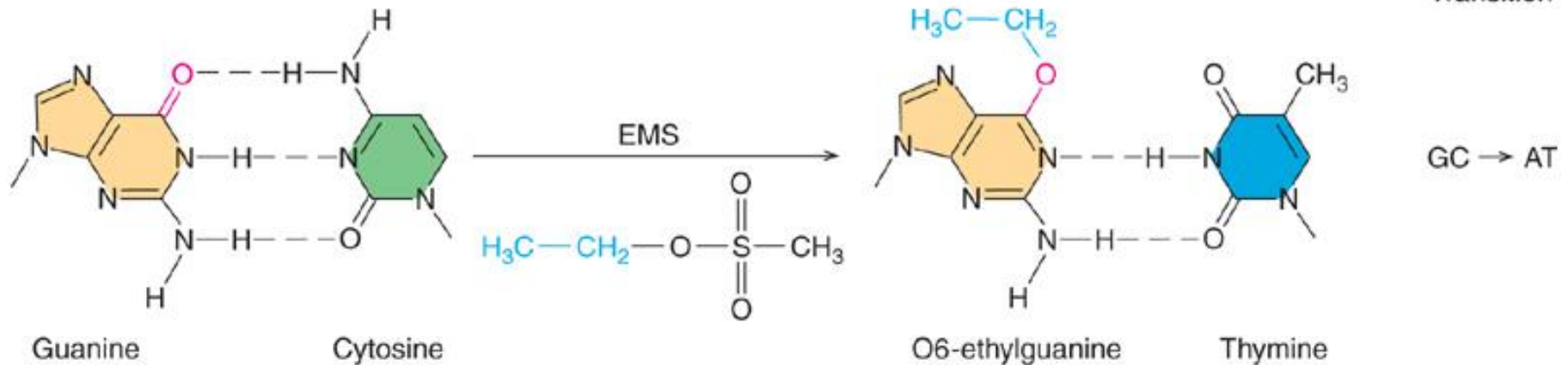
3-methyladenine



O⁶-methylguanine

Ethylation of Guanine O₆ by EMS

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Alkylating agent: ethylmethane sulfonate (EMS)

Effect: Hydrogen bonding potential of the O₆ position has been altered

A class of chemotherapeutics (anti-cancer drugs) work by alkylating DNA bases and thus can:

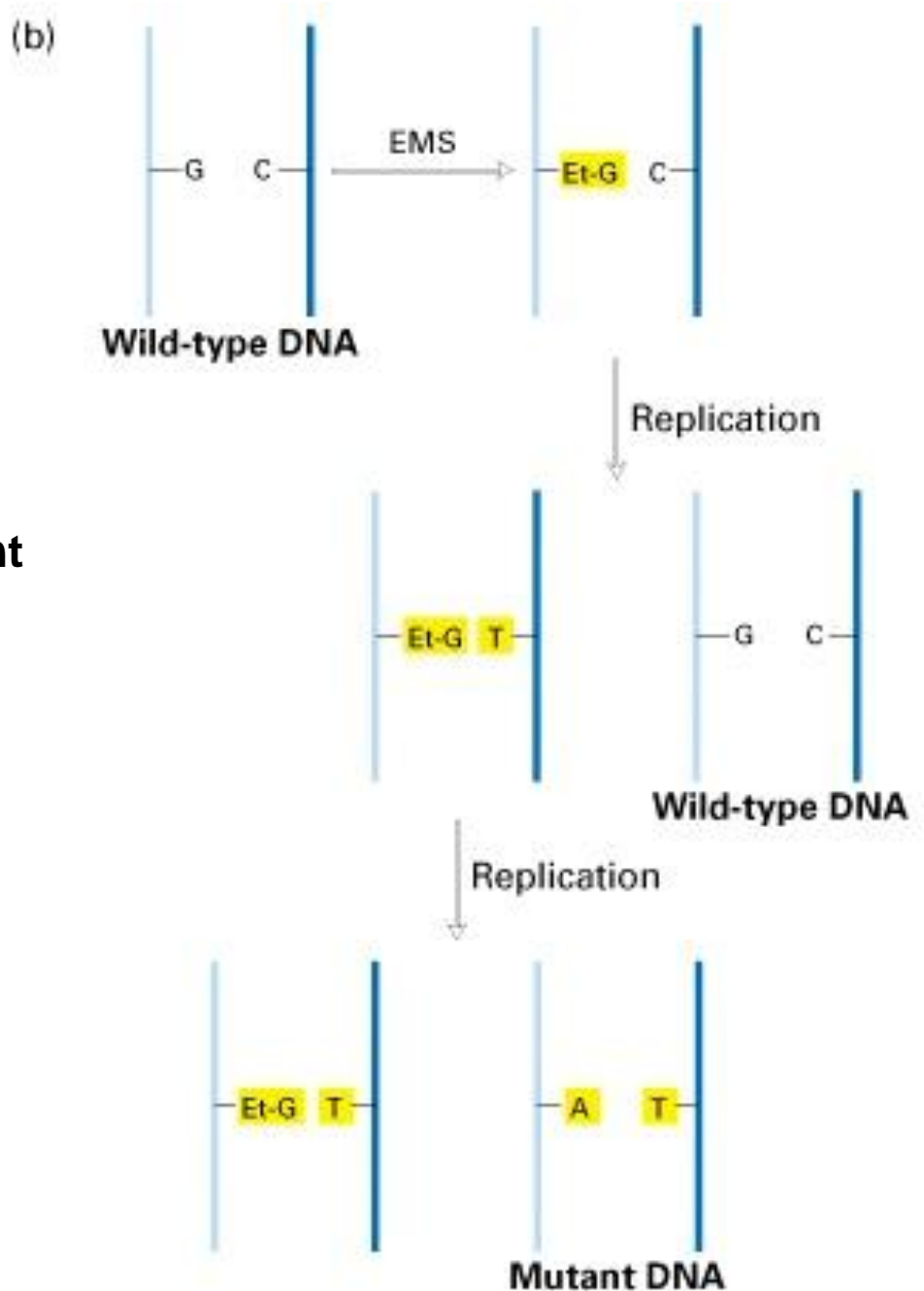
1) inhibit DNA replication (replication stalling) and 2) cause hypermutations in actively dividing cells

- both result in cytotoxicity (cell death)

Induction of point mutations by EMS

Scientists may induce mutations in experimental organisms by treatment with chemical mutagens (e.g. EMS) or exposure to ionizing radiation

GC→AT mutation (transition)



Base deamination

There is a certain amount of base deamination occurring spontaneously, but the rate increases with certain chemicals – **nitrous acid** – it deaminates A,C and G and **sodium disulphate** that acts only on C.

Deamination of G is not mutagenic – as resulting base – xanthine – blocks replication when it appears in the template polynucleotide.

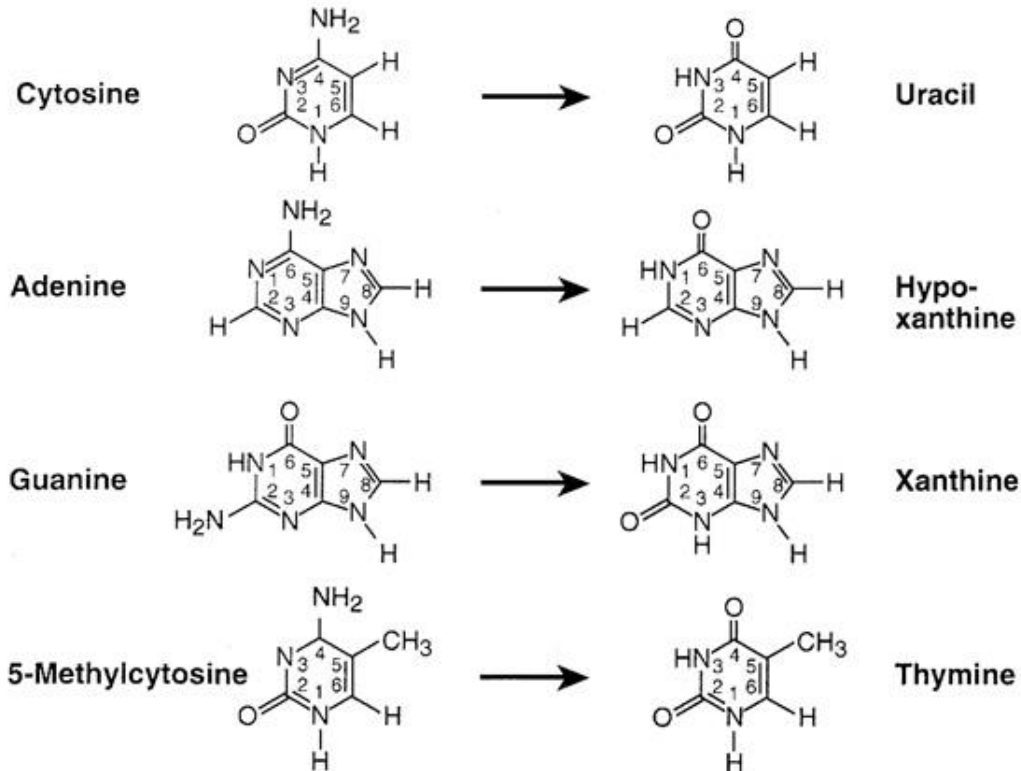
Deamination of A gives hypoxanthine – it pairs with C

Deamination of C give uracil – which pairs with A rather than with G

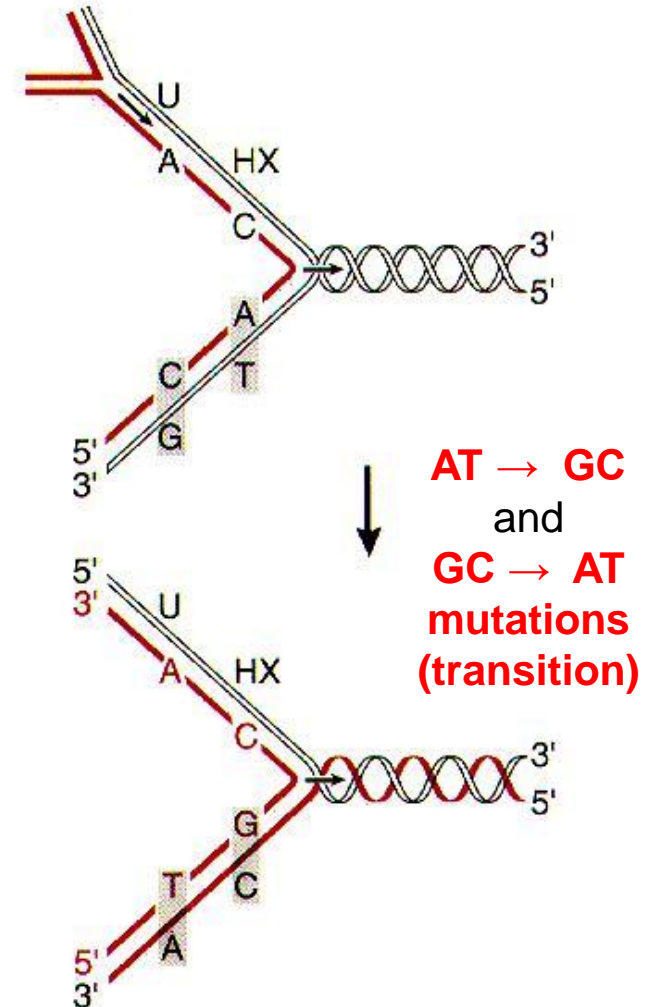
Deaminations of these two bases therefore result in point mutations when template strand is copied.

Base deamination and its mutagenic effect

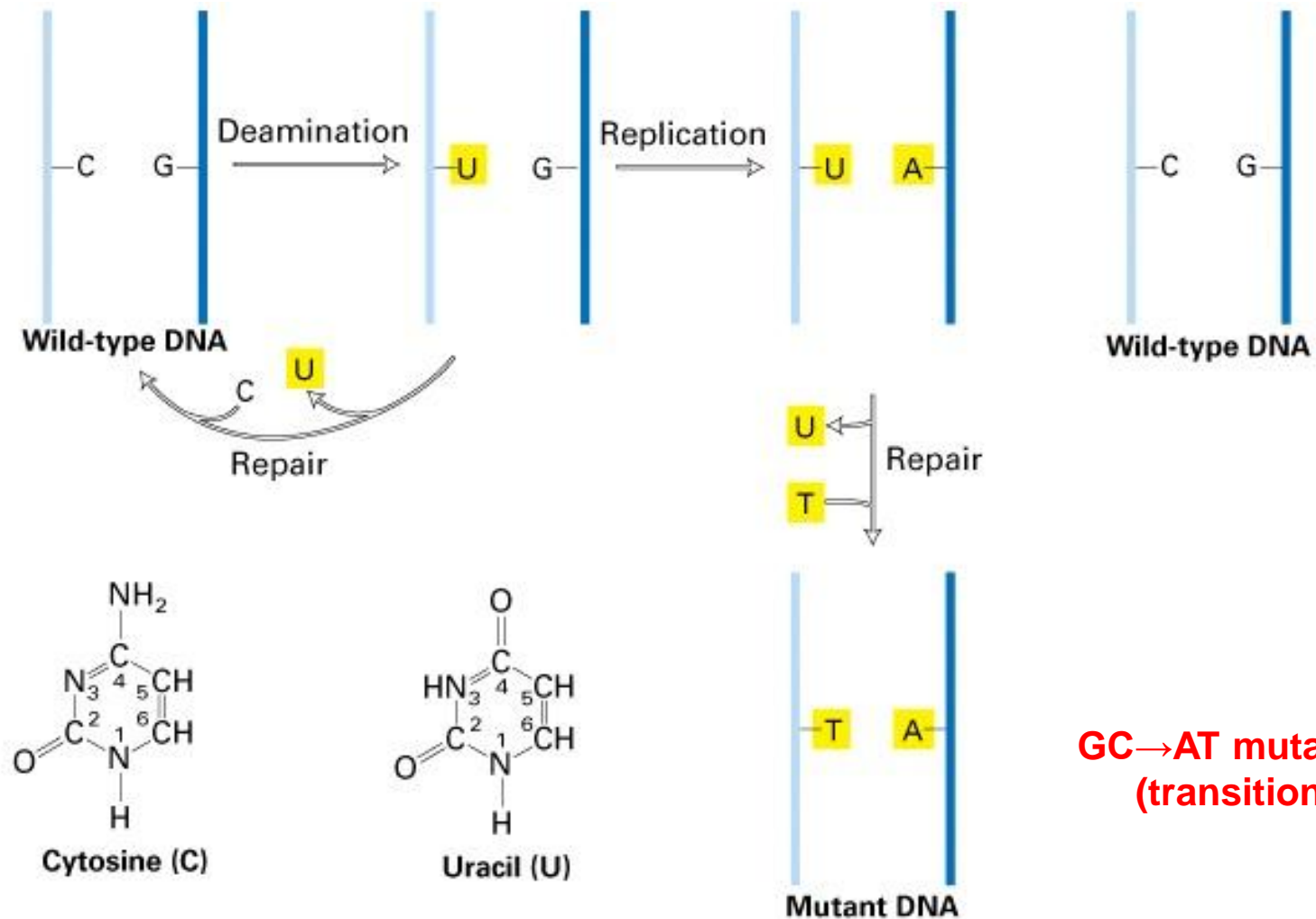
Mispaired DNA: deamination



Mutations caused by base deamination



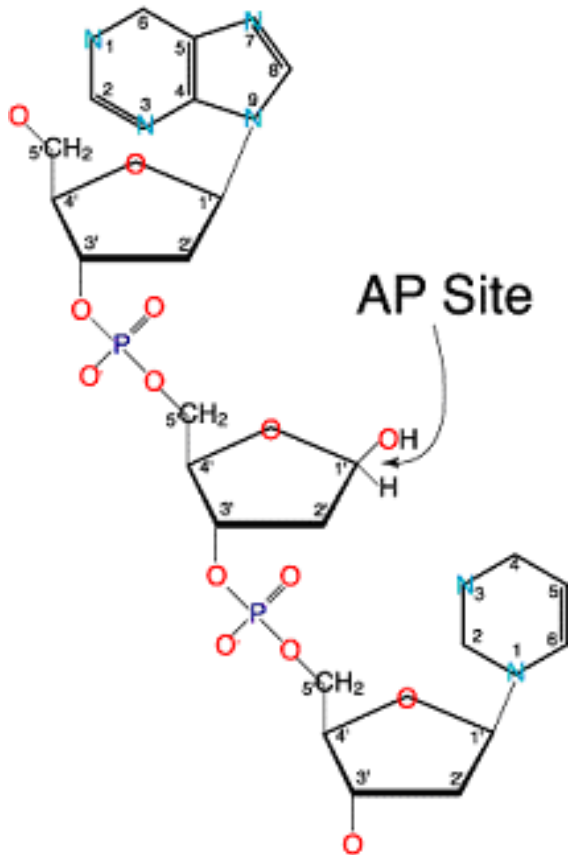
Base deamination leads to point mutations



**GC → AT mutation
(transition)**

DNA depurination (base loss)

Cleavage of the beta-N-glycosidic bond that attaches the base to the sugar component



Base loss

The glycosyl bond linking DNA bases with deoxyribose is labile under physiological conditions. Within a typical mammalian cell, several thousand purines and several hundred pyrimidines are spontaneously lost per haploid genome per day. Loss of a purine or pyrimidine base creates an **apurinic/apyrimidinic (AP)** site (also called an **abasic** site).

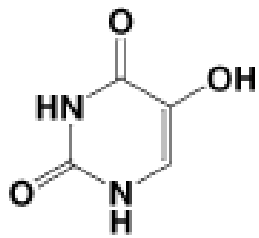
DNA depurination is usually a spontaneous modification.

Reactive oxygen species

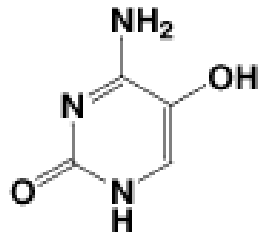
Mitochondrial respiration generates free radicals and reactive oxygen species. They are also produced as result of ionizing and non-ionizing radiation.

- **ROS (*reactive oxygen species*):**
 - **superoxide ($\cdot\text{O}_2^-$)**
 - **Hydrogen peroxide (H_2O_2)**
 - **Hydroxyl radicals ($\cdot\text{OH}$)**
- **Effects:**
 - **single-strand breaks**
 - **base modification (hydroxylation) and damage**

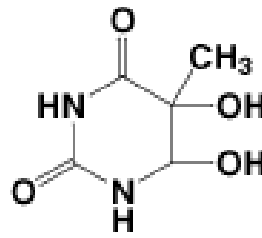
Base modifications caused by hydroxyl radicals



5-hydroxy-dU

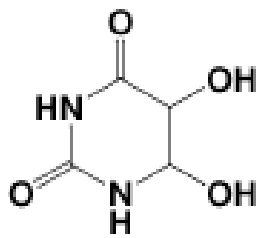


5-hydroxy-dC

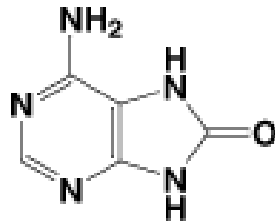


thymine glycol

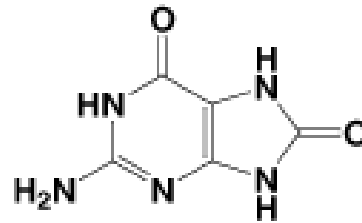
pyrimidine C₅=C₆ is particularly susceptible to hydroxylation



uracil glycol

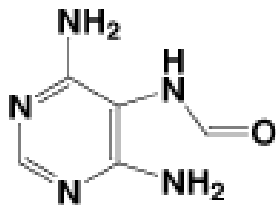


8-oxo-dA

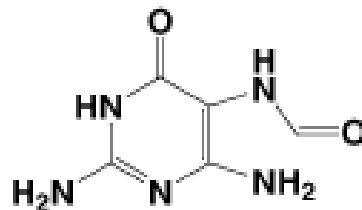


8-oxo-dG

purine C₈ is susceptible to hydroxylation and these species can tautomerize (transfer of H between the N₇ and oxygen)



Fapy-dA

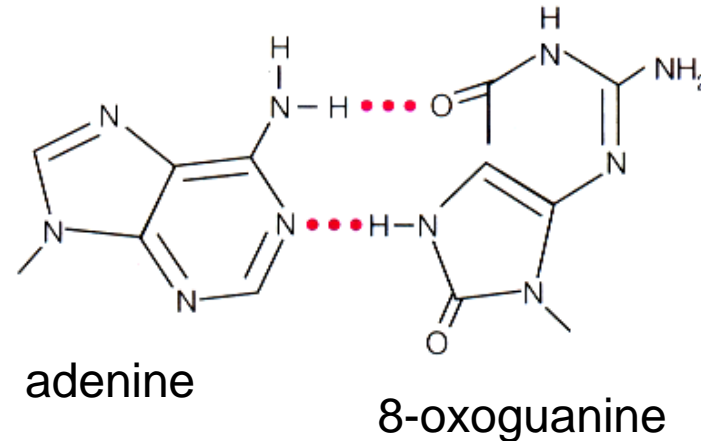


Fapy-dG

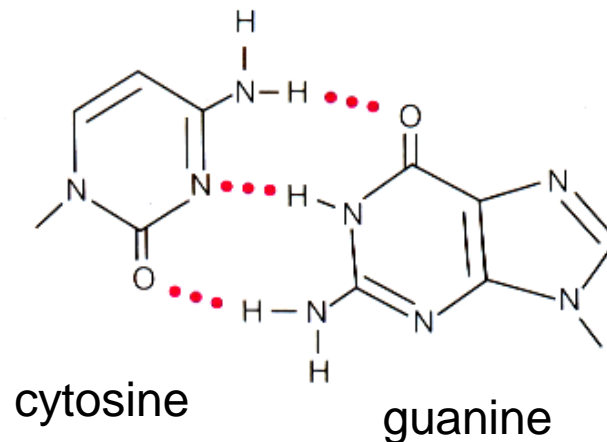
Base damage: breaks in the purine rings

Fapy = formamidopyrimidine

Mutagenic effects of base hydroxylation



**GC→TA mutation
(transversion)**

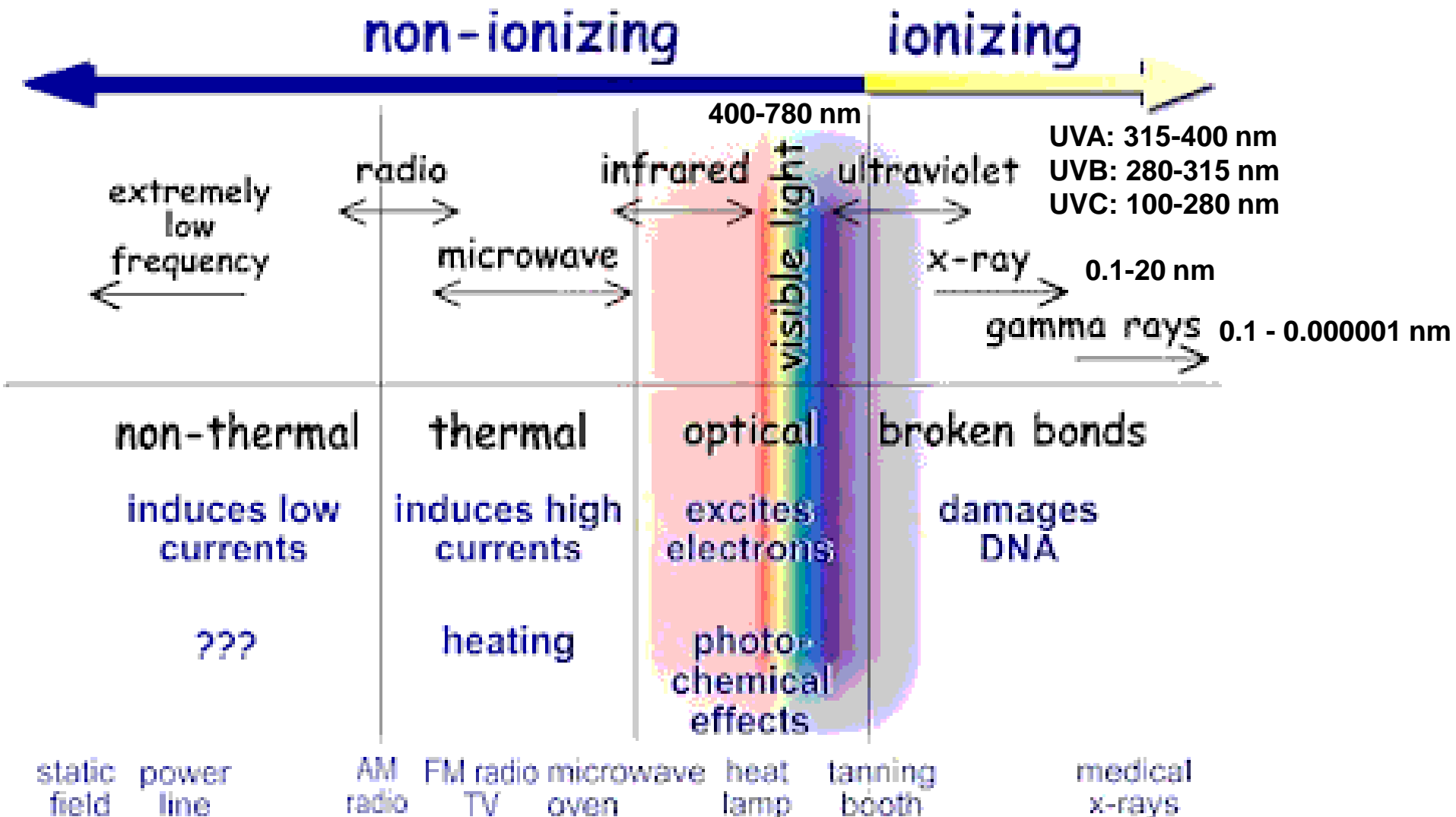


Effect: Altered base-pairing interactions are induced such as **oxoG–A** pairs during DNA replication which can eventually lead to a G-C base pair (original) converting to a T-A base pair (mutant)

Physical mutagens

- **Ionizing radiation** (X and γ radiation)
- **Non-ionizing radiation** (UV radiation)
- **Heat**

Different types of electromagnetic radiation



Ionizing radiation

- **High energy radiation:**
 - X and γ radiation
- **Effect:**
 - nonspecific: ionization of cellular compounds, leads to generation of ROS
- **DNA damage by ionization:**
 - Base and sugars damage
 - Crosslinks
 - Strand breaks

Damage by ionizing radiation - *Base damage*

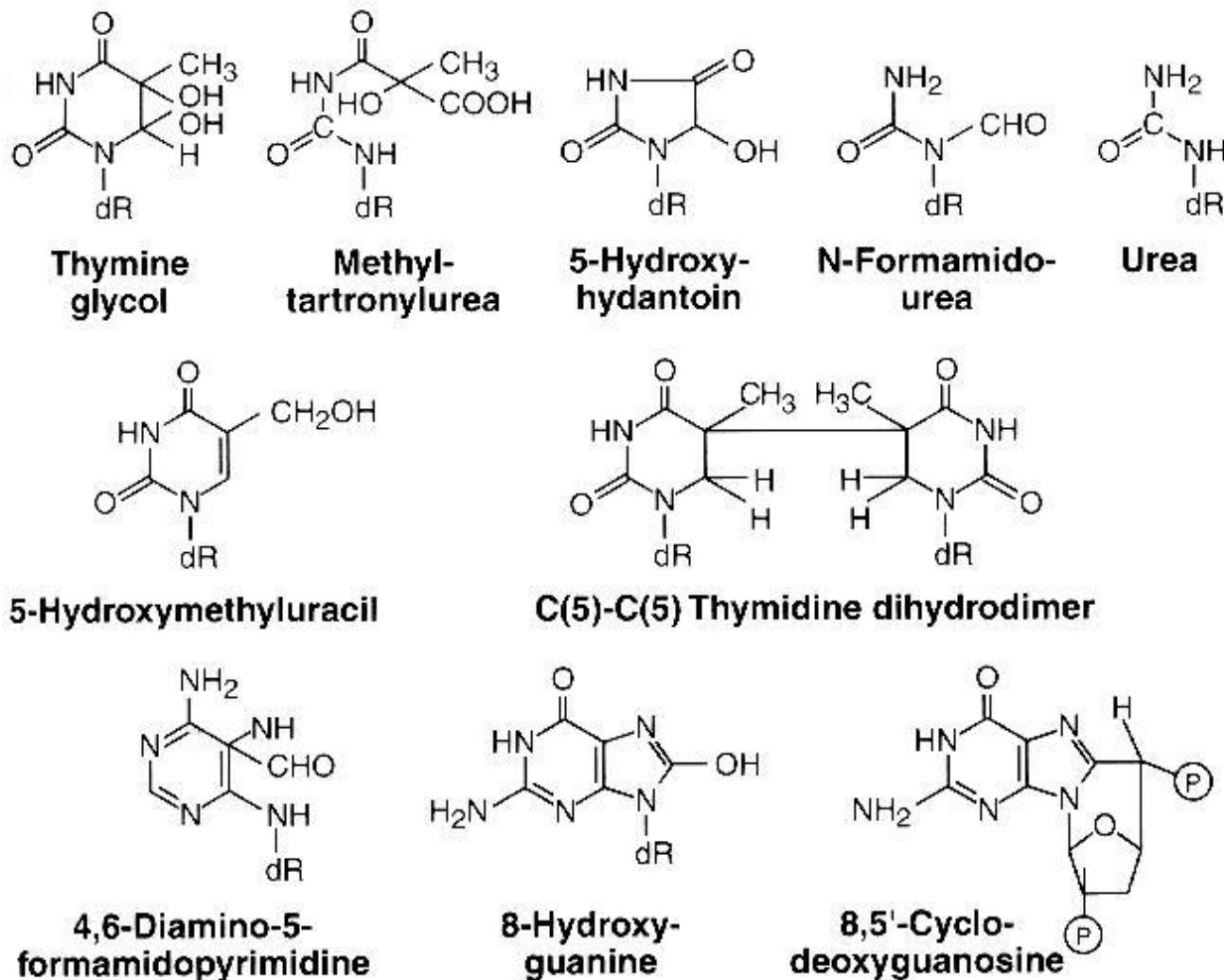


Figure 1-19 Examples of base damage induced by ionizing radiation.

Damage by ionizing radiation

- Backbone damage

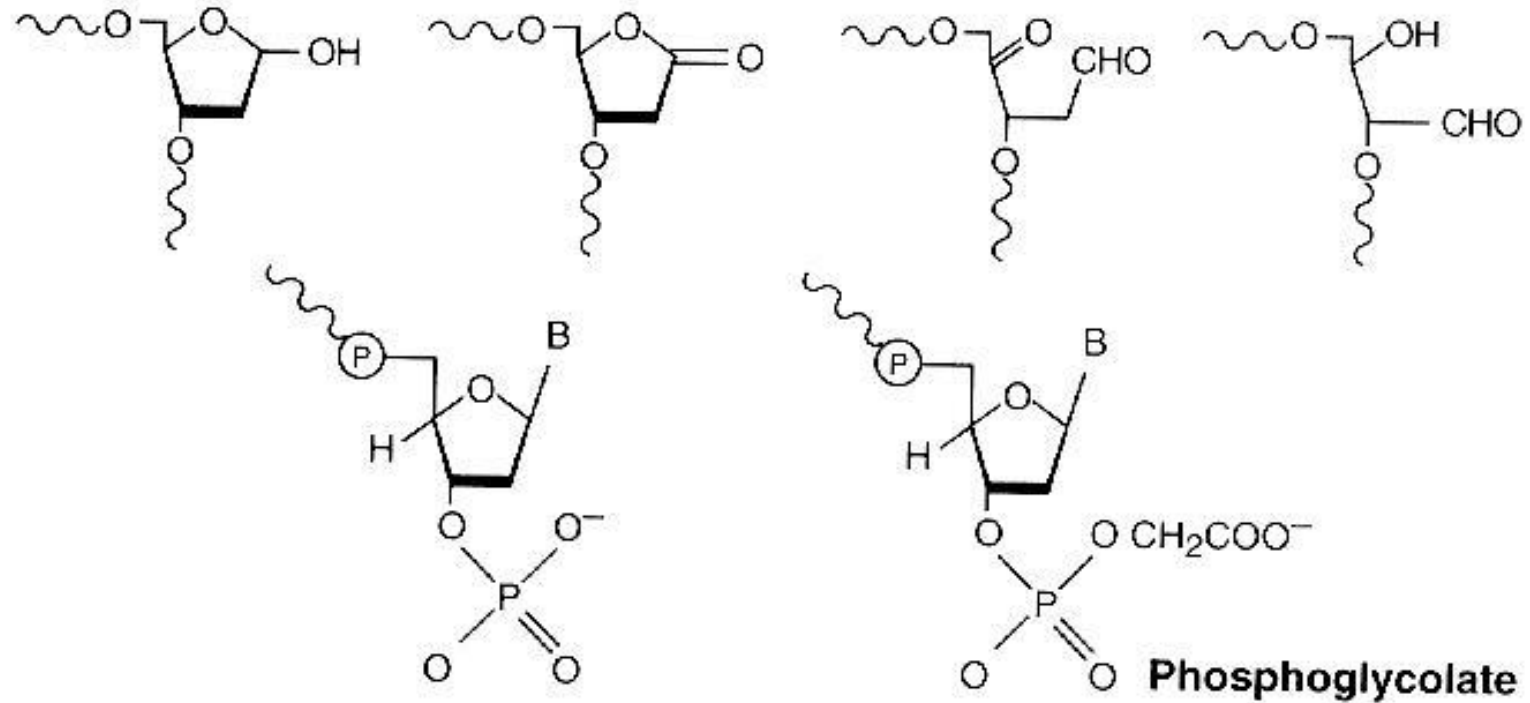


Figure 1-20 Examples of damage to the sugar-phosphate moieties induced by ionizing radiation.

Damage by ionizing radiation

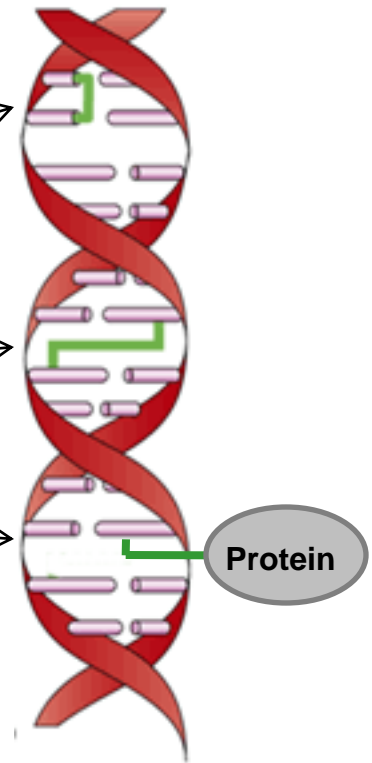
- *Crosslinks and strand breaks*

- **Crosslinks:**

- Intra-strand crosslinks
- Inter-strand crosslinks
- DNA-protein crosslinks

Crosslinks may be also generated by UV light and bifunctional alkylating agents (e.g. psoralen)

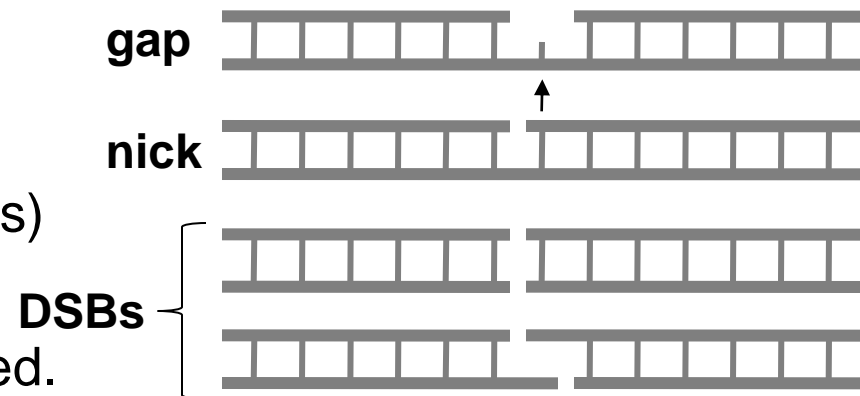
Cisplatin has similar effect on DNA as ionizing radiation (used for cancer therapy and in DNA damage/repair studies)



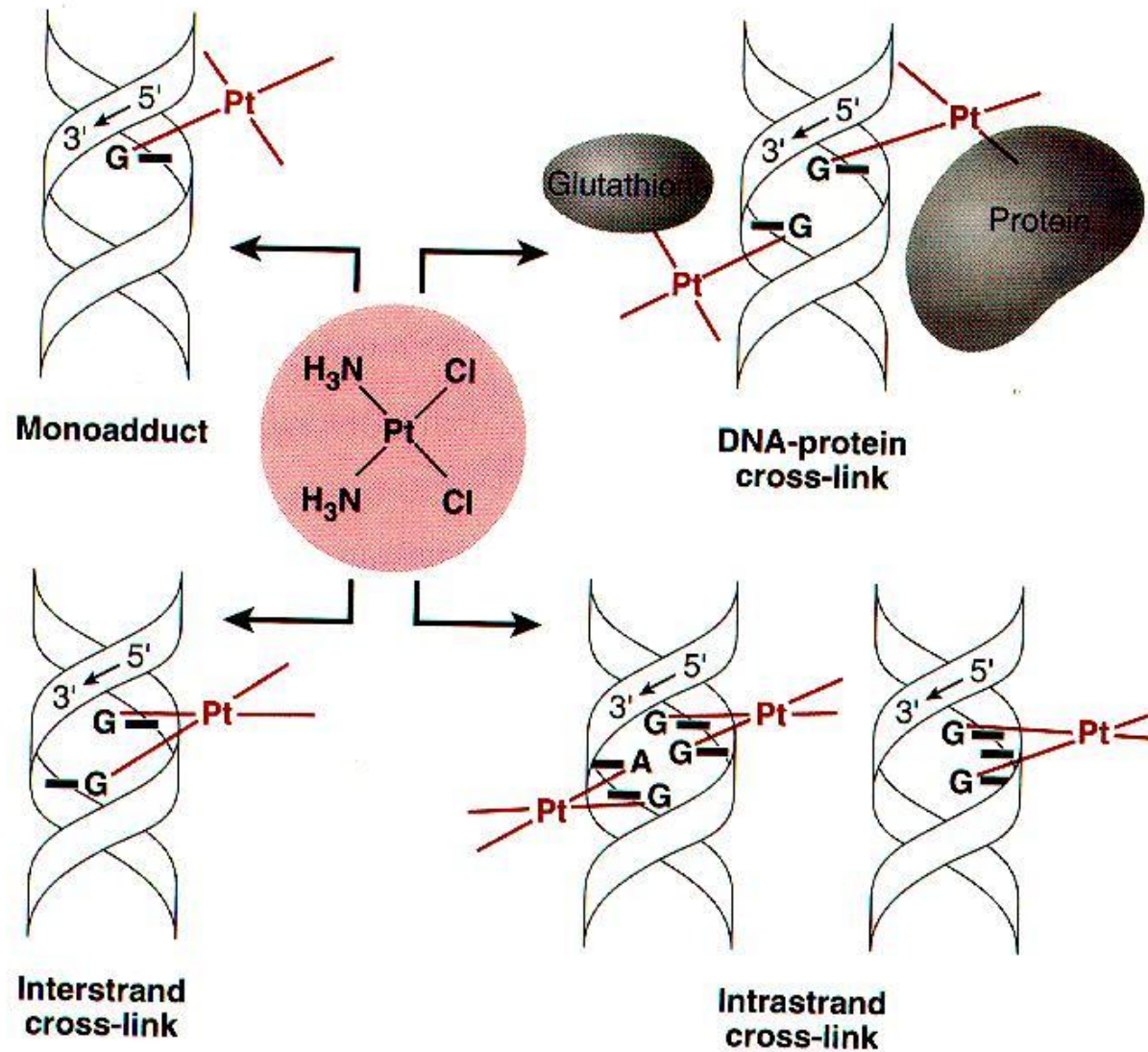
- **Strand breaks:**

- single-strand breaks (SSBs, nicks)
- double-strand breaks (DSBs)

Both types of break must be repaired.



Cisplatin crosslinks



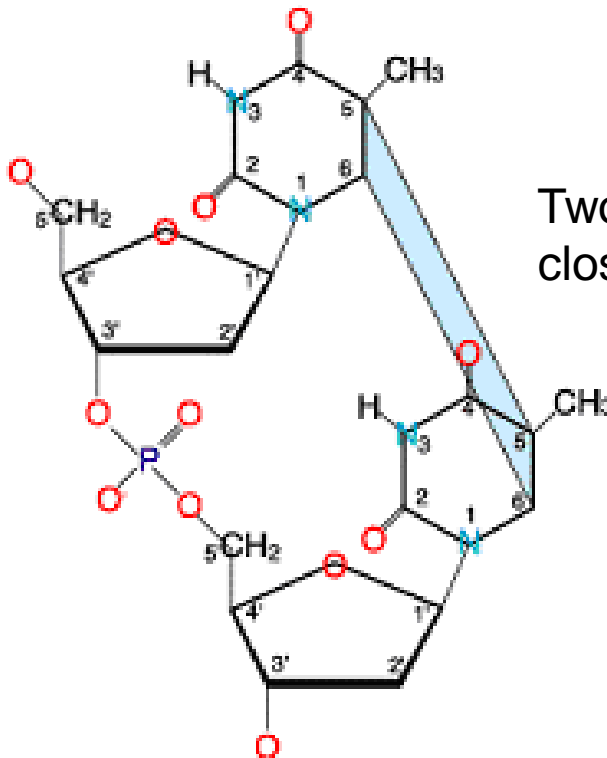
UV radiation

- **Ultraviolet radiation (UV light):**
 - **UVA (320-400 nm)** – non-ionizing
 - **UVB (280-320 nm)**
 - **UVC (200-280 nm)** } ionizing
 - UVC - absorbed by ozone
- **Effect: generation of ROS**
 - UVA – chemical modification of bases:
 - **8-oxo-7,8-dihydroguanine**
 - UVB and UVC – DNA breaks, chemical and structural changes of bases (intrastrand crosslinks)
 - **Thymine dimers**
 - **(6-4) Photoproducts**

Photodamage: CPDs

UV is absorbed by the nucleic acid bases, and the resulting influx of energy can induce chemical changes. The most frequent **photoproducts** are the consequences of bond formation between adjacent pyrimidines within one strand, and, of these, the most frequent are **cyclobutane pyrimidine dimers (CPDs)**. T-T CPDs are formed most readily, followed by T-C or C-T; C-C dimers are least abundant.

T-T dimer:

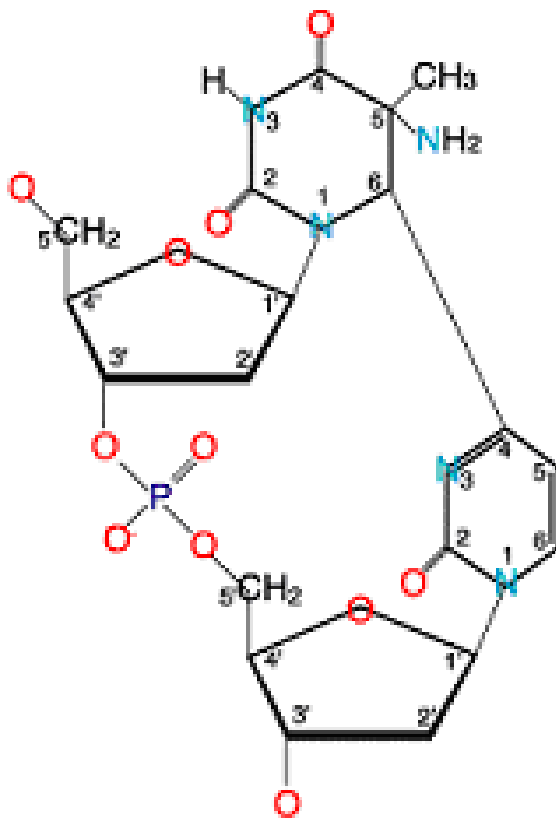


Two adjacent pyrimidines must be pulled closer to each other than in normal DNA.

Photodamage: (6-4) photoproducts

Dimers can also be produced by formation of a single covalent bond between the 6 position of one pyrimidine and the 4 position of the adjacent pyrimidine on the 3' side. The order of abundance of such **pyrimidine-(6-4)-pyrimidone photoproducts (6-4PPs)** is T-C>>C-C>T-T>C-T. Although only one bond attaches the adjacent pyrimidines, there is nevertheless extensive distortion of the normal DNA structure.

T-C 6-4PP:



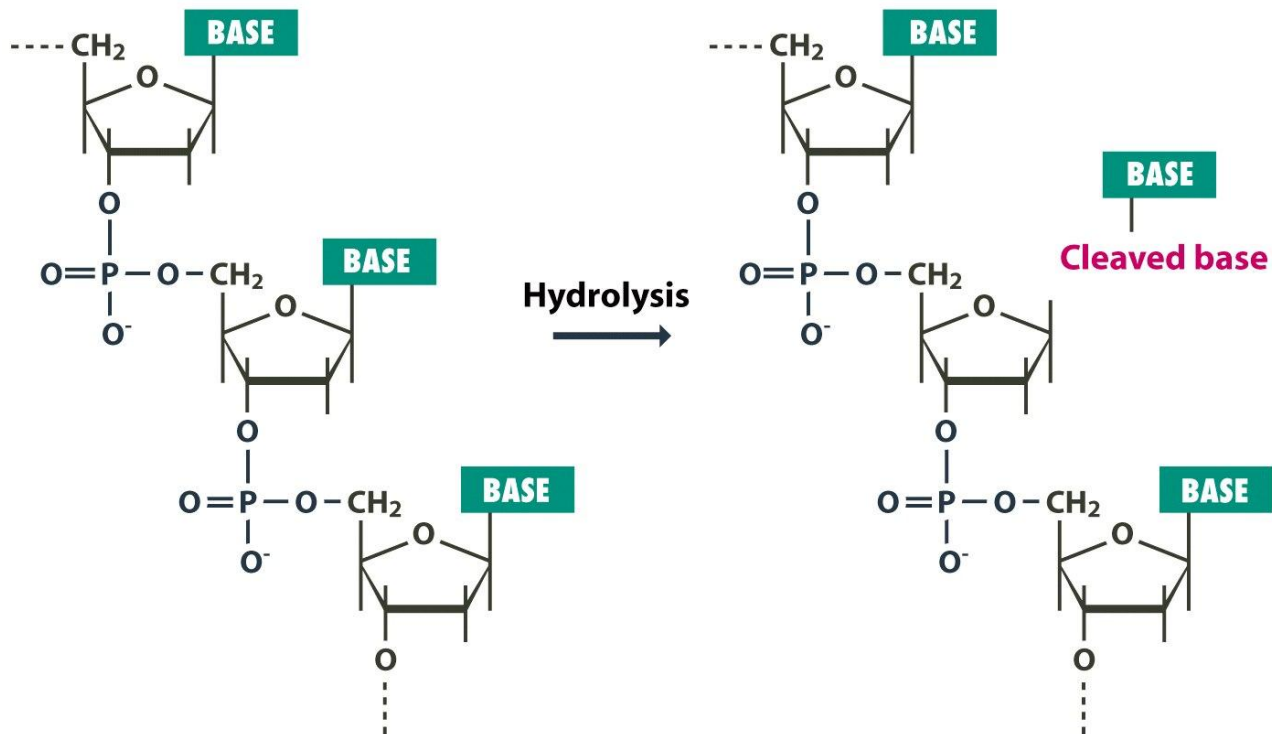
Amino group, originally from the 4 position of the cytosine, ends up at the 5 position of the thymine.

The structural distortion generated by 6-4PPs (a DNA bend of 44 degrees) is significantly greater than that produced by CPDs (bend of 7-9 degrees).

Heat and its effect on DNA (1)

1. Base loss: generation of an AP site:

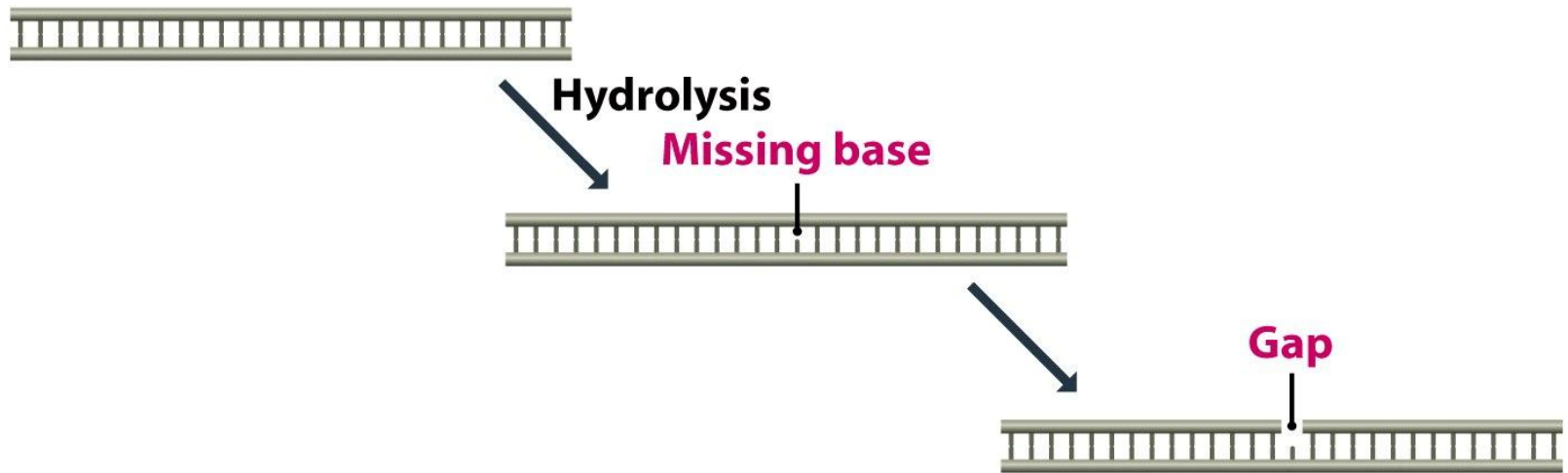
Heat-induced hydrolysis of a β -N-glycosidic bond



Heat and its effect on DNA (2)

2. Further degradation of the AP nucleotide leaves a **gap** in one strand

The effect of hydrolysis on double-stranded DNA



Normally, heat is not mutagenic, because cells have effective systems for repairing AP sites and gaps. Under certain circumstances (e.g. when the SOS response is activated in *E. coli*) gaps are filled with As; this may result in point mutations.

Summary

- ❑ DNA molecules, like all other biomolecules, can be damaged in numerous ways.
- ❑ Spontaneous damage due to replication errors, deamination, depurination and oxidation is compounded in the real world by the additional effects of radiation and environmental chemicals.
- ❑ If unrepaired, DNA damages may lead to mutation
- ❑ Since repair systems must be capable of recognizing and dealing with each type of damage, it is not surprising that there is a large number of different types of repair system.

References:

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3. Lodish et al., Molecular cell biology, 2001
4. B. Lewin. Genes VII.
5. J. Huberman. DNA repair. Roswell Park Cancer Institute.
(<http://asajj.roswellpark.org/huberman>)
6. E. Friedberg, G. Walker, W. Siede. DNA repair and mutagenesis, ASM press, Washington DC, 1995

Reading:

Chapter 16; Section 16.1: Mutations

Next:

Chapter 16; Section 16.2: DNA repair