B.Sc (Hons) Microbiology (CBCS Structure)

C-7: Molecular Biology

Unit 2: Replication of DNA

The Mutability and Repair of DNA

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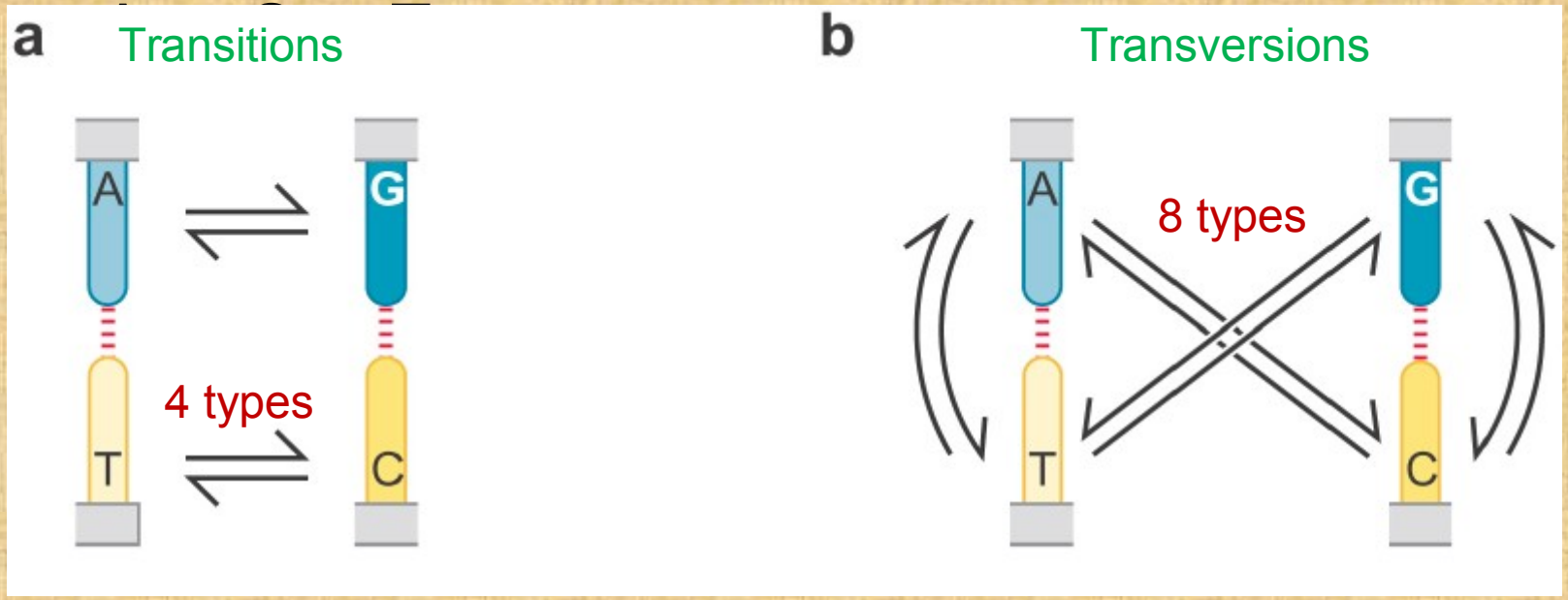
Reference – Molecular Biology of the Gene (6th Edition) by Watson et. al. Pearson education, Inc
Principles of Genetics (8th Edition) by D. Peter Snustad, D. Snustad, Eldon Gardner, and Michael J. Simmons, Wiley publications

Replication errors and their repair

The Nature of Mutations:

- Transitions: A kind of the simplest mutation which is pyrimidine-to-pyrimidine and purine-to-purine substitutions such as T to C and A to G
- Transversions: The other kind of mutation which are pyrimidine-to-purine and purine-to-pyrimidine substitutions such as T to A or G and



Synonymous substitution

Non synonymous substitution

- Point mutations: Mutations that alter a single nucleotide

Other kinds of mutations (which cause more drastic changes in DNA):

- Insertions
- Deletions
- Gross rearrangements of chromosome

These mutations might be caused by insertion by transposon or by aberrant action of cellular recombination processes.

Mutation

- Substitution, deletion, or insertion of a base pair.
- Chromosomal deletion, insertion, or rearrangement.

Somatic mutations occur in somatic cells and only affect the individual in which the mutation arises.

Germ-line mutations alter gametes and passed to the next generation.

Mutations are quantified in two ways:

1. Mutation rate = probability of a particular type of mutation per unit time (or generation).
2. Mutation frequency = number of times a particular mutation occurs in a population of cells or individuals.

Types of mutations in ORFs:

Nonsynonymous/missense mutation : Base pair substitution results in substitution of a different amino acid.

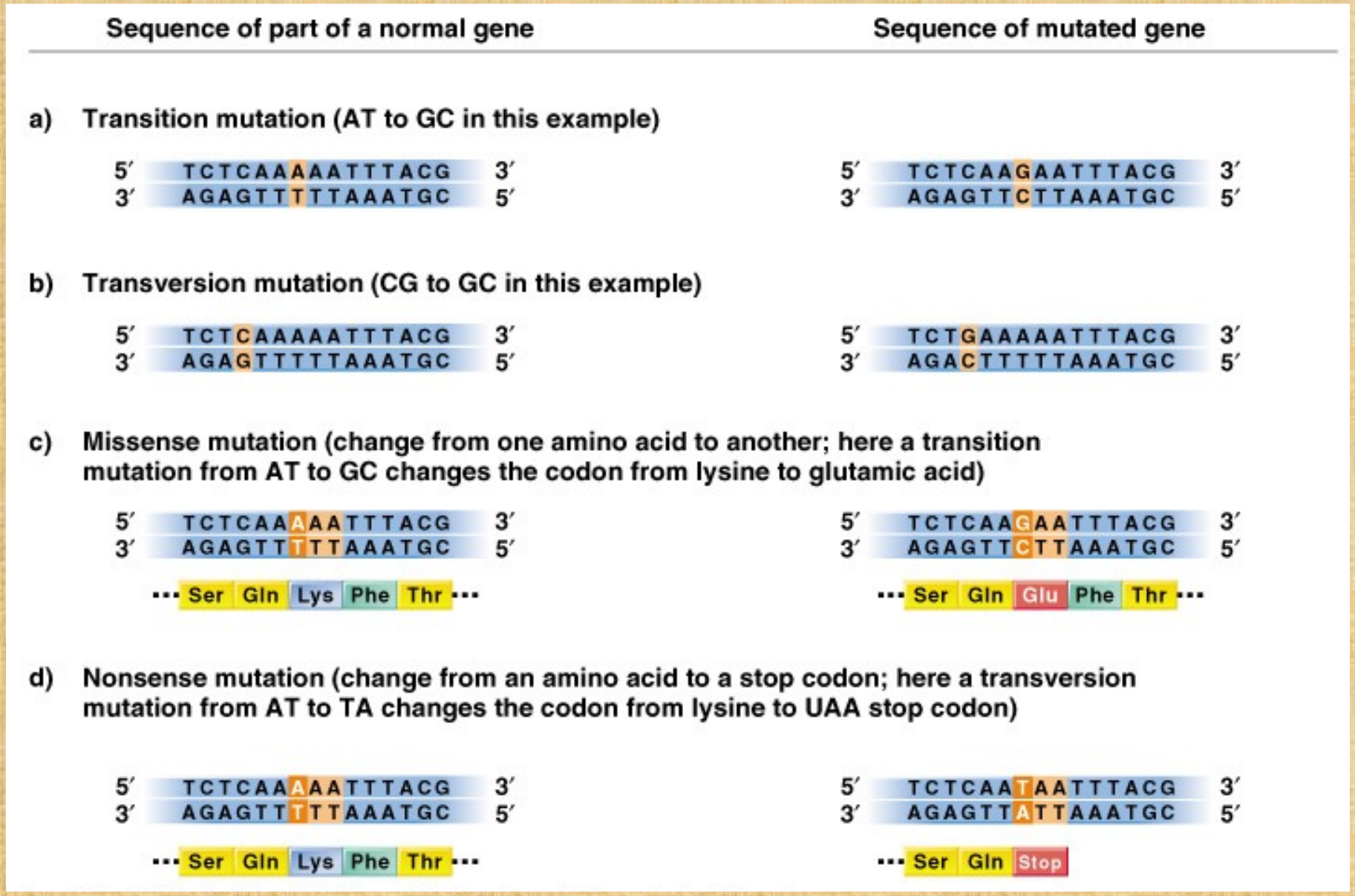
Nonsense mutation: Base pair substitution results in a stop codon (and shorter polypeptide).

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

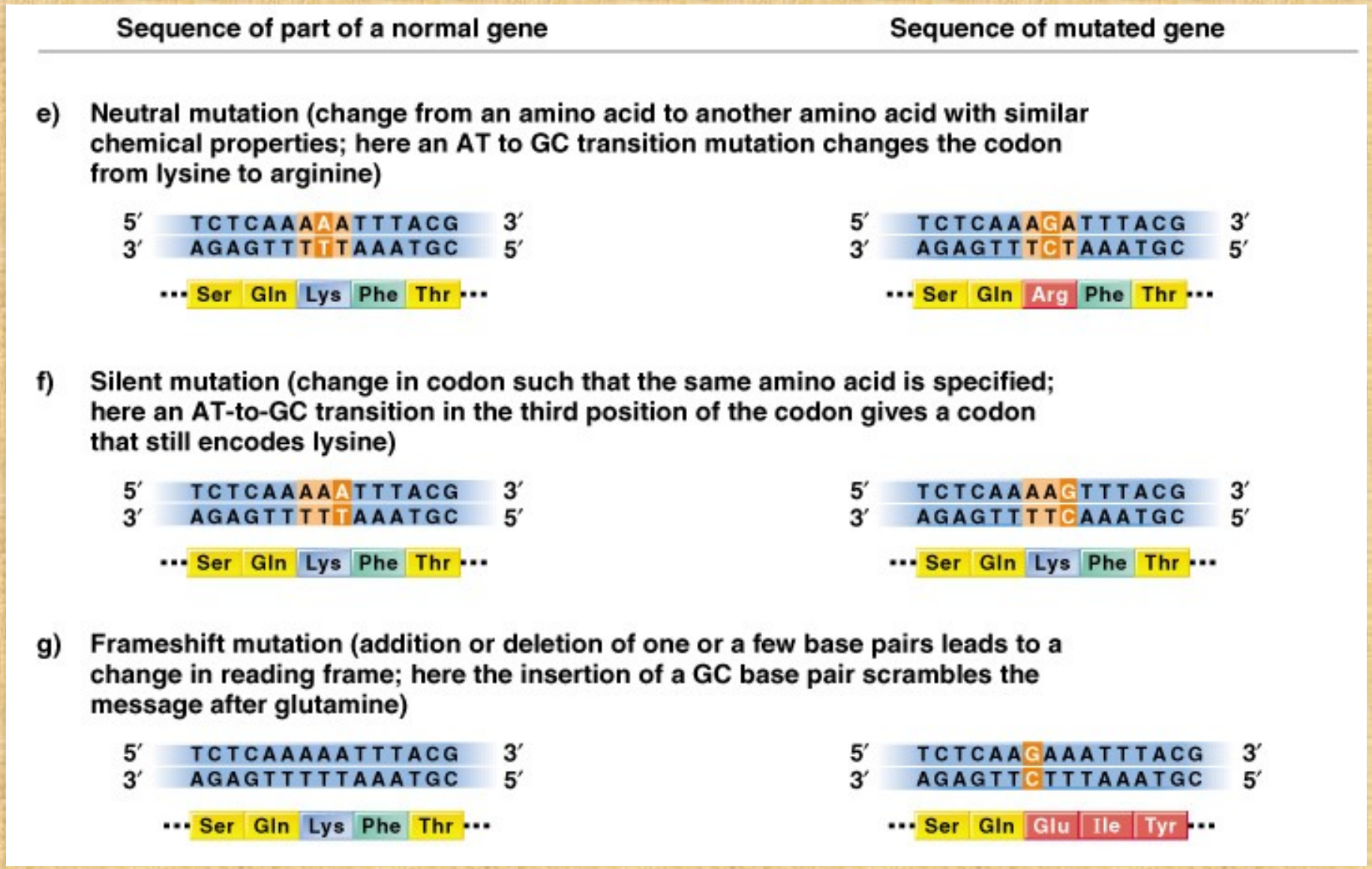
Synonymous/silent mutation: Base pair substitution results in the same amino acid.

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

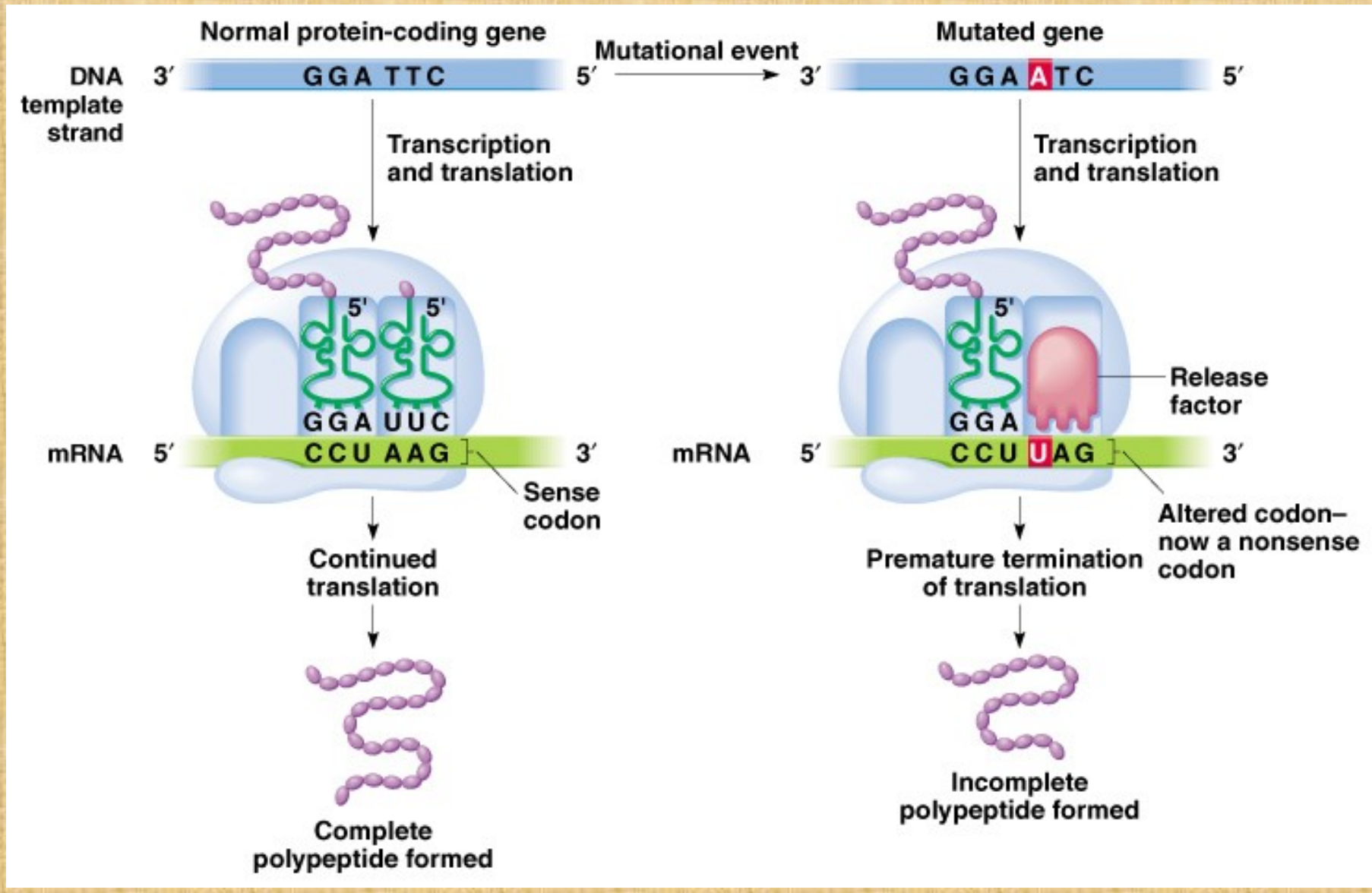
Types of base pair substitutions and mutations.



Types of base pair substitutions and mutations.



Effect of a nonsense mutation on translation



Reverse mutations and suppressor mutations:

Forward mutation : Mutation changes wild type to mutant.

Reverse mutation (back mutation) : Mutation changes mutant to wild type.

- Reversion to the wild type amino acid restores function.
- Reversion to another amino acid partly or fully restores function.

Suppressor mutation : Occur at sites different from the original mutation and mask or compensate for the initial mutation without reversing it.

- Intragenic suppressors occur on the same codon; e.g., nearby addition restores a deletion
- Intergenic suppressors occur on a different gene.

Spontaneous and induced mutations:

Spontaneous mutations

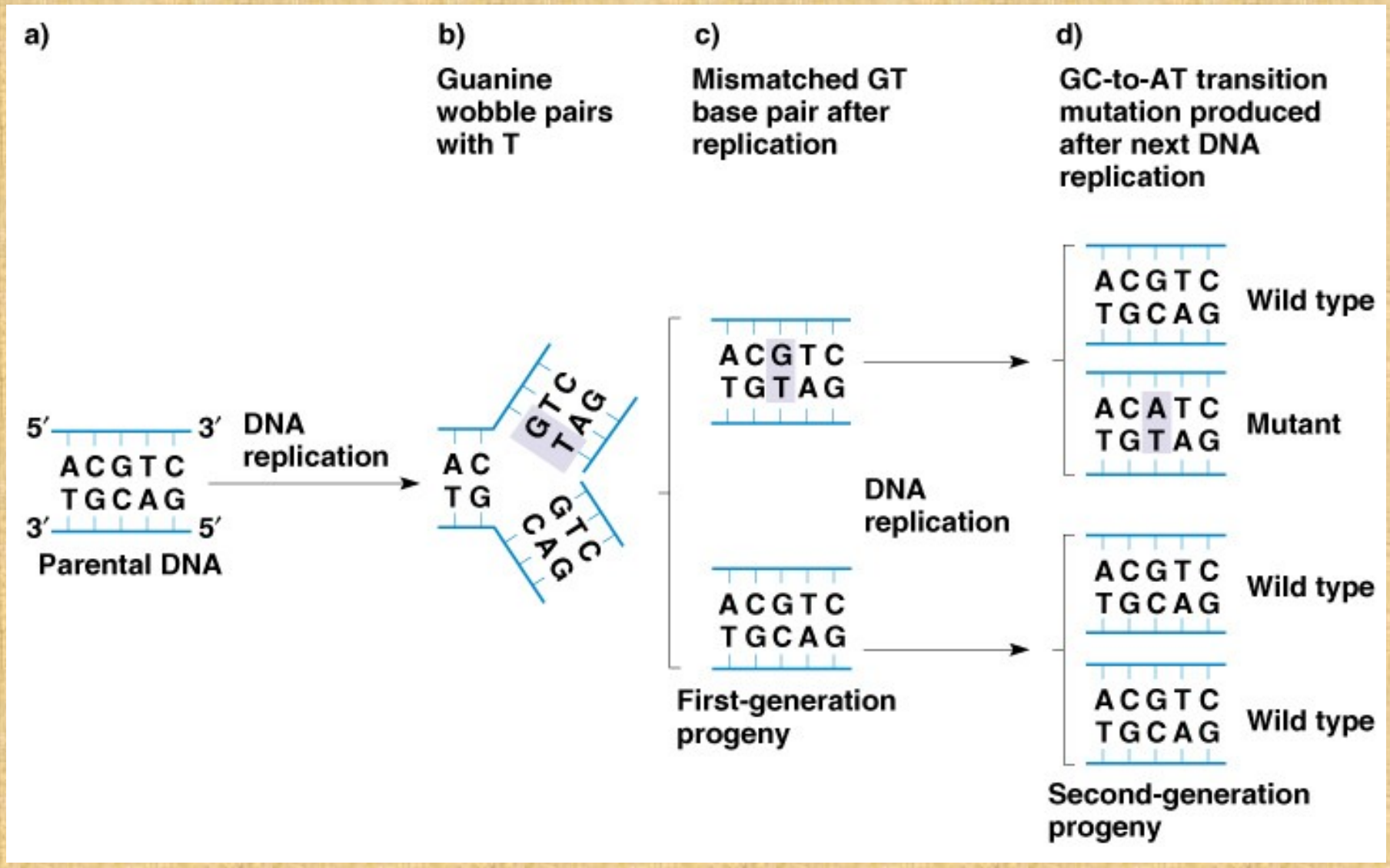
- Spontaneous mutations can occur at any point of the cell cycle.
- Movement of transposons (mobile genetic elements) causes spontaneous mutations.
- Mutation rate = ~10^{-4} to 10^{-6} mutations/gene/generation
- Rates vary by lineage, and many spontaneous errors are repaired.

Different types of DNA replication errors

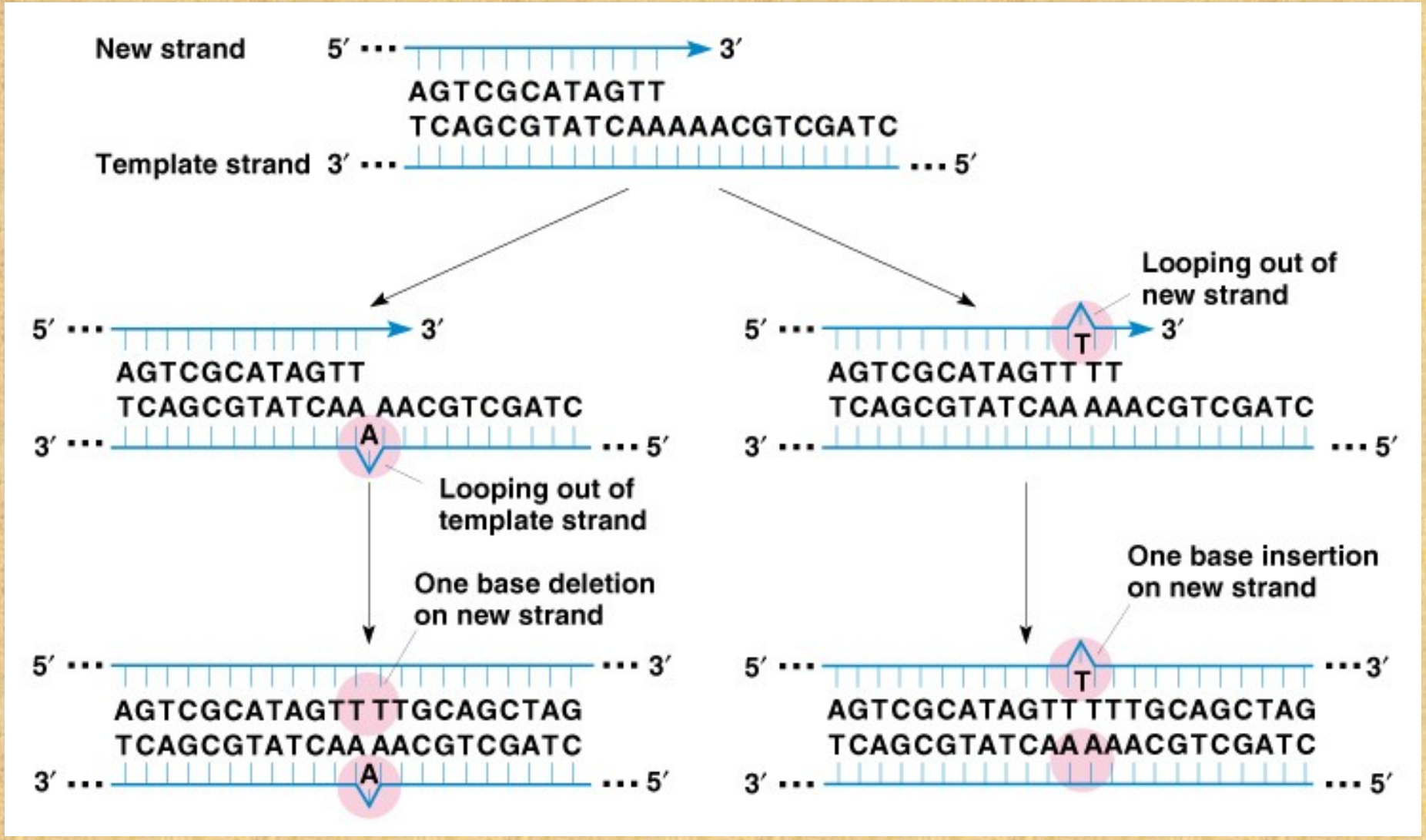
Wobble-pairing : T-G, C-A, A-G, T-C: Normal pairing typically occurs in the next round of replication; frequency of mutants in F_2 is 1/4. GT pairs are targets for correction by proofreading and other repair systems.

Additions and deletions : DNA loops out on template strand, DNA polymerase skips bases, and deletion occurs. Or DNA loops out on new strand, DNA polymerase adds untemplated bases.

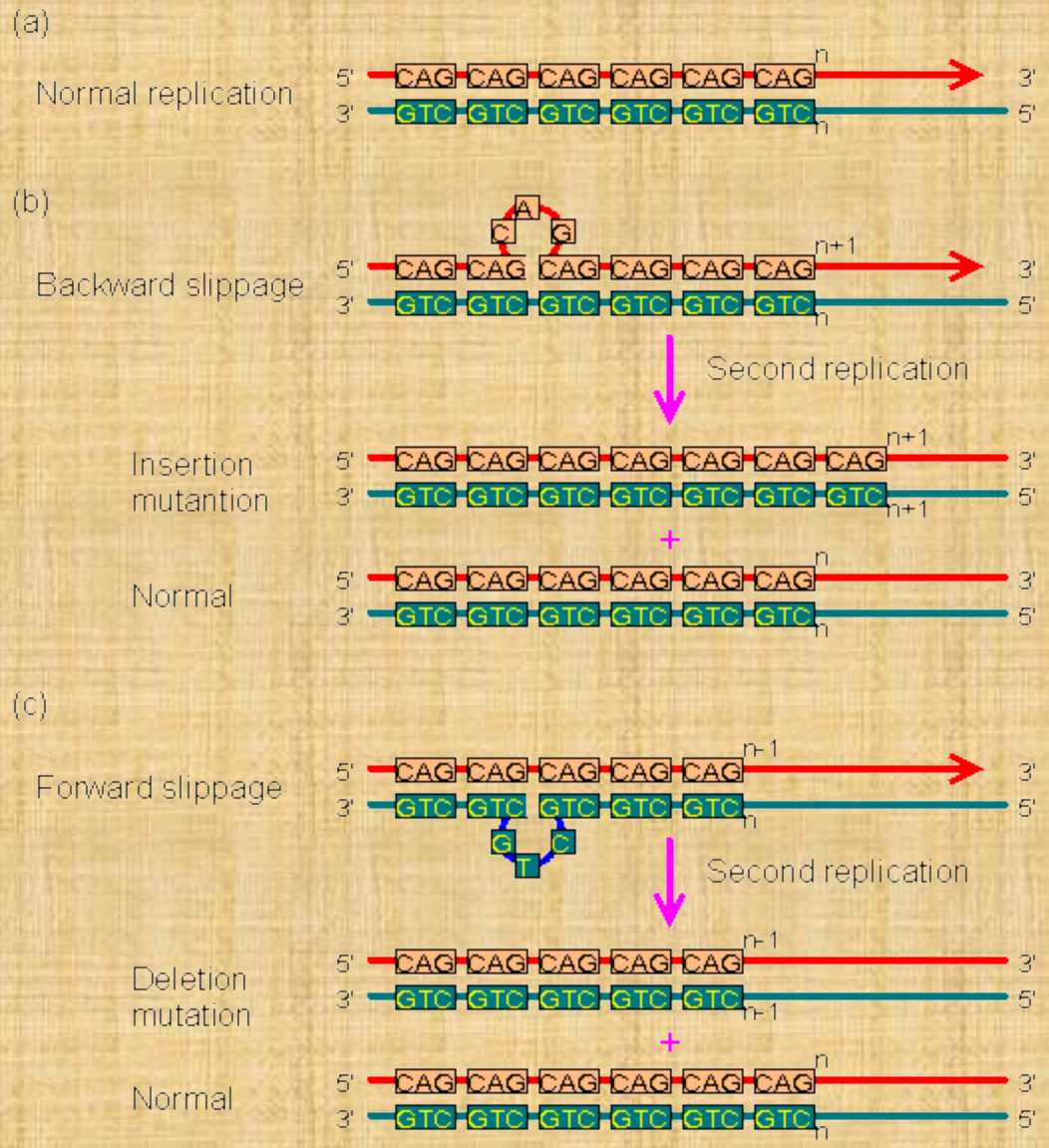
Mutation caused by mismatch wobble base pairing.



Addition and deletion by DNA looping-out.



Replication Slippage

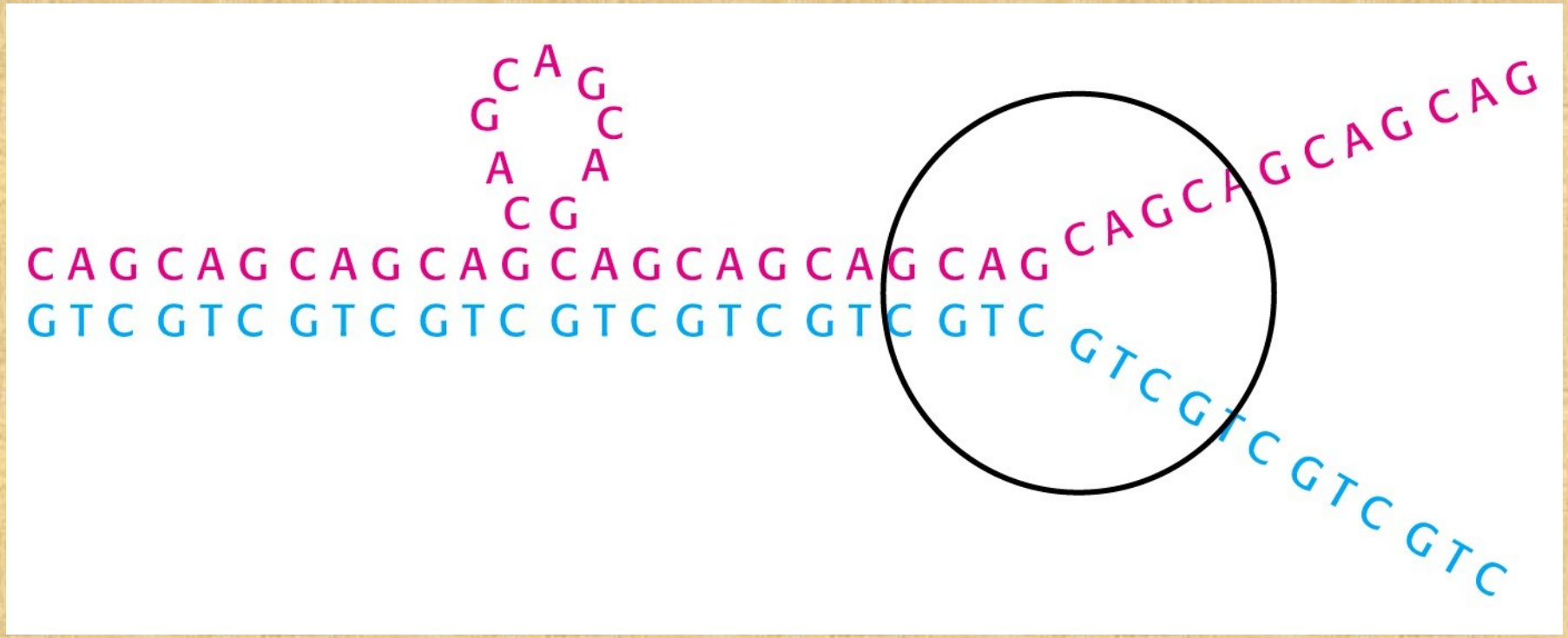


- DNA microsatellites: Mutation-prone sequence in human genome are repeats of simple di-, tri- or tetranucleotide sequences, known as DNA microsatellites

- **In particular, trinucleotide repeats cause difficulties which can lead to expansion of these sequences.**

- **Depending where the repeat is located expansion of the sequence can have severe effects on the expression of a gene**

Several mechanisms for the expansion of trinucleotide repeats have been proposed, but the precise mechanism is unknown.



Several inherited diseases are associated with expa trinucleotide repeat sequences.

Table 3.1. Trinucleotide repeat disorders. The major hereditary neurologic diseases associated with repeat expansions are shown. XR, X-linked recessive; AD, autosomal dominant; AR, autosomal recessive; UTR, untranslated region

Syndrome	Mode of inheritance	Affected gene	Chromosome locus	Insertion site	Repeat sequence	Repeat size (normal)	Repeat size (disease)	Effect on function
Huntington	AD	*Huntingtin*	4p16.3	coding region	CAG	10–35	40–150	gain
Spinocerebellar ataxia (SCA) type 1 (2,3,7)	AD	*Ataxin-1 (2,3,7)*	6p22-23	coding region	CAG	25–35	40–80	gain
Machado-Joseph	AD		14q24.3-q32	coding region	CAG		50–100	gain
Dentatorubral and pallido-luysian atrophy (DRPLA)	AD	*Atrophin-1*	12p12-ter	coding region	CAG			gain
Kennedy	XR	*Androgen receptor*	Xq21.3	coding region	CAG	15–30	40–80	loss
Fragile X	XR	*FMR-1 (FRAXA)*	Xq27.3	5′-UTR	CGG	5–50	100–4000	loss
Myotonic dystrophy	AD	*Myotonin*	19q13.2	3′-UTR	CTG	5–40	50–3000	loss
Friedreich ataxia	AR	*Frataxin*	9q13-q21.1	intron	GAA		100–2000	loss
(Fragile) XE	XR		Xq28		GCC			loss

Very different disorders, but they share the characteristic of becoming more severe in succeeding generations due to pro expansion of the repeats

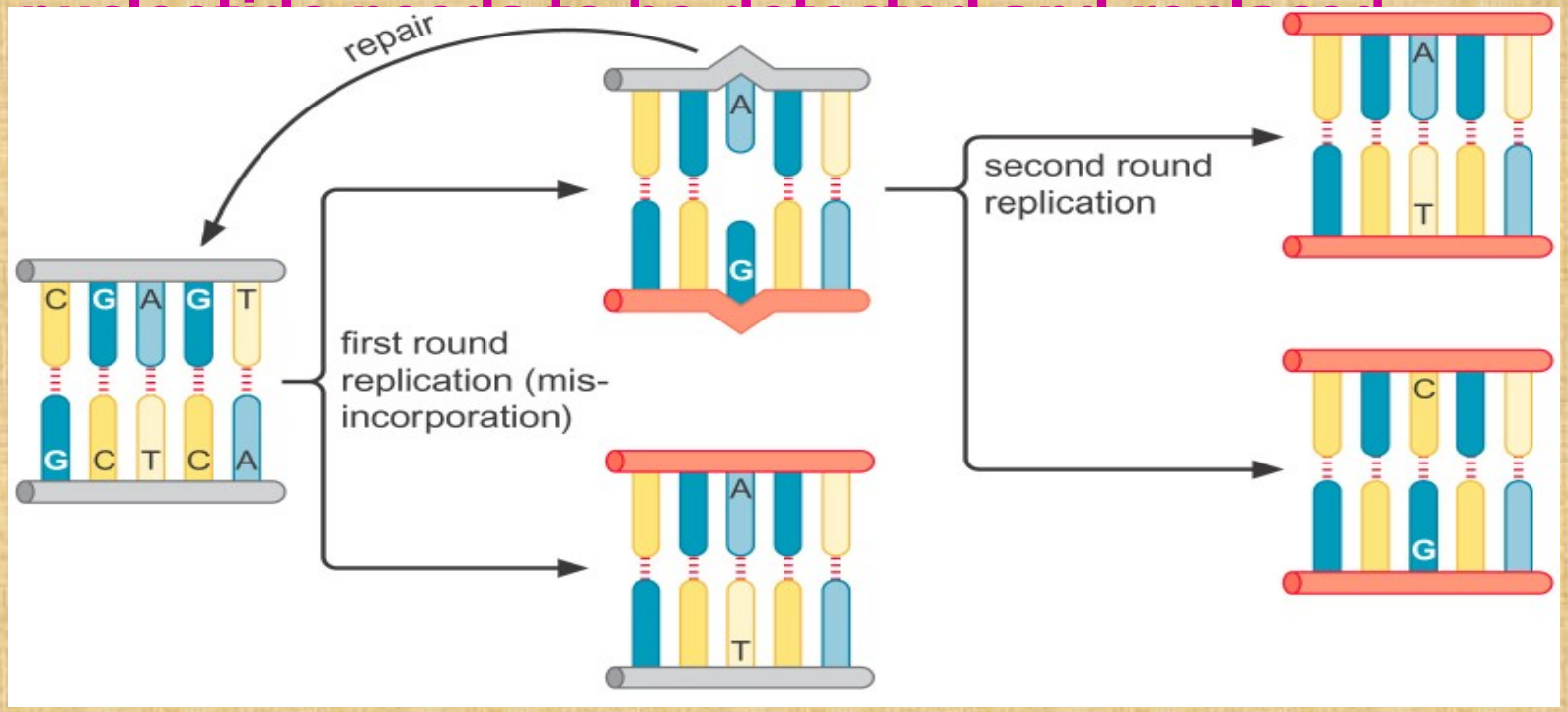
DNA Repair Mechanisms of Various Types of Damage

TYPE OF DAMAGE	DAMAGE MECH.	REPAIR MECH.
Single Base Alteration	Depurination	BER
	Deamination	BER
	Alkylation	BER
	Base Analogue Incorporation	BER
	Mismatch Base	MMR
Double Base Alteration	Pyrimidine dimer	NER
	Purine dimer	NER
Chain Break	Single stranded break	HR,NHEJ
	Double stranded break	HR,NHEJ
Cross linking	Between DNA-DNA	HR
	Between DNA-Protein	?
Polymerase slippage	Replication error in microsettelite	MMR & NER

Some Replication Errors Escape Proofreading

The 3'-5' exonuclease component of replisome only improves the fidelity of DNA replication by a factor of about 100.

But, that's not enough : The misincorporated



A mutation may be introduced by mis-incorporation of a base in the first round of replication. In the second round of replication the mutation becomes permanently incorporated in the DNA sequence.

Mismatch Repair Removes Errors That Escape Proofreading

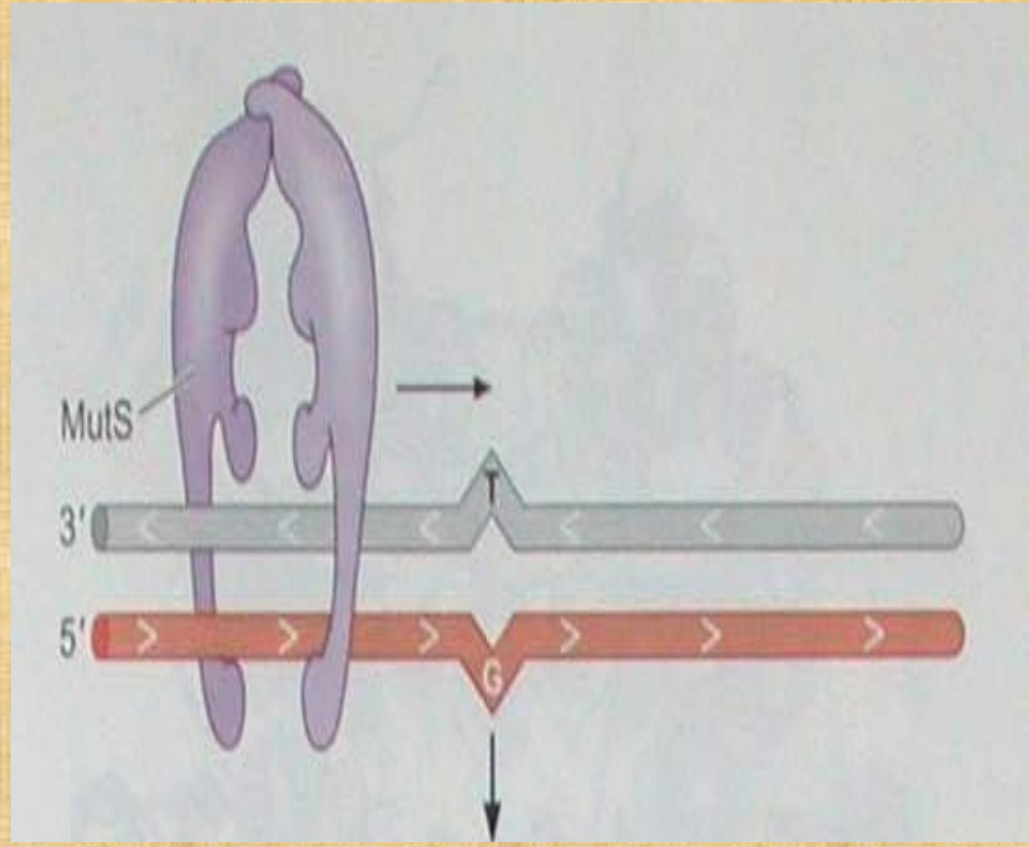
- **Mismatch repair system:** A system that increases the accuracy of DNA synthesis by an additional two to three orders of magnitude.
- This system faces **2 challenges**:

(1) Rapidly find the mismatches/mispairs and repair before second round of replication

(2) Accurately correct the mismatch

Important parts of mismatch repair system

MutS: A dimer of the mismatch repair protein which detects mismatches



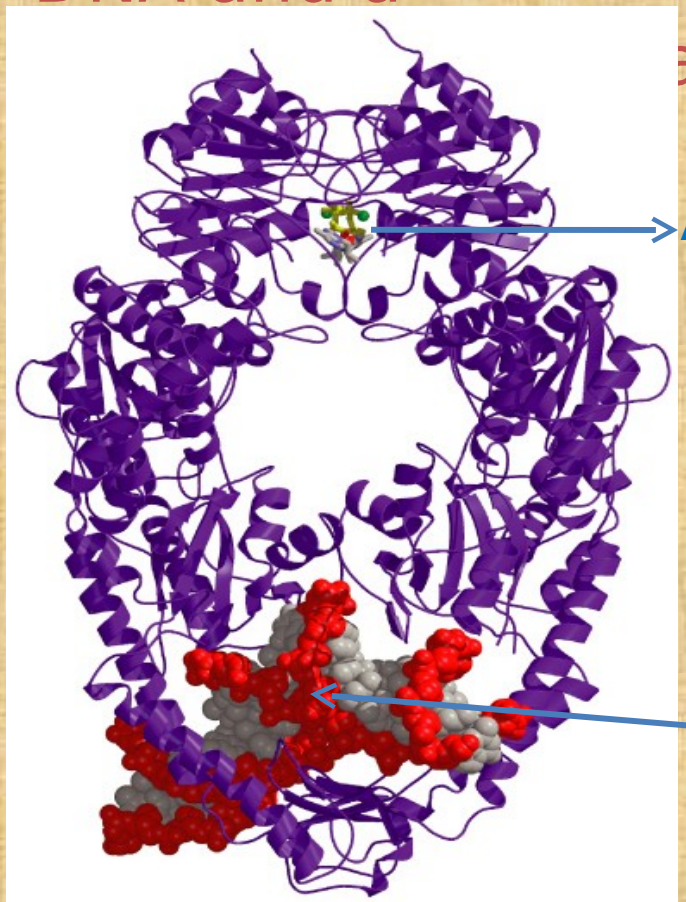
Fuctions of MutS:

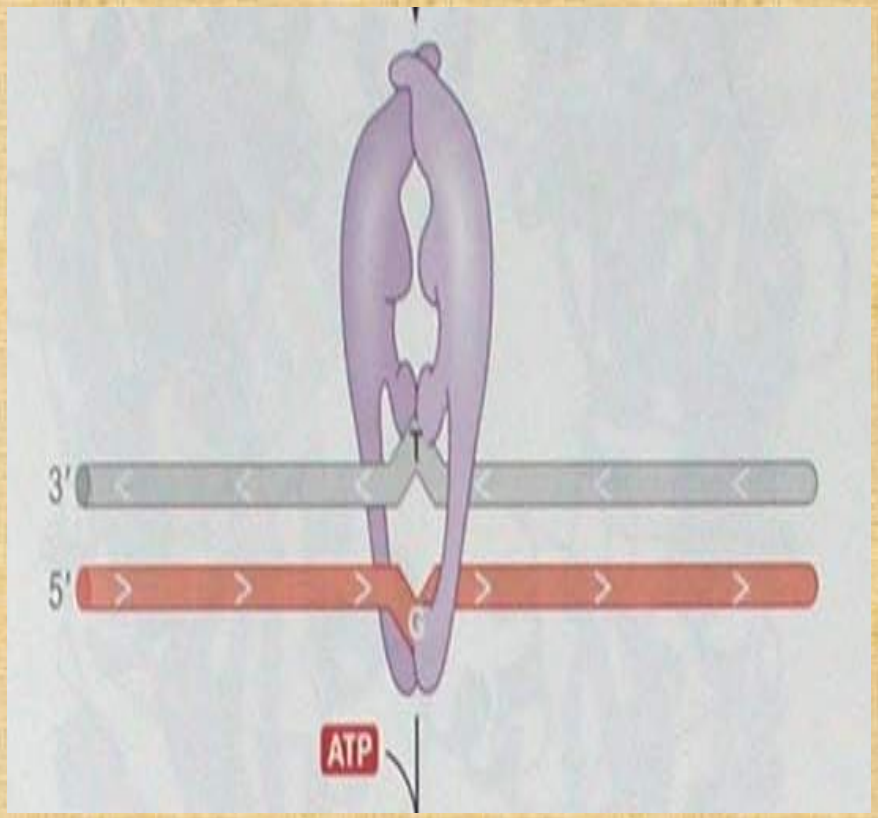
1. MutS scans the DNA, recognizes the mismatch from the distortion they cause in the DNA backbone

Functions of MutS

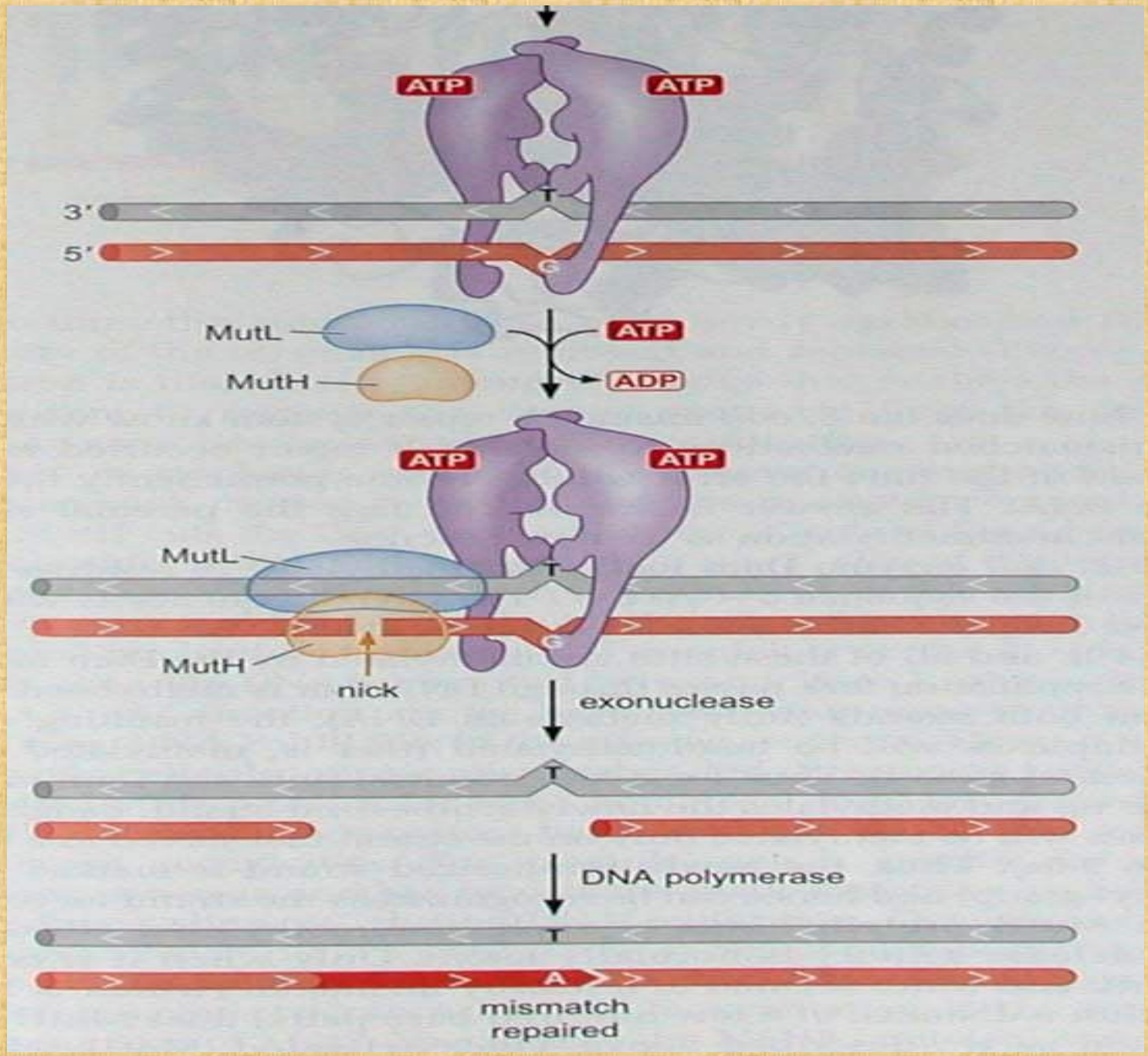
MutS embraces the mismatch-containing DNA, inducing a pronounced kink in the DNA and a

ange





MutL and MutH



How these three parts interact

MutS-mismatch-containing DNA complex recruits MutL, MutL in turn activates MutH, an enzyme causing an incision or nick on one strand near the site of the mismatch. Nicking is followed by the specific helicase and one of three exonucleases.

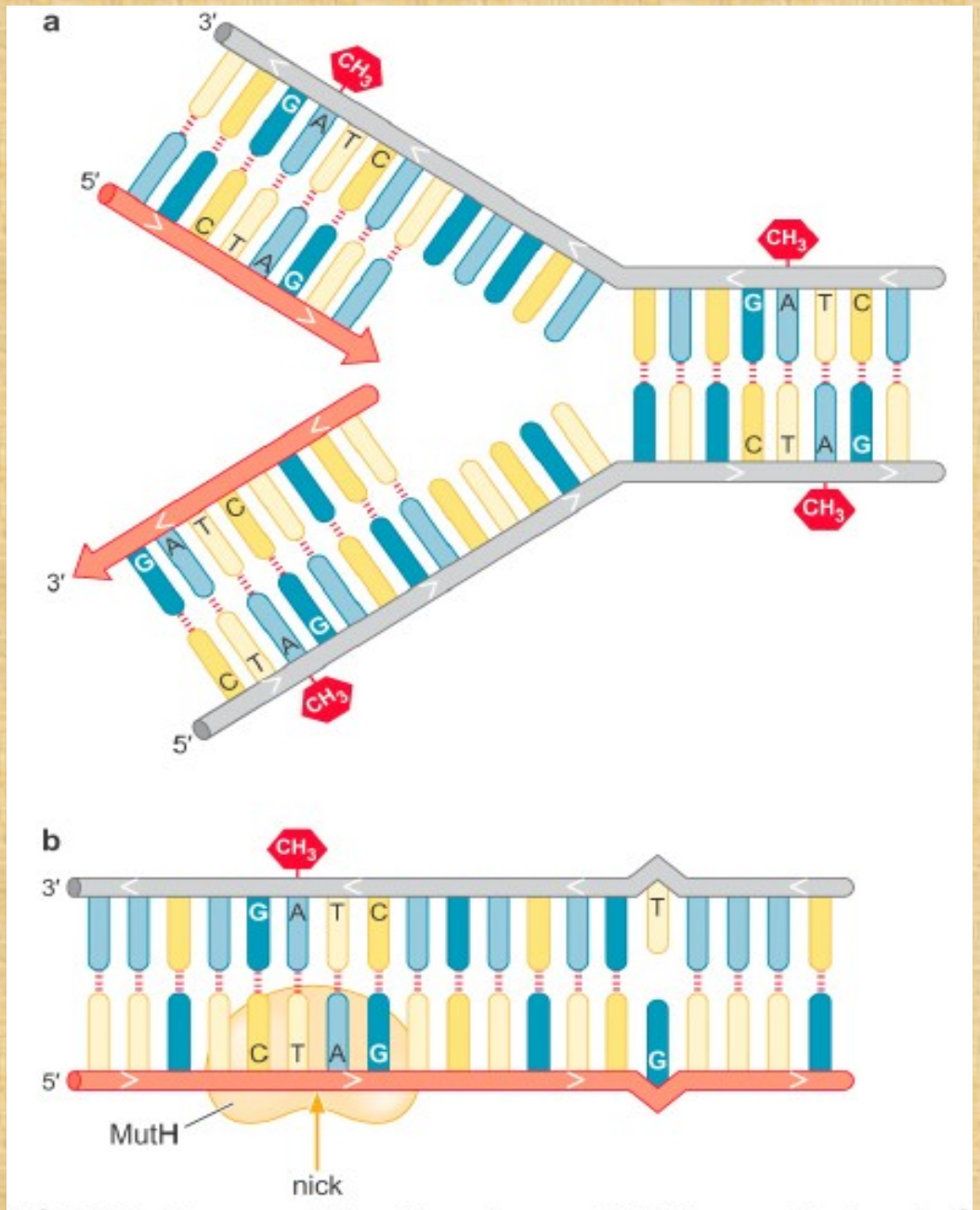
How does the *E.coli* mismatch repair system know which of the two mismatched nucleotides to replace?

Dam Methylase

- **Dam methylase:** The *E. coli* enzyme that methylases **A** residues on both strands of the sequence **5`-GATC-3`.**

The newly synthesized strand is not methylated by Dam methylase in a few minutes after the synthesis.

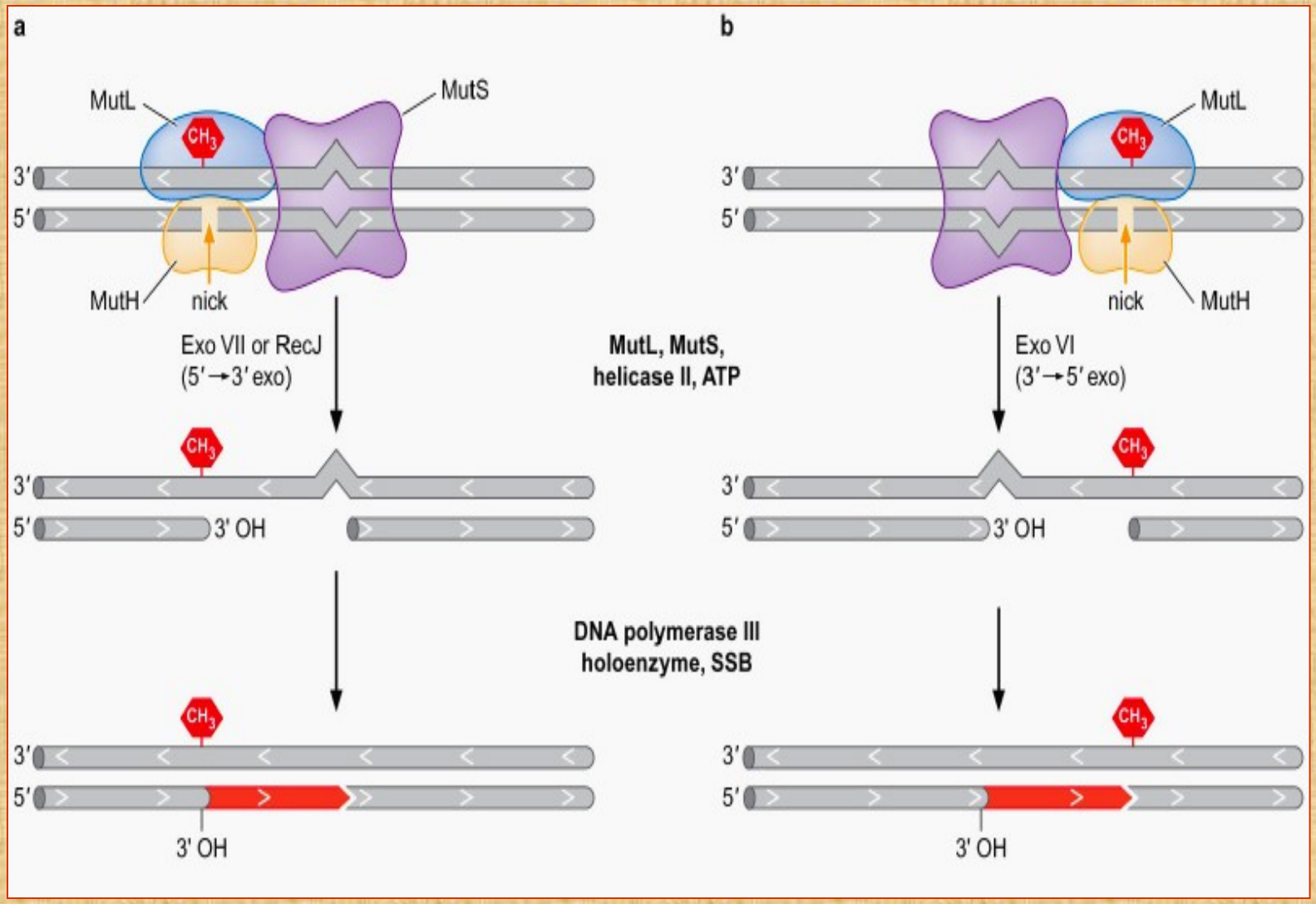
Dam methylation at replication fork



a. Replication generates hemimethylated DNA in E.coli.

b. MutH makes incision in unmethylated daughter strand.

Different exonucleases are used to remove single-strand DNA between the nick created by MutH and the mismatch.



DNA Mismatch repair in Eukaryotic cells

- **In fact, eukaryotes have multiple MutS-like proteins with different specificities.**
- **MSH proteins for MutS homologs.**
- **MLH and PMS proteins for MutL**

Repair of DNA Damage

There are two consequences of DNA damage:

- Some kinds of damage create impediments to replication or transcription

- Other kinds of damage create altered bases that cause mispairing which results a permanent alternation to DNA

Systems that repair damage to DNA

- A repair **enzyme** simply reverses the damage
- **Excision repair systems,** in which damaged nucleotide is not repaired but removed from DNA(more elaborate step),composed of **base excision repair** and **nucleotide excision repair**

Repair System	Enzymes/proteins	Repair System	Enzymes/proteins
Base excision	DNA glycosylase	Mismatch	Dam methylase
	AP endonuclease		MutS, MutL, MutH
	DNA polymerase I		Exonuclease
	DNA ligase		DNA helicase II
Nucleotide excision	Uvr-A, Uvr-B, Uvr-C		SSB protein
	DNA polymerase I		DNA polymerase III
	DNA ligase		DNA ligase

Systems that repair damage to DNA

- **Recombinational repair**,which is employed when both strands of DNA are damaged,also known as double-strand break repair.(more elaborate)
- **Translesion DNA synthesis**,the last way cells choose

Base excision repair systems

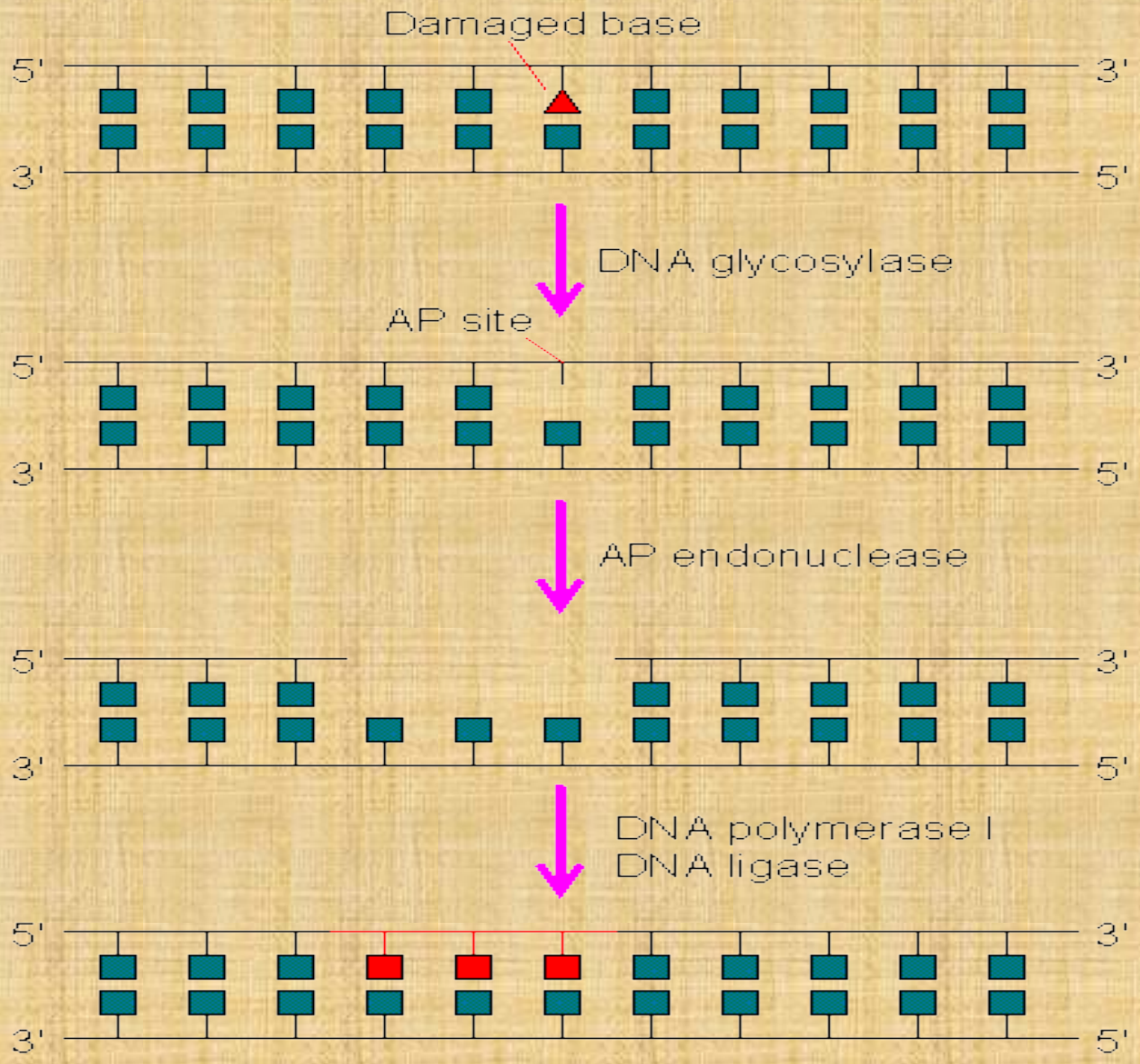
Base excision repair enzymes—**glycosylase** recognize and remove damaged bases by a base-flipping mechanism by entering along the minor groove,hydrolyzing the glycosidic bond.

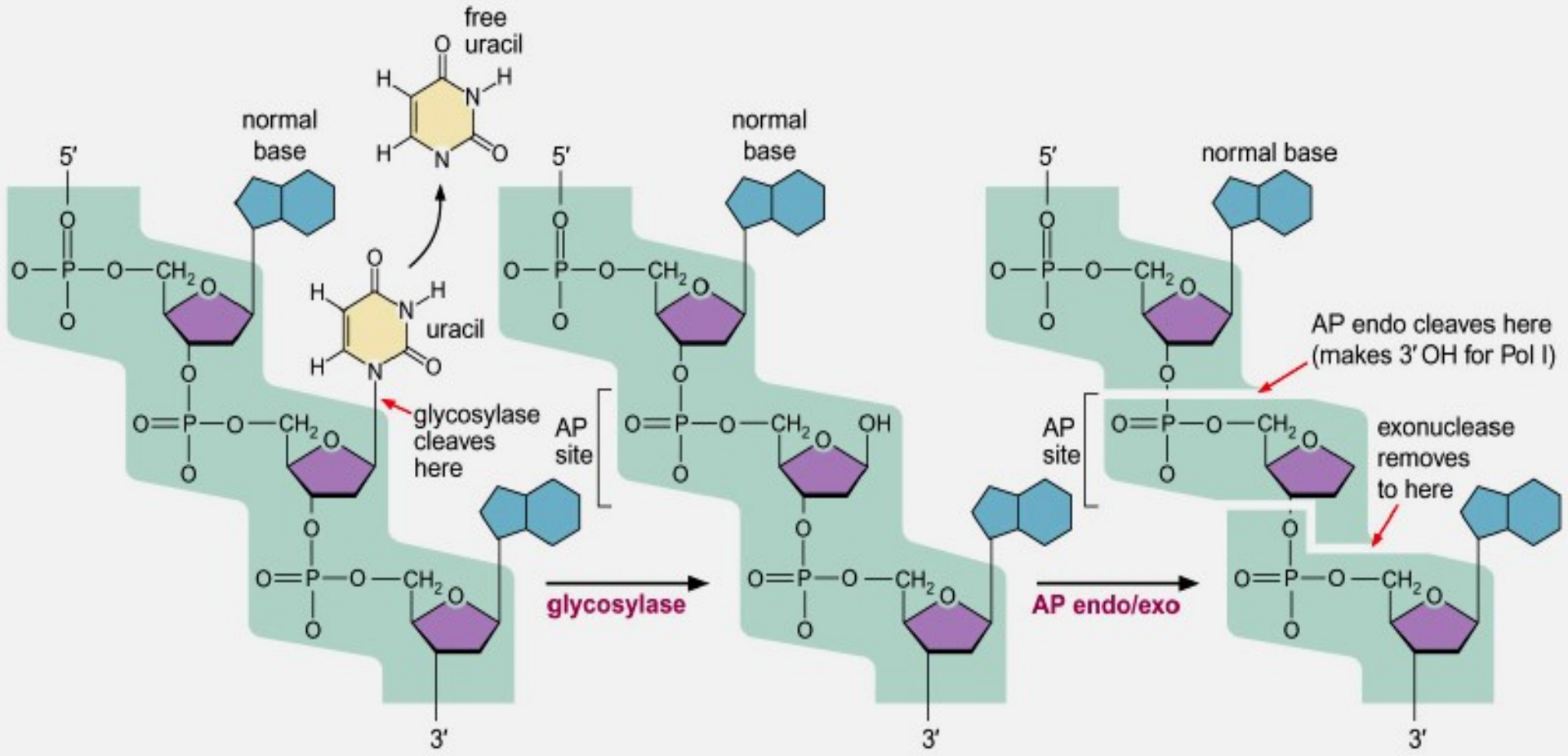
DNA glycosylases are lesion-specific. Separate glycosylases for repairing uracil or oxoG. 8 in human nuclei

Types of DNA Glycosylases

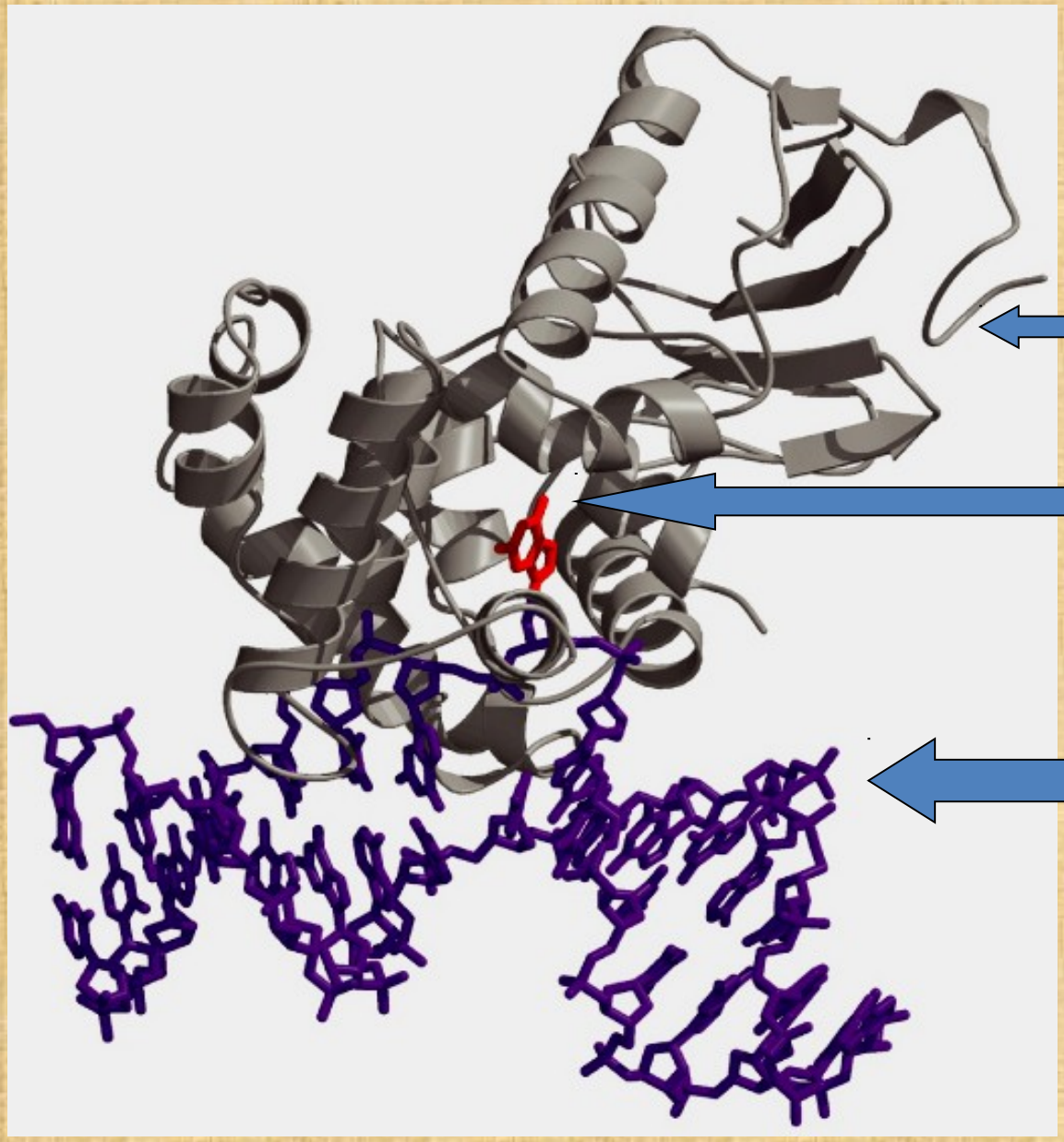
DNA Glycosylases	Type of Removal Lesions
Alkyladenine DNA Glycosylase (AAG)	3-meA. 7-meG. 3-meG. Hypoxanthine (Hx). 1,N^6-ethenoadenine (εA). 3,N^2-ethenoguanine. Oxanine.
Uracil DNA Glycosylases: UNG SMUG1 Thymine DNA Glycosylase (TDG) MBD4	Uracil. Thymine glycols (opposite G) by TDG and MBD4.
MutY DNA Glycosylase (MYH)	Adenine (opposite 8-oxoG). 2-Hydroxyadenine (2-OH-A).

Base excision repair systems





1. The AP site is created by the hydrolysis of glycosylase bond.
2. AP endonuclease cuts DNA backbone at the 5’ position of AP site leaving a 3’OH group; exonuclease cut out the 3’ position of AP site leaving a 5’ PO4.
3. DNA polymerase 1 fill in the gap.



The enzyme

The damaged base which is filpped out

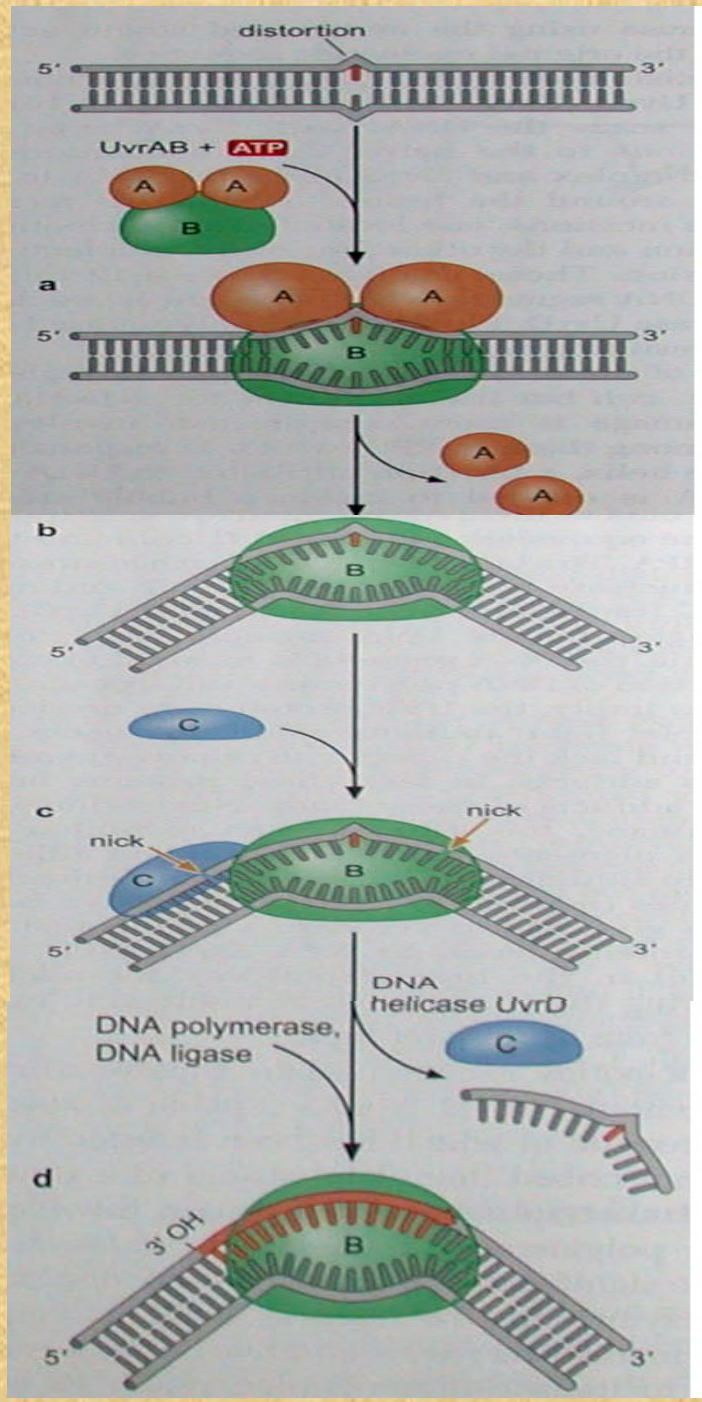
The DNA

Nucleotide excision repair systems

What is the difference between the two kinds of excision repair systems?

Also,how does the NER system work?

- **Recognize distortions to the shape of the DNA double helix**
- **Remove a short single-stranded segment that includes the lesion.**
- **DNA polymerase/ligase fill in the gap.**

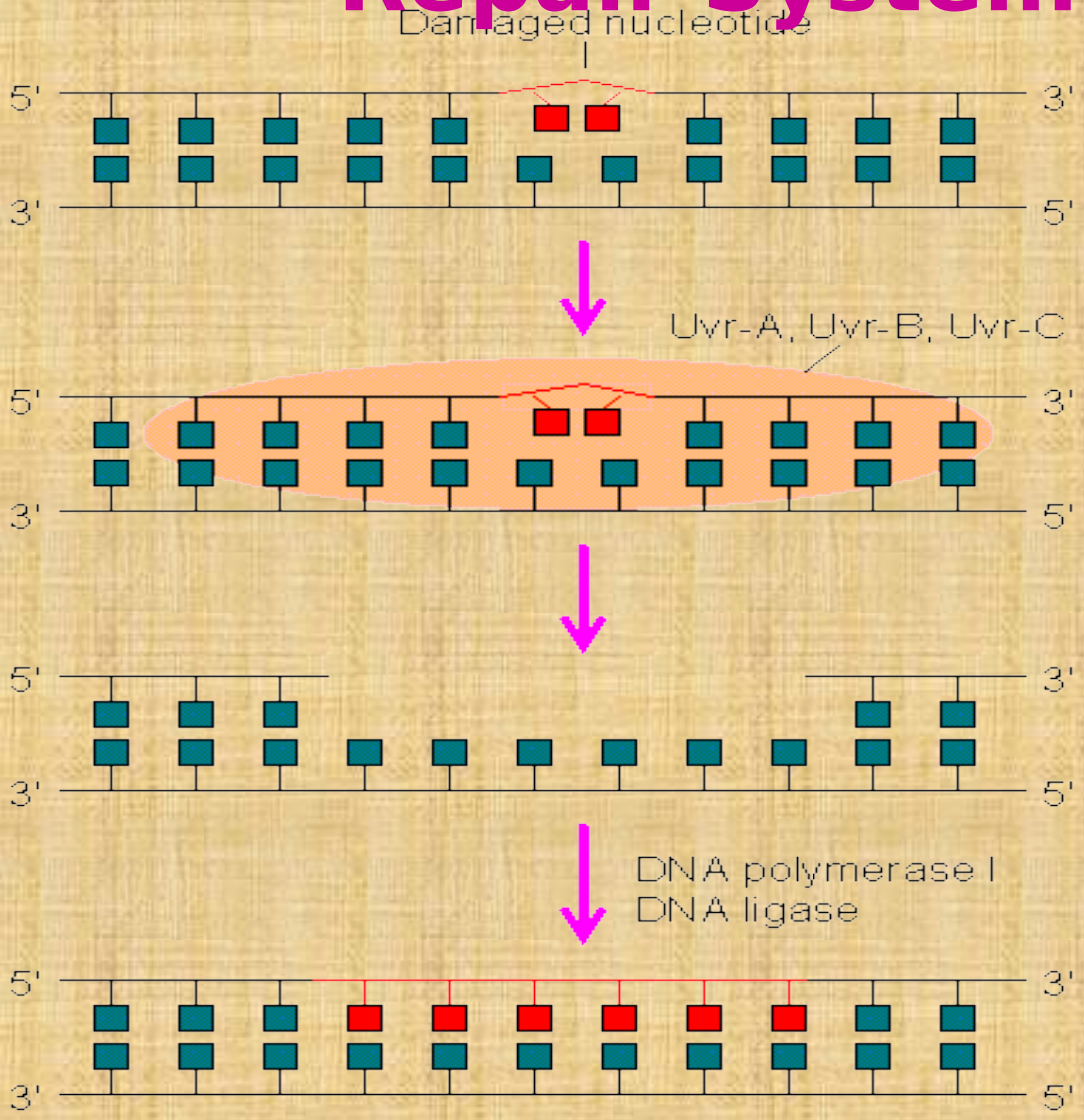


Once encountering a distortion UvrA exits the complex and UvrB melts the DNA to create a single-strand bubble around the lesion.

Next,UvrB recruits UvrC,and UvrC creates two incisions in different positions on one strand.

Finally,DNA polymerase and ligase fill in the gap.

Nucleotide excision repair (Dark Repair System)



T=T dimers due to exposure to UV light