MUTATION & MUTAGENESIS

WHAT IS MUTATION?

➢ **Any change in the DNA sequence of an organism is a mutation.**

     - Mutation is the ultimate source of the altered versions of genes that provide the raw material for evolution.

➢ **An organism exhibiting a novel phenotype as a result of the presence of a mutation is referred to as a "MUTANT".**

     - Most mutations have no effect on the organism, especially among the eukaryotes, because a large portion of the DNA is not in genes and thus does not affect the organism's phenotype.

     - Of the mutations that do affect the phenotype, the most common effect of mutations is lethality, because most genes are necessary for life. Only a small percentage of mutations causes a visible but non-lethal change in the phenotype.

*** Without mutation, all genes would exist in only one form- alleles would not exist and thus genetic analysis would not be possible. Organisms will not be able to evolve and adapt to environmental changes.

Mutations can be :

- **Substitution, deletion, or insertion of a base pair(s) in DNA.**

- **Chromosomal deletion, insertion, or rearrangement.**

Somatic mutations **occur in somatic cells and only affect the individual in which the mutation arises.** For e.g., the Delicious apple & Navel orange, originally were mosaics in somatic tissues. The desirable traits were due to spontaneous mutation in single cells. In each case the cell carrying the mutant gene reproduced, eventually producing an entire branch with the mutant phenotype. Fortunately vegetative propagation was feasible for both, and today numerous progenies from grafts and buds have perpetuated the original mutation. Both the varieties are now world famous.

Germ-line mutations **may be dominant and effects may be expressed in immediate progenies. If the mutation is recessive effects are often obscured in diploids.** If the mutation arises in a gamete, only a single member of the progeny is likely to be affected. If mutations occur in gonial cells, several mutant gametes may be produced, increasing the chance of perpetuation. **Dominance of the mutant allele and stage in the reproductive cycle, determines the likelihood of manifestation**.

Mutations are quantified in two ways:

Mutation rate = probability of a particular type of mutation per unit time (or generation).

Mutation frequency = number of times a particular mutation occurs in a population of cells or individuals.

- The simplest mutations are base changes, where <u>one base</u> is converted to another **(substitution).**

  - best e.g., Transition & Transversion

- Another simple type of mutation is the gain or loss of one or a few bases.

- Larger mutations include **insertion** of whole new sequences, often due to movements of transposable elements in the DNA.

- **Deletions** of large segments of DNA also occurs.

*** Genotypic changes are also caused by changes in chromosome number (euploidy & aneuploidy) and gross change in chromosome structure (chromosomal aberrations)

TYPES OF MUTATIONS

Types of mutations

1. **Based on source of the mutation**

2. **Based on effect the mutation has on the gene product**

Spontaneous or Induced

Silent, Missense, Nonsense, Sense mutations

SPONTANEOUS vs INDUCED MUTATIONS

Spontaneous mutations are those that occur without a known cause.

- Could be due to low levels of metabolic errors, i.e. mistakes during replication or,

- caused by mutagenic agents present in the environment.

Induced mutations are those that result from exposure of organisms to mutagenic agents such as ionizing radiations, ultraviolet rays or various chemicals that react with DNA or RNA, in RNA viruses.

*** In reality it is impossible to detect whether a particular mutation occurred spontaneously or was induced.

Mutations classified according to their effects on the protein (or mRNA) produced by the mutant gene (mutations in ORFs)

1. **Silent Mutations (synonymous mutations).** Since the genetic code is degenerate, several codons produce the same amino acid. Especially, third base changes often have no effect on the amino acid sequence of the protein (Wobble hypothesis). These mutations affect the DNA but not the protein. **Therefore they have no effect on the organism's phenotype.**

2. **Missense mutations (non-synonymous).** Missense mutations substitute one amino acid for another. Some missense mutations have very large effects, while others have minimal or no effect. It depends on where the mutation occurs in the protein's structure, and how big a change in the type of amino acid it is.

   - Example: HbS, **sickle cell hemoglobin**, is a change in the beta-globin gene, where a GAG codon is converted to GUG. GAG codes for glutamic acid, which is a hydrophilic amino acid that carries a -1 charge, and GUG codes for valine, a hydrophobic amino acid. This amino acid is on the surface of the globin molecule, exposed to water. Under low oxygen conditions, valine's affinity for hydrophobic environments causes the hemoglobin to crystallize out of solution.

Sequence of part of a normal gene	Sequence of mutated gene

e) Neutral mutation (change from an amino acid to another amino acid with similar chemical properties; here an AT to GC transition mutation changes the codon from lysine to arginine)

5′ TCTCAAA A ATTTACG 3′
3′ AGAGTTT T TAAATGC 5′

··· Ser Gln Lys Phe Thr ···

5′ TCTCAAA G ATTTACG 3′
3′ AGAGTTT C TAAATGC 5′

··· Ser Gln Arg Phe Thr ···

f) Silent mutation (change in codon such that the same amino acid is specified; here an AT-to-GC transition in the third position of the codon gives a codon that still encodes lysine)

5′ TCTCAAAA A TTTACG 3′
3′ AGAGTTTT T AAATGC 5′

··· Ser Gln Lys Phe Thr ···

5′ TCTCAAAA G TTTACG 3′
3′ AGAGTTTT C AAATGC 5′

··· Ser Gln Lys Phe Thr ···

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3. **<u>Nonsense mutations</u>** convert an amino acid into a stop codon. The effect is to shorten the resulting protein. Sometimes this has only a little effect, as the ends of proteins are often relatively unimportant to function. However, often nonsense mutations result in completely non-functional proteins.

   – Example: Normal beta-globin is 146 amino acids long. In this mutation, codon 145 UAU (codes for tyrosine) is mutated to UAA (stop). The final protein is thus 143 amino acids long. The clinical effect is to cause overproduction of red blood cells, resulting in thick blood subject to abnormal clotting and bleeding.

4. **<u>Sense mutations</u>** are the opposite of nonsense mutations. Here, a stop codon is converted into an amino acid codon. Since DNA outside of protein-coding regions contains an average of 3 stop codons per 64, the translation process usually stops after producing a slightly longer protein.

   – Example: Normal alpha-globin is normally 141 amino acids long. In this mutation, the stop codon UAA is converted to CAA (glutamine). The resulting protein gains 31 additional amino acids before it reaches the next stop codon. This results in **thalassemia**, a severe form of anemia.

Sequence of part of a normal gene — Sequence of mutated gene

a) Transition mutation (AT to GC in this example)

```
5′  TCTCAAAAATTTACG  3′        5′  TCTCAAGAATTTACG  3′
3′  AGAGTTTTTAAATGC  5′        3′  AGAGTTCTTAAATGC  5′
```

b) Transversion mutation (CG to GC in this example)

```
5′  TCTCAAAAATTTACG  3′        5′  TCTGAAAAATTTACG  3′
3′  AGAGTTTTTAAATGC  5′        3′  AGACTTTTTAAATGC  5′
```

c) Missense mutation (change from one amino acid to another; here a transition mutation from AT to GC changes the codon from lysine to glutamic acid)

```
5′  TCTCAAAAATTTACG  3′        5′  TCTCAAGAATTTACG  3′
3′  AGAGTTTTTAAATGC  5′        3′  AGAGTTCTTAAATGC  5′
```

··· Ser | Gln | Lys | Phe | Thr ···   ··· Ser | Gln | Glu | Phe | Thr ···

d) Nonsense mutation (change from an amino acid to a stop codon; here a transversion mutation from AT to TA changes the codon from lysine to UAA stop codon)

```
5′  TCTCAAAAATTTACG  3′        5′  TCTCAATAATTTACG  3′
3′  AGAGTTTTTAAATGC  5′        3′  AGAGTTATTAAATGC  5′
```

··· Ser | Gln | Lys | Phe | Thr ···   ··· Ser | Gln | Stop

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Effect of a nonsense mutation on translation.


10.10
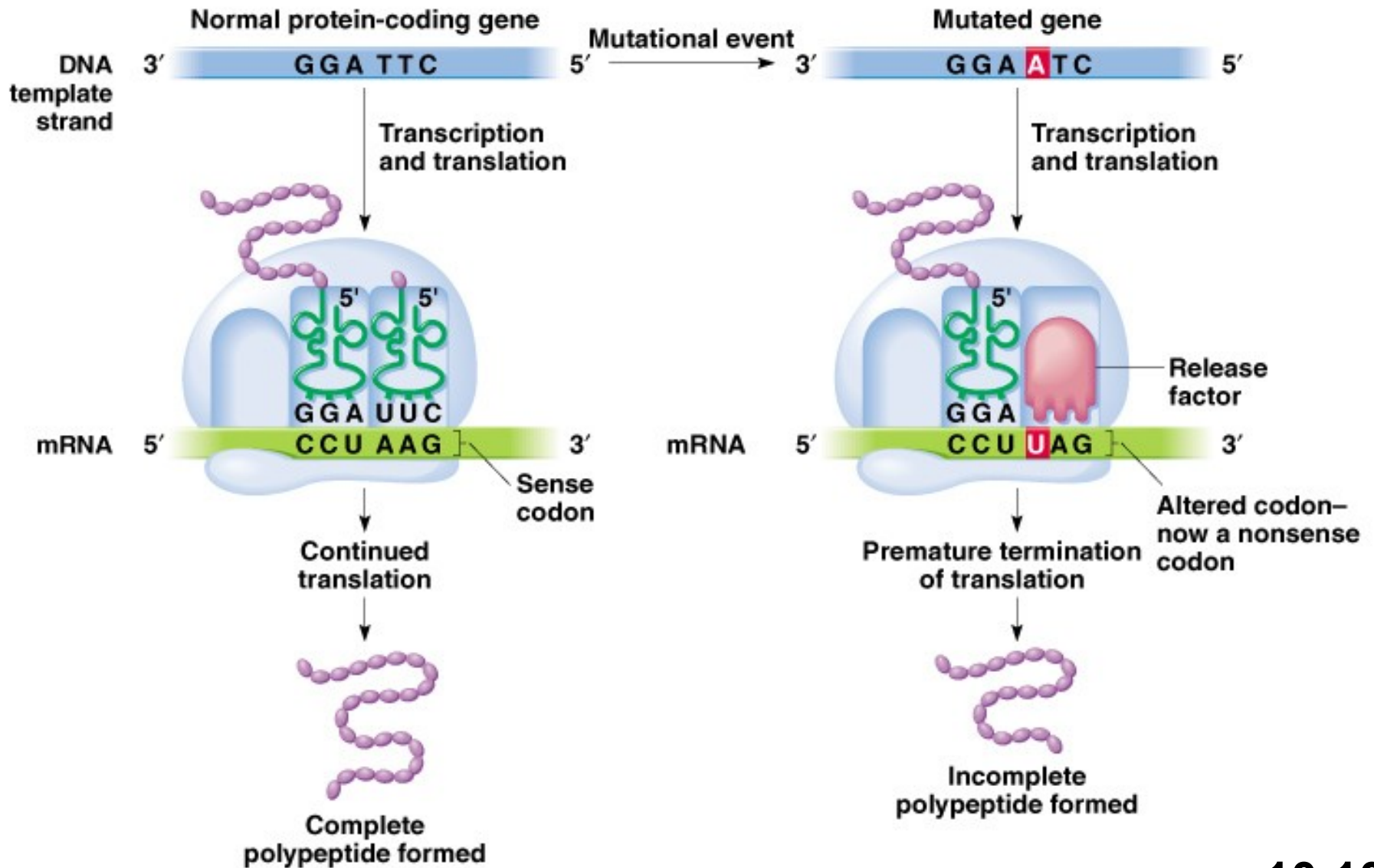
Frameshift mutations:

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).

g) Frameshift mutation (addition or deletion of one or a few base pairs leads to a change in reading frame; here the insertion of a GC base pair scrambles the message after glutamine)

| 5′ | TCTCAAAAATTTACG | 3′ |
| 3′ | AGAGTTTTTAAATGC | 5′ |

··· Ser Gln Lys Phe Thr ···

| 5′ | TCTCAAGAAATTTACG | 3′ |
| 3′ | AGAGTTCTTTAAATGC | 5′ |

··· Ser Gln Glu Ile Tyr ···

Streisinger Model

In the mid-1960s, George Streisinger and his coworkers deduced the **nucleotide** sequence surrounding different sites of frameshift **mutations** in the lysozyme **gene** of **phage** T4. They found that these **mutations** often occurred at repeated sequences and formulated a model to account for frameshifts in DNA synthesis.
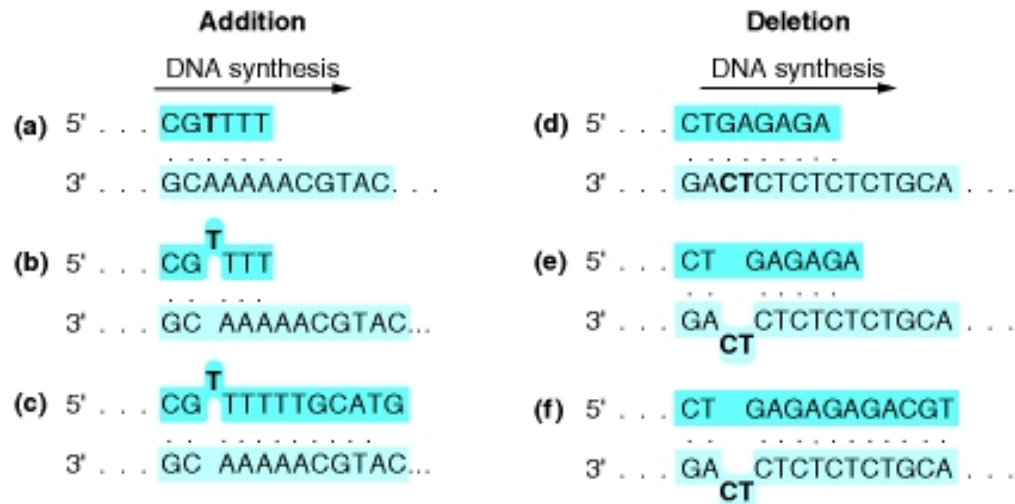
A simplified version of the Streisinger model for frameshift formation. (a–c) In DNA synthesis, the newly synthesized strand slips, looping out one or several bases. This loop is stabilized by the pairing afforded by the repetitive-sequence unit (the A bases in this case). An addition of one base pair, A–T, will result at the next round of replication in this example. (d–f) If, instead of the newly synthesized strand, the template strand slips, then a deletion results. Here the repeating unit is a CT dinucleotide. After slippage, a deletion of two base pairs (C–G and T–A) would result at the next round of replication.

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
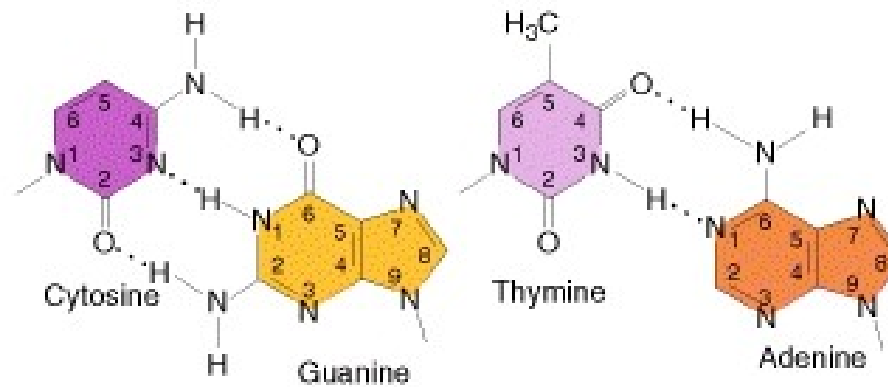
Intergenic suppressors occur on a different gene.

*** If the wild type phenotype is restored by suppressor mutation, the original mutation will still be present and can be separated from the suppressor mutation by backcrossing the revertants.

Molecular Basis of Mutation

During 1950s when **Watson & Crick** proposed the double helix structure and the semi-conservative mode of DNA replication, they **also suggested the possible cause of Spontaneous Mutation:**

➢ Structure of the bases in DNA are not static.

➢ Hydrogen atoms can move from one position in a purine or pyrimidine to another position, for e.g., from an amino group to a ring nitrogen.

➢ Such chemical fluctuations are called **Tautomeric Shifts**. Although tautomeric shifts occur at a very low rate, they are of considerable importance in DNA metabolism, as they can alter the pairing potential of the bases.

➢ **The more stable "keto" forms of thymine and guanine, and "amino" forms of adenine and cytosine may infrequently undergo tautomeric shifts to less stable enol and imino forms.**

➢ Though the tautomeric forms exist for very short period, if this form exists during replication, base pairing gets altered leading to mutation. In their rare imino or enol states, they can form **adenine-cytosine** and **guanine-thymine** base pairing.
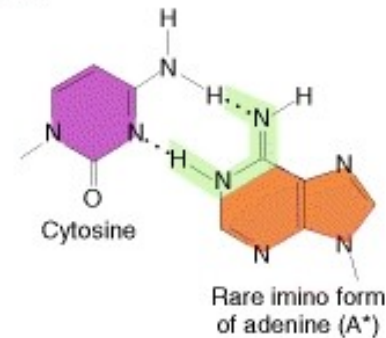
Normal base-pairing

Base-pairing in rare forms

Rare imino form of cytosine (C*) — Adenine

Rare enol form of thymine (T*) — Guanine

(a)

Cytosine — Rare imino form of adenine (A*)

Thymine — Rare enol form of guanine (G*)
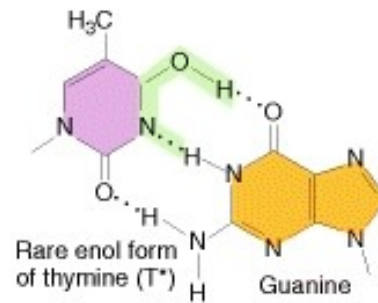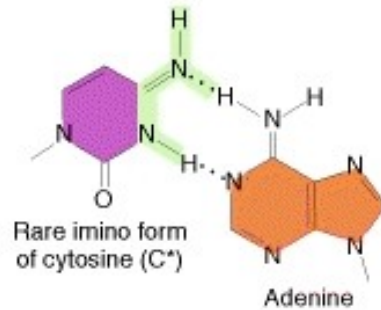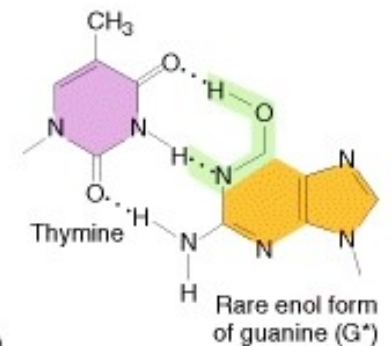
(b)

Two types of point mutations:

1. **Base pair substitutions.**
   ### A. Transitions

   - **Conversion of a purine-pyrimidine to the other purine-pyrimidine.**

   - **4 types of transitions; A $\leftrightarrow$ G and T $\leftrightarrow$ C**

   - **Most transitions results in synonymous substitution.**

   ### B. Transversions

   - **Conversion a purine-pyrimidine to a pyrimidine-purine.**

   - **8 types of transversions; A $\leftrightarrow$ T, G $\leftrightarrow$ C, A $\leftrightarrow$ C, and G $\leftrightarrow$ T**

   - **Transversion more likely to result in nonsynonymous substitution.**

Base pair substitutions:
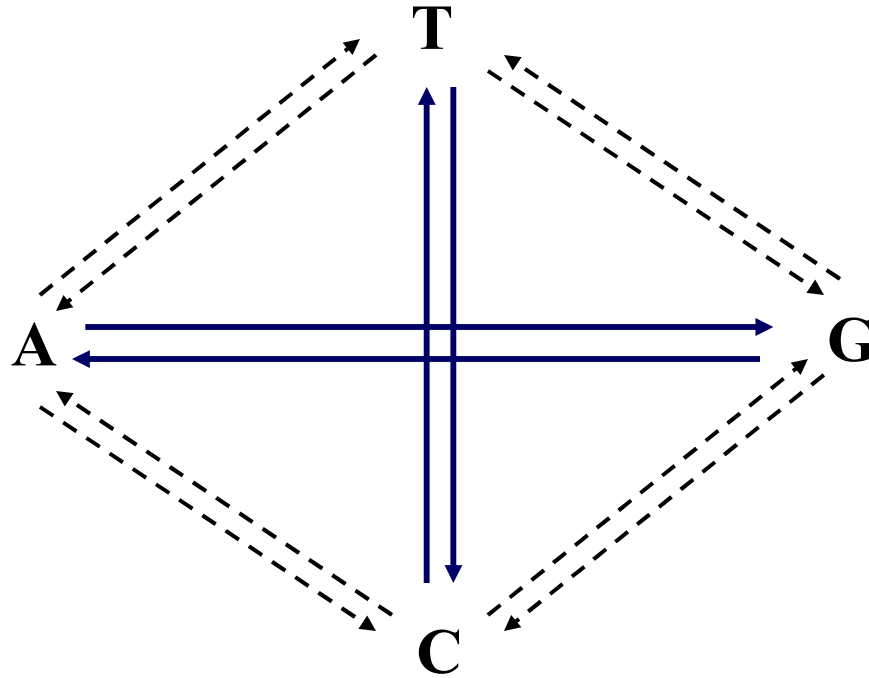
Four possible transition mutations i.e., purine to purine or pyrimidine to pyirimidine changes (indicated by solid arrow)

Eight possible transversion mutations i.e., purine to pyrimidine and pyrimdine to purine changes

*** **The repair machineries can recognize and edit such mismatches. Therefore the amount of mismatches observed are much less than what occurs.**

Types of base pair substitution

Transition
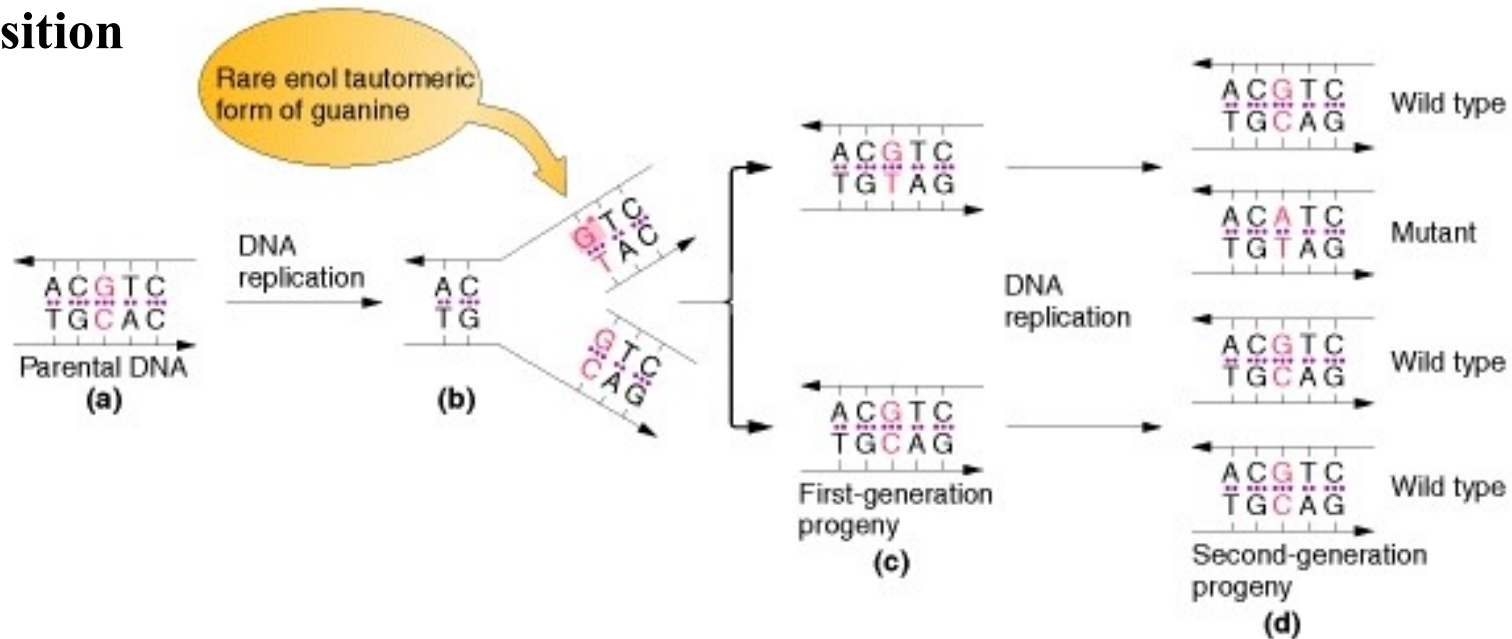
Mutation by tautomeric shifts in the bases of DNA. (a) In the example diagrammed, a guanine undergoes a tautomeric shift to its rare enol form (G*) at the time of replication. (b) In its enol form, it pairs with thymine. (c and d) In the next replication, the guanine shifts back to its more stable keto form. The thymine incorporated opposite the enol form of guanine, seen in part b, directs the incorporation of adenine in the subsequent replication, shown in parts c and d. The net result is a GC→AT mutation. If a guanine undergoes a tautomeric shift from the common keto form to the rare enol form at the time of incorporation (as a nucleoside triphosphate, rather than in the template strand diagrammed here), it will be incorporated opposite thymine in the template strand and cause an AT→GC mutation. (From E. J. Gardner and D. P. Snustad, *Principles of Genetics,* 5th ed. Copyright © 1984 by John Wiley & Sons, New York.)

SPONTANEOUS & INDUCED MUTATIONS:

Spontaneous mutations

- Spontaneous mutations can occur at any point of the cell cycle.

- Movement of <u>transposons</u> (mobile genetic elements) causes spontaneous mutations.

- Mutation rate = ~10^{-4} to 10^{-6} mutations/gene/generation

- Rates vary by lineage, and most spontaneous errors are repaired.

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Spontaneous mutations:

Different types 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 <u>template strand</u>, DNA polymerase <u>skips bases</u>, and deletion occurs.

DNA loops out on <u>new strand</u>, DNA polymerase <u>adds untemplated bases</u>.

Mutation caused by mismatch wobble base pairing.

10.15
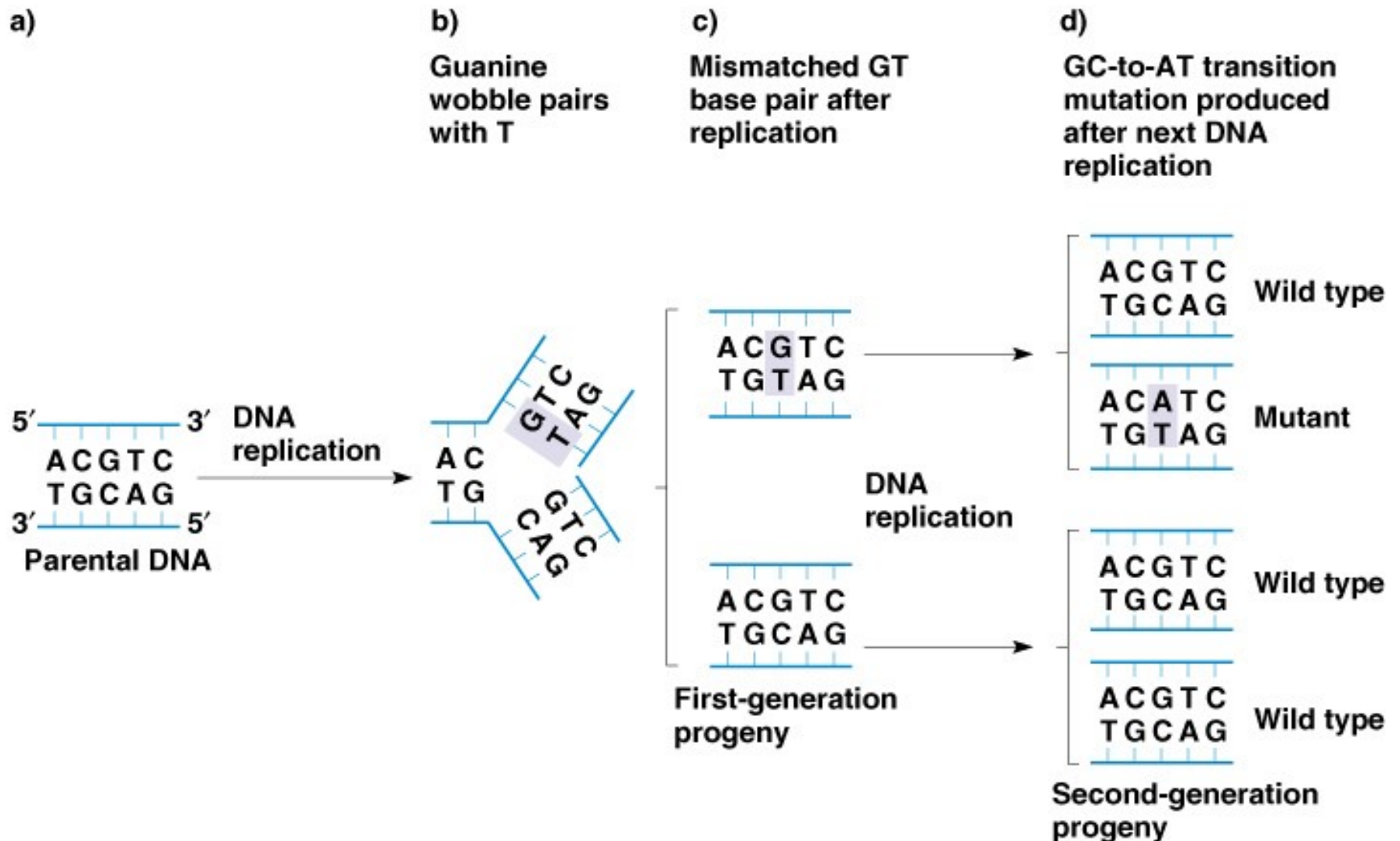
Addition and deletion by DNA looping-out.

10.16
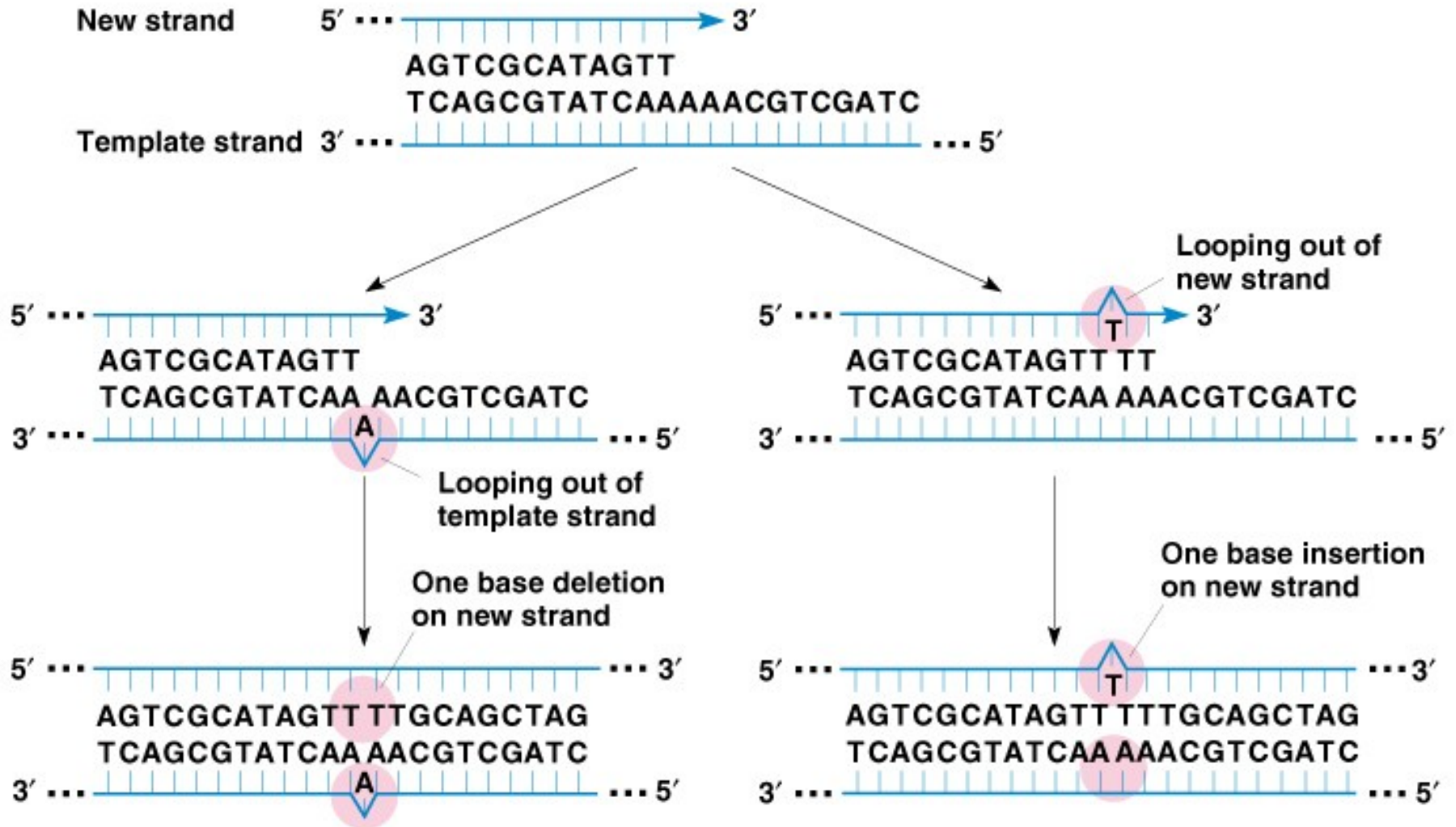
Spontaneous mutations:

Spontaneous chemical changes: Frequent spontaneous lesions also result from
          i) depurination and
          ii) deamination

Depurination

➤ More common; involves interruption of the glycosidic bond between the base and the deoxyribose backbone, leading to loss of A or G residues from the DNA.

➤ A mammalian cell spontaneously loses ~ 10,000 purines during a 20 hr generation time at 37°C.

➤ During replication an apurinic site cannot specify for a base complementary to the original purine.

➤ Sometimes random bases might get inserted across a apurinic site.

➤ Efficient repair machineries can remove apurinic sites.

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Deamination

➢ Deamination of cytosine generates uracil (C → U); if not replaced U pairs with A in next round of replication, thus GC pair gets converted to AT.

➢ Deamination at certain cytosine residues were found to be the cause of mutational hotspots.

➢ Sequence analysis of GC to AT transition hotspots in *lacI* gene showed presence of 5-methyl cytosine in each spot; deamination of $5^{M}C$ produces T (CG → TA).

➢ Regions with high levels of $5^{M}C$ are mutation hot spots because:

One of the repair enzyme UDG (uracil DNA-glycosylase) recognize and removes uracil residues.

However, deamination of 5-methyl cytosine will generate thymine cannot be recognized by UDG and thus remains unrepaired.

Deamination.

a)

Cytosine → Deamination → Uracil

b)

5-methylcytosine (5mC) → Deamination → Thymine (T)

10.18
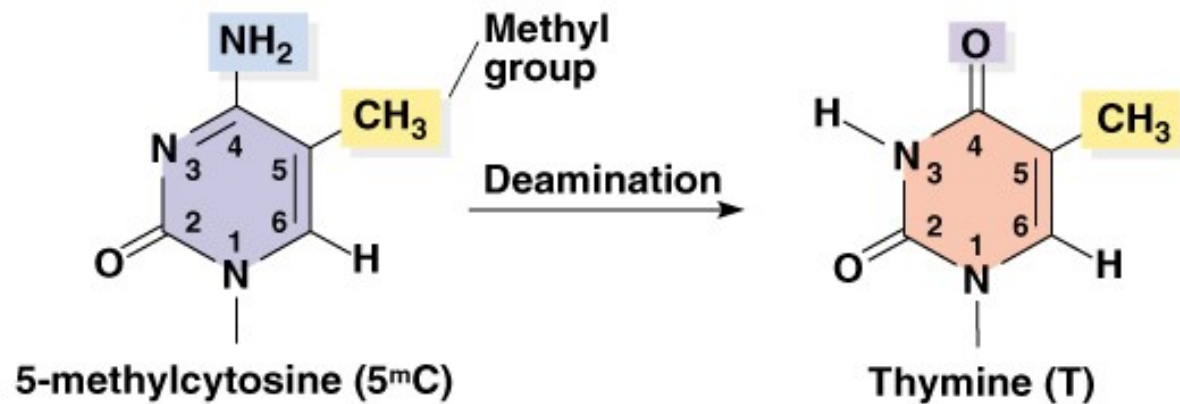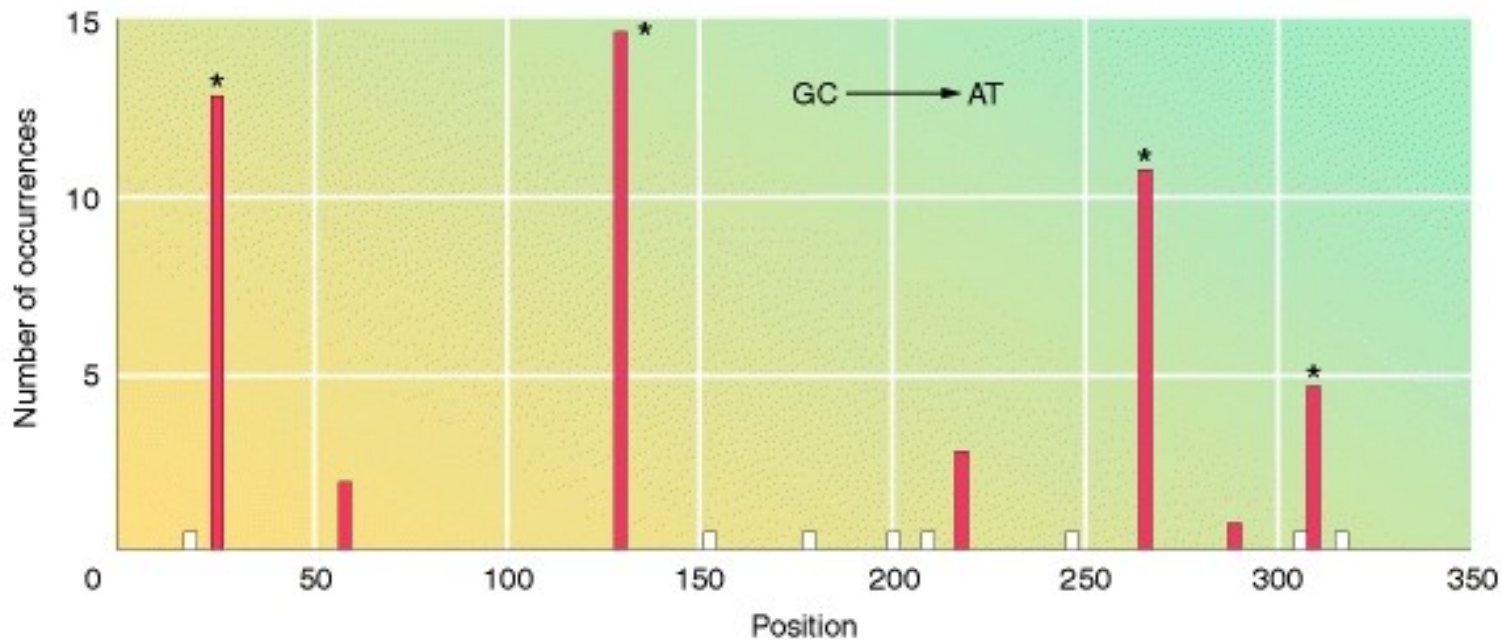
5-Methylcytosine hot spots in *E. coli*. Nonsense mutations occurring at 15 different sites in *lacI* were scored. All result from the GC→AT transition. The asterisks (*) mark the positions of 5-methylcytosines. Open bars depict sites at which the GC→AT change could be detected but at which no mutations occurred in this particular collection. It can be seen that 5-methylcytosine residues are hot spots for the GC→AT transition. Of 50 independently occurring mutations, 44 were at the 4 methylated cytosine sites and only 6 were at the 11 unmethylated cytosines. (From C. Coulondre et al., *Nature* 274, 1978, 775.).

Induced mutations

Radiation (e.g., X-rays, UV)

Ionizing radiation breaks covalent bonds including those in DNA and is the leading cause of DNA mutations.

Ionizing radiation has a cumulative effect and kills cells at high doses.

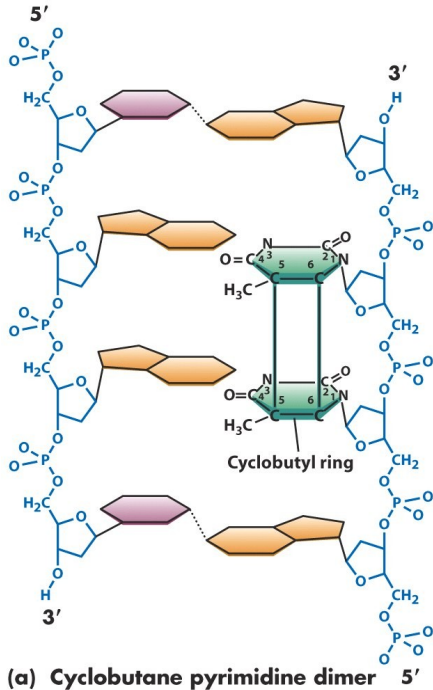**UV (254-260 nm) causes purines and pyrimidines to form abnormal dimer bonds and bulges in the DNA strands.**
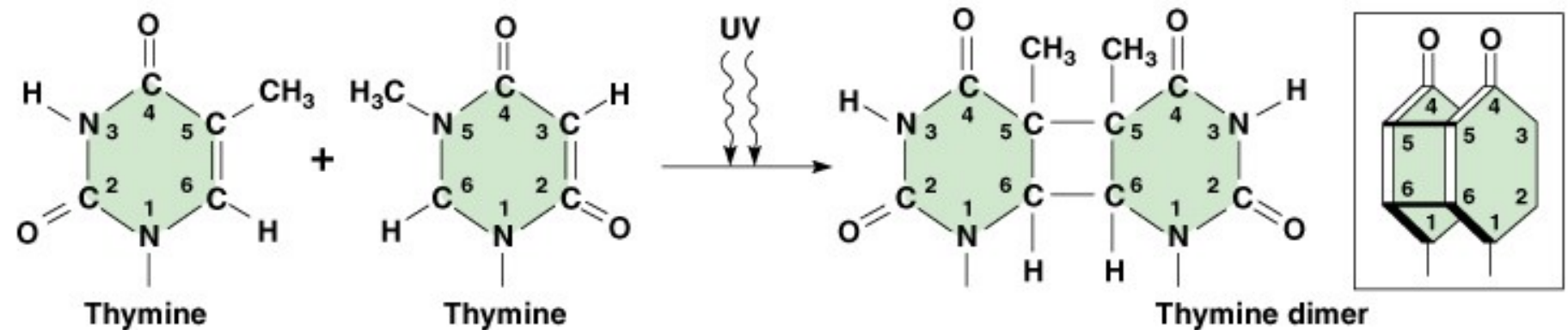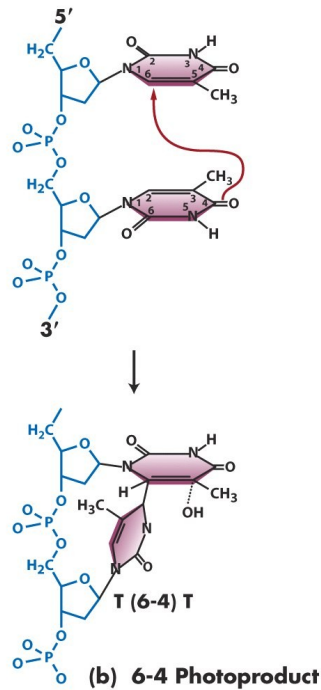

Thymine dimers induced by UV light.
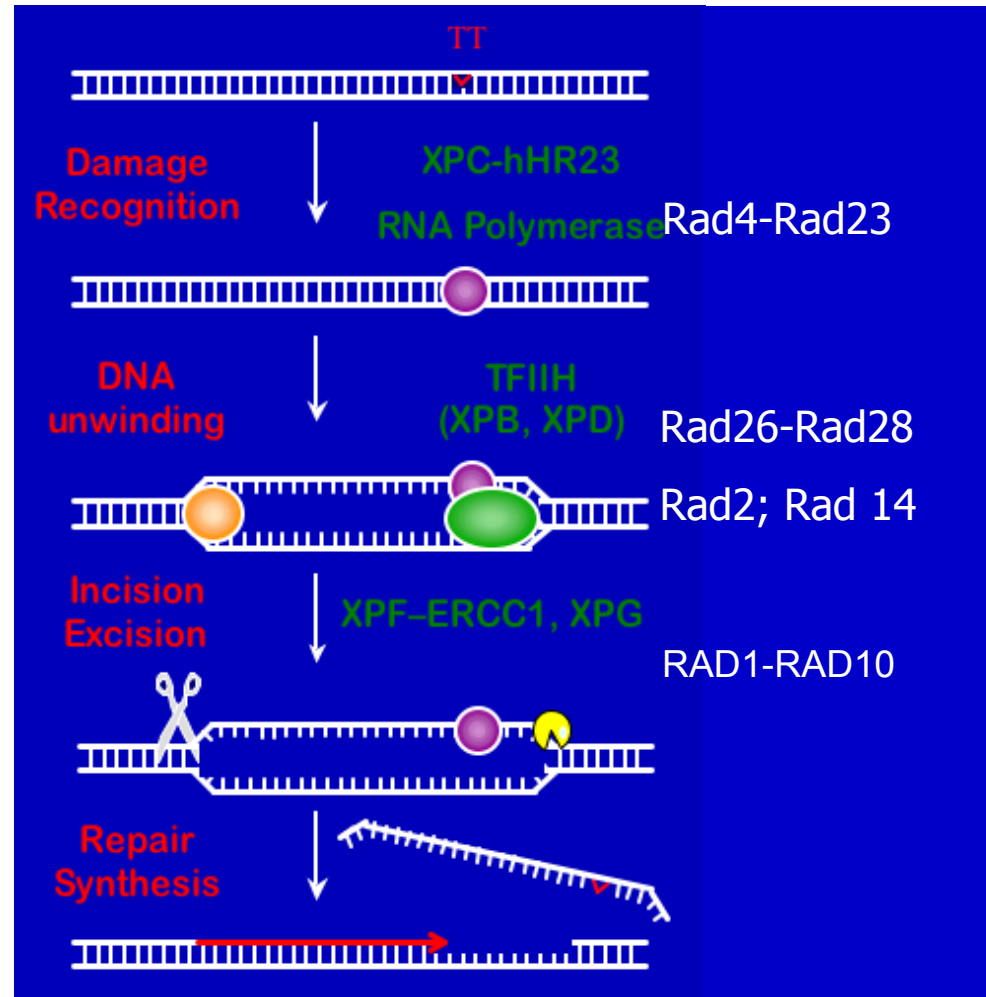
DNA Damage and Consequences

Spontaneous Point Mutations

Replication Errors

Metabolism

UV Radiation

Ionizing Radiation

Extensive Chemical Agents

Endogenous

Exogenous

Proteins

DNA

Lipids

Degraded and Resynthesized

Apoptosis

Mutation Fixation

Repaired

Degraded and Resynthesized

Cancer

Genetic Disorders
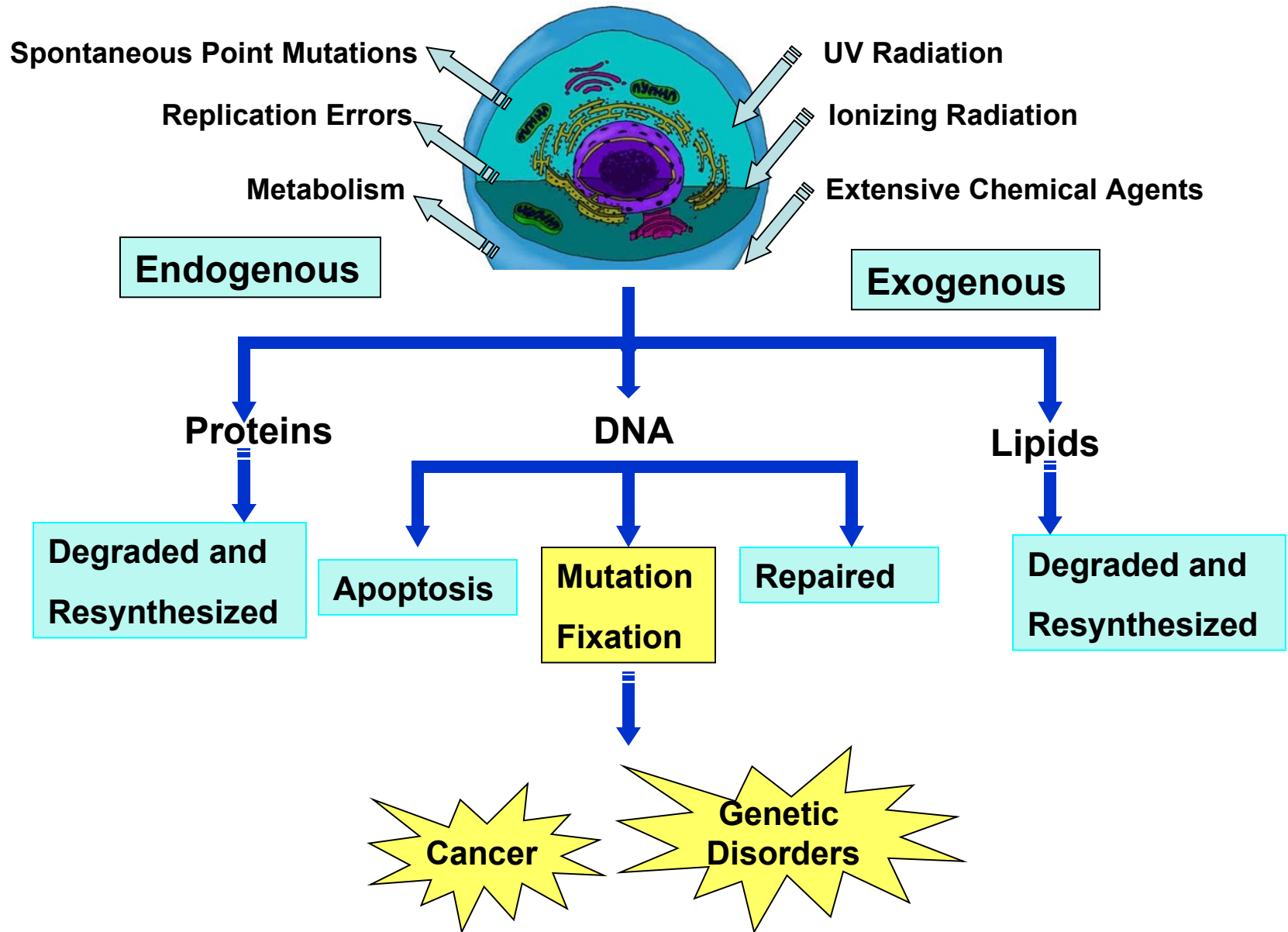
DNA damages induced by UV light

(a) Cyclobutane pyrimidine dimer

(b) 6-4 Photoproduct

CPD

6-4 PP

Rad4-Rad23

Rad26-Rad28

Rad2; Rad 14

RAD1-RAD10

NER in eukaryotic cells

Diseases with Defective Nucleotide Excision Repair

XP: Xeroderma Pigmentosum →

CS: Cockayne's Syndrome

TTD: Triochothiodystrophy

(a)

- extreme photosensitivity
- strong predisposition to skin cancer
- sometimes mental retardation
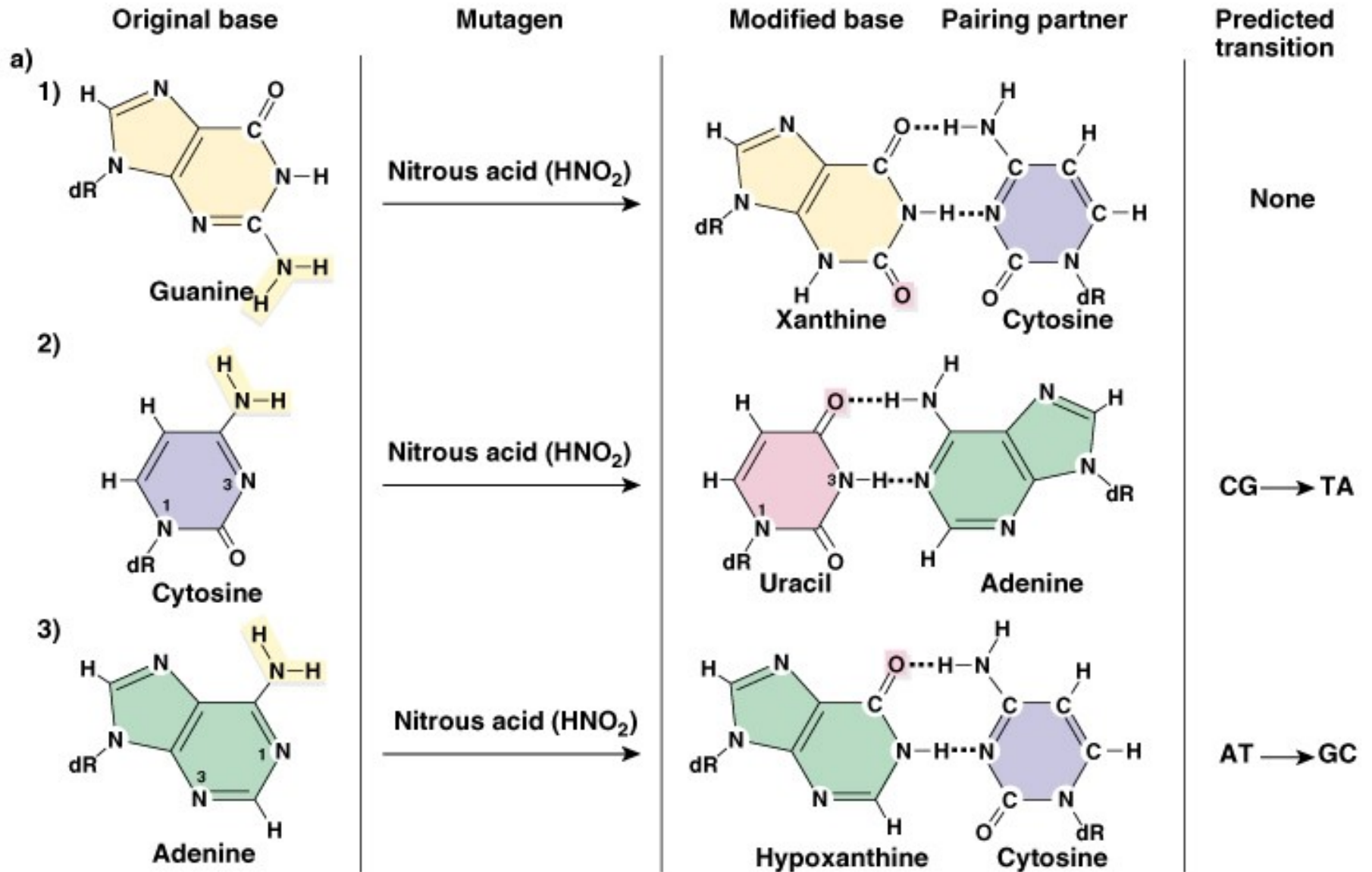- predisposition to various cancers

Induced mutations: chemical mutagens

Base analogs

- Similar to normal bases, incorporated into DNA during replication.

- Some cause mis-pairing (e.g., 5-bromouracil).

- Not all are mutagenic.

Mutagenic effects of 5-bromouracil

a) Base-pairing of 5-bromouracil in its normal state

5-bromouracil (behaves like thymine; normal state)

Adenine (normal state)

Attachment of base to sugar

Attachment of base to sugar
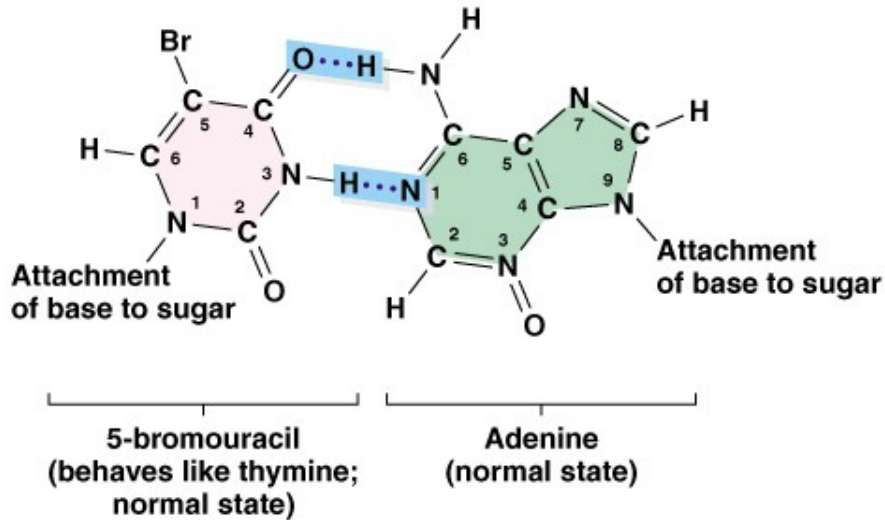
b) Base-pairing of 5-bromouracil in its rare state

5-bromouracil (behaves like cytosine; rare state)

Guanine (normal state)

10.21
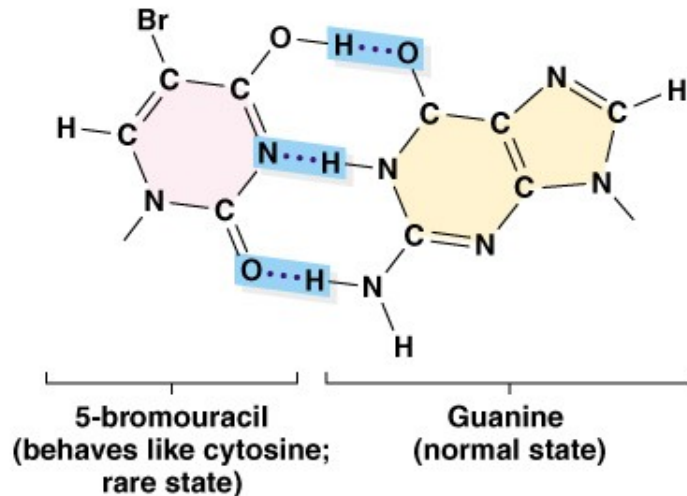
Mutagenic effects of 5-bromouracil

c) Mutagenic action of 5BU

10.22
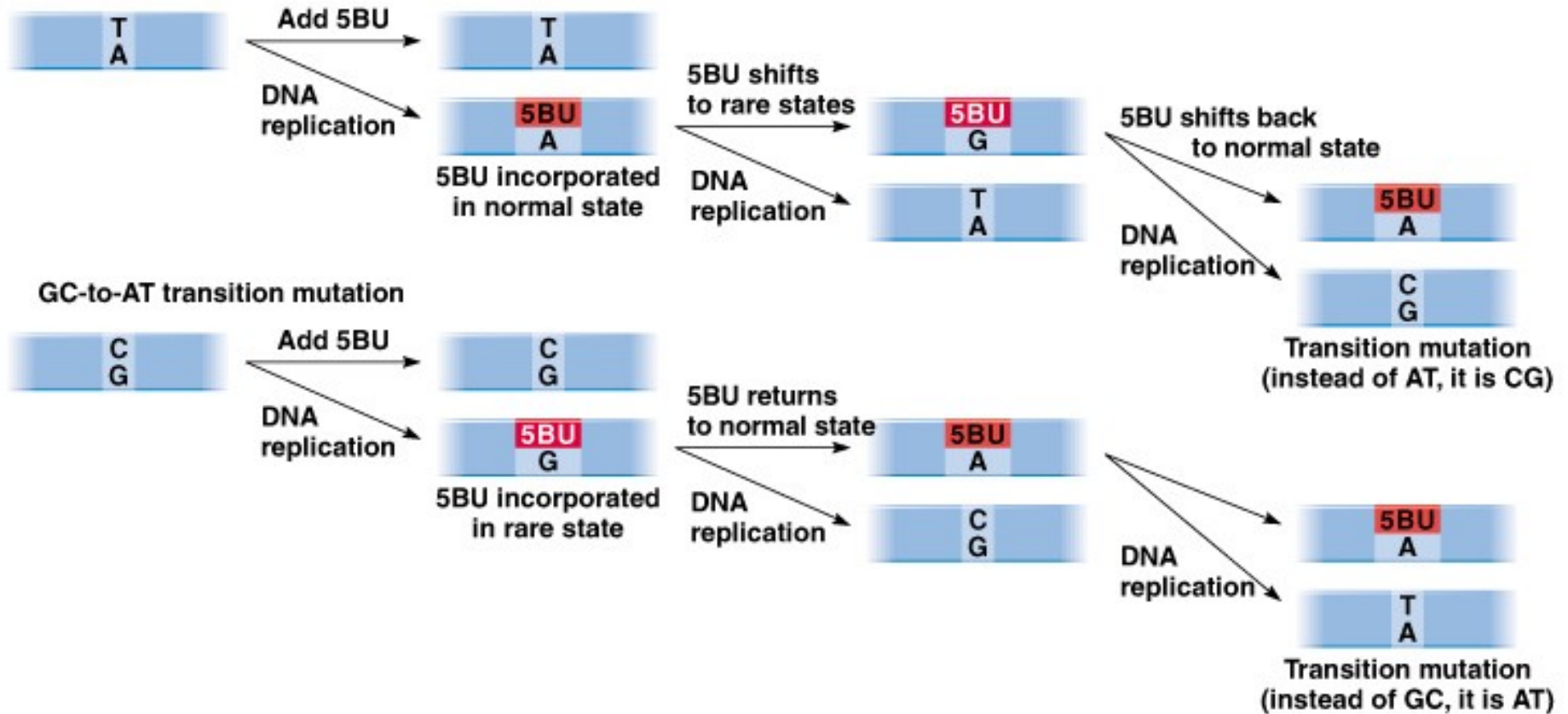
Induced mutations: Chemical mutagens
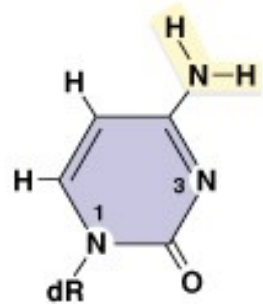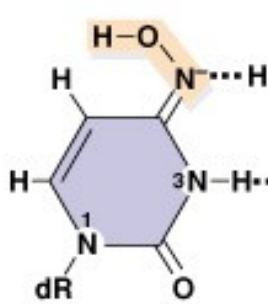
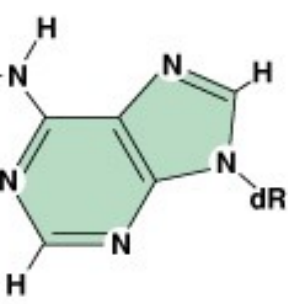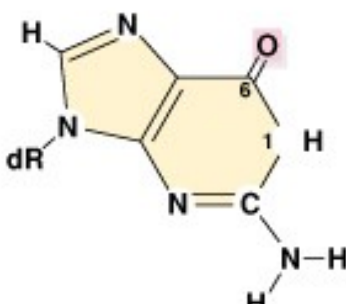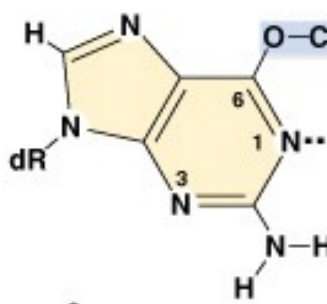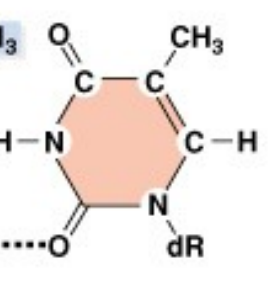**Base modifying agents, act at any stage of the cell cycle:**

- **Deaminating agents**

- **Hydroxylating agents**

- **Alkylating agents**

10.23

Base-modifying agents.
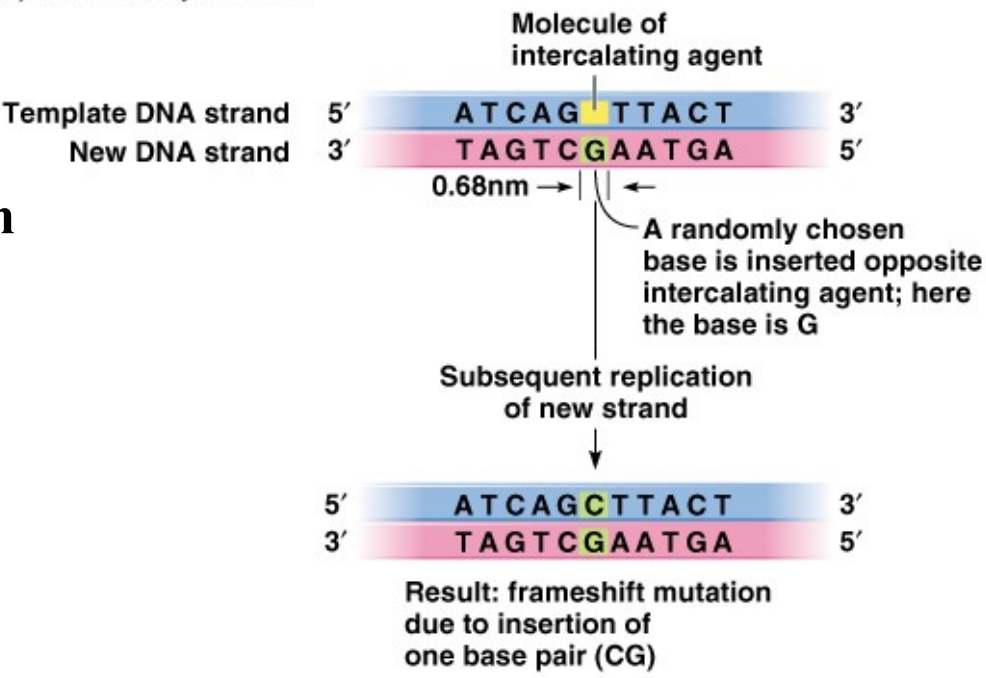

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Base-modifying agents (cont.).

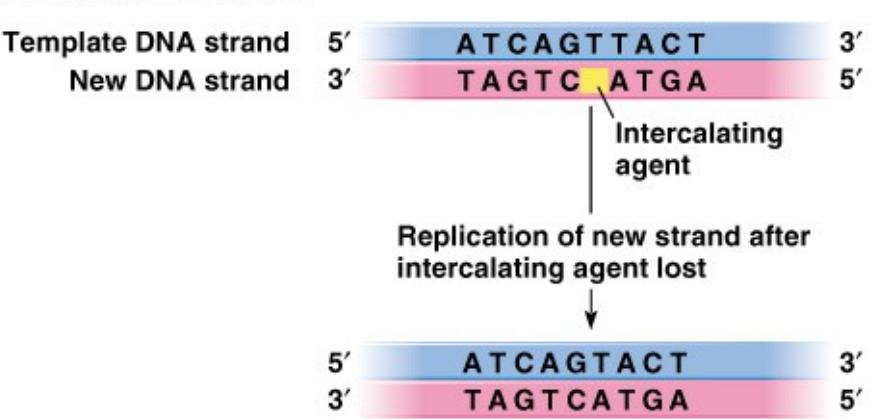**10.25**

Induced mutations: chemical mutagens

Intercalating agents:

- **Thin, plate-like hydrophobic molecules insert themselves between adjacent base-pairs,**

- **Mutagenic intercalating agents cause insertions during DNA replication.**

- **Loss of intercalating agent can result in deletion.**

- **Examples: proflavin, ethidium bromide**

a) Mutation by addition

Molecule of intercalating agent

Template DNA strand   5′   A T C A G [ ] T T A C T   3′
New DNA strand   3′   T A G T C G A A T G A   5′

0.68nm →| | ←

A randomly chosen base is inserted opposite intercalating agent; here the base is G

Subsequent replication of new strand

5′   A T C A G C T T A C T   3′
3′   T A G T C G A A T G A   5′

Result: frameshift mutation due to insertion of one base pair (CG)

b) Mutation by deletion

Template DNA strand   5′   A T C A G T T A C T   3′
New DNA strand   3′   T A G T C [ ] A T G A   5′

Intercalating agent

Replication of new strand after intercalating agent lost

5′   A T C A G T A C T   3′
3′   T A G T C A T G A   5′

10.26

Detecting Mutagens

- Radiation and certain chemical compounds are "mutagens": they cause mutation.

- Cancer is caused by somatic mutations, and so mutagens are also carcinogens.

- Testing for mutagenicity is a key step in development of pharmaceutical drugs.

- Simple test using bacteria (Salmonella, a close relative of E. coli) developed by Bruce Ames: the **"Ames test".**

Ames Test

- Start with Salmonella that are his-, auxotrophs unable to make their own histidine. They will only grow if histidine is added to the growth medium.

- Add compound to be tested to growth medium, count number of colonies growing. These are revertants, which have been mutated back to wild type.

- In many cases, mutagens need to be activated, converted to mutagenic state, by enzymes in the liver that are meant to detoxify dangerous compounds. Liver extracts are often added to the growth medium to accomplish this.

- Test isn't perfect: Salmonella are prokaryotes, and we have complex biochemistries that modify foreign compounds. But, it is a good initial screen.