DNA Mutation

Molecular Biology

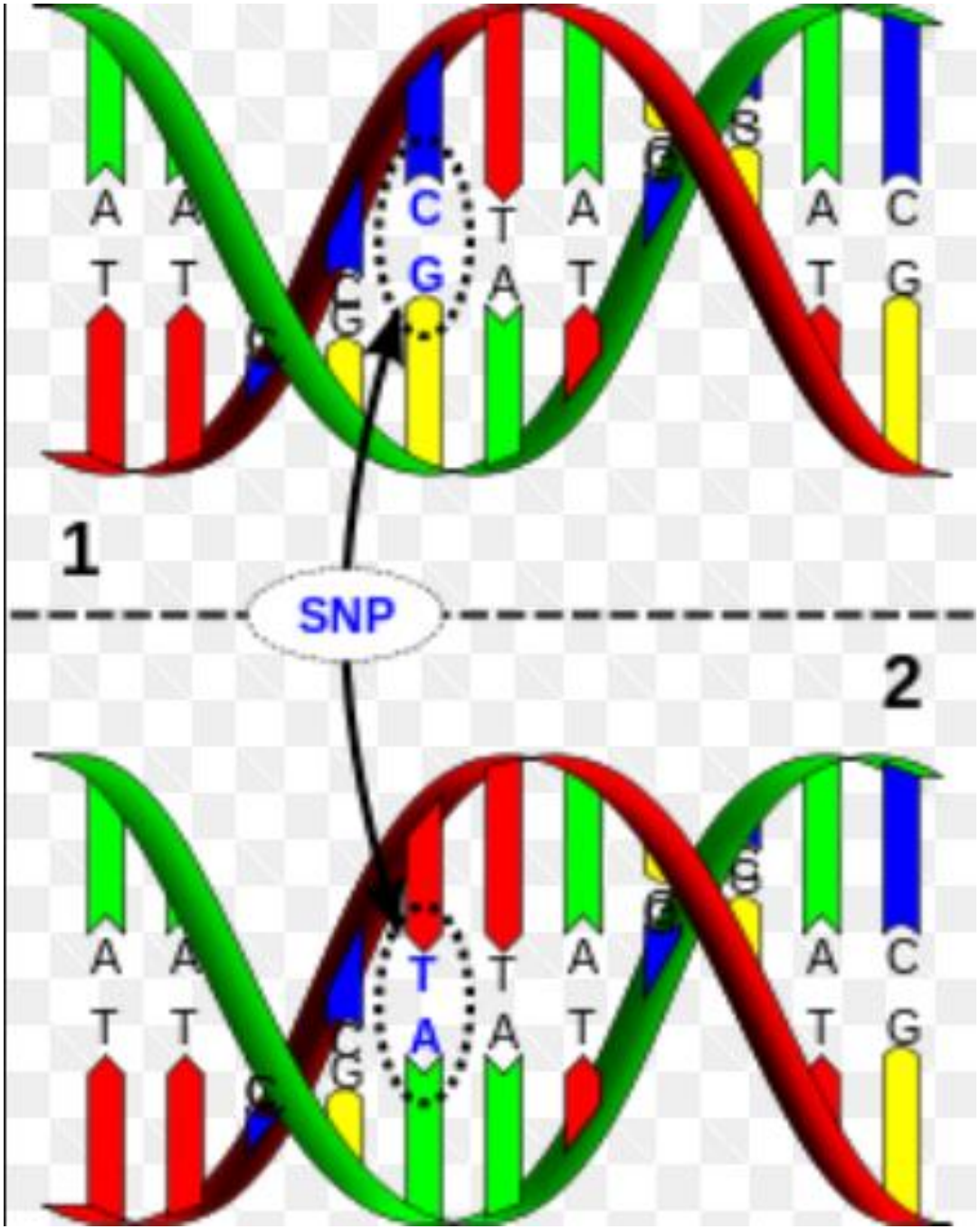
Why are mutations so important to living organisms?

- Most mutations are harmful to an organism. Random changes in the gene sequence may result in the malformation and subsequent loss of function of a protein.
- Humans suffer from over **3000 genetic diseases**. Every one of these is caused by a mutation. Some mutations however are neutral. The protein may be identical or, if changed, works equally well.
- A very **few mutations are beneficial** to an organism. A different protein may alter or create a trait that better adapts an organism to its niche in life.
- All living organisms today are the result of the accumulation of beneficial mutations over the past 3.5 billion years.

Mutations Defined

1. A mutation is a change in a DNA base-pair or a chromosome.
   a. Somatic mutations affect only the individual in which they arise.
   b. Germ-line mutations alter gametes, affecting the next generation.
2. Mutations are quantified in two different ways:
   a. Mutation rate is the probability of a particular kind of mutation as a function of time (e.g., number per gene per generation).
   b. Mutation frequency is number of times a particular mutation occurs in proportion to the number of cells or individuals in a population (e.g., number per 100,000 organisms).

- A mutation is a change in the sequence of bases in a DNA molecule. A mutation can occur in any cell but the most important ones happen in the gamete-making cells because they are passed onto the next generation.


1
SNP
2

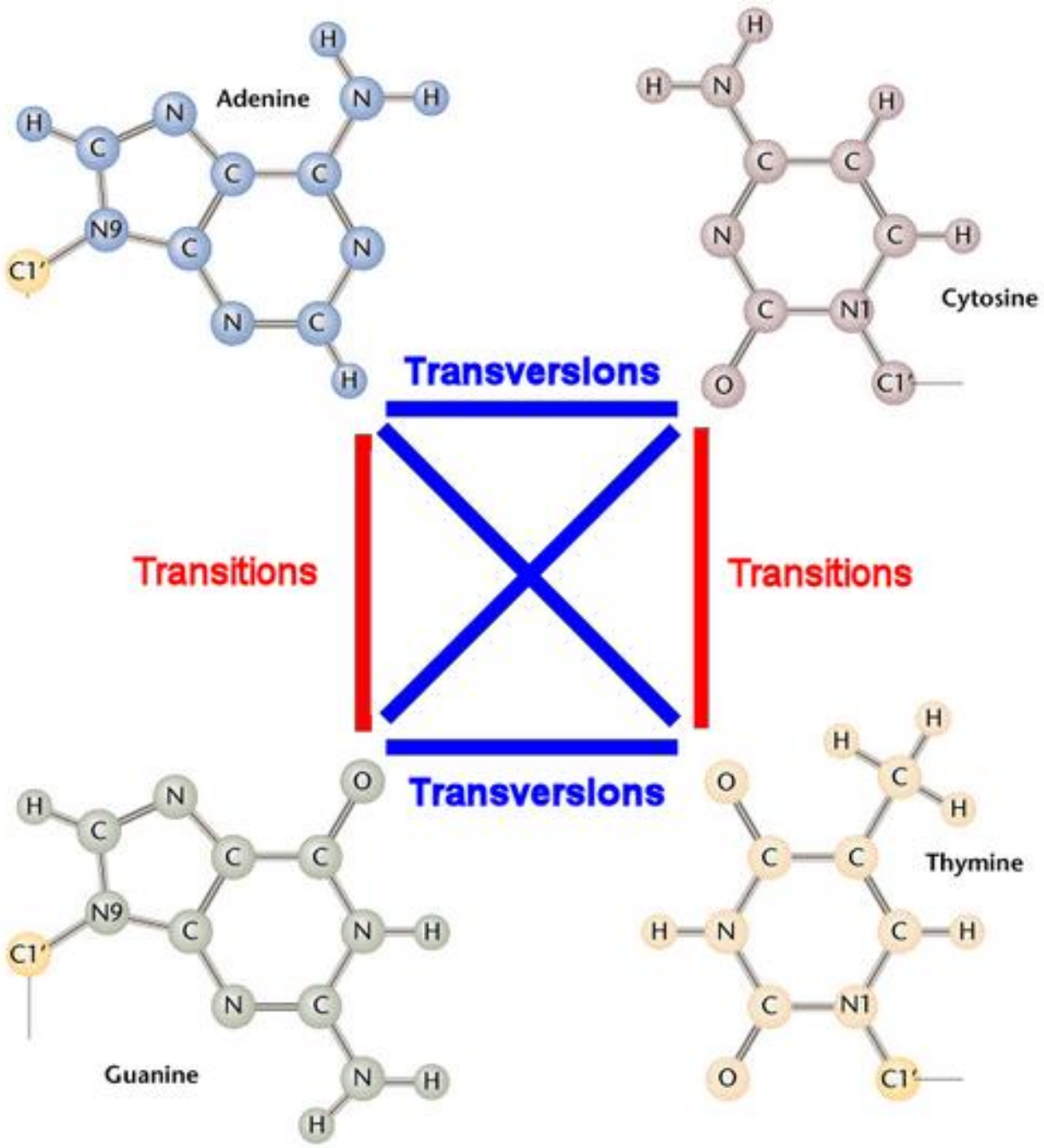
Point Mutations

There are two general categories of point mutations: base-pair substitutions and base-pair deletions or insertions.

1. A base-pair substitution replaces 1 base-pair with another. There are two types:

   a. **Transitions** convert a purine-pyrimidine pair to the other purine-pyrimidine pair (e.g., AT to GC or TA to CG).

   b. **Transversions** convert a purine-pyrimidine pair to a pyrimidine-purine pair (e.g., AT to TA, or AT to CG).


Adenine
Cytosine
Transversions
Transitions
Transitions
Transversions
Guanine
Thymine

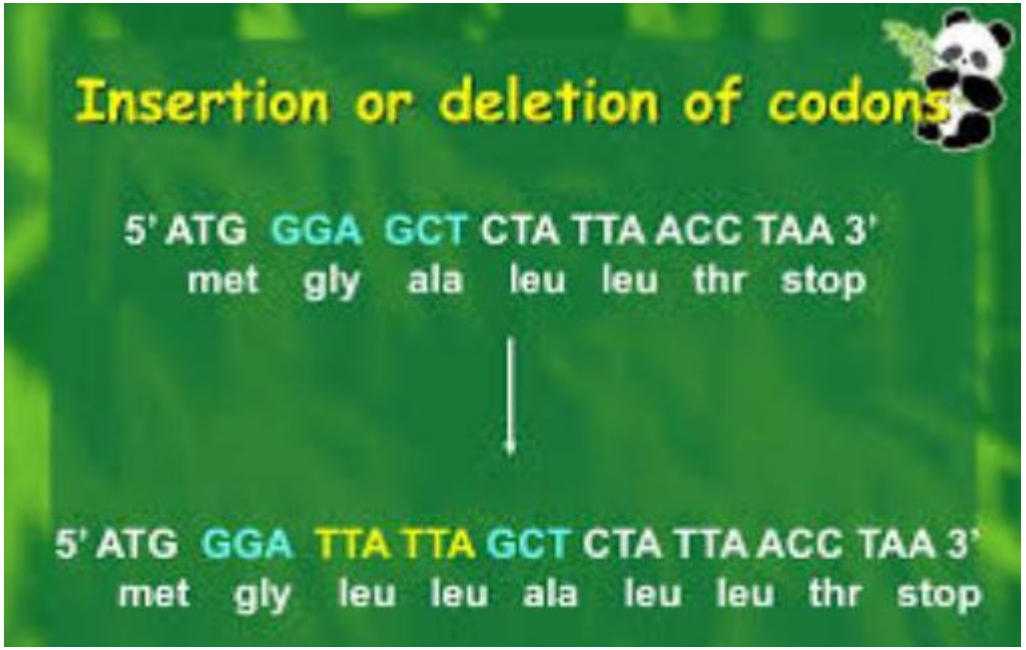
Point Mutations Cont.

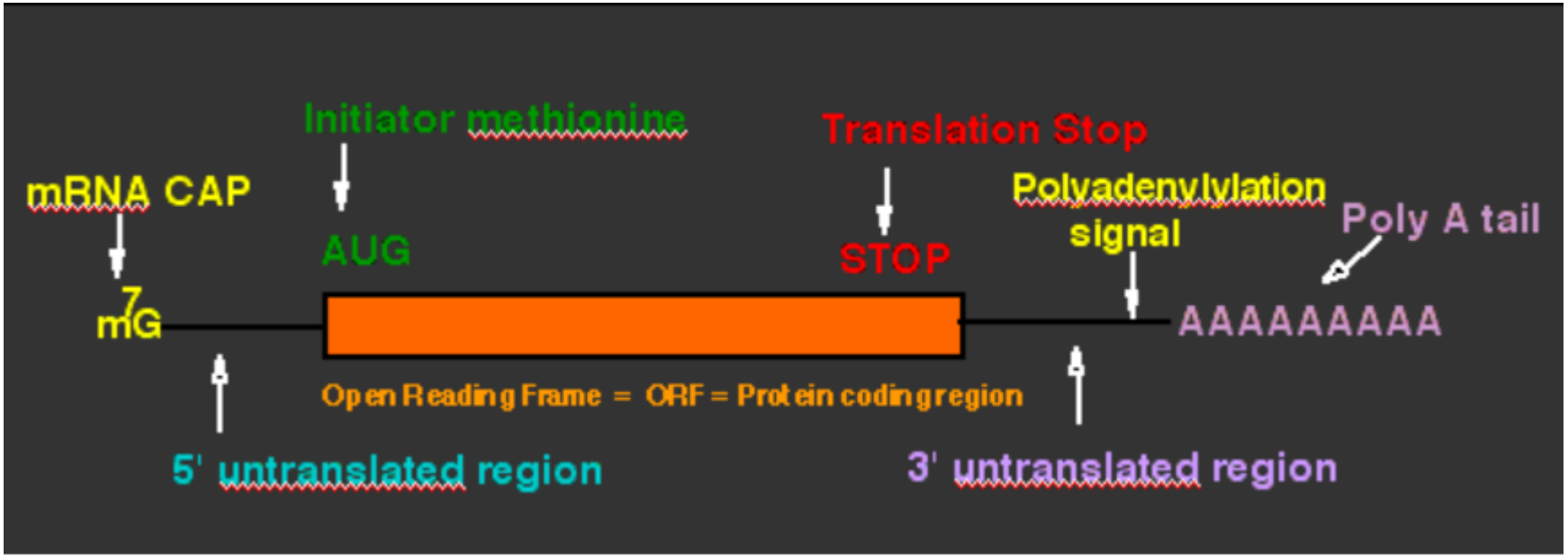
2. **Deletions and insertions** can change the reading frame of the mRNA downstream of the mutation, resulting in a frameshift mutation.
   a. When the reading frame is shifted, incorrect amino acids are usually incorporated.
   b. Frameshifts may bring stop codons into the reading frame, creating a shortened protein.
   c. Frameshifts may also result in read-through of stop codons, resulting in a longer protein.
   d. Frameshift mutations result from insertions or deletions when the number of affected base pairs is not divisible by three.

Open reading Frame (ORF)

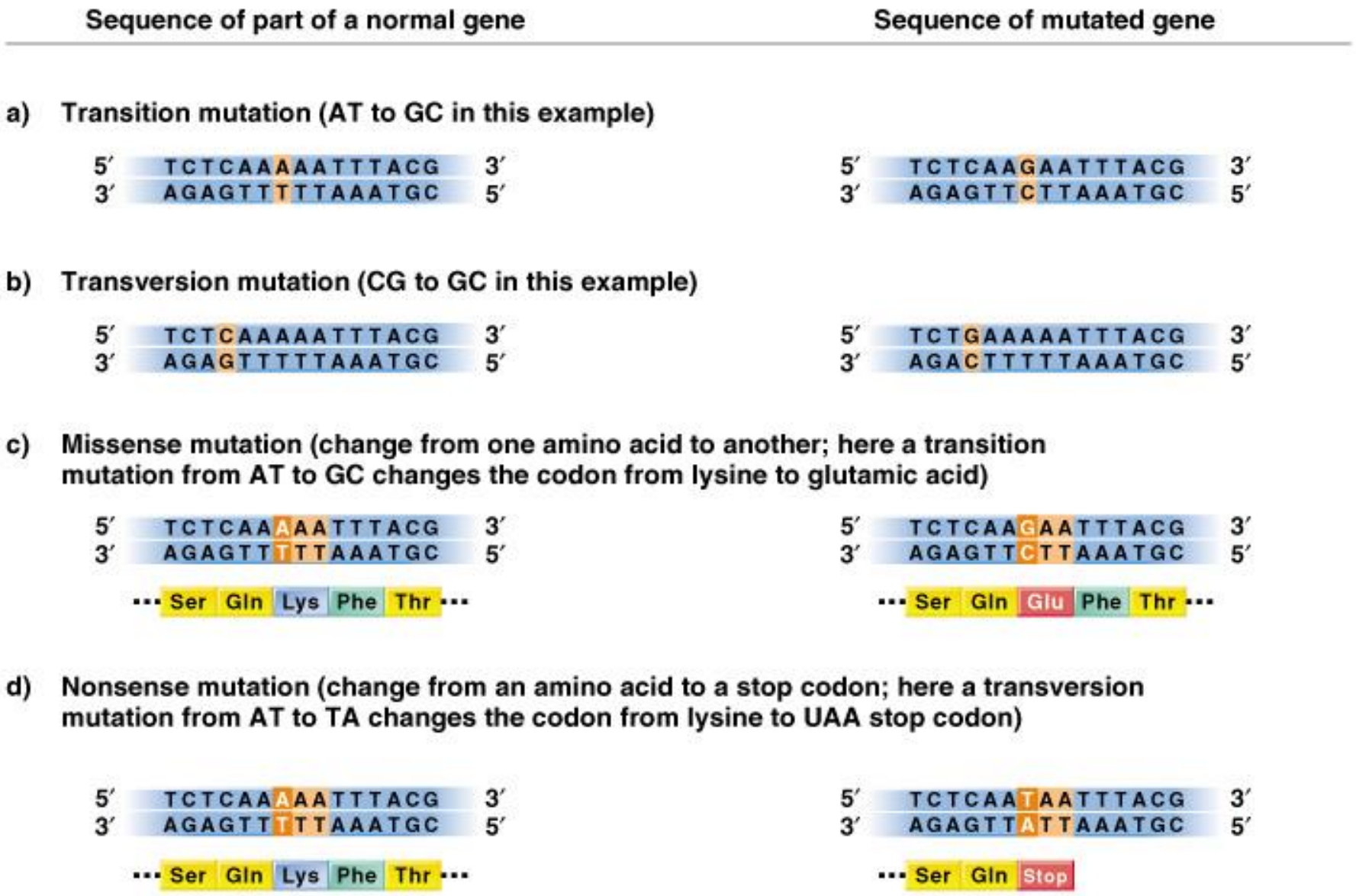
Base-pair substitutions in ORFs are also defined by their effect on the protein sequence.

Nonsense mutations change a codon in the ORF to a stop (nonsense) codon, resulting in premature termination of translation, and a truncated (often nonfunctional) protein.

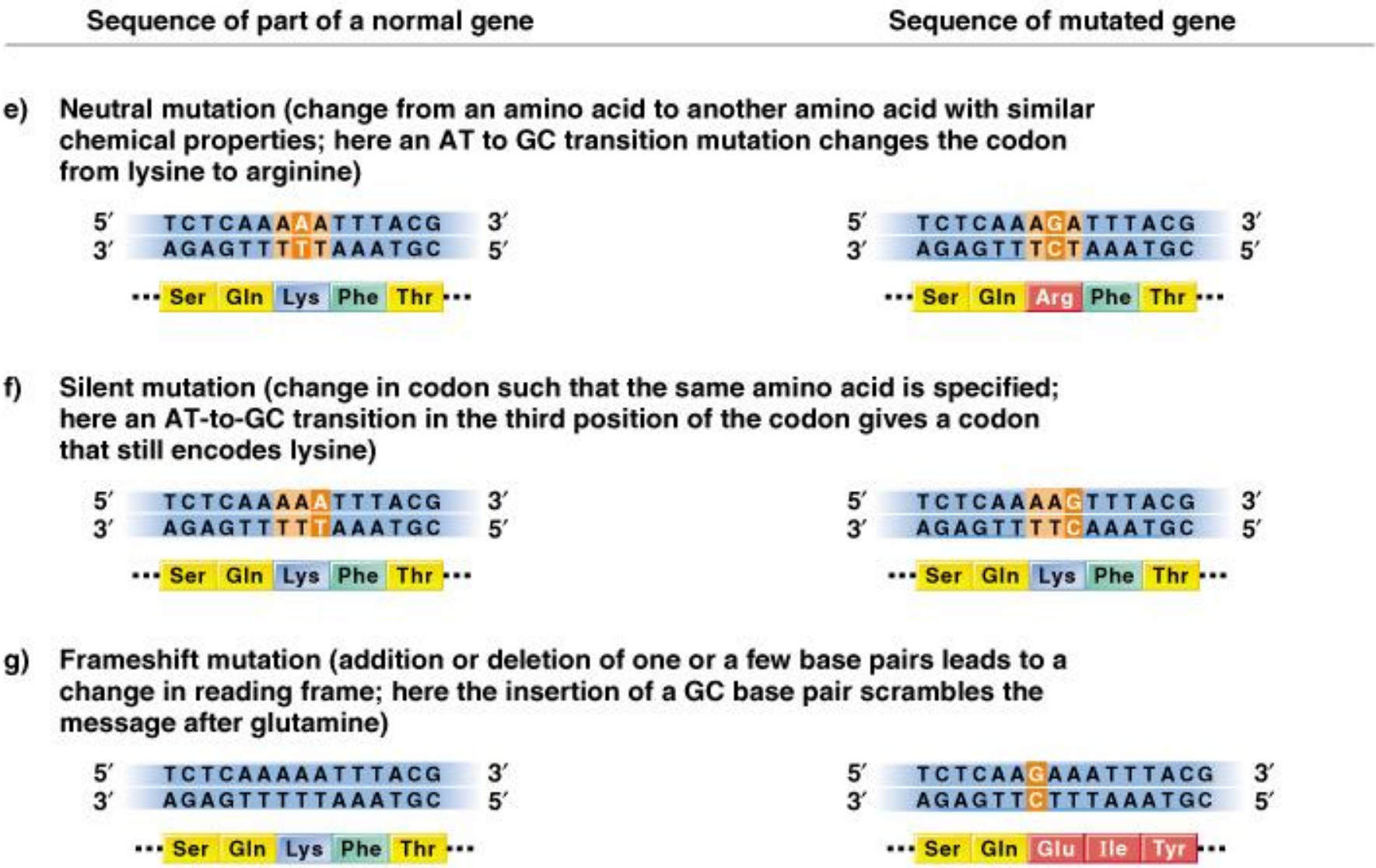

Insertion or deletion of codons
5' ATG GGA GCT CTA TTA ACC TAA 3'
met gly ala leu leu thr stop
5' ATG GGA TTA TTA GCT CTA TTA ACC TAA 3'
met gly leu leu ala leu leu thr stop


Initiator methionine
Translation Stop
mRNA CAP
Polyadenylylation signal
Poly A tail
AUG
STOP
7
mG
AAAAAAAAA
Open Reading Frame = ORF = Protein coding region
5' untranslated region
3' untranslated region

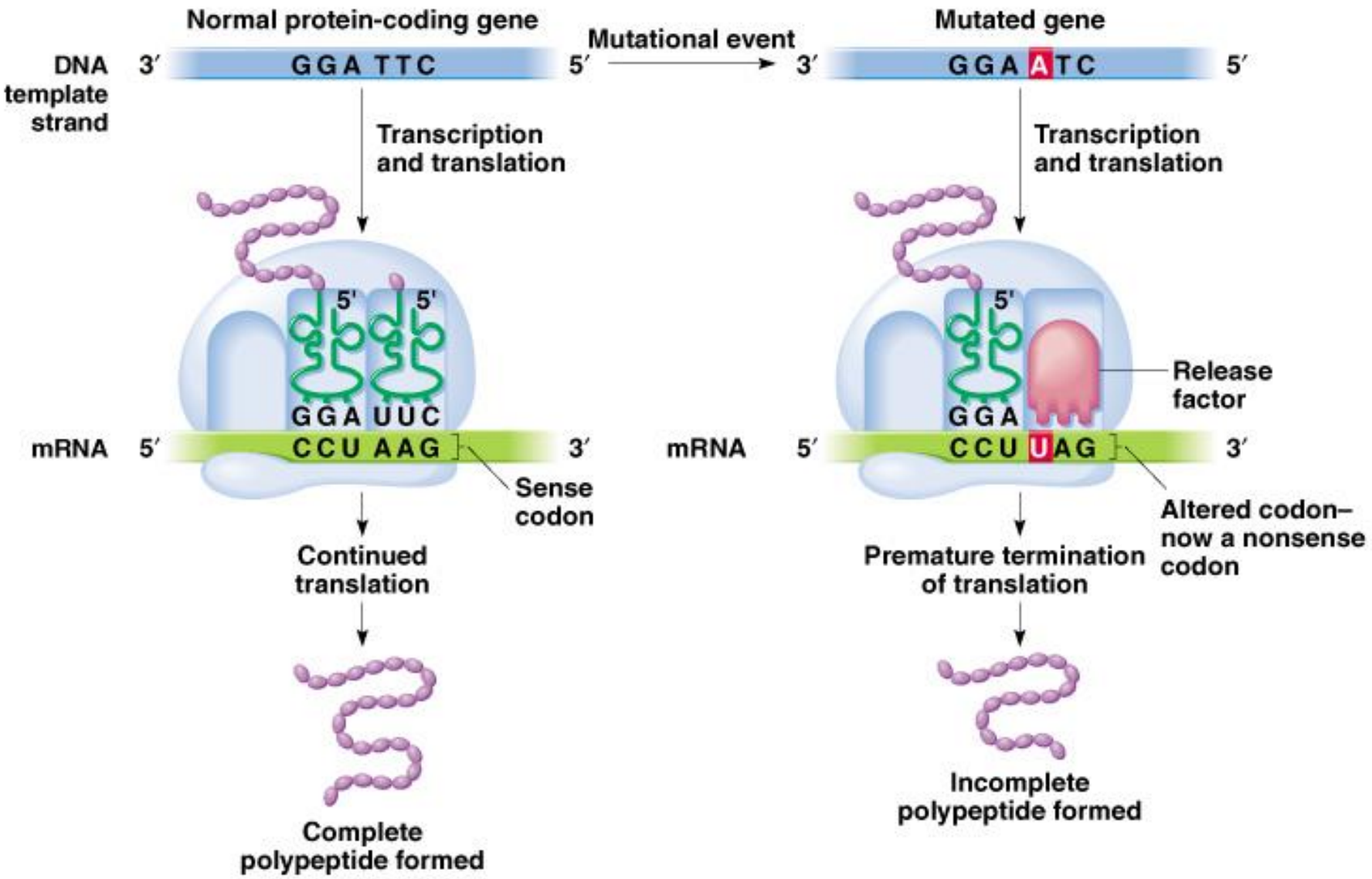
Types of base-pair substitution mutations



Types of base-pair substitution mutations



A nonsense mutation and its effect on translation



b. **Missense mutations** have a base-pair change resulting in a different mRNA codon, and therefore a different amino acid in the protein.

Phenotypic effects may or may not occur, depending on the specific amino acid change.

i. Neutral mutations change a codon in the ORF, but the resulting amino acid substitution produces no detectable change in the function of the protein (e.g., AAA to AGA substitutes arginine for lysine. The amino acids have similar properties, so the protein's function may not be altered).

ii. Silent mutations occur when the mutant codon encodes the same amino acid as the wild-type gene, so that no change occurs in the protein produced (e.g., AAA and AAG both encode lysine, so this transition would be silent).

Reverse Mutations and Suppressor Mutations

Point mutations are divided into two classes based on their effect on phenotype:

1a. Forward mutations change the genotype from wild type to mutant.

1b. Reverse mutations (reversions or back mutations) change the genotype from mutant to wild-type or partially wild-type.

i. A reversion to the wild-type amino acid in the affected protein is a true reversion.

ii. A reversion to some other amino acid that fully or partly restores protein function is a partial reversion.

2. Suppressor mutations occur at sites different from the original mutation, and mask or compensate for the initial mutation without actually reversing it. Suppressor mutations have different mechanisms depending on the site at which they occur.
    a. **Intragenic suppressors** occur within the same gene as the original mutation, but at a different site. Two different types occur:
        i. A different nucleotide is altered in the same codon as the original mutation.
        ii. A nucleotide in a different codon is altered (e.g., an insertion frameshift is suppressed by a nearby deletion event).

b. **Intergenic suppressors** occur in a different gene (the suppressor gene) from the original mutation. Many work by changing mRNA translation.

i. Each suppressor gene works on only one type of nonsense, missense or frameshift mutation.

ii. A given suppressor gene suppresses all mutations for which it is specific.

iii. Suppressor genes often encode tRNAs that recognize stop codons and insert an amino acid, preventing premature termination of translation.

(1) Full or partial function of the polypeptide may be restored.

(2) The effect depends on how compatible the substituted amino acid is with protein function.

iv. Nonsense suppressors fall into three classes, one for each stop codon (UAG, UAA and UGA).

v. Typical tRNA suppressor mutations are in redundant tRNA genes, so the wild-type tRNA activity is not lost.

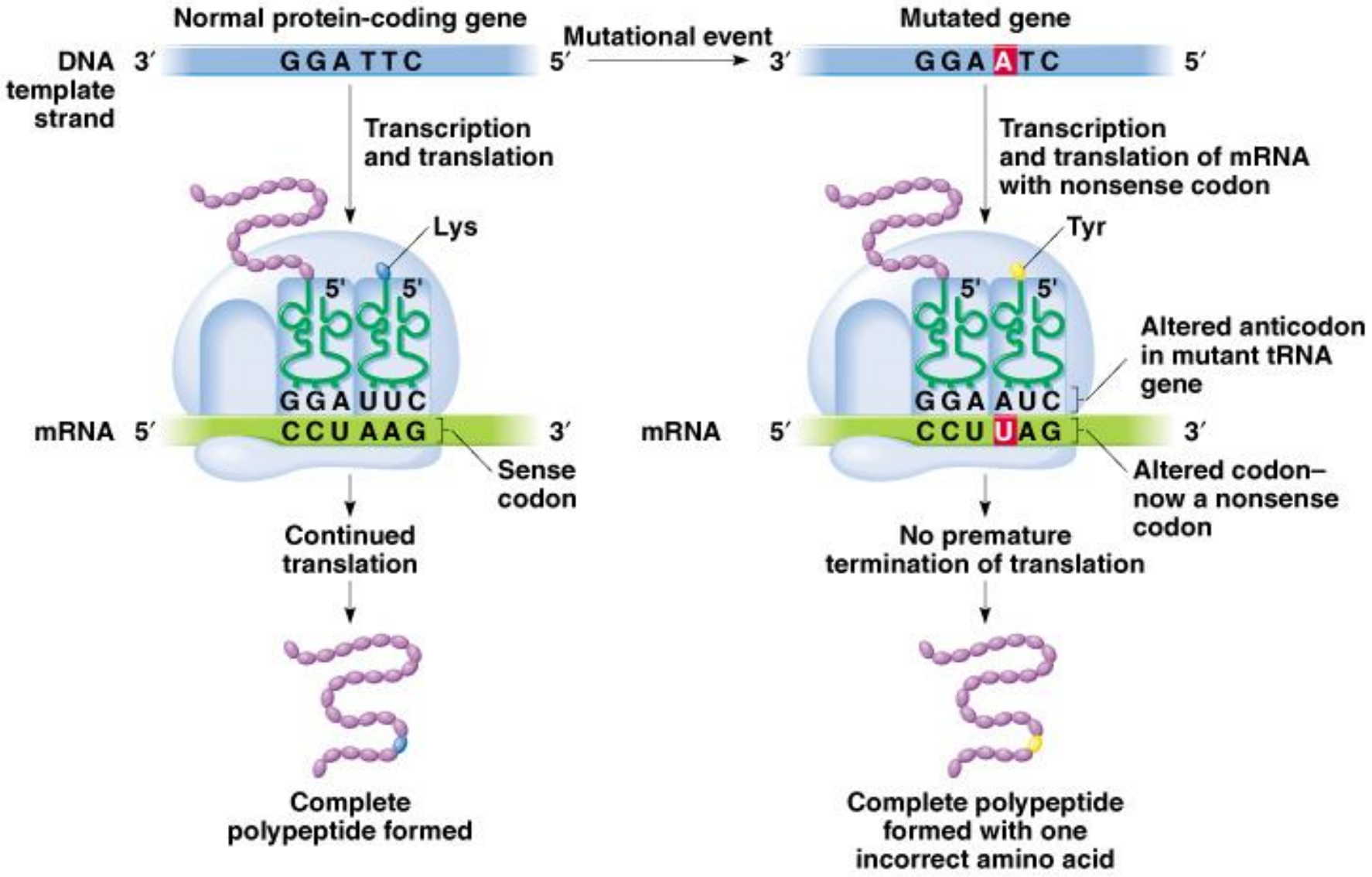
vi. Nonsense suppression occurs by competition between release factors and suppressor tRNAs.

(1) UAG and UGA suppressor tRNAs do well in competition with release factors.

(2) UAA suppressor tRNAs are only 1–5% efficient.

vii. Suppression by a tRNA occurs at all of its specific stop codons (e.g., UGA or UAG), not just the mutant one. This may produce read-through proteins, but they are not as common as expected, possibly due to tandem stop codons (e.g., UAGUGA).

Mechanism of action of an intergenic nonsense suppressor mutation that results from mutation of a tRNA gene



Occurrence of Mutations/Causes!

Spontaneous and Induced Mutations

Spontaneous Mutations

1. All types of point mutations can occur spontaneously, during S, G_1 and G_2 phases of the cell cycle, or by the movement of transposons.

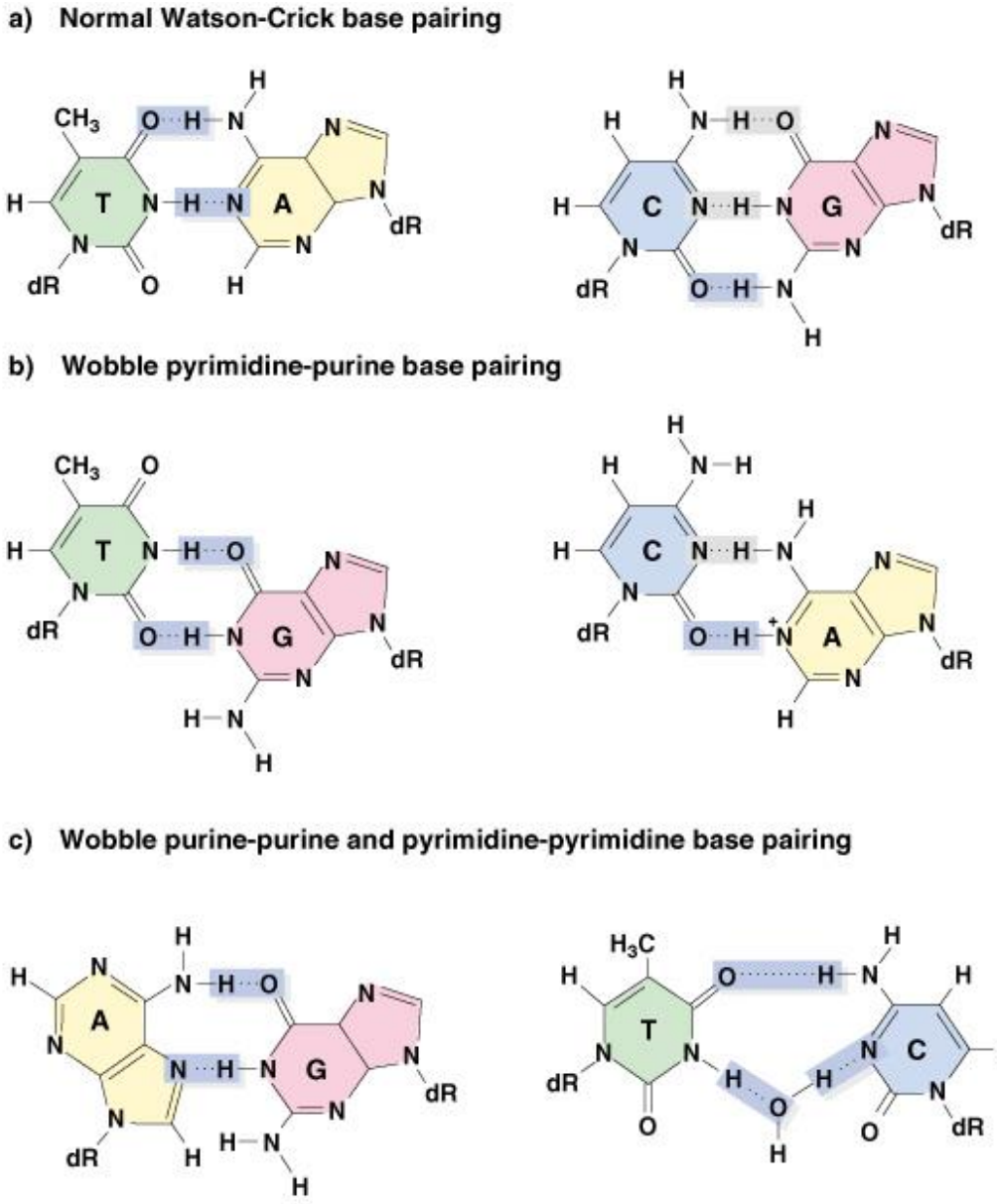
2. The spontaneous mutation rate in eukaryotes is between 10^{-4}-to-10^{-6} per gene per generation, and in bacteria and phages 10^{-5}-to-10^{-7}/ gene/generation.
   a. Genetic constitution of the organism affects its mutation rate.
   b. Many spontaneous errors are corrected by the cellular repair systems, and so do not become fixed in DNA.

DNA Replication Errors

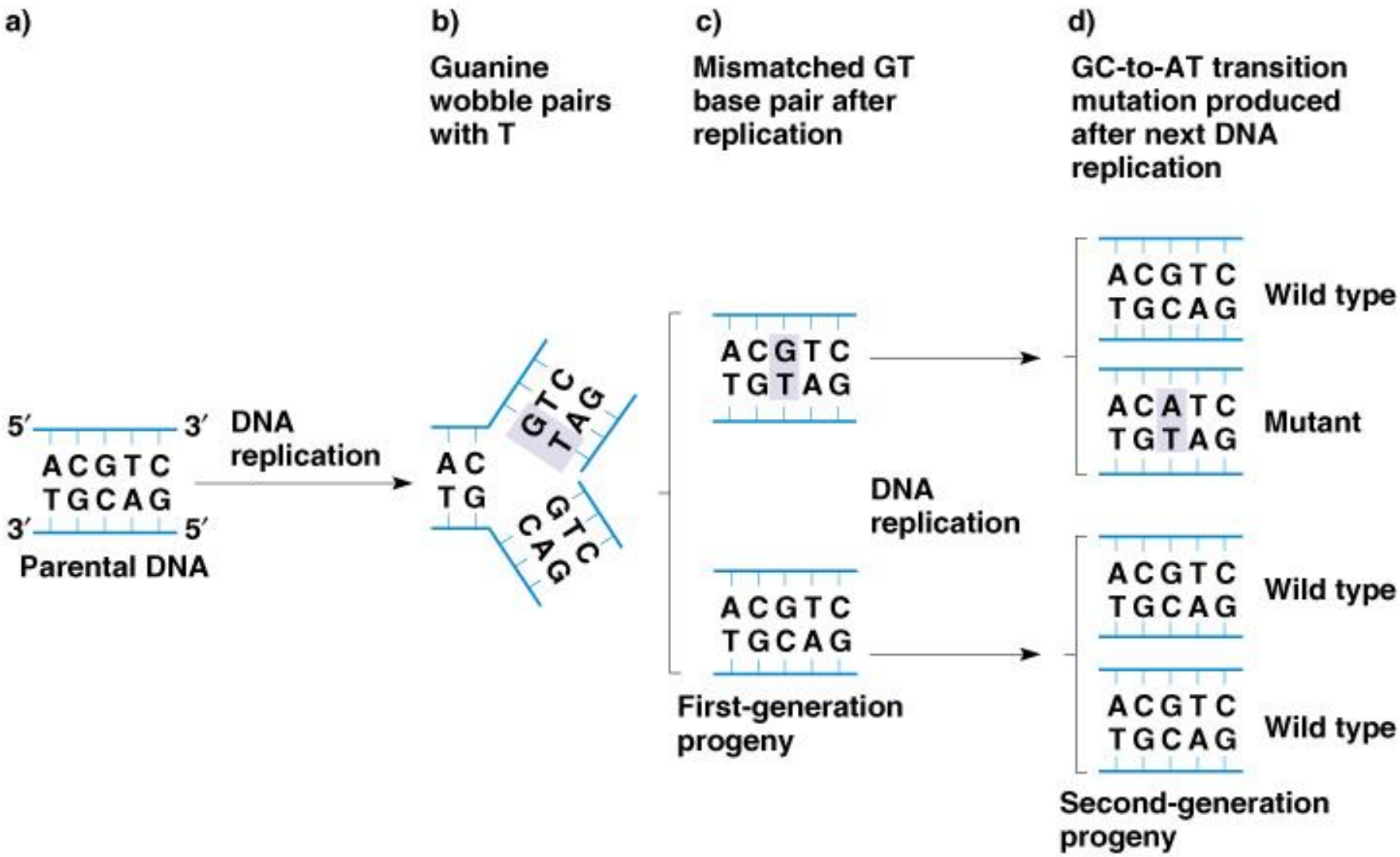
1. DNA replication errors can be either point mutations, or small insertions or deletions.

2. Base-pair substitution mutations can result from "wobble" pairing. A normal form of the base-pairs with an incorrect partner due to different spatial positioning of the atoms involved in H-bonding …….. An example is a GC-to-AT transition:
   a. During DNA replication, G could wobble pair with T, producing a GT pair.
   b. In the next round of replication, G and A are likely to pair normally, producing one progeny DNA with a GC pair, and another with an AT pair.
   c. GT pairs are targets for correction by proofreading during replication, and by other repair systems. Only mismatches uncorrected before the next round of replication lead to mutations.

Normal and wobble base pairing in DNA



Production of a mutation as a result of a mismatch caused by wobble base pairing



3. Additions and deletions can occur spontaneously during replication.

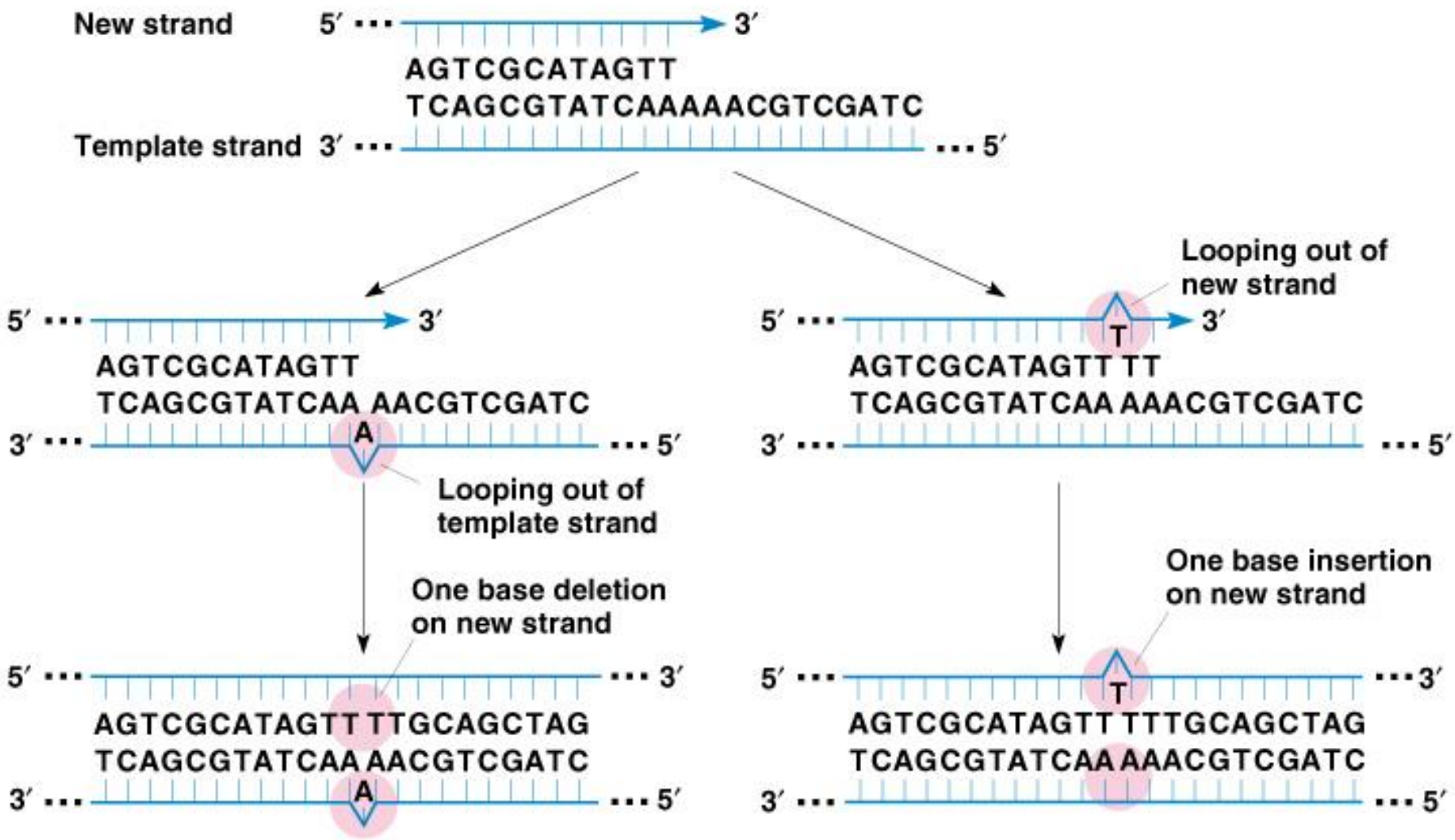
   a. DNA loops out from the template strand, generally in a run of the same base.

   b. DNA polymerase skips the looped out bases, creating a deletion mutation.

   c. If DNA polymerase adds untemplated base(s), new DNA looping occurs, resulting in additional mutation.

   d. Insertions and deletions in structural genes generate frameshift mutations (especially if they are not multiples of three).

Spontaneous generation of addition and deletion mutants by DNA looping-out errors during replication



4. Spontaneous chemical changes include Depurination and deamination of particular bases, creating lesions in the DNA.

a. **Depurination** removes the purine (A or G) from DNA by breaking the bond with its deoxyribose in the backbone.

i. Depurination is common.

ii. If not repaired before the next round of replication, it will result in a random base at that site.

b. **Deamination** removes an amino group from a base (e.g., cytosine to uracil)

i. Uracil is an abnormal base in DNA, usually might be repaired.

ii. If uracil is not replaced, it will pair with an A during replication, resulting in a CG-to-TA transition.

iii. Both prokaryotic and eukaryotic DNA have small amounts of 5-methylcytosine ($5^{m}C$) in place of the normal C.

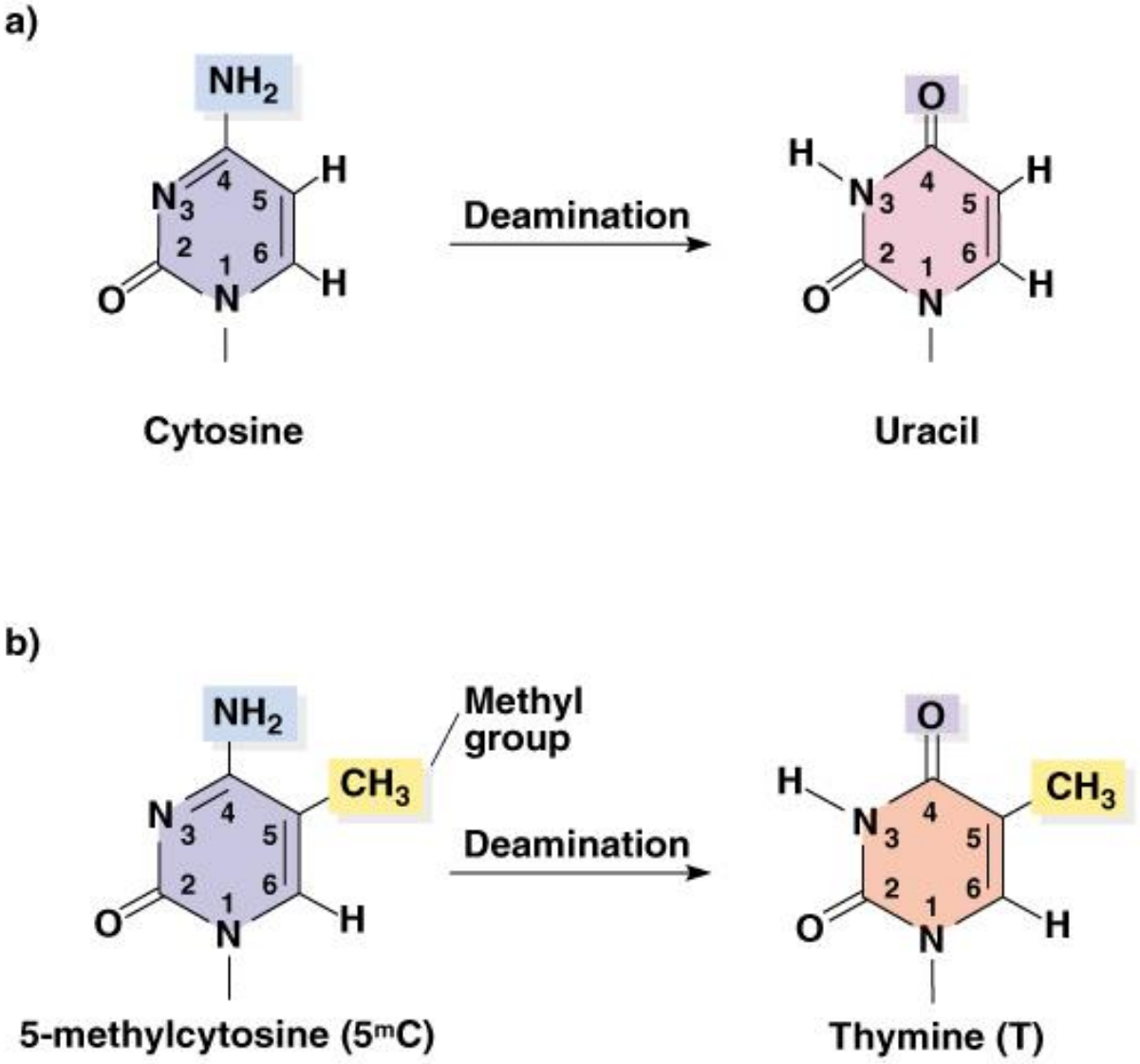
(1) Deamination of $5^{m}C$ produces T.

(2) T is a normal nucleotide in DNA, so it is not detected by repair mechanisms.

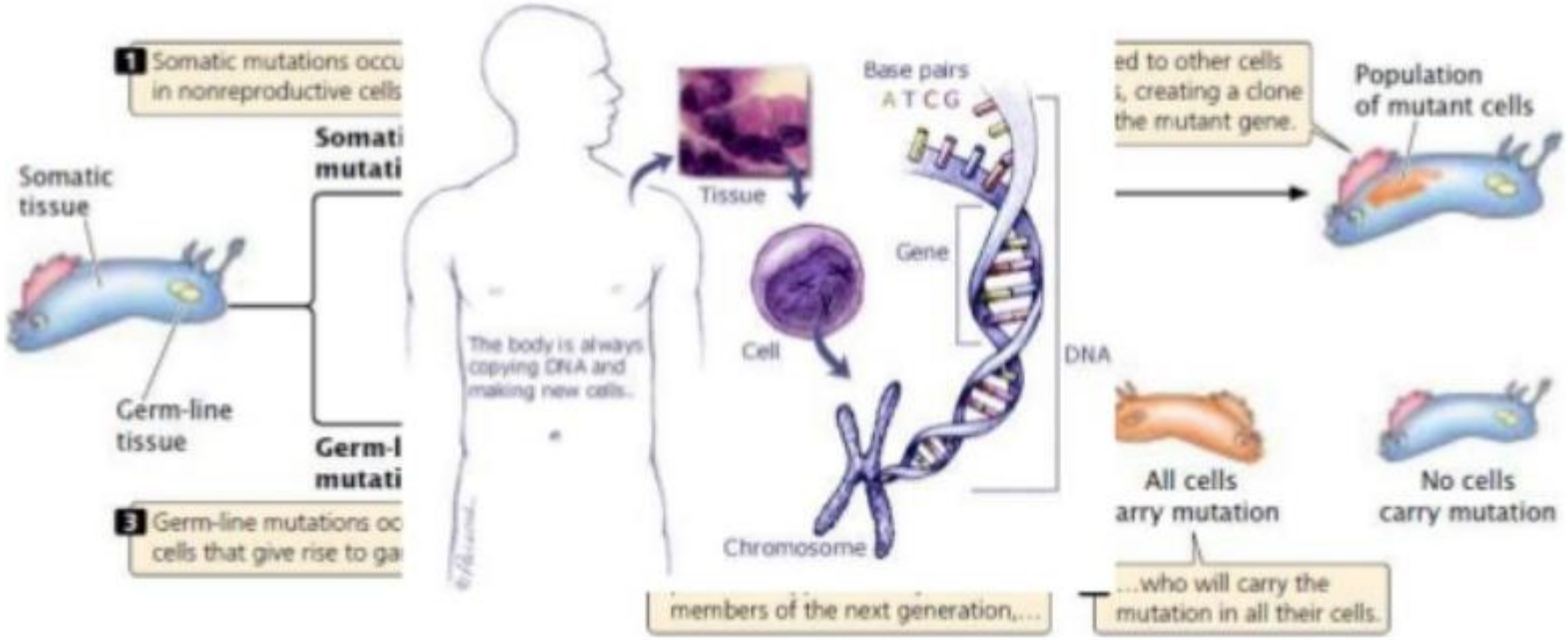
(3) Deamination of $5^{m}C$ results in CG-to-TA transitions.

(4) Locations of $5^{m}C$ in the chromosome are often detected as mutational hot spots.

Deamination of cytosine to uracil (a); deamination of 5-methylcytosine to thymine



Induced Mutations



Physical mutagens

Plays a role in genetic research, where they are used to increase mutation frequencies to provide mutant organisms for study.

1. **Radiation** (e.g., X rays and UV) induces mutations.
   a. **X rays are an example of ionizing radiation**, which penetrates tissue and collides with molecules, knocking electrons out of orbits and creating ions.
      i. Ions can break covalent bonds, including those in the DNA sugar-phosphate backbone.
      ii. **Ionizing radiation is the leading cause of human gross chromosomal mutations.**
      iii. Ionizing radiation kills cells at high doses, and lower doses produce point mutations.
      iv. Ionizing radiation has a cumulative effect. A particular dose of radiation results in the same number of mutations whether it is received over a short or a long period of time.

b. **Ultraviolet (UV)** causes photochemical changes in the DNA.

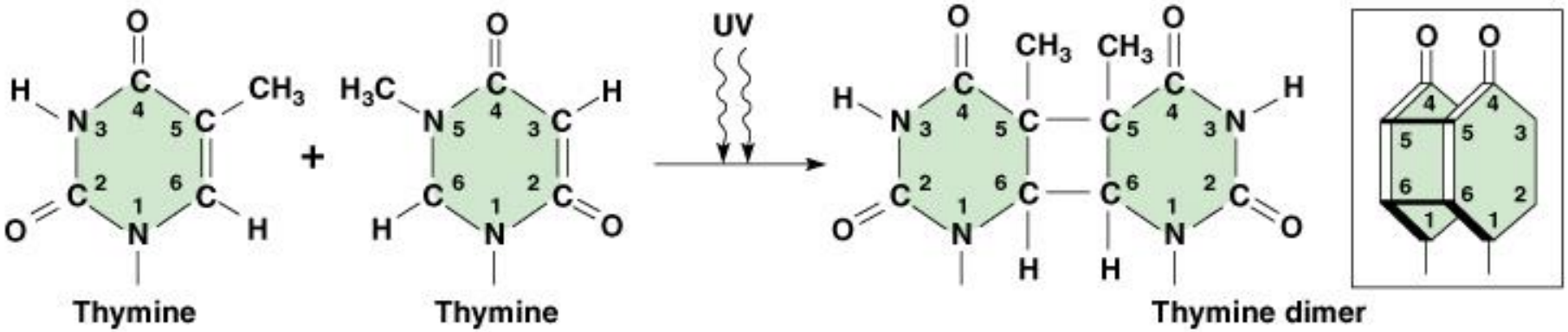
UV in the 254–260 nm range is strongly absorbed by purines and pyrimidines, forming abnormal chemical bonds.

(1) A common effect is **dimer formation** between adjacent pyrimidines, commonly thymine's (designated T^T).

(2) C^C, C^T and T^C dimers also occur, but at lower frequency. Any pyrimidine dimer can cause problems during DNA replication.

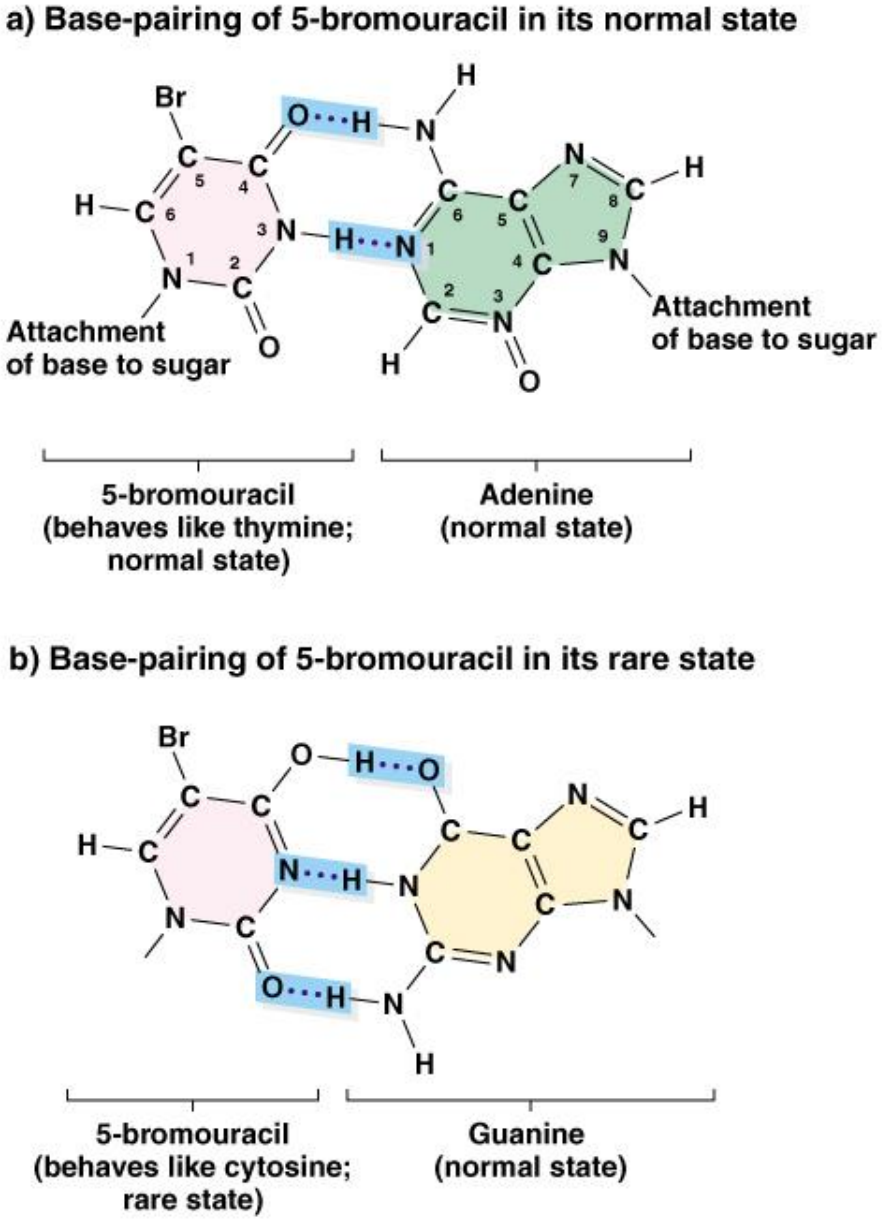
(3) Most pyrimidine dimers are repaired, because they produce a bulge in the DNA helix. If enough are unrepaired, cell death may result.

Production of thymine dimers by ultraviolet light irradiation

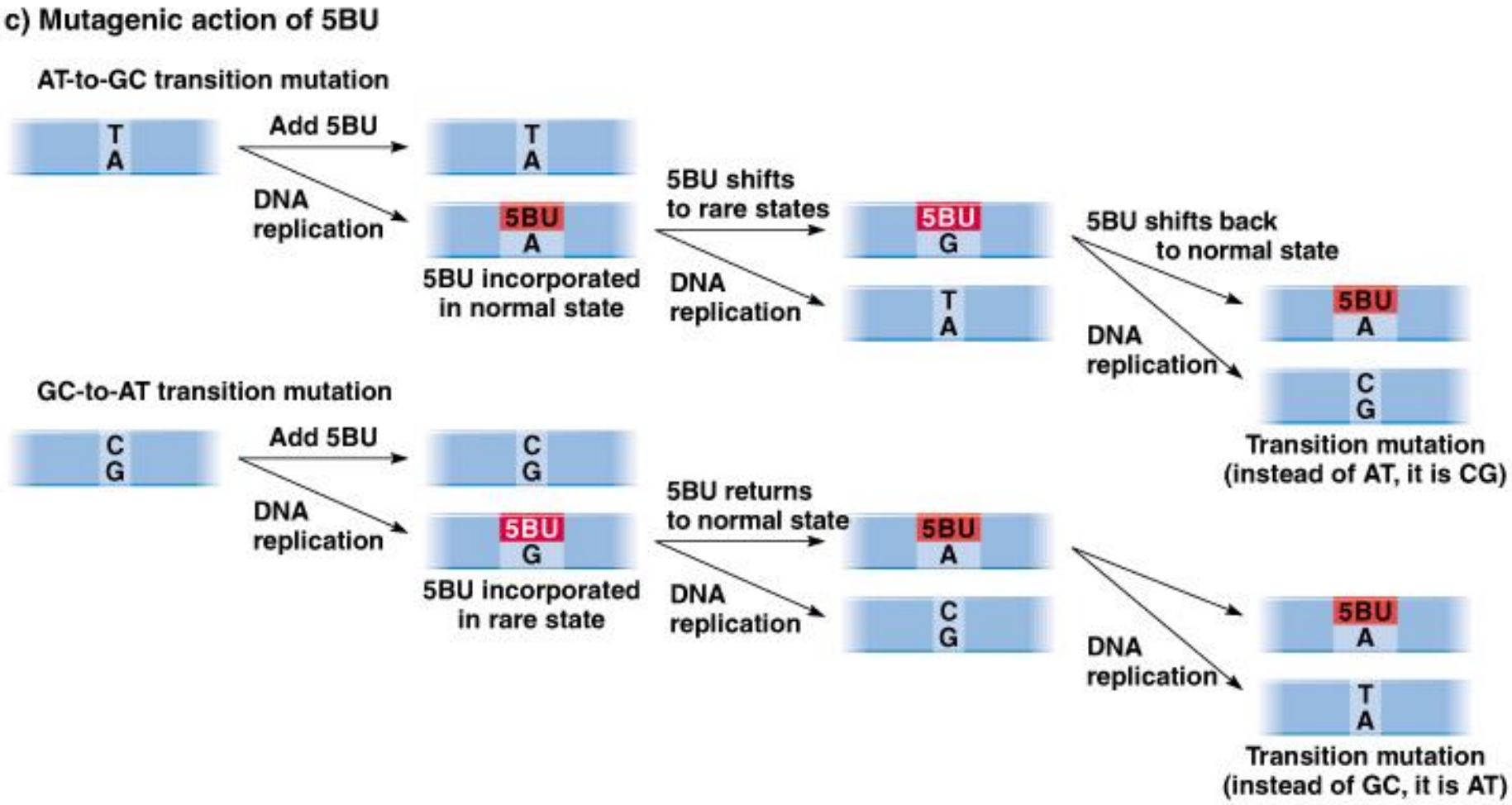


2. **Chemical mutagens** may be naturally occurring, or synthetic. They form different groups based on their mechanism of action:
    a. **Base analogs** depend upon replication, which incorporates a base with alternate states that allow it to base pair in alternate ways, depending on its state.
        i. Analogs are similar to normal nitrogen bases, and so are incorporated into DNA readily.
        ii. Once in the DNA, a shift in the analog's form will cause incorrect base pairing during replication, leading to mutation.
        iii. 5-bromouracil (5BU) is an example. 5BU has a bromine residue instead of the methyl group of thymine.
            (1) Normally **5BU resembles thymine**, pairs with adenine and is incorporated into DNA during replication.
            (2) In its rare state, 5BU pairs only with guanine, resulting in a TA-to-CG transition mutation.
            (3) If 5BU is incorporated in its rare form, the switch to its normal state results in a CG-to-TA transition. Thus 5BU- induced mutations may be reverted by another exposure to 5BU.
        iv. Not all base analogs are mutagens, only those that cause base- pair changes (e.g. AZT is a stable analog that does not shift).

Mutagenic effects of the base analog 5-bromouracil (5BU)



Mutagenic effects of the base analog 5-bromouracil (5BU)



b. **Base-modifying agents** can induce mutations at any stage of the cell cycle. They work by modifying the chemical structure and properties of the bases. Three types are (Figure 19.13):

i. Deaminating agents remove amino groups. An example is nitrous acid (HNO_2), which deaminates G, C and A.

(1) HNO_2 deaminates guanine to produce xanthine, which has the same base pairing as G. No mutation results.

(2) HNO_2 deaminates cytosine to produce uracil, which produces a CG-to-TA transition.

(3) HNO_2 deaminates adenine to produce hypoxanthine, which pairs with cytosine, causing an AT-to-GC transition.

(4) Mutations induced by HNO_2 can revert with a second treatment.

ii. **Hydroxylating agents** include hydroxylamine (NH_2OH).

(1) NH_2OH specifically modifies C with a hydroxyl group (OH), so that it pairs only with A instead of with G.

(2) NH_2OH produces only CG-to-TA transitions, and so revertants do not occur with a second treatment.

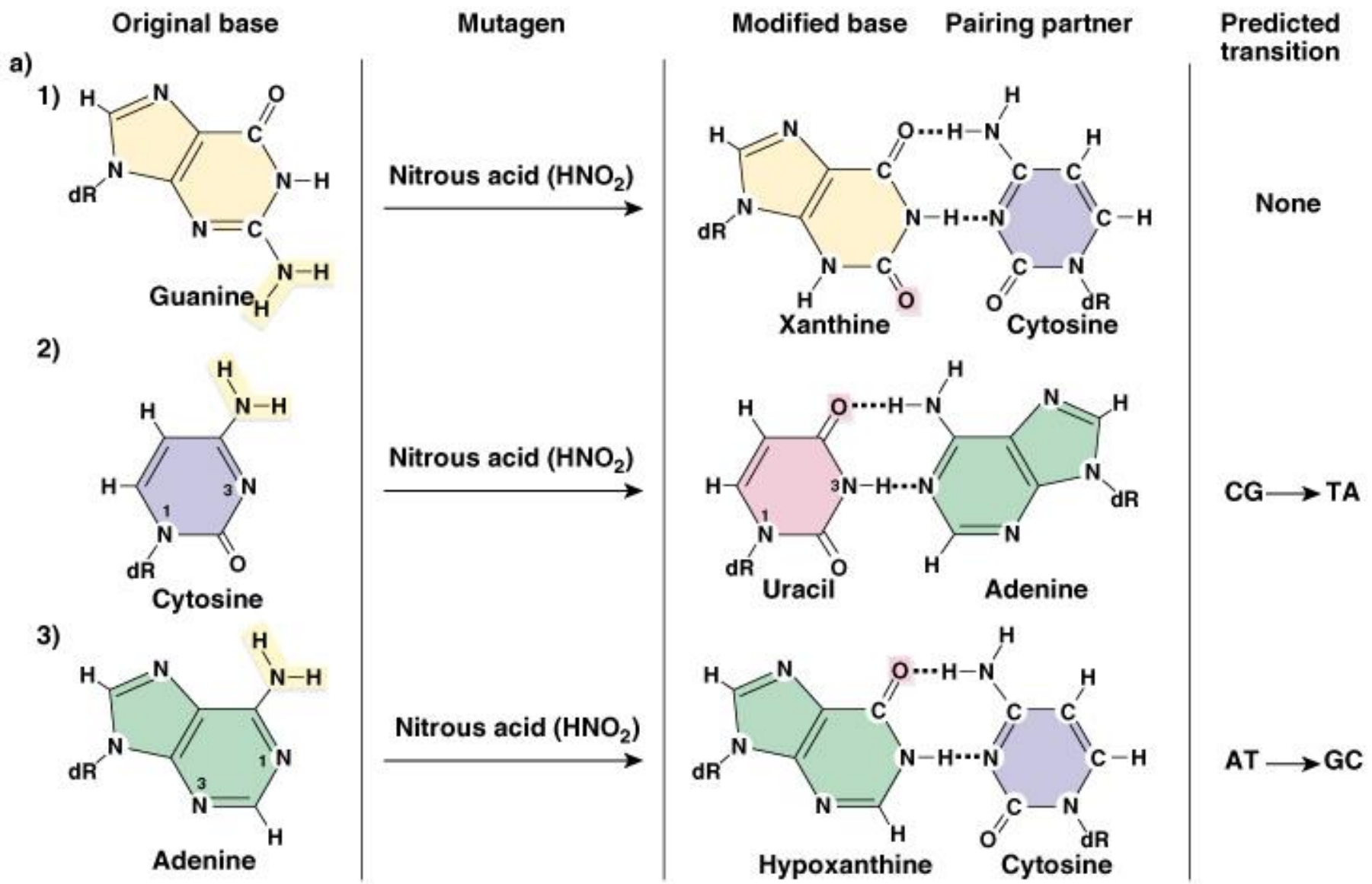
(3) NH_2OH mutants, however, can be reverted by agents that do cause TA-to-CG transitions (e.g., 5BU and HNO_2).

iii. **Alkylating agents** are a diverse group that add alkyl groups to bases. Usually alkylation occurs at the 6-oxygen of G, producing O^6-allcylguanine.

(1) An example is methylmethane sulfonate (MMS), which methylates G to produce O^6-alkyl G.

(2) O^6-aIkylG pairs with T rather than C, causing GC-to-AT transitions.

Action of three base-modifying agents



c. **Intercalating agents** insert themselves between adjacent bases in dsDNA. They are generally thin, plate-like hydrophobic molecules.

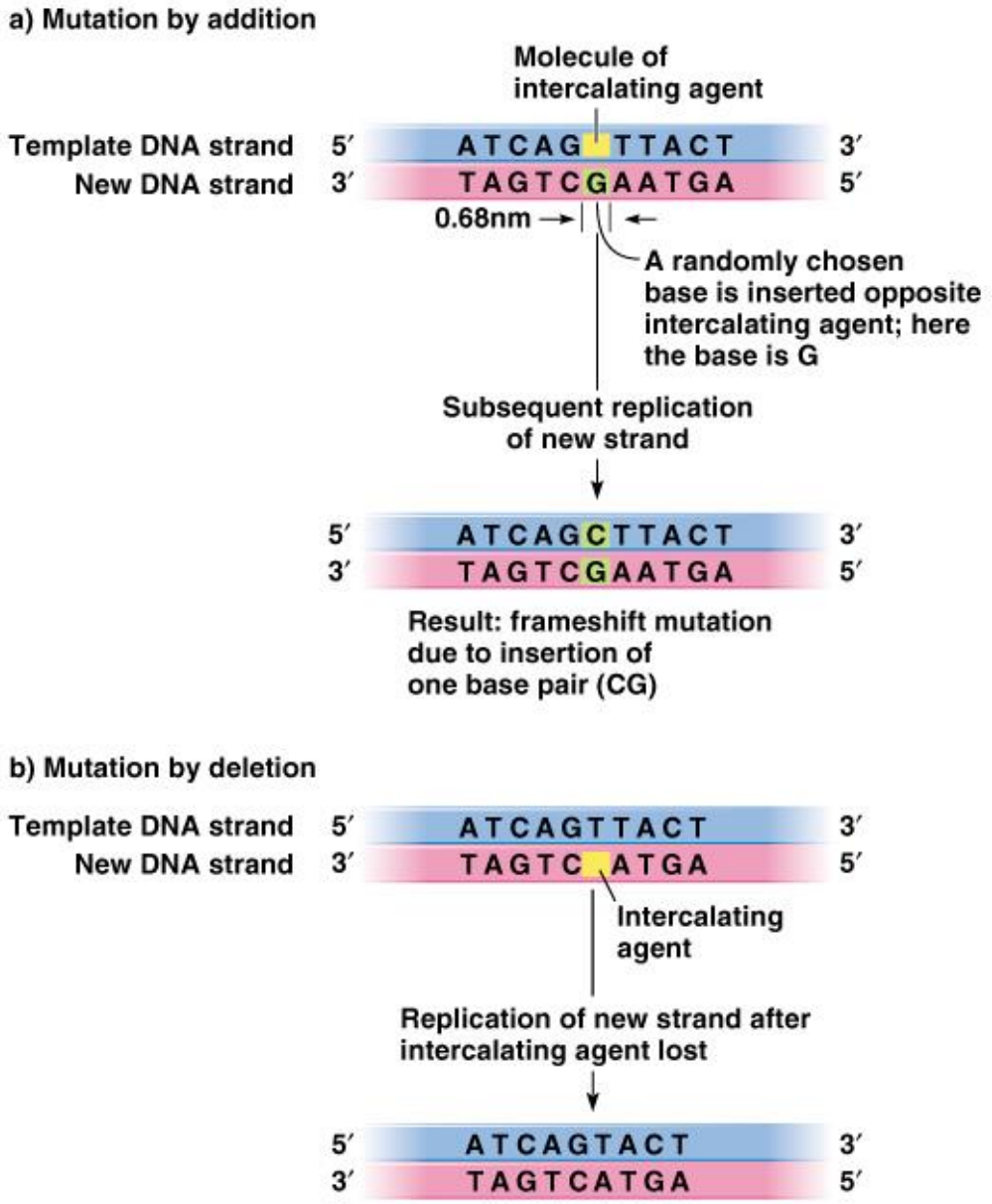
i. At replication, a template that contains an intercalated agent will cause insertion of a random extra base.

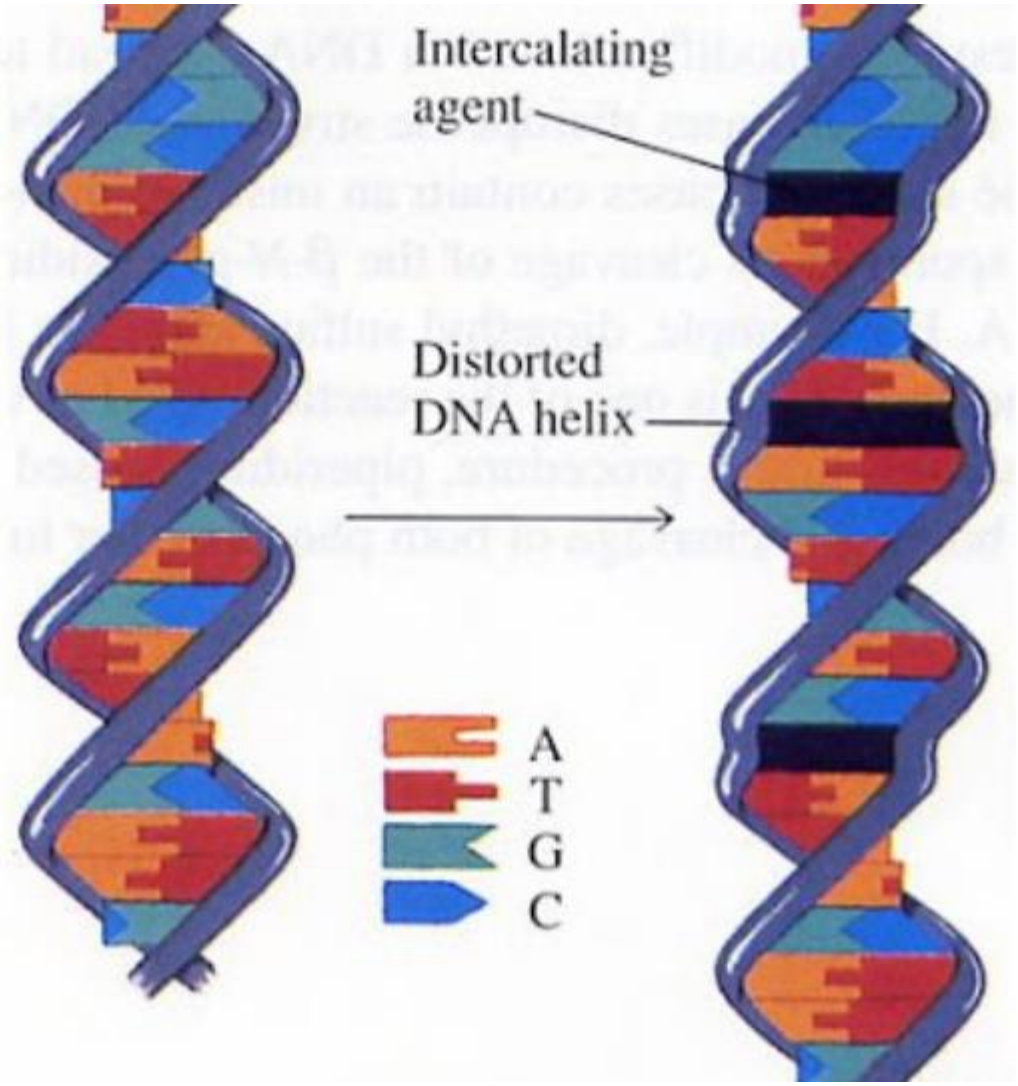
ii. The base-pair addition is complete after another round of replication, during which the intercalating agent is lost.

iii. If an intercalating agent inserts into new DNA in place of a normal base, the next round of replication will result in a deletion mutation.

iv. Point deletions and insertions in ORFs result in frameshift mutations. These mutations show reversion with a second treatment.

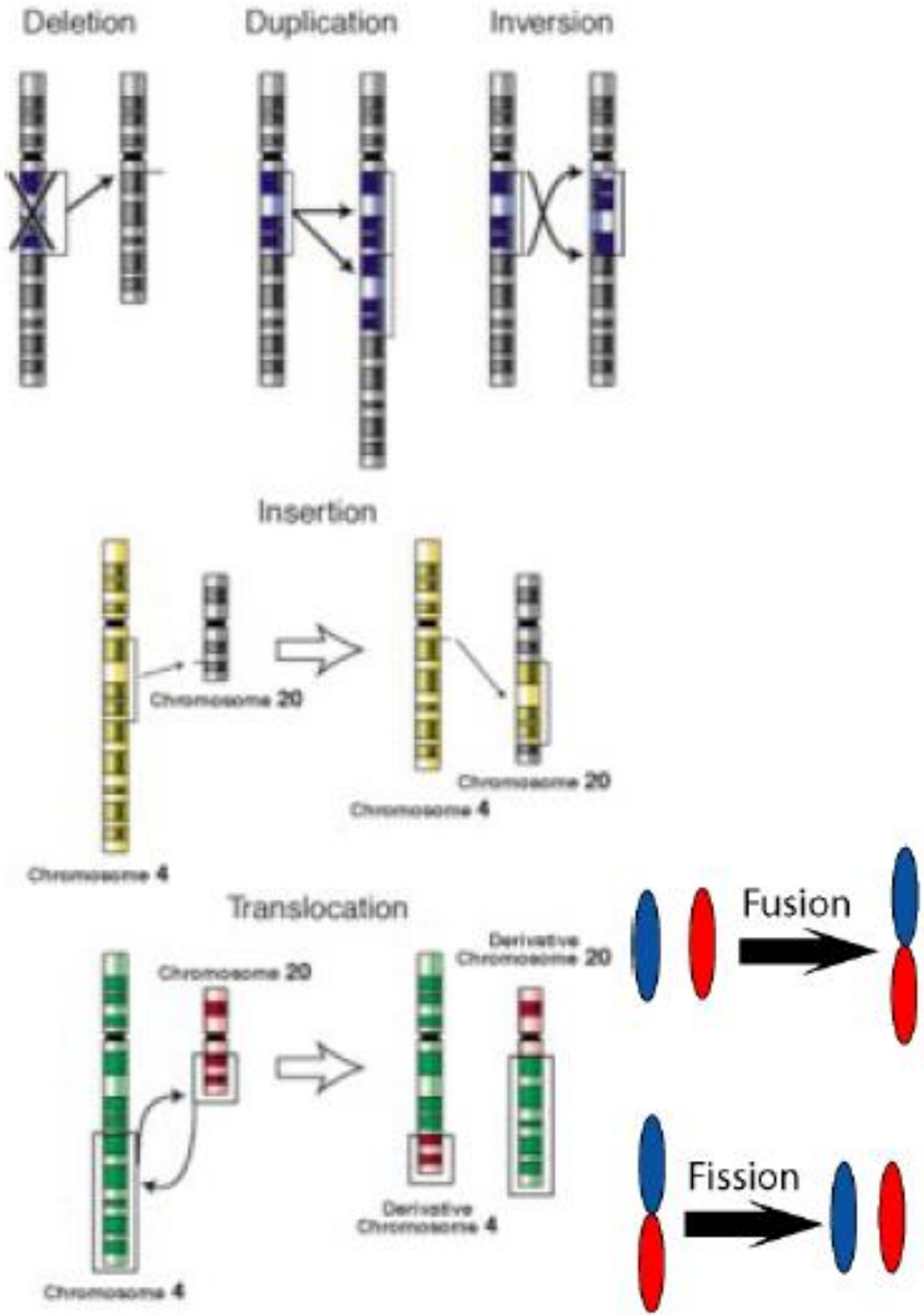
Intercalating mutations

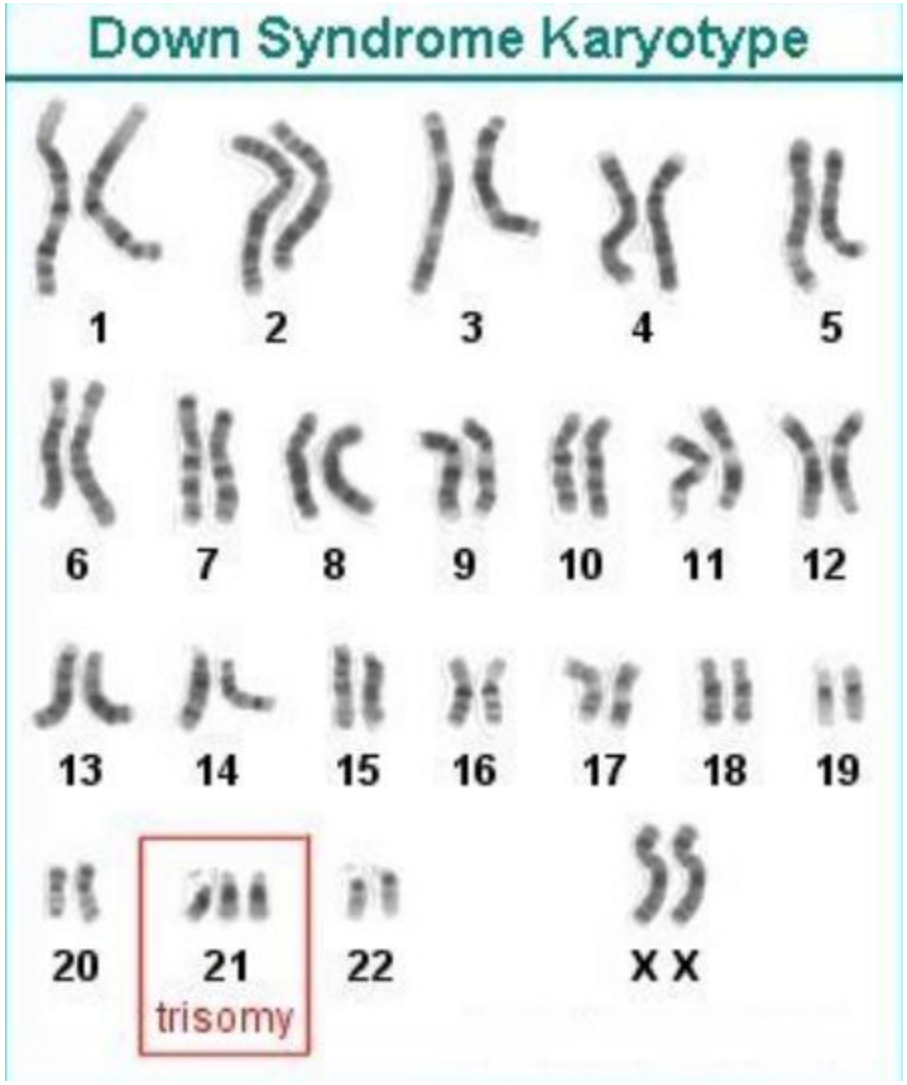



Intercalating agent
Distorted DNA helix
A
T
G
C

Chromosomal mutations

- Mutations range from a change in a single nucleotide pair up to a change in vast regions of a chromosome.
- Chromosomal mutations are detected by comparing the banding pattern of chromosomes (Karyotyping)
- Chromosomal mutations cause several genetic disorders but also are extremely useful in tracing evolutionary change in a related group of organisms.
- Various types of chromosomal mutations including: Inversions, deletions, duplications, fusions, fissions, and translocations.


Deletion
Duplication
Inversion
Insertion
Chromosome 20
Chromosome 4
Chromosome 20
Chromosome 4
Translocation
Chromosome 20
Chromosome 4
Derivative Chromosome 20
Derivative Chromosome 4
Fusion
Fission


Down Syndrome Karyotype
1
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trisomy
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X X

Review questions

1. What is a mutation?
2. What causes mutations?
3. Why are mutations so important to living organisms?
4. Name the two basic kinds of point mutations.
5. What is the difference between a missense mutation and a nonsense mutation?
6. Describe some common chromosomal mutations.
7. What is a frameshift mutation?
8. Describe in detail what happens when an individual is exposed to X-Ray over a period of time.