INTRODUCTION

- **Mutation, an alteration in the genetic material (the genome) of a cell of a living organism or of a virus that is more or less permanent and that can be transmitted to the cell's or the virus's descendants.**
- Mutations provide allelic variations
  - On the positive side, mutations are the foundation for evolutionary change
  - On the negative side, mutations are the cause of many diseases
- Since mutations can be quite harmful, organisms have developed ways to repair damaged DNA

Mutations are the ultimate source of all genetic change

- **Gene mutation**:
- mutational events (changes in the DNA sequence) that take place within individual genes.
- These changes may or may not result in altering the spatial or functional state of the protein or the level of activity or specificity of the protein.

- **Chromosome mutation**:
- mutational events that affect the entire chromosome or large pieces of the chromosome.
- These affects will mainly result in gene dosage defects.

Random vs Adaptive Mutation

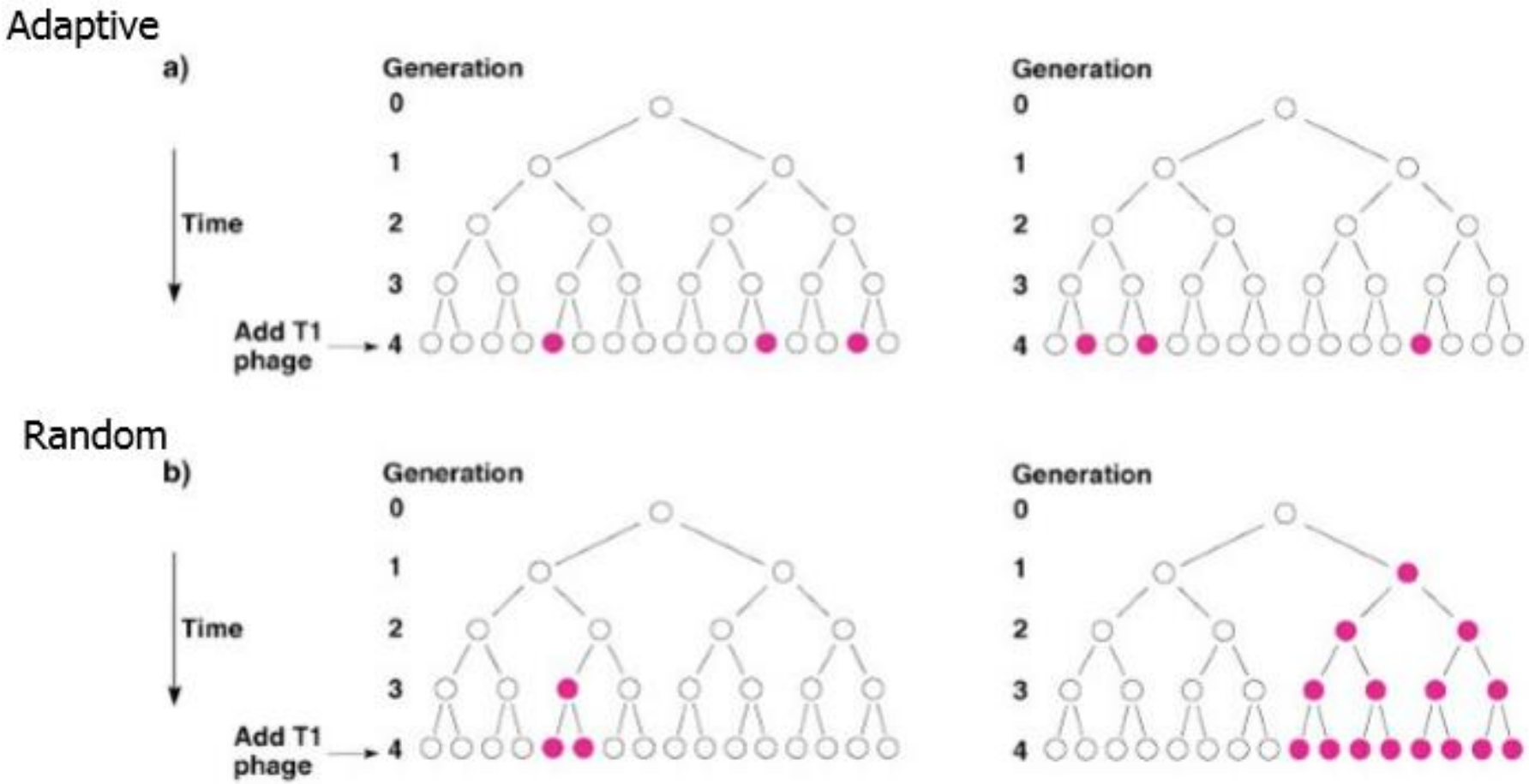
- Does mutation cause random variation leading to adaptation, or does the environment induces heritable adaptations?????
  - Lamarckism is the doctrine of inheritance of acquired characteristics.
  - The random mutation doctrine says that sometimes chance changes happen to be adaptive, thus altering phenotype by changing a protein
  - The observation that phage T1–resistant *E. coli* arise could be interpreted to support either of these theories.

Random vs Adaptive Mutation

- An *E. coli* population that started from one cell would show different patterns of T1 resistance depending on which model is correct.
  - The adaptive theory says that cells are induced to become resistant to T1 when it is added.
    - Therefore, the proportion of resistant cells would be the same for all cultures with the same genetic background.
  - The mutation theory says that random events confer resistance to T1, whether the phage is present or not.
    - Cultures will therefore show different numbers of T1–resistant cells, depending on when the resistance mutation(s) occurred.

Representation of a dividing population of T1 phage-sensitive wild-type *E. coli*

(Luria-Delbruck fluctuation test)



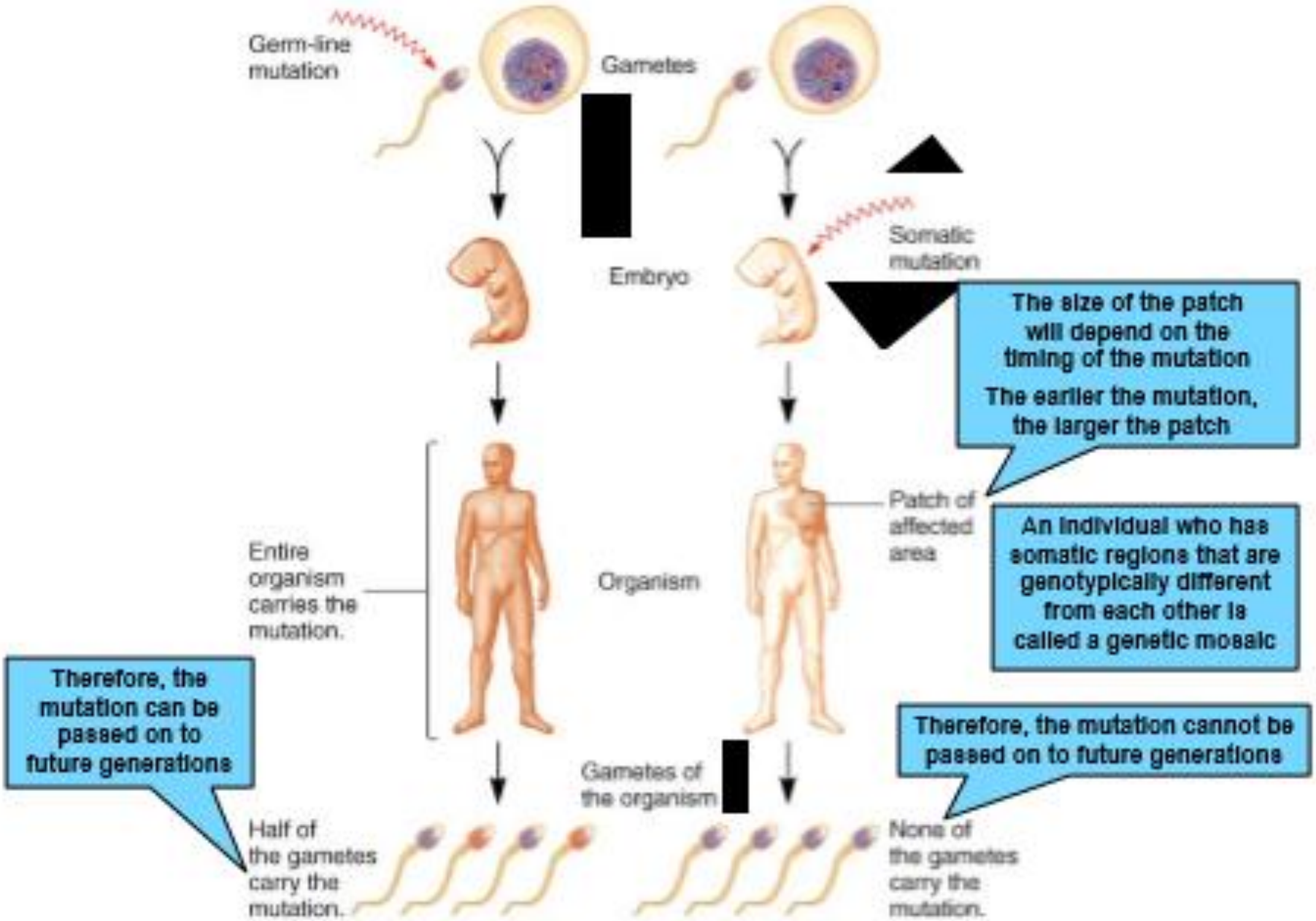
- Results:

  Luria and Delbrück observed fluctuating numbers of resistant bacteria from *E. coli* cultures

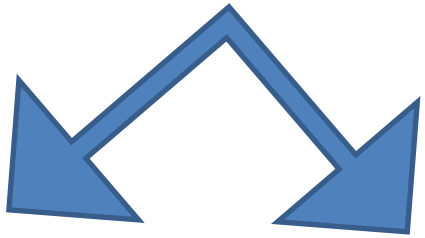
  – indicating that the random mutation model is correct.

Mutations Defined

- A mutation is a change in a DNA base pair or a chromosome.
  1. Somatic mutations affect only the individual in which they arise.
  2. Germ-line mutations alter gametes, affecting the next generation.

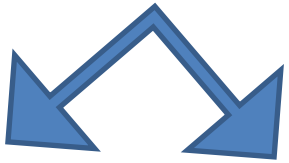

Germ-line mutation
Gametes
Somatic mutation
Embryo
The size of the patch will depend on the timing of the mutation
The earlier the mutation, the larger the patch
Patch of affected area
An individual who has somatic regions that are genotypically different from each other is called a genetic mosaic
Entire organism carries the mutation.
Organism
Therefore, the mutation can be passed on to future generations
Therefore, the mutation cannot be passed on to future generations
Gametes of the organism
Half of the gametes carry the mutation.
None of the gametes carry the mutation.
(a) Germ-line mutation
(b) Somatic cell mutation

TYPES OF POINT MUTATIONS



Base pair substitutions

(A change in the DNA such that one base pair is replaced by another base pair)



Transition mutation

Transversion mutation

- Transition -
  - purine-purine and pyrimidine-pyrimidine
  - Only one choice
  - C to T or G to A and vice versa
- Transversion -
  - purine-pyrimidine
  - Two choices
  - C or T to G or A; and vice versa

Base pair insertions or deletions

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′

What Point Mutations/Base Substitutions Do to the Protein

- Mutations in the coding sequence of a structural gene can have various effects on the polypeptide
  - Silent mutations are those base substitutions that do not alter the amino acid sequence of the polypeptide
    - Due to the degeneracy of the genetic code
  - Missense mutations are those base substitutions in which an amino acid change does occur
    - Example: Hemoglobin in sickle-cell anemia
    - NOTE: If the substituted amino acids have similar chemistry, the mutation is said to be neutral
  - Nonsense mutations are those base substitutions that change a normal codon to a termination codon

Sequence of part of a normal gene | Sequence of mutated gene

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)

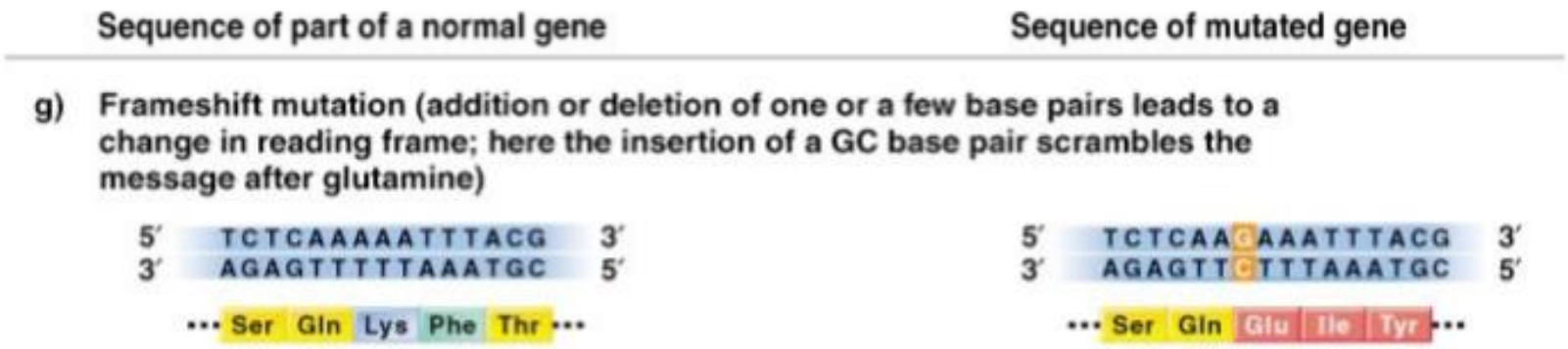
d) Nonsense mutation (change from an amino acid to a stop codon; here a transversion mutation from AT to TA changes the codon from lysine to UAA stop codon)

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)

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)

Point Mutations: Deletions & Insertions

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



Point Mutations: Phenotypic Effect

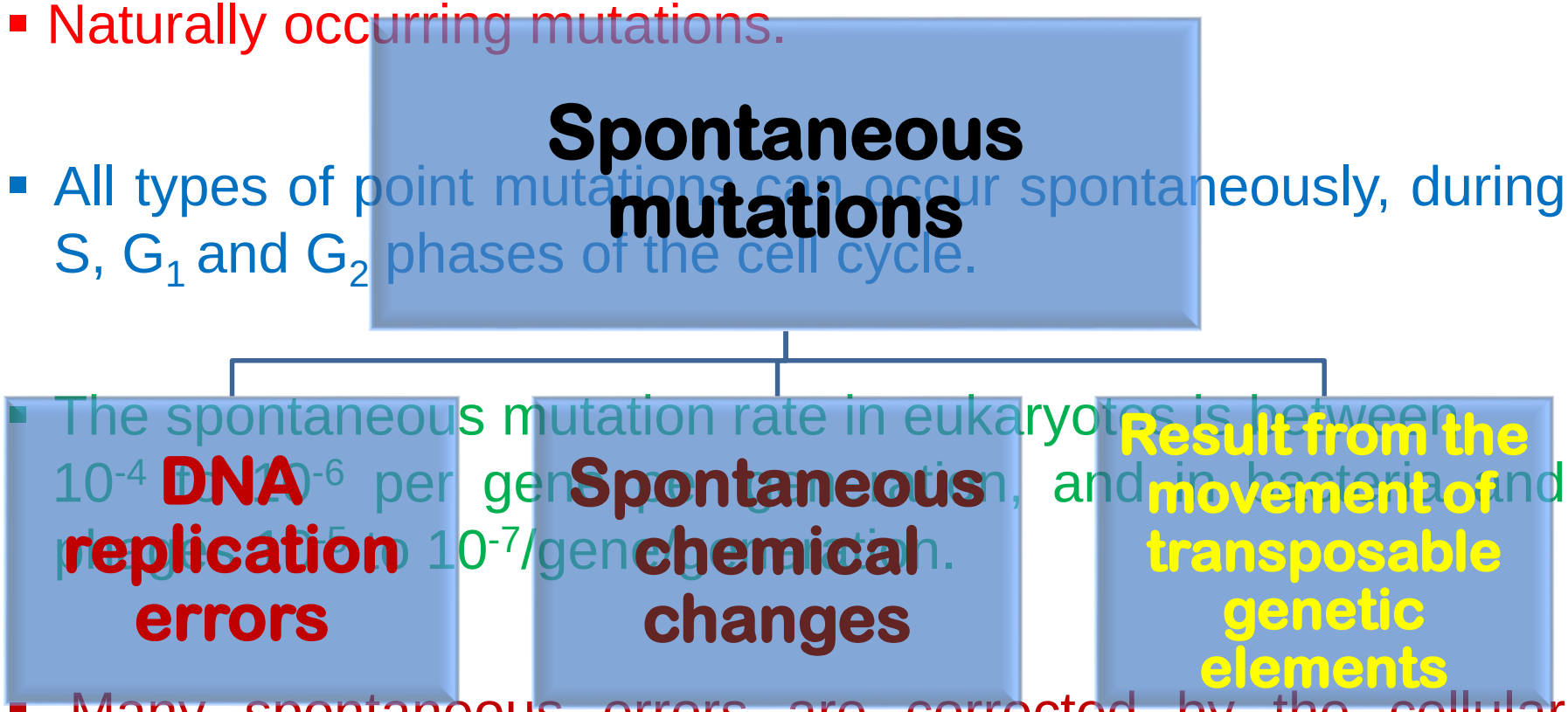
- Point mutations are divided into two classes based on their effect on phenotype:

1. Forward mutations change the genotype from wild type to mutant.
2. Reverse mutations (reversions or back mutations) change the genotype from mutant to wild type or partially wild type.
    - A reversion to the wild-type amino acid in the affected protein is a true reversion.
    - A reversion to some other amino acid that fully or partly restores protein function is a partial reversion.

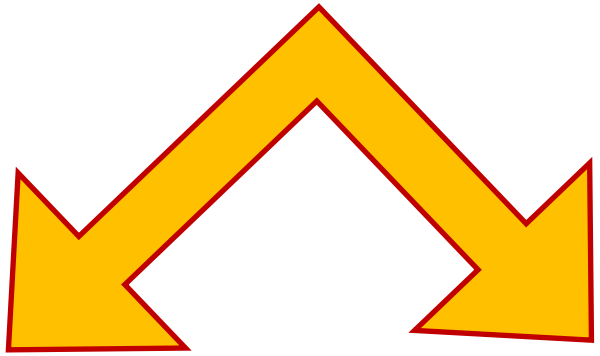
Spontaneous and Induced Mutations

SPONTANEOUS MUTATION

- Naturally occurring mutations.
- All types of point mutations can occur spontaneously, during S, G_1 and G_2 phases of the cell cycle.
- 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.
- Many spontaneous errors are corrected by the cellular repair systems, and so do not become fixed in DNA.
- Spontaneous are more frequent than induced mutations.



DNA replication errors



Base-pair substitution mutations

Small additions & Small deletions (Indel)

Base-pair substitution mutations

Wobble base pairing

- **Pyrimidine-Purine pairing**

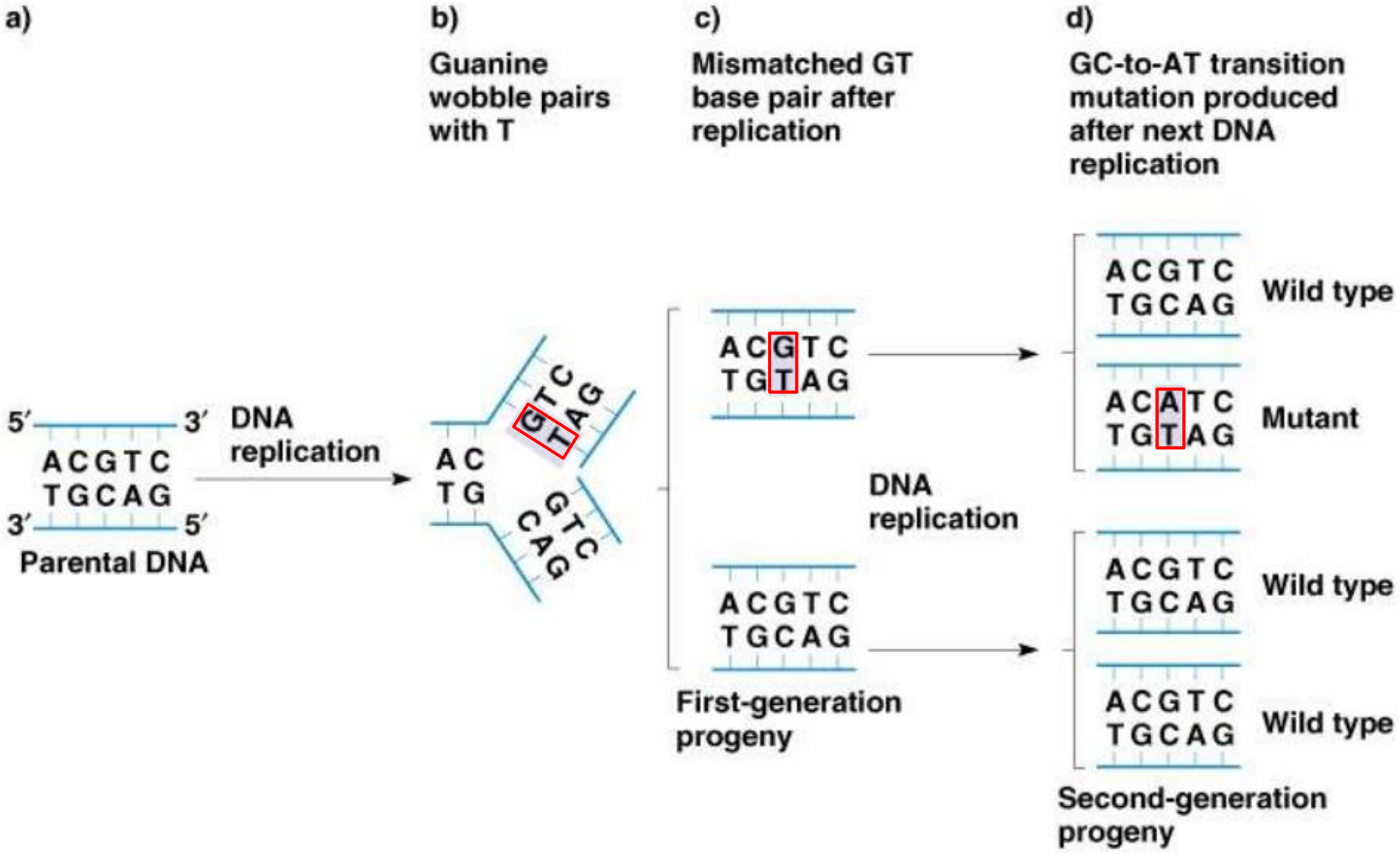
T-G
C-A

- **Purine-Purine and Pyrimidine-Pyrimidine pairing**

A-G
T-C

- GT base 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

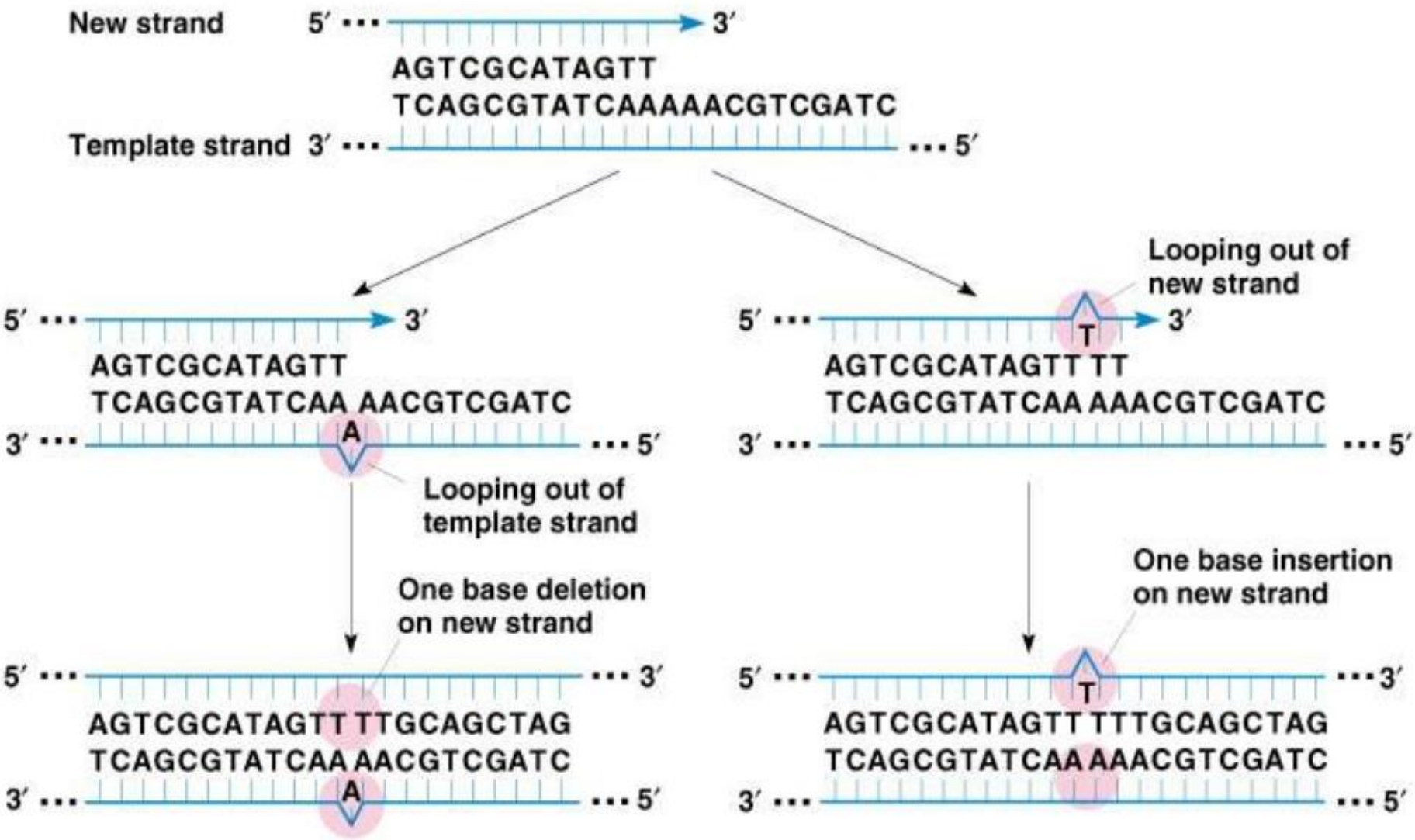
Production of a mutation as a result of a mismatch caused by wobble base pairing



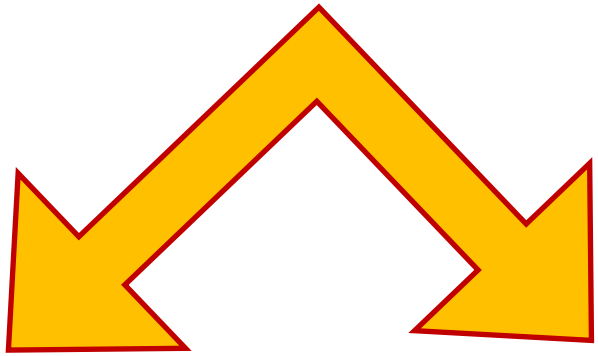
Small additions & Small deletions mutations

- Additions and deletions can occur spontaneously during replication
  - DNA loops out from the template strand
    - generally in a run of the same base.
  - DNA polymerase skips the looped-out bases
    - creating a deletion mutation.
  - If DNA polymerase adds untemplated base(s)
    - new DNA looping occurs, resulting in additional mutation.
  - Insertions and deletions in structural genes generate frameshift mutations
    - if they are not in multiples of three.

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



Spontaneous chemical changes

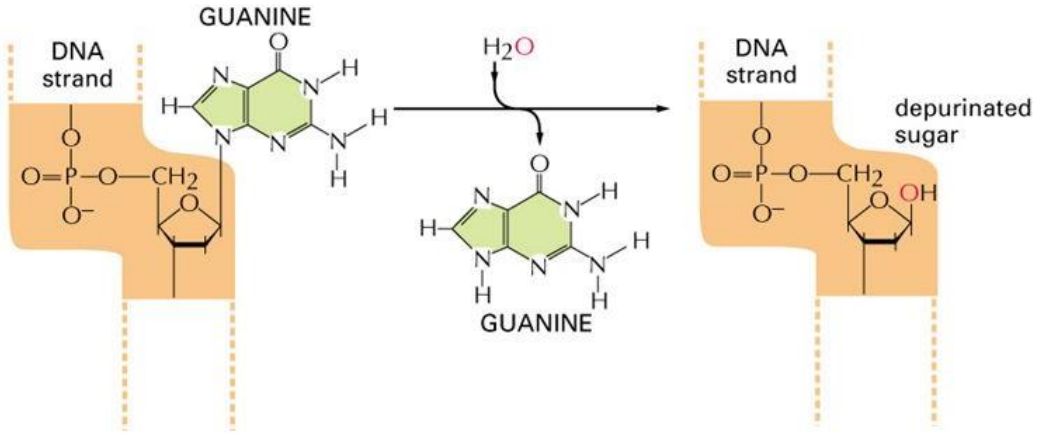


Most common chemical changes

Depurination

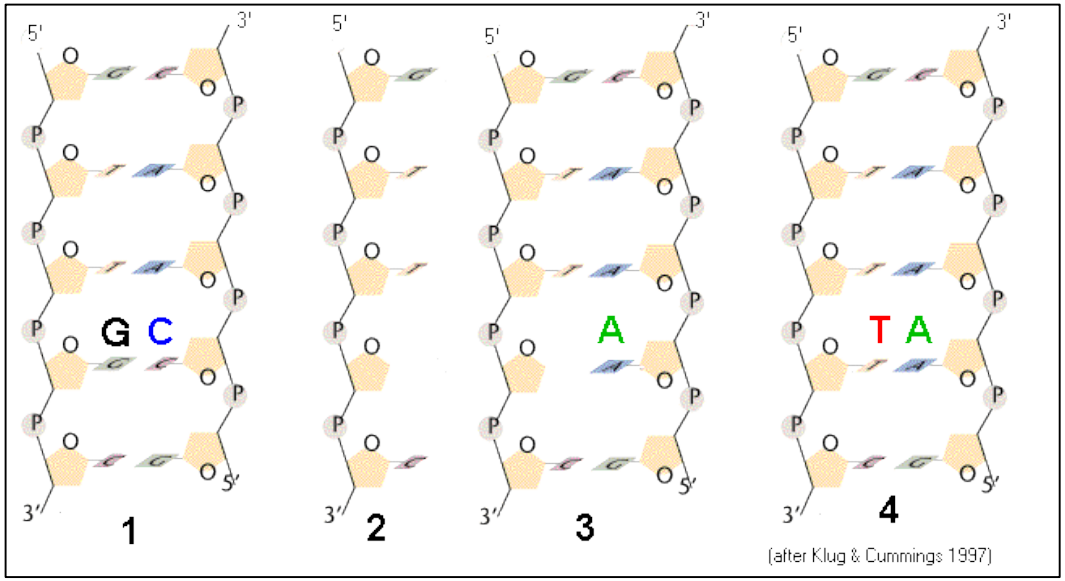
Deamination

Depurination



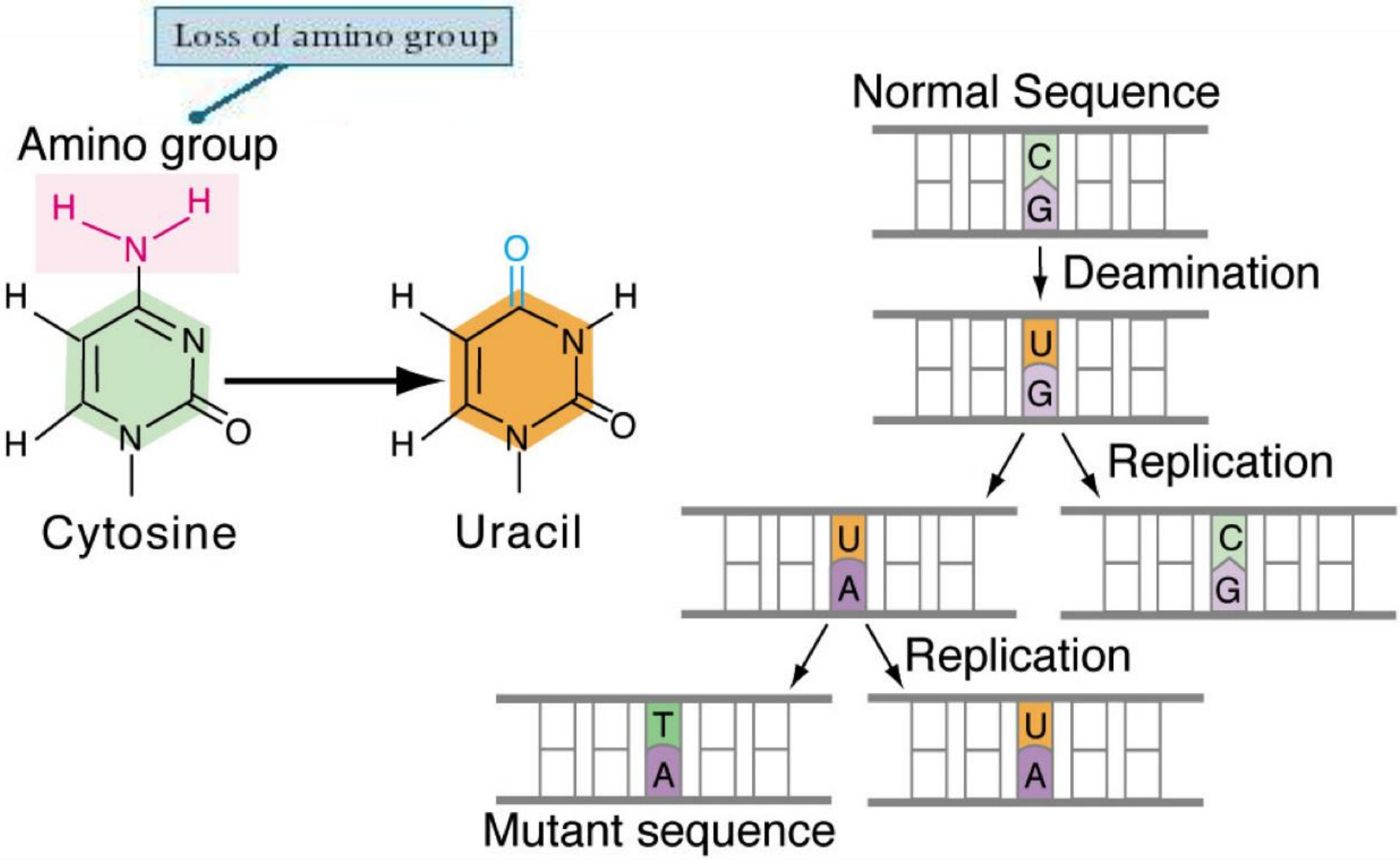
- Release of adenine or guanine bases

A mammalian cell typically loses thousands of purines in an average cell generation period.
If such lesions are not repaired, there is no base to specify a complementary base during DNA replication. Instead, a randomly chosen base is inserted, which can result in a mutation.



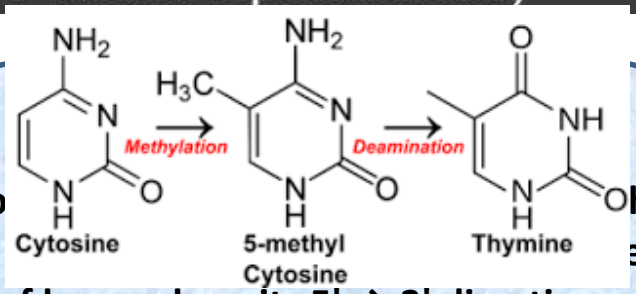
Depurination can produce transversion mutations

Deamination



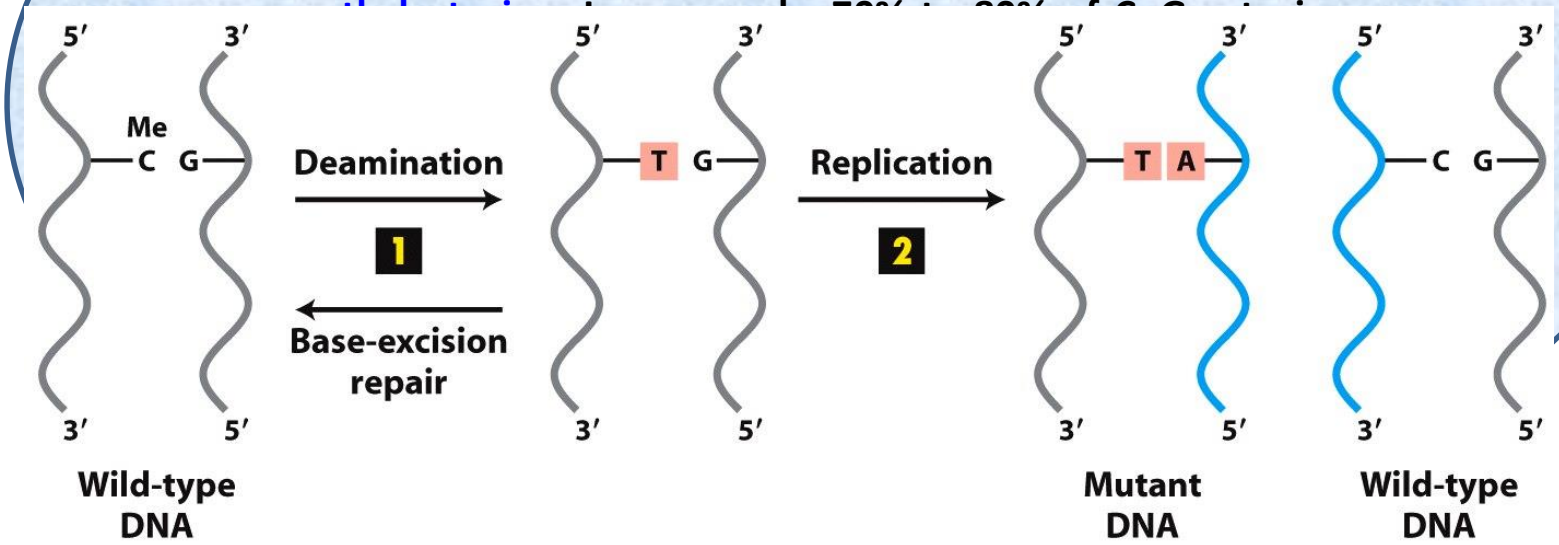
Mutational Hot-Spot

- The CG doublet represents a true 'hotspot' for mutation in human genome.
- Transitions occur more frequently at CpG islands, because the cytosine is prone to methylation at position 5, & spontaneous deamination of 5-methylcytosine to thymine follows [C→ 5-MethylC→T]



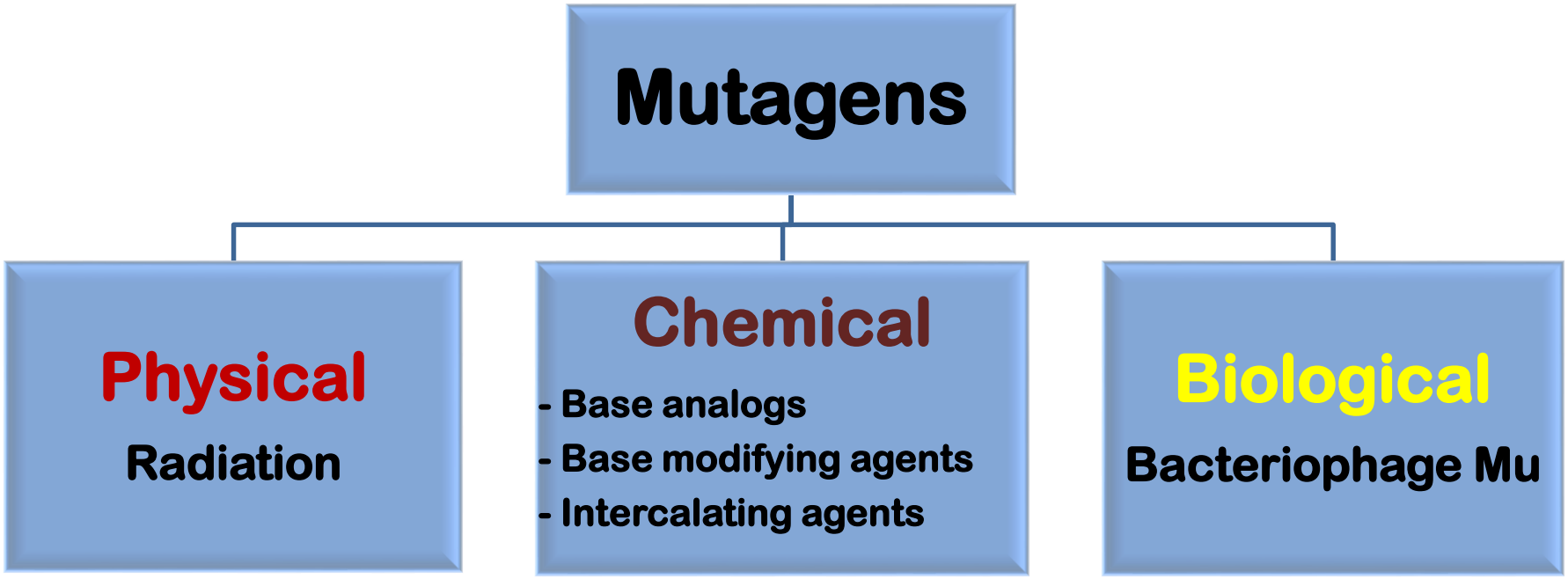
The CpG sites o… here a cytosine nucleotide is … eotide in the linear sequence of bases along its 5' → 3' direction.

❖ Cytosines in CpG dinucleoti… can be methylated to form 5-…



INDUCED MUTATION

- Mutations can be induced by exposing organisms to physical mutagens such as radiation or chemical mutagens.



Radiation

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

i. UV is not energetic enough to induce ionization.

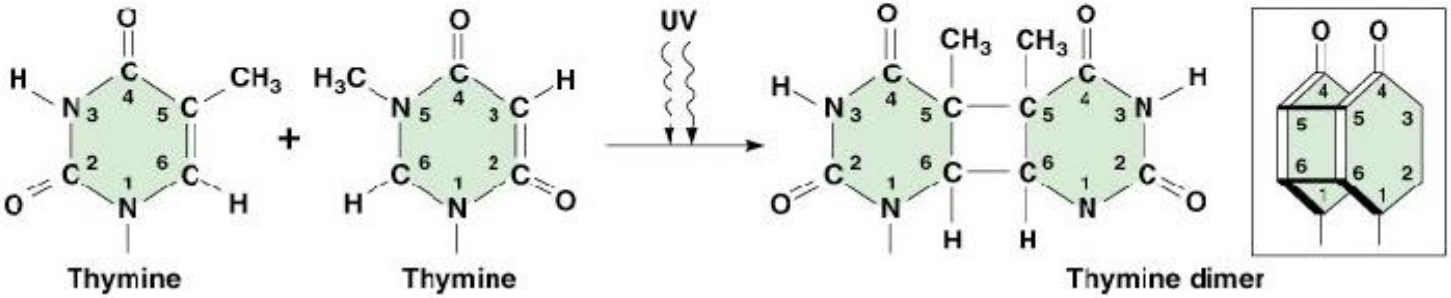
ii. UV has lower-energy wavelengths than X rays, and so has limited penetrating power.

iii. However, 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 thymines (designated T^T)**

Thimine dimer is also called as Cyclobutane photodimer or CPD because it structurally ressembles of cyclobutane nucleus

Production of thymine dimers by ultraviolet light irradiation



(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.

Chemical mutagens

A wide variety of chemicals exist in our environment, and many can have mutagenic effects that can lead to genetic diseases and cancer. Examples include:

- Drugs
- Cosmetics
- Food additives
- Pesticides
- Industrial compounds
- Chemical warfare agents such as mustard gas

Chemical mutagens

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 incorpocates a base with alternate states (tautomers) 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.

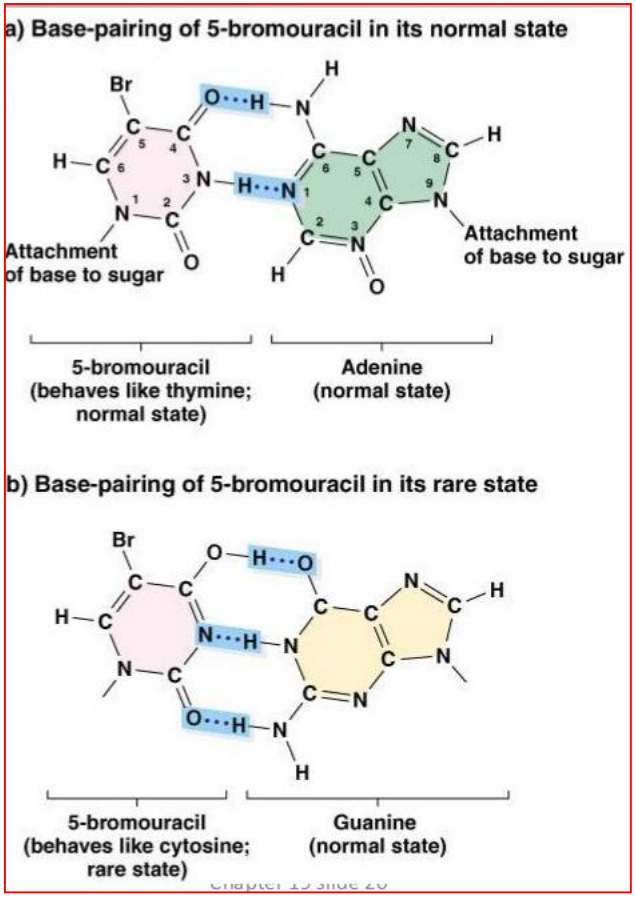
iii. 5-bromouradil (5BU) is an example. 5BU has a bromine residue instead of the methyl group of thymine

(1) Normally 5BU resembles thymine, pairs with adenine and is incorporated into DNA during replication.

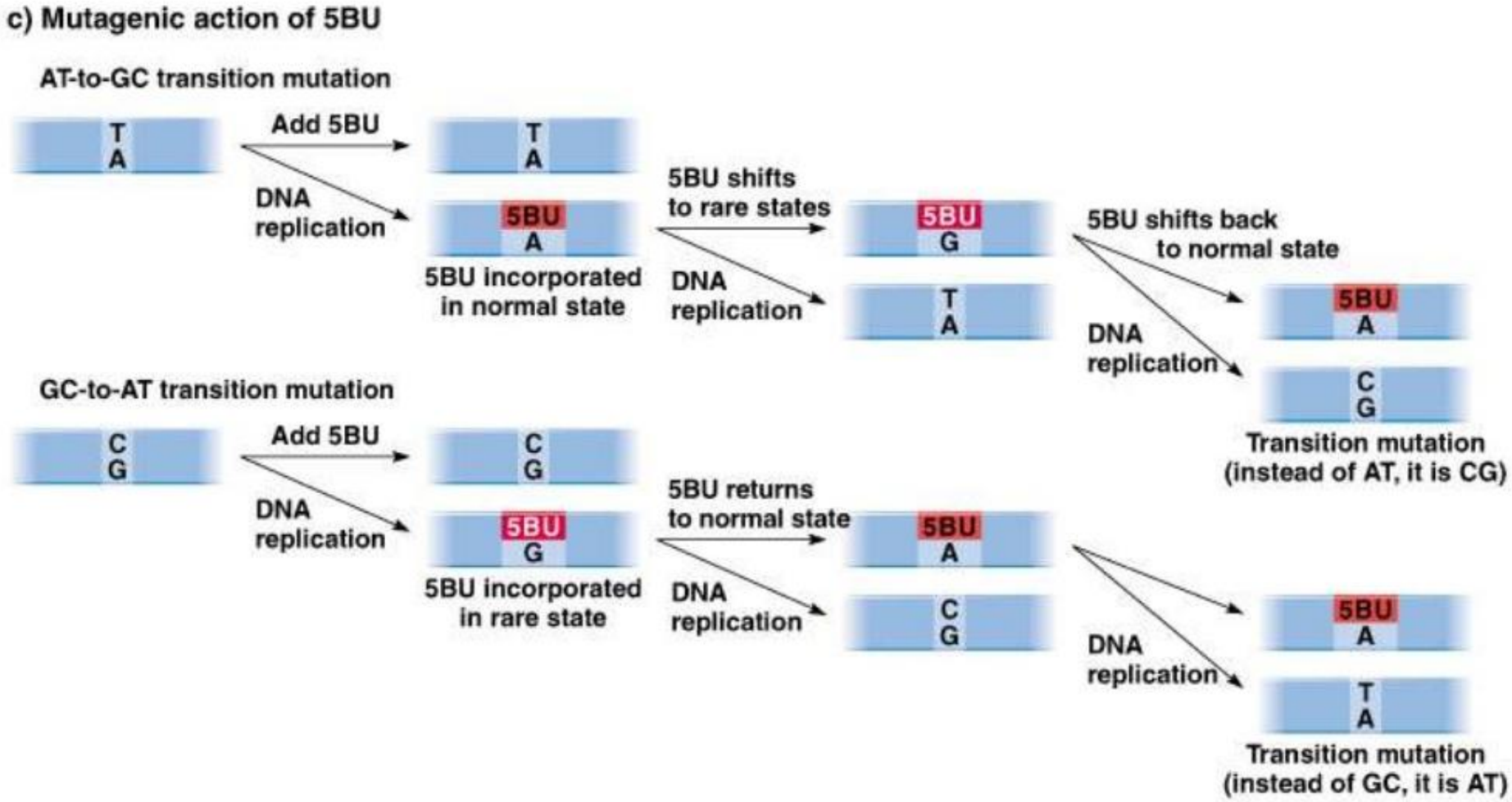
(2) In its rare state, 5BU pairs only with guanine, resulting in a TA-to-CG transition mutation.

(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)



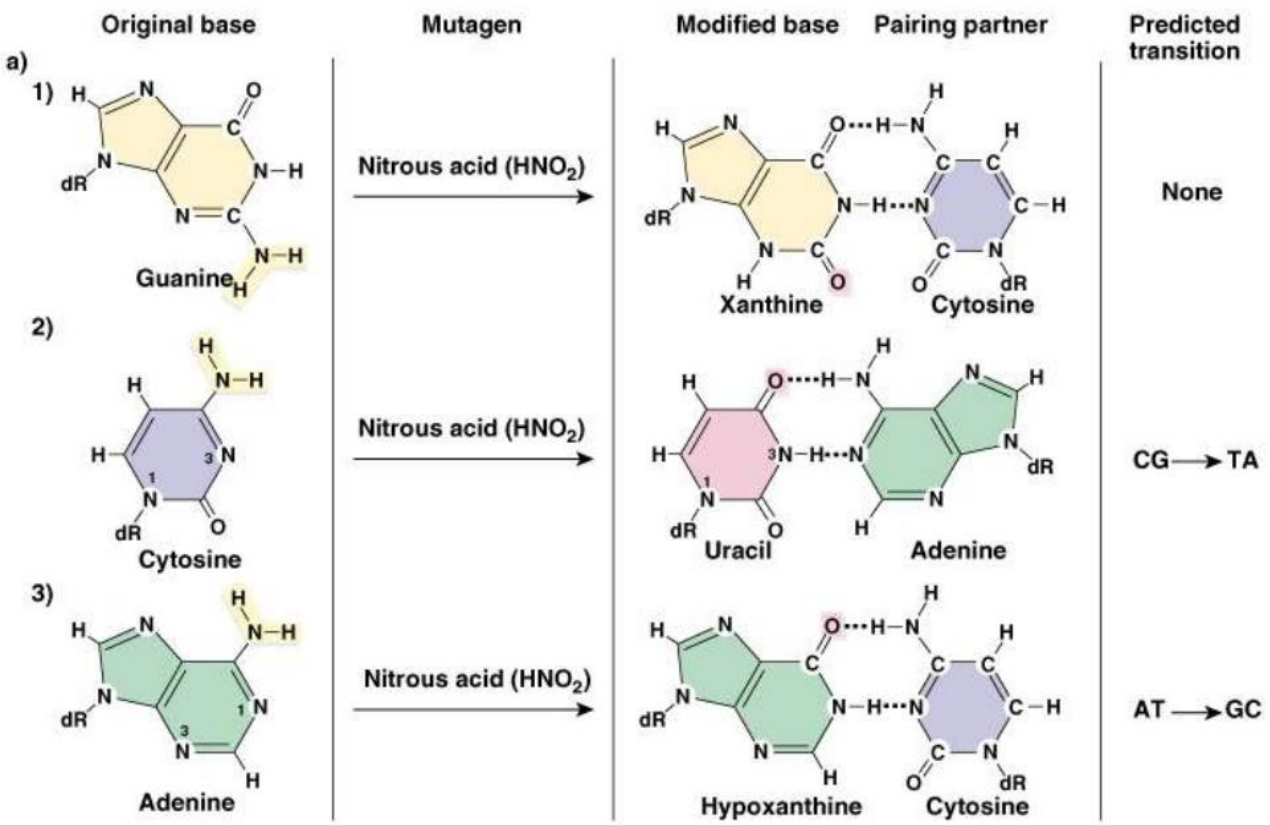
b Base-modifying agents can induce mutations at any stage of the cell cycle. They work by modifying the chemical structure and properties of the bases. Three types are

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 uradil, which produces a CG-to-TA transition.

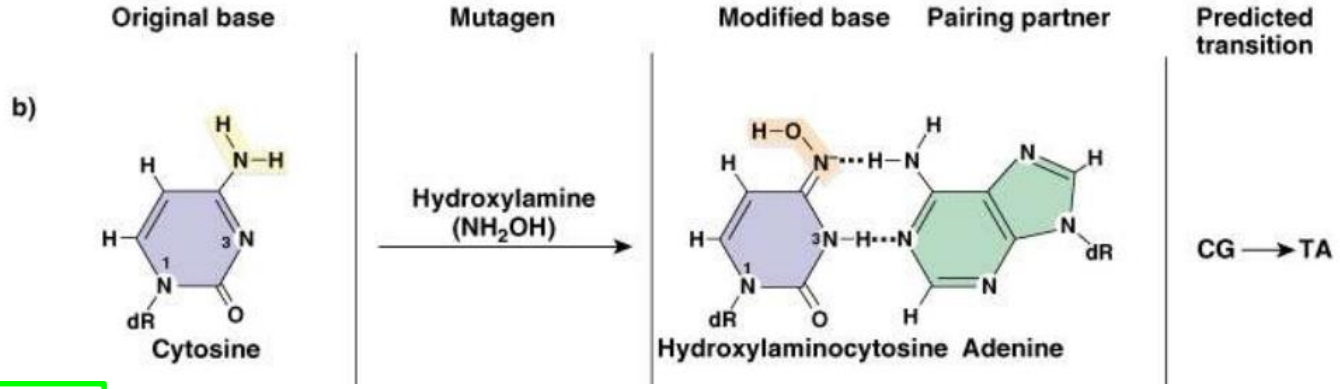
(3) HNO_2 deaminates adenine to produce hypoxanthine, which pairs with cytosine, causing an AT-to-GC transition.



b. Base-modifying agents

ii. Hydroxylating agents include hydroxylamine (NH_2OH).

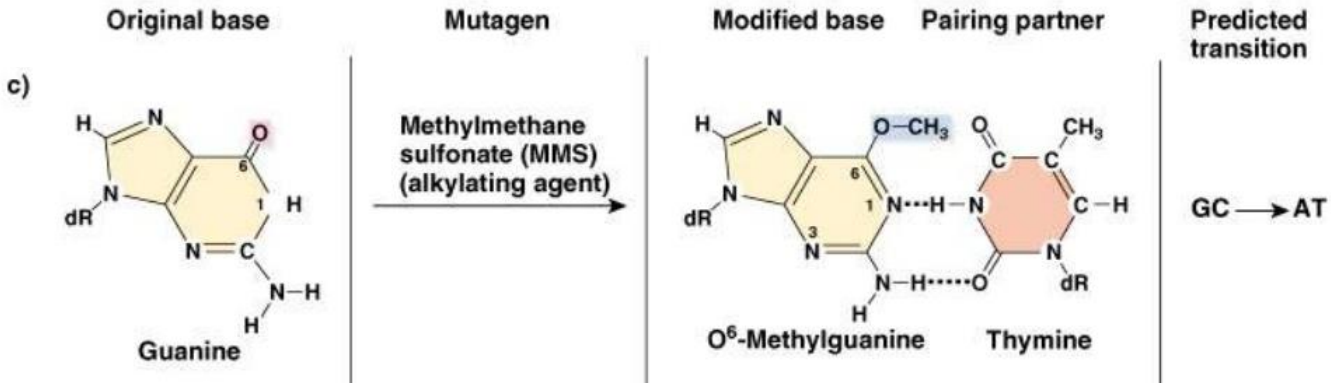
(1) NH_2OH specifically modifies C with a hydroxyl group (OH), so that it pairs only with A instead of with G.



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.



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

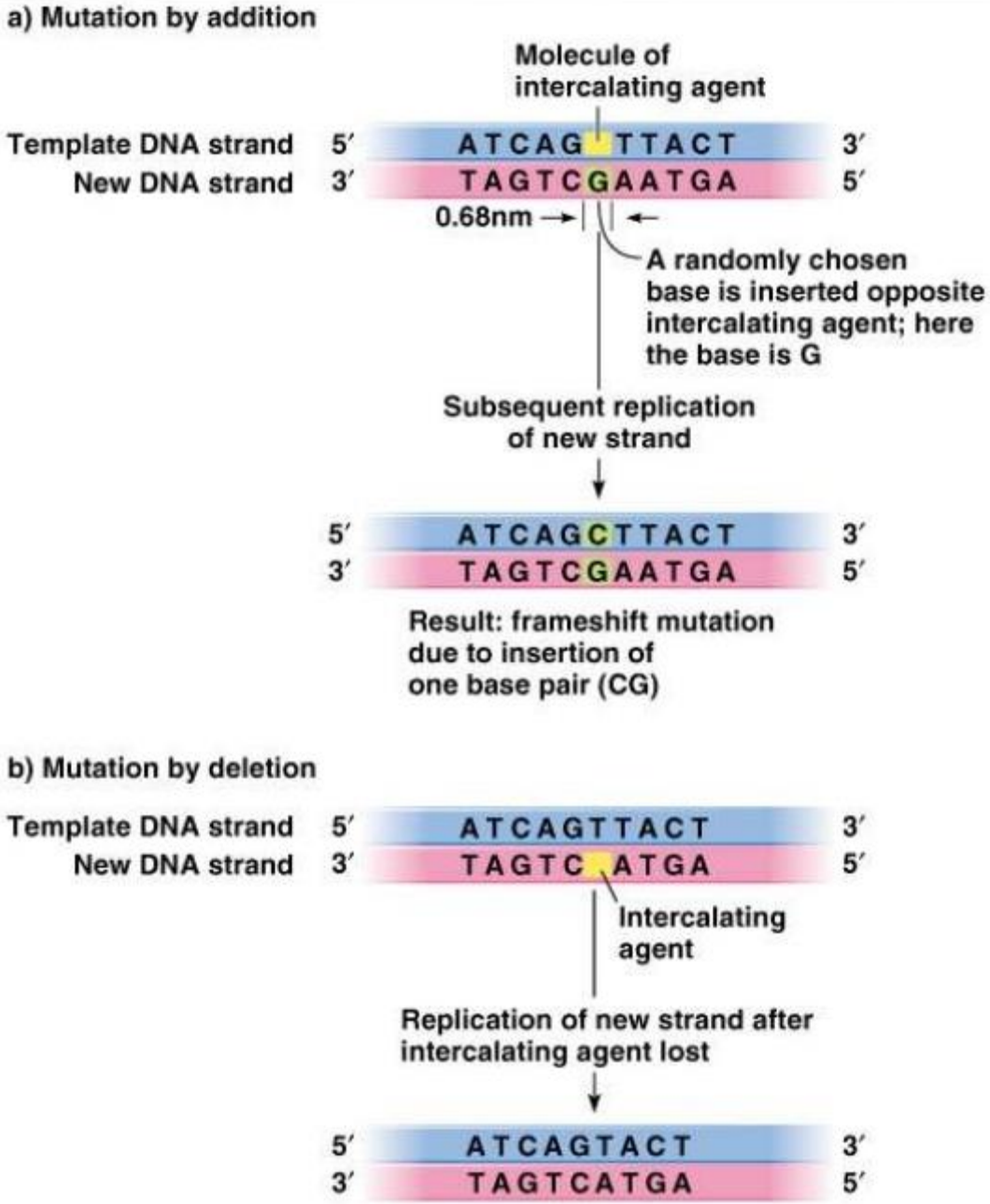
i. At replication, a template that contains an intercalated agent will cause insertion of a random extra base.

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.

Intercalating mutations

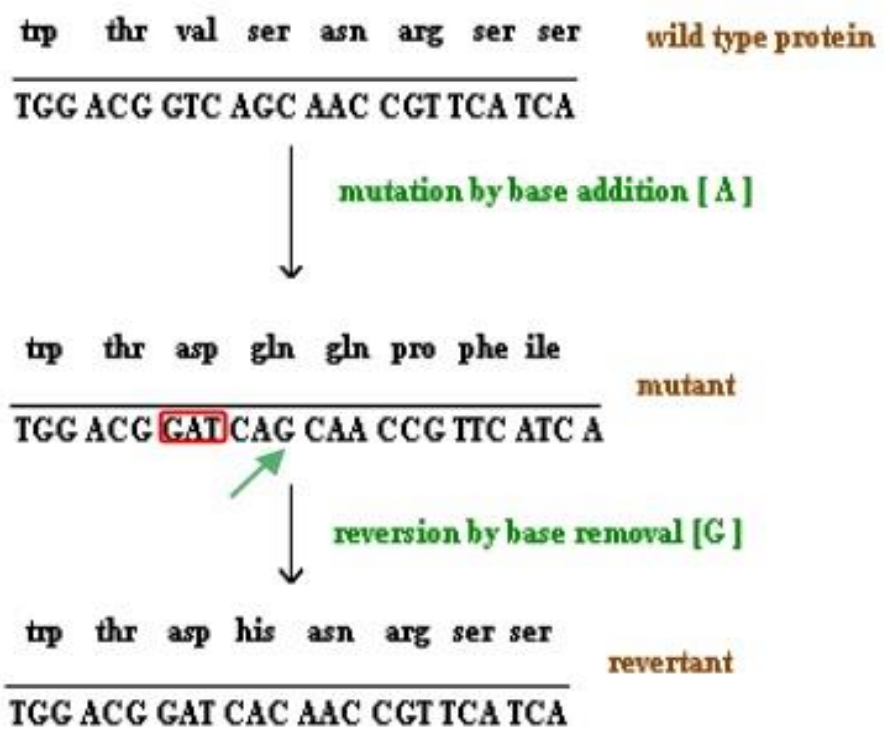


Reverse Mutation

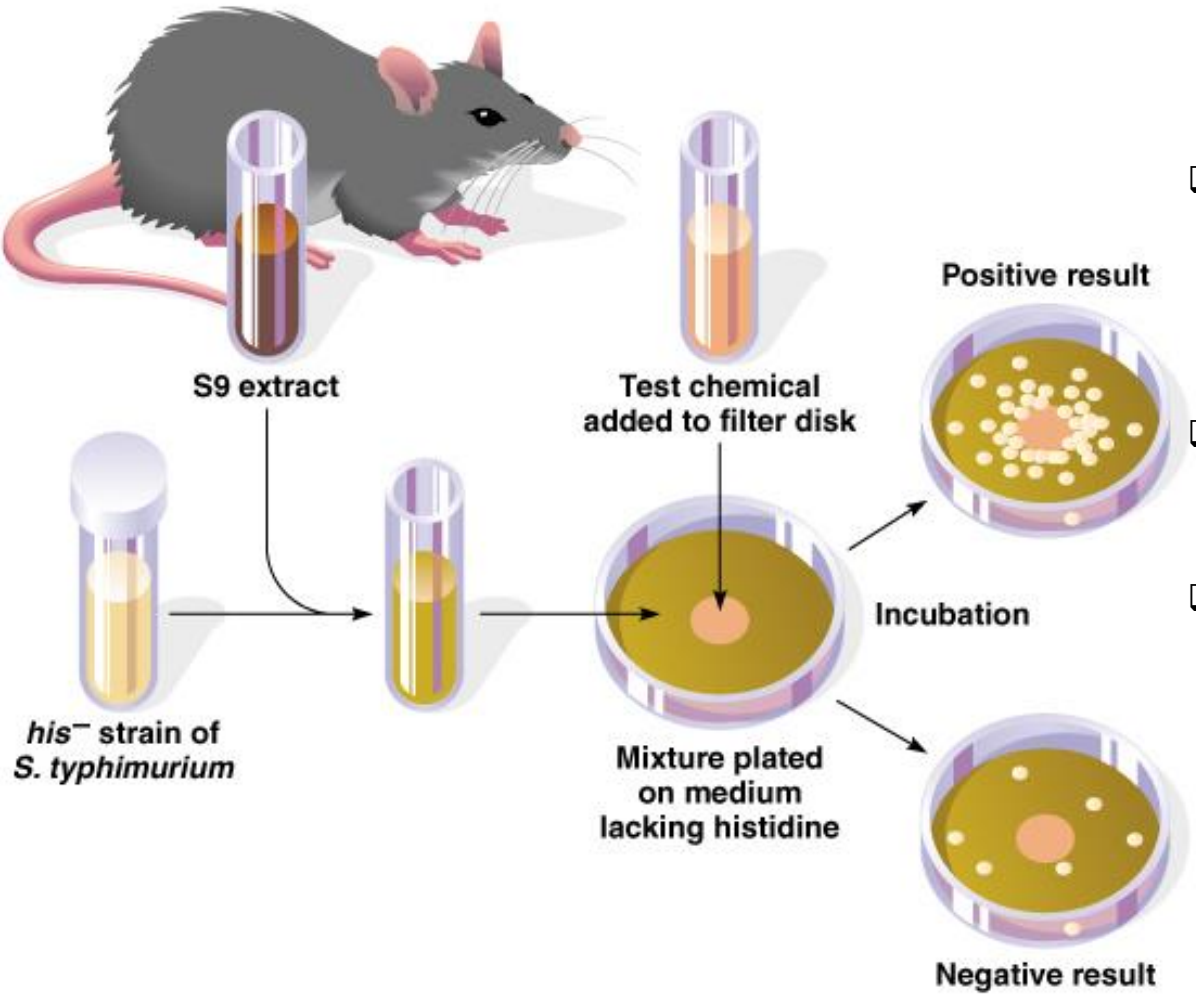
- Reverse mutation (reversion) is the production by further mutation of a permutated gene from a mutant gene.
- Reversion is the correction of a mutation, i.e. it occurs at the same site; more loosely, though, the term is applied also to a mutation at another site that masks or suppresses the effect of the first mutation.
- In fact, such organisms are not non-mutant, but are double mutants with the same phenotype.

- Two types:-

1. True reversion is the reversal of the original nucleotide change.
2. Phenotypic reversion can result from changes that restore a different amino acid with properties identical to the original. Second-site changes within a protein can also restore normal function.



The Ames test for assaying the potential mutagenicity of chemicals



- The tester strains also carry mutations in the genes responsible for lipopolysaccharide synthesis, making the cell wall of the bacteria more permeable
- and also mutation in the excision repair system to make the test more sensitive.
- Rat liver extract is optionally added to simulate the effect of metabolism, as some compounds, like benzo[*a*]pyrene, are not mutagenic themselves but their metabolic products are.

A list of commonly used Ames bacterial strains and detailed assay guidelines can be obtained in OECD test 471 guideline (OECD 471, 1997; Mortelmans and Zeiger, 2000). (Organisation for Economic Co-operation and Development)