Mutations in Microorganism

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LEARNING OBJECTIVES

- Compare point mutations and frameshift mutations
- Describe the differences between missense, nonsense, and silent mutations
- Describe the differences between light and dark repair
- Explain how different mutagens act
- Explain why the Ames test can be used to detect carcinogens
- Analyze sequences of DNA and identify examples of types of mutations

- A **mutation** is a heritable change in the DNA sequence of an organism. The resulting organism, called a **mutant**, may have a recognizable change in **phenotype** compared to the **wild type**, which is the phenotype most commonly observed in nature. A change in the DNA sequence is conferred to mRNA through transcription, and may lead to an altered amino acid sequence in a protein on translation. Because proteins carry out the vast majority of cellular functions, a change in amino acid sequence in a protein may lead to an altered phenotype for the cell and organism.

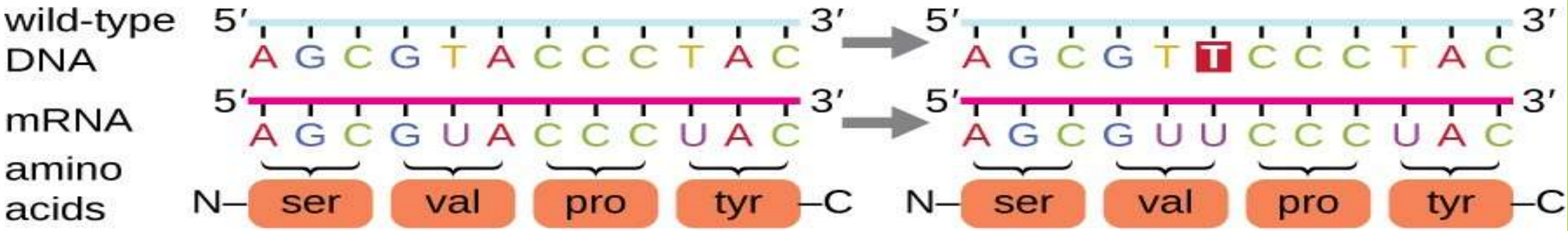
Effects of Mutations on DNA Sequence

- There are several types of mutations that are classified according to how the DNA molecule is altered. One type, called a **point mutation**, affects a single base and most commonly occurs when one base is **substituted or replaced** by another. Mutations also result from the addition of one or more bases, known as an **insertion**, or the removal of one or more bases, known as a **deletion**.

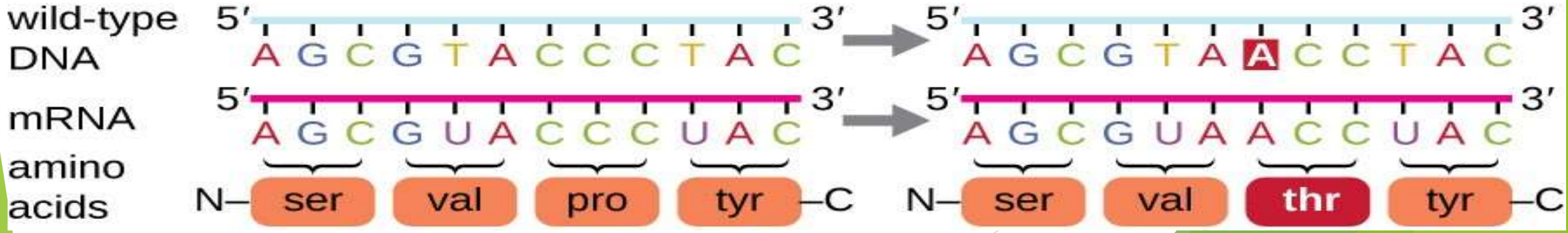
Effects of Mutations on Protein Structure and Function

point mutation: substitution of a single base

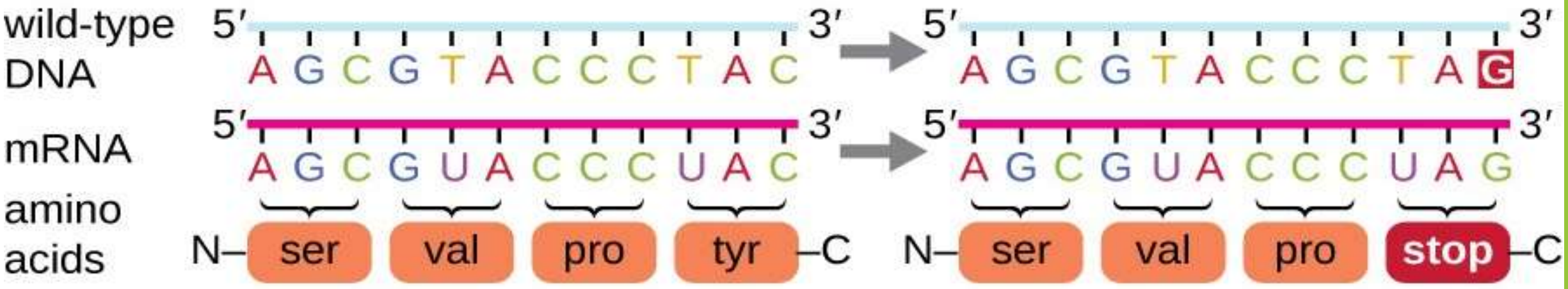
silent: has no effect on the protein sequence



missense: results in an amino acid substitution

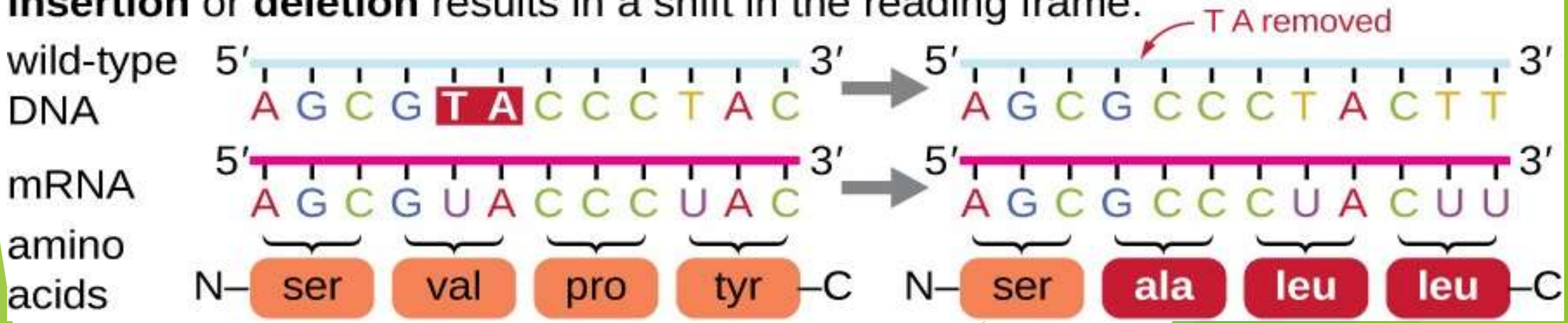


nonsense: substitutes a stop codon for an amino acid



frameshift mutation: insertion or deletion of one or more bases

Insertion or **deletion** results in a shift in the reading frame.



A BENEFICIAL MUTATION

- In recent years, scientific interest has been piqued by the discovery of a few individuals from northern Europe who are **resistant** to **HIV infection**. In **1998,** American geneticist **Stephen J. O'Brien** at the National Institutes of Health (**NIH**) and colleagues published the results of their genetic analysis of more than **4,000** individuals.
- These indicated that many individuals of Eurasian descent (up to 14% in some ethnic groups) have a deletion mutation, called CCR5-delta 32, in the gene encoding CCR5. CCR5 is a coreceptor found on the surface of T cells that is necessary for many strains of the virus to enter the host cell. The mutation leads to the production of a receptor to which HIV cannot effectively bind and thus blocks viral entry. People homozygous for this mutation have greatly reduced susceptibility to HIV infection, and those who are heterozygous have some protection from infection as well.

- This mutation may protect individuals from plague (caused by the bacterium *Yersinia pestis*) and smallpox (caused by the variola virus) because this receptor may also be involved in these diseases. The age of this mutation is a matter of debate, but estimates suggest it appeared between 1875 years to 225 years ago, and may have been spread from Northern Europe through Viking invasions.

- This exciting finding has led to new avenues in HIV research, including looking for drugs to block CCR5 binding to HIV in individuals who lack the mutation. Although DNA testing to determine which individuals carry the CCR5-delta 32 mutation is possible, there are documented cases of individuals homozygous for the mutation contracting HIV. For this reason, DNA testing for the mutation is not widely recommended by public health officials so as not to encourage risky behavior in those who carry the mutation. Nevertheless, inhibiting the binding of HIV to CCR5 continues to be a valid strategy for the development of drug therapies for those infected with HIV.

Causes of Mutations

- Mistakes in the process of DNA replication can cause **spontaneous mutations to occur.** The error rate of **DNA polymerase** is one incorrect base per billion base pairs replicated. Exposure to **mutagens** can cause **induced mutations**, which are various types of chemical agents or radiation (Table 1). Exposure to a mutagen can increase the rate of mutation more than 1000-fold. Mutagens are often also **carcinogens**, agents that cause cancer. However, whereas nearly

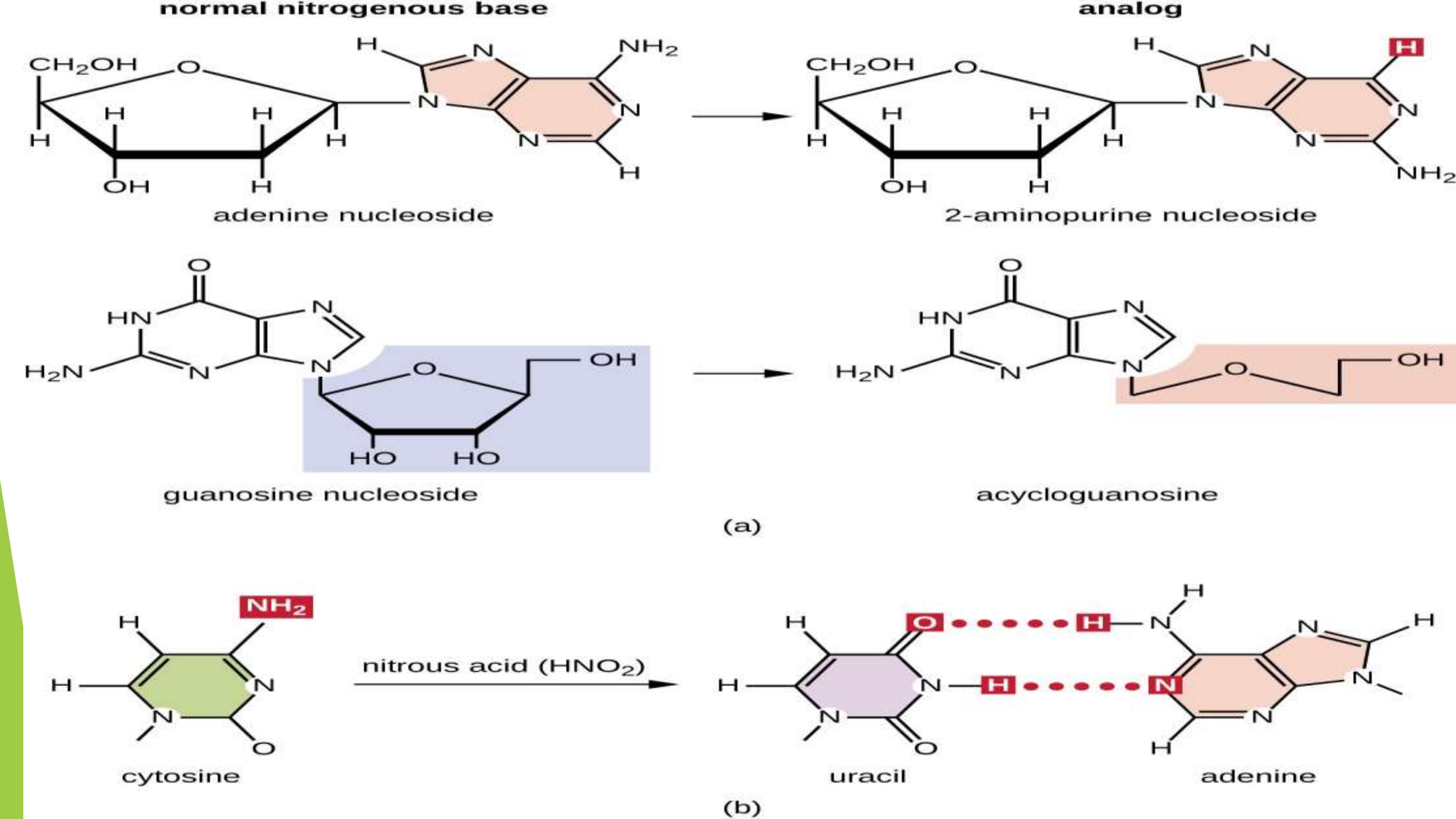
- ***all carcinogens are mutagenic, not all mutagens are necessarily carcinogens.***

Table 1. A Summary of Mutagenic Agents

Mutagenic Agents	Mode of Action	Effect on DNA	Resulting Type of Mutation
Nucleoside analogs			
2-aminopurine	Is inserted in place of A but base pairs with C	Converts AT to GC base pair	Point
5-bromouracil	Is inserted in place of T but base pairs with G	Converts AT to GC base pair	Point
Nucleotide-modifying agent			
Nitrous oxide	Deaminates C to U	Converts GC to AT base pair	Point
Intercalating agents			
Acridine orange, ethidium bromide, polycyclic aromatic hydrocarbons	Distorts double helix, creates unusual spacing between nucleotides	Introduces small deletions and insertions	Frameshift
Ionizing radiation			
X-rays, γ-rays	Forms hydroxyl radicals	Causes single- and double-strand DNA breaks	Repair mechanisms may introduce mutations
X-rays, γ-rays	Modifies bases (e.g., deaminating C to U)	Converts GC to AT base pair	Point
Nonionizing radiation			
Ultraviolet	Forms pyrimidine (usually thymine) dimers	Causes DNA replication errors	Frameshift or point

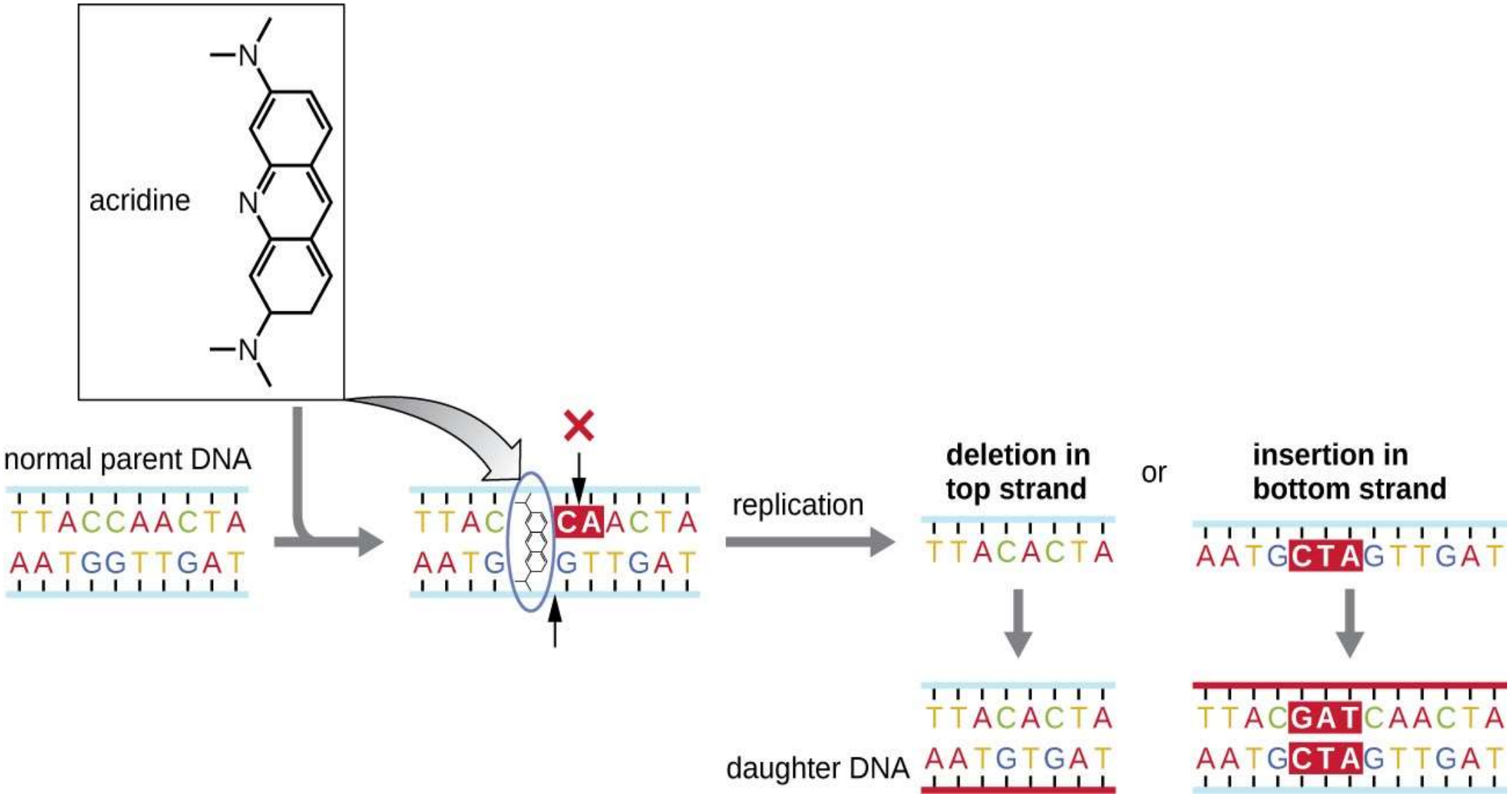
Chemical Mutagens

- Various types of chemical mutagens interact directly with DNA either by acting as nucleoside analogs or by modifying nucleotide bases. Chemicals called **nucleoside analogs** are *structurally similar to normal nucleotide bases and can be incorporated into DNA during replication*. These base analogs induce mutations because they often have different base-pairing rules than the bases they replace. Other chemical mutagens can modify normal DNA bases, resulting in different base-pairing rules, nitrous acid deaminates cytosine, converting it to uracil. Uracil then pairs with adenine in a subsequent round of replication, resulting in the conversion of a GC base pair to an AT base pair. Nitrous acid also deaminates adenine to hypoxanthine, which base pairs with cytosine instead of thymine, resulting in the conversion of a TA base pair to a CG base pair.


normal nitrogenous base
analog
adenine nucleoside
2-aminopurine nucleoside
guanosine nucleoside
acycloguanosine
(a)
cytosine
nitrous acid (HNO_2)
uracil
adenine
(b)

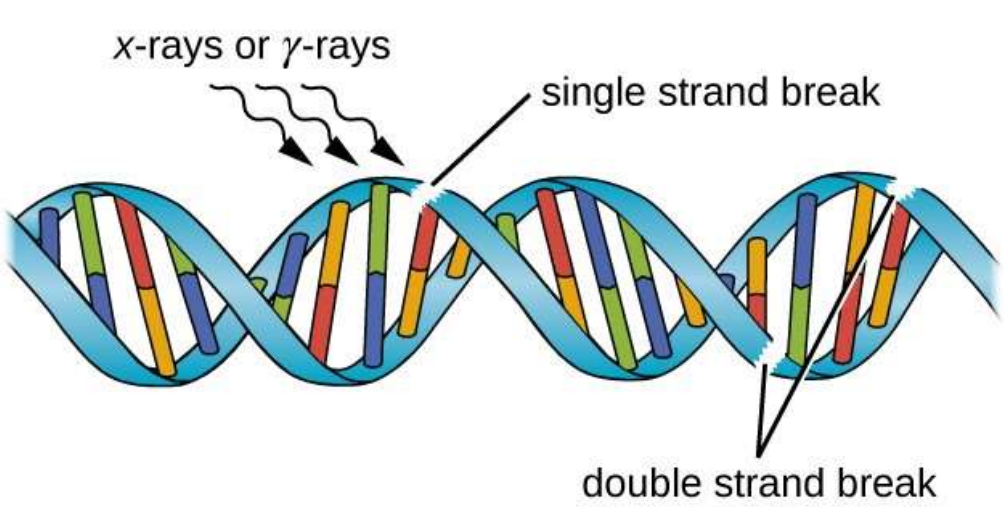
intercalating agents:-

- These molecules slide between the stacked nitrogenous bases of the DNA double helix,
- Distorting the molecule and creating atypical spacing between nucleotide base pairs
- As a result, during DNA replication, DNA polymerase may either **skip** replicating several nucleotides **(creating a deletion)** or **insert** extra nucleotides (creating an **insertion**).
- Either outcome may lead to a **frameshift mutation**.
- Combustion products like polycyclic aromatic hydrocarbons are particularly dangerous intercalating agents that can lead to mutation-caused cancers.
- The intercalating agents **ethidium bromide**and **acridine orange** are commonly used in the laboratory to stain DNA for visualization and are potential mutagens.

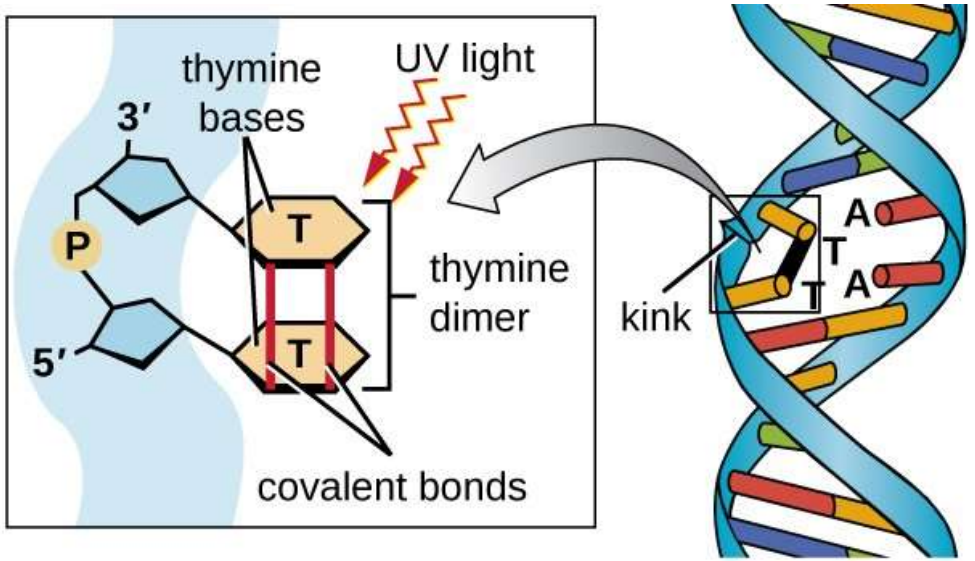

acridine
N
N
N
normal parent DNA
TTACCAACTA
AATGGTTGAT
TTAC
CA
ACTA
AATG
GTTGAT
replication
deletion in
top strand
TTACACTA
daughter DNA
TTACACTA
AATGTGAT
or
insertion in
bottom strand
AATGCTAGTTGAT
TTACGATCAACTA
AATGCTAGTTGAT

Radiation

- Strong **ionizing radiation** like **X-rays** and **gamma rays** can cause single- and double-stranded breaks in the DNA backbone through the formation of hydroxyl radicals on radiation exposure
- Ionizing radiation can also modify bases; for example, the deamination of cytosine to uracil, analogous to the action of nitrous acid.
- Ionizing radiation exposure is used to kill microbes to sterilize medical devices and foods, because of its dramatic nonspecific effect in damaging DNA, proteins, and other cellular components
- **Nonionizing radiation,** like **ultraviolet light**, *is not energetic enough to initiate these types of chemical changes*. However, **nonionizing radiation** can induce *dimer formation between two adjacent pyrimidine bases,*
- commonly two thymines, within a nucleotide strand. During **thymine dimer** formation, the two adjacent thymines become *covalently linked and*, if left unrepaired, both DNA replication and transcription are stalled at this point.
- DNA polymerase may proceed and replicate the dimer incorrectly, potentially leading to frameshift or point mutations.



(a) Ionizing radiation



(b) Non-ionizing radiation

DNA Repair

- The process of **DNA replication** is highly accurate, but mistakes can occur spontaneously or be induced by mutagens. Uncorrected mistakes can lead to serious consequences for the phenotype. Cells have developed several repair mechanisms to minimize the number of mutations that persist.
- **Proofreading**
- Most of the mistakes introduced during DNA replication are promptly corrected by most **DNA polymerases** through a function called proofreading. In **proofreading**, the DNA polymerase reads the newly added base, ensuring that it is complementary to the corresponding base in the template strand before adding the next one. If an incorrect base has been added, the enzyme makes a cut to release the wrong nucleotide and a new base is added.

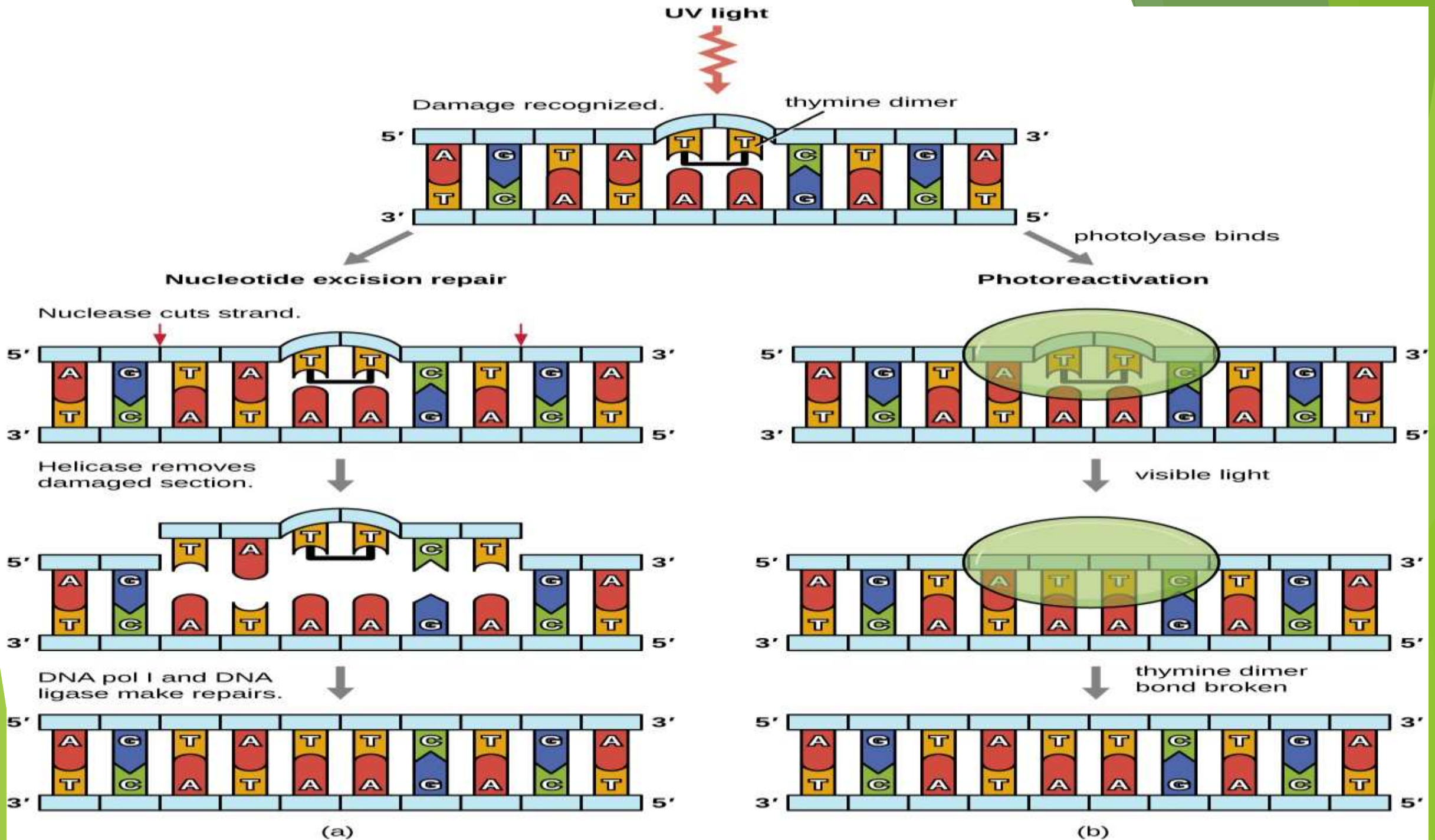
Mismatch Repair

Some errors introduced during replication are corrected shortly after the replication machinery has moved. This mechanism is called **mismatch repair**. The enzymes involved in this mechanism recognize the incorrectly added nucleotide, excise it, and replace it with the correct base. One example is the methyl-directed mismatch repair in ***E. coli***. The DNA is hemimethylated. This means that the parental strand is methylated while the newly synthesized daughter strand is not. It takes several minutes before the new strand is methylated. Proteins MutS, MutL, and MutH bind to the hemimethylated site where the incorrect nucleotide is found. MutH cuts the nonmethylated strand (the new strand). An exonuclease removes a portion of the strand (including the incorrect nucleotide). The gap formed is then filled in by DNA pol III and ligase.

Repair of Thymine Dimers

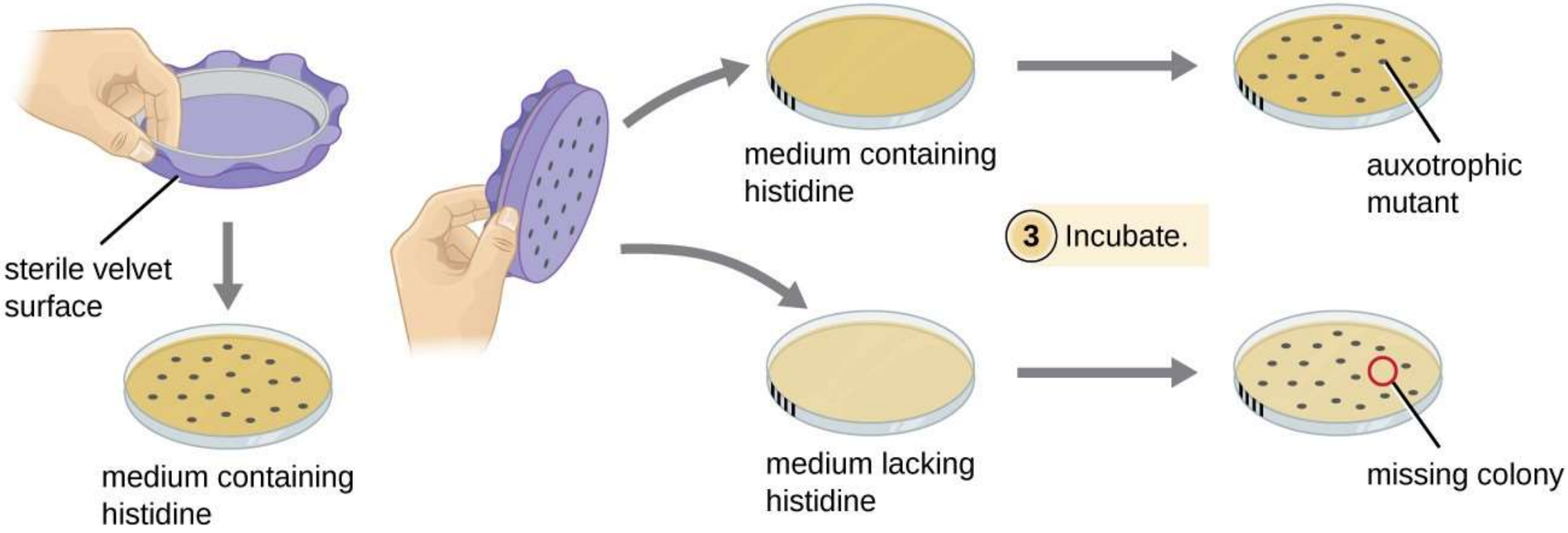
Because the production of **thymine dimers** is common (many organisms cannot avoid ultraviolet light), mechanisms have evolve to repair these lesions. In **nucleotide excision repair** (also called dark repair), enzymes remove the pyrimidine dimer and replace it with the correct nucleotides (Figure 6). In *E. coli*, the DNA is scanned by an enzyme complex. If a distortion in the double helix is found that was introduced by the pyrimidine dimer, the enzyme complex cuts the sugar-phosphate backbone several bases upstream and downstream of the dimer, and the segment of DNA between these two cuts is then enzymatically removed. DNA pol I replaces the missing nucleotides with the correct ones and DNA ligase seals the gap in the sugar-phosphate backbone.

The **direct repair** (also called light repair) of thymine dimers occurs through the process of **photoreactivation** in the presence of visible light. An enzyme called **photolyase** recognizes the distortion in the DNA helix caused by the thymine dimer and binds to the dimer. Then, in the presence of visible light, the photolyase enzyme changes conformation and breaks apart the thymine dimer, allowing the thymines to again correctly base pair with the adenines on the complementary strand. Photoreactivation appears to be present in all organisms, with the exception of placental mammals, including humans. Photoreactivation is particularly important for organisms chronically exposed to **ultraviolet radiation**, like plants, photosynthetic bacteria, algae, and corals, to prevent the accumulation of mutations caused by thymine dimer formation.


UV light
Damage recognized.
thymine dimer
5′
3′
3′
5′
photolyase binds
Nucleotide excision repair
Photoreactivation
Nuclease cuts strand.
Helicase removes damaged section.
DNA pol I and DNA ligase make repairs.
visible light
thymine dimer bond broken
(a)
(b)

Identifying Bacterial Mutants

One common technique used to identify bacterial mutants is called **replica plating**. This technique is used to detect nutritional mutants, called **auxotrophs**, which have a mutation in a gene encoding an enzyme in the biosynthesis pathway of a specific nutrient, such as an amino acid. As a result, whereas wild-type cells retain the ability to grow normally on a medium lacking the specific nutrient, auxotrophs are unable to grow on such a medium. During replica plating (Figure 7), a population of bacterial cells is mutagenized and then plated as individual cells on a complex nutritionally complete plate and allowed to grow into colonies. Cells from these colonies are removed from this master plate, often using sterile velvet. This velvet, containing cells, is then pressed in the same orientation onto plates of various media. At least one plate should also be nutritionally complete to ensure that cells are being properly transferred between the plates. The other plates lack specific nutrients, allowing the researcher to discover various auxotrophic mutants unable to produce specific nutrients. Cells from the corresponding colony on the nutritionally complete plate can be used to recover the mutant for further study.


sterile velvet surface
medium containing histidine
medium containing histidine
auxotrophic mutant
3 Incubate.
medium lacking histidine
missing colony
1 Press sterile velvet onto plate to pick up cells from bacterial colonies.
2 Transfer cells to new plates.
4 Compare growth on plates to identify auxotrophic mutants that grow on medium containing histidine but do not grow on medium lacking histidine.

The Ames Test

The **Ames test**, developed by Bruce **Ames** (1928–) in the 1970s, is a method that uses bacteria for rapid, inexpensive screening of the carcinogenic potential of new chemical compounds. The test measures the mutation rate associated with exposure to the compound, which, if elevated, may indicate that exposure to this compound is associated with greater cancer risk. The Ames test uses as the test organism a strain of ***Salmonella typhimurium*** that is a histidine auxotroph, unable to synthesize its own histidine because of a mutation in an essential gene required for its synthesis. After exposure to a potential mutagen, these bacteria are plated onto a medium lacking histidine, and the number of mutants regaining the ability to synthesize histidine is recorded and compared with the number of such mutants that arise in the absence of the potential mutagen (Figure 8). Chemicals that are more mutagenic will bring about more mutants with restored histidine synthesis in the Ames test. Because many chemicals are not directly mutagenic but are metabolized to mutagenic forms by liver enzymes, rat liver extract is commonly included at the start of this experiment to mimic liver metabolism. After the Ames test is conducted, compounds identified as mutagenic are further tested for their potential carcinogenic properties by using other models, including animal models like mice and rats.

1. Add rat liver extract and *Salmonella* to top control tube; add rat liver extract, possible mutagen, and *Salmonella* to bottom experimental tube. Plate and incubate both samples using medium lacking histidine.

2. Compare growth on plates to identify revertants, which suggest mutagen causes mutations.