LS2202

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Bacterial genetics - Mutations, Transformation, Conjugation, Transduction,

Basics of Lac operon.

Phages - Small SS-DNA phages, Alpha phage, P1 phage.

Gene manipulation and recombinant DNA technology.

The origin of point mutations

Genetic variation among individuals provides the raw material for evolution.

Two major processes are responsible for genetic variation, *mutation and recombination.*

Mutation is a change in the DNA sequence of a gene.

In the cellular environment, DNA molecules are not absolutely stable; each base pair in a DNA double helix has a certain probability of mutating.

Mutational events take place *within individual genes,* such events are called *gene mutations.*

Two general classes of gene mutation:
- Mutations affecting single base pairs of DNA
- Mutations altering the number of copies of a small repeated sequence within a gene

Point mutations typically refer to alterations of single base pairs of DNA or of a small number of adjacent base pairs—that is, mutations that map to a single location, or "point," within a gene.

Newly arising mutations are categorized as **induced or spontaneous**.

Induced mutations *are defined as those that* arise after purposeful treatment with mutagens, environmental agents that are known to increase the rate of mutations.

Spontaneous mutations are those that arise in the absence of *known mutagen treatment. They account* for the "background rate" of mutation.

The frequency at which spontaneous mutations occur is low, generally in the range of one cell in 10^5 to 10^8. Therefore, if a large number of mutants are required for genetic analysis, mutations must be induced.

The induction of mutations is accomplished by treating cells with mutagens.

The production of mutations through exposure to mutagens is called **mutagenesis,** and the organism is said to be ***mutagenized.***

The most commonly used mutagens are high-energy radiation or specific chemicals;

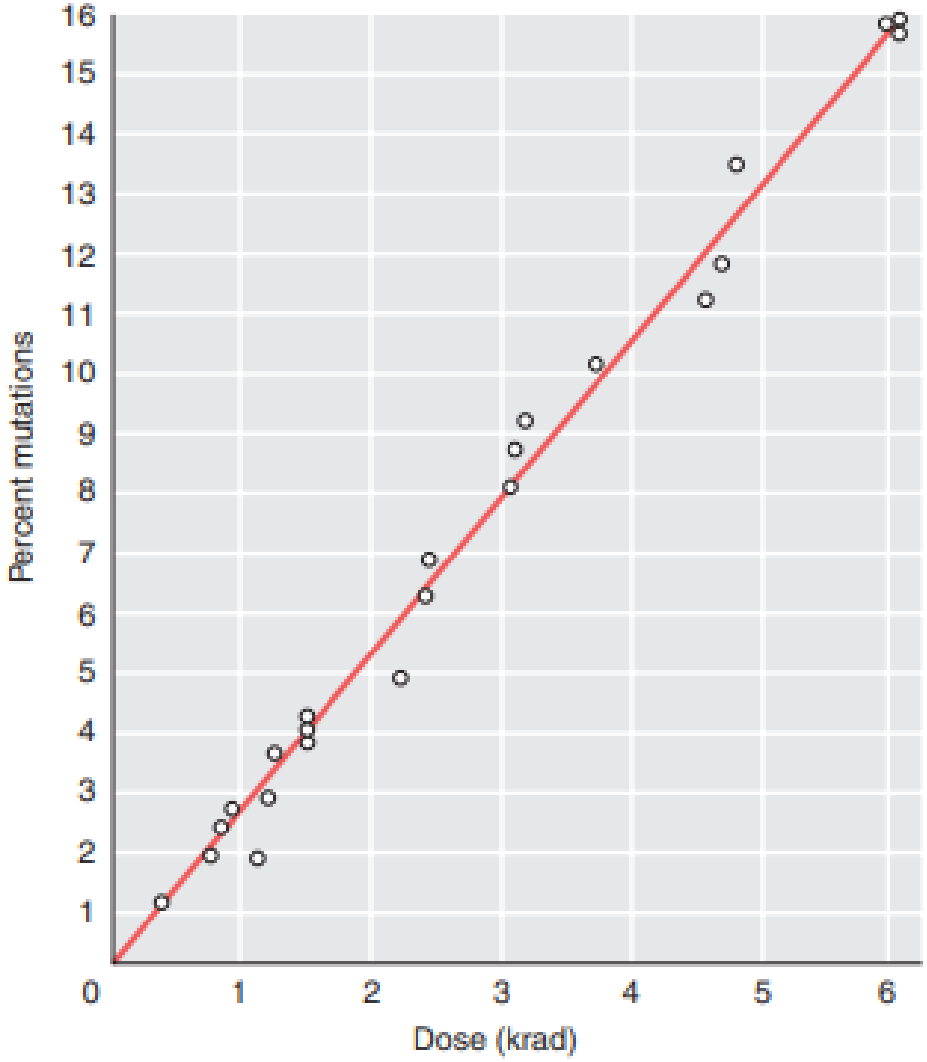


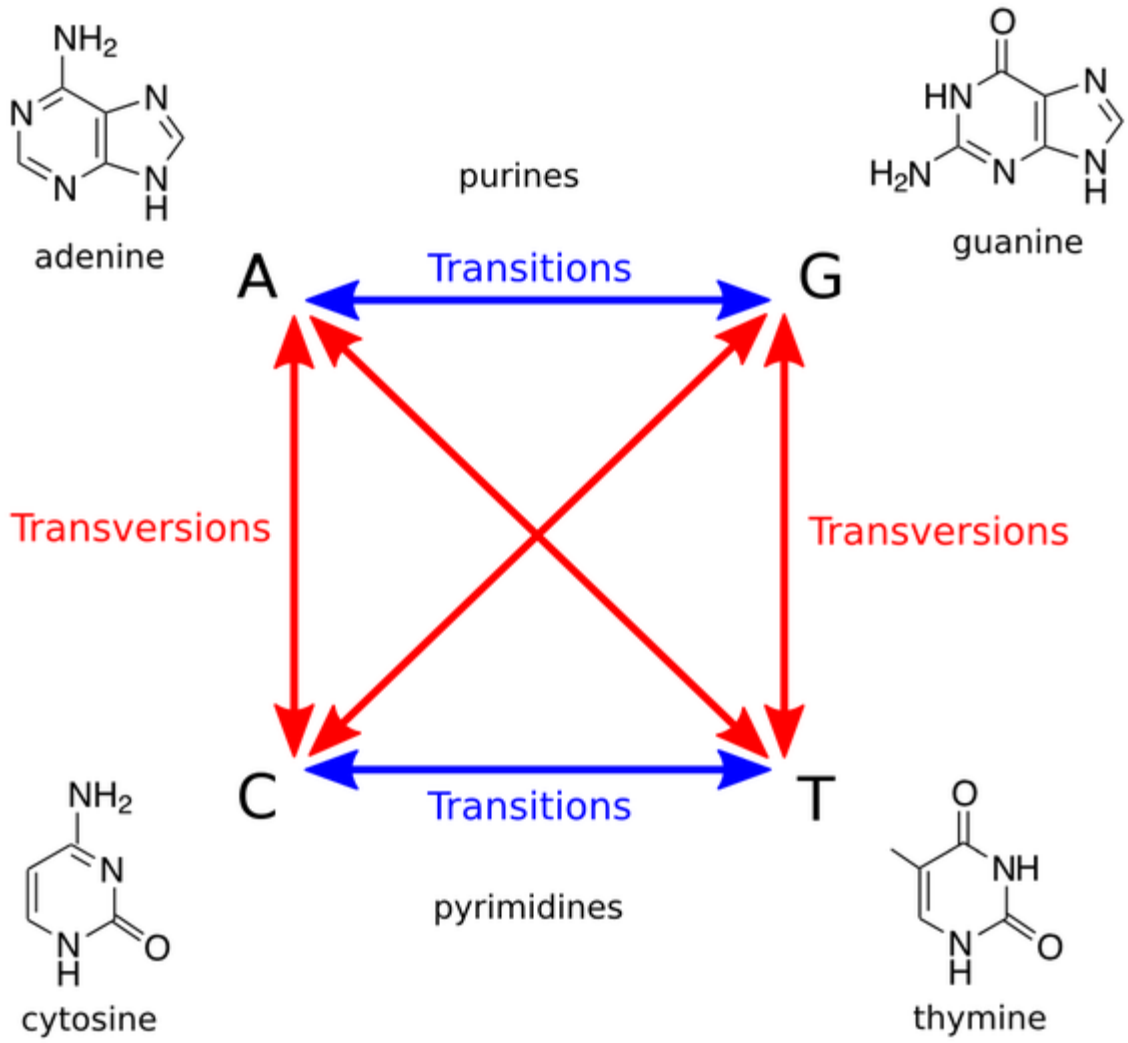
Figure 14-2 Linear relationship between X-ray dose and mutation. The relationship is measured by the induction of sex-linked recessive lethals in *Drosophila*.

The two main types of point mutation in DNA are ***base substitutions and base additions or deletions.***

Base substitutions are mutations in which one base pair is replaced by another. Base substitutions also can be divided into two subtypes: transitions and transversions.

A **transition is the replacement** of a base by the other base of the same chemical category (purine replaced by purine: either A to G or G to A; pyrimidine replaced by pyrimidine: either C to T or T to C).

A **transversion is the opposite—the replacement of a** base of one chemical category by a base of the other (pyrimidine replaced by purine: C to A, C to G, T to A, T to G; purine replaced by pyrimidine: A to C, A to T, G to C, G to T).


adenine
purines
guanine
A
Transitions
G
Transversions
Transversions
C
Transitions
T
pyrimidines
cytosine
thymine

Addition or deletion mutations are actually additions or deletions of *nucleotide pairs; nevertheless, the convention* is to call them *base-pair additions or deletions.*

Collectively, they are termed ***indel mutations*** *(for insertiondeletion).*

The simplest of these mutations are single base-pair additions or single-base-pair deletions.

Purines consist of a six-membered and a five-membered nitrogen-containing ring, fused together.
Adenine and guanine are found in both DNA and RNA.

Pyridmidines have only a six-membered nitrogen-containing ring.
Cytosine is found in both DNA and RNA. Uracil is found only in RNA. Thymine is normally found in DNA.

Table 14-2 Point Mutations at the Molecular Level

Type of mutation	Result and examples
At DNA level	
Transition	Purine replaced by a different purine, or pyrimidine replaced by a different pyrimidine: A · T ⟶ G · C ⟶ G · C ⟶ A · T C · G ⟶ T · A T · A ⟶ C · G
Transversion	Purine replaced by a pyrimidine, or pyrimidine replaced by a purine: A · T ⟶ C · G A · T ⟶ T · A G · C ⟶ T · A G · C ⟶ C · G T · A ⟶ G · C T · A ⟶ A · T C · G ⟶ A · T C · G ⟶ G · C
Indel	Addition or deletion of one or more base pairs of DNA (inserted or deleted bases are underlined): AAGACTCCT ⟶ AAGA<u>G</u>CTCCT AA<u>G</u>ACTCCT ⟶ AAACTCCT

The molecular consequences of point mutations on gene structure and expression

What are the functional consequences of these different types of point mutations? First, consider what happens when a mutation arises in a polypeptide-coding part of a gene. For single-base substitutions, there are several possible outcomes,

Synonymous mutations. The mutation changes one codon for an amino acid into another codon for that same amino acid. Synonymous mutations are also referred to as *silent mutations.*

- **Missense mutations.** The codon for one amino acid is changed into a codon for another amino acid. Missense mutations are sometimes called *onsynonymous* mutations.

- **Nonsense mutations.** The codon for one amino acid is changed into a translation termination (stop) codon.

- The sequence of mRNA is “read” by the translational apparatus in register (“in frame”), three bases (one codon) at a time.

- The addition or deletion of a single base pair of DNA changes the reading frame for the remainder of the translation process, from the site of the base-pair mutation to the next stop codon in the new reading frame.

- Hence, these lesions are called **frameshift mutations.**

- These mutations cause the entire amino acid sequence translationally downstream of the mutant site to bear no relation to the original amino acid sequence.

At protein level

Synonymous mutation	Codons specify the same amino acid: AGG ⟶ CGG Arg Arg
Missense mutation	Codon specifies a different amino acid
Conservative missense mutation	Codon specifies chemically similar amino acid: AAA ⟶ AGA Lys (basic) Arg (basic) Does not alter protein function in many cases
Nonconservative missense mutation	Codon specifies chemically dissimilar amino acid: UUU ⟶ UCU Hydrophobic phenylalanine Polar serine
Nonsense mutation	Codon signals chain termination: CAG ⟶ UAG Gln Amber termination codon
Frameshift mutation	One base-pair addition (underlined) AAG ACT CCT ⟶ AAG AGC TCC T... One base-pair deletion (underlined) AAG ACT CCT ⟶ AAA CTC CT...

Mutations that occur in regulatory and other noncoding sequences.

Those parts of a gene that do not directly encode a protein contain many crucial DNA binding sites for proteins interspersed among sequences that are nonessential to gene expression or gene activity.

At the DNA level, the docking sites include the sites to which RNA polymerase and its associated factors bind, as well as sites to which specific transcription-regulating proteins bind.

At the RNA level, additional important docking sites include the ribosome-binding sites of bacterial mRNAs, and sites that regulate translation and localize the mRNA to particular areas and compartments within the cell.

It is much harder to predict the ramifications of mutations in parts of a gene other than the polypeptide coding segments. In general, the functional consequences of any point mutation in such a region depend on whether it disrupts (or creates) a binding site. Mutations that disrupt these sites have the potential to change the expression pattern of a gene by altering the amount of product expressed at a certain time or in a certain tissue or by altering the response to certain environmental cues.

Such regulatory mutations will alter the amount of the protein product produced but *not the structure of the protein*.

The effects of some common types of mutations at the RNA and protein levels.

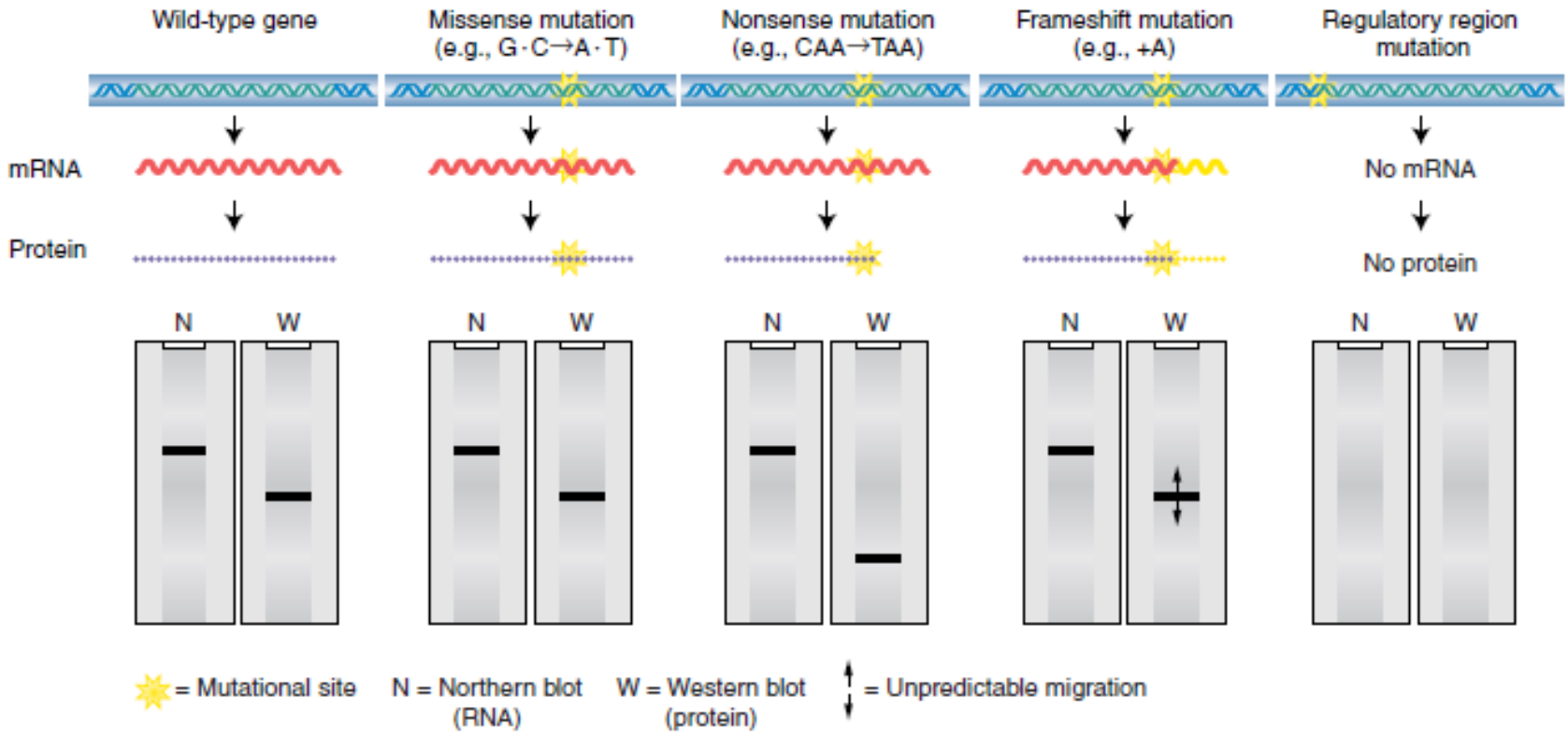


Figure 14-4 Effects of common mutations. The effects of some common types of mutations at the RNA and protein levels.

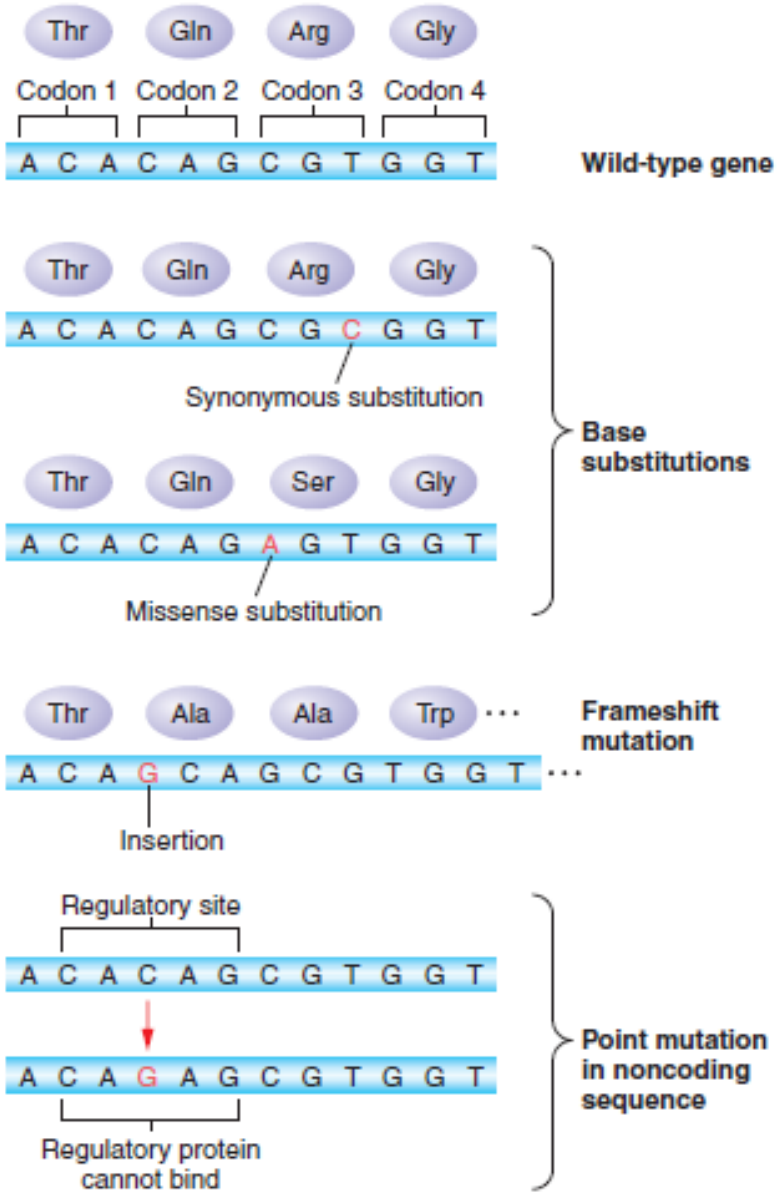


Figure 14-3 Consequences of point mutations within genes. In the top four panels, codons numbered 1–4 are located within the coding region of a gene.