

S1. There are mutant tRNAs that act as nonsense and missense suppressors. At the molecular level, explain how you think these suppressors work.

Answer: A suppressor is a second-site mutation that suppresses the phenotypic effects of a first mutation. Intergenic suppressor mutations in tRNA genes can act as nonsense or missense suppressors. For example, let's suppose a first mutation puts a stop codon into a structural gene. A second mutation in a tRNA gene can alter the anticodon region of a tRNA so that the anticodon recognizes a stop codon but inserts an amino acid at this site. A missense suppressor is a mutation in a tRNA gene that changes the anticodon so that it puts in the wrong amino acid at a normal codon that is not a stop codon. These mutant tRNAs are termed missense tRNAs. For example, a tRNA that normally recognizes glutamic acid may incur a mutation that changes its anticodon sequence so that it recognizes a glycine codon instead. Like nonsense suppressors, missense suppressors can be produced by mutations in the anticodon region of tRNAs so that the tRNA recognizes an incorrect codon. Alternatively, missense suppressors can also be produced by mutations in aminoacyl-tRNA synthetases that cause them to attach the incorrect amino acid to a tRNA.

S2. If the rate of mutation is 10^{-5} , how many new mutations would you expect in a population of one million bacteria?

Answer: If we multiply the mutation rate times the number of bacteria (10^{-5} times 10^6), we obtain a value of 10 new mutations in this population. This answer is correct, but it is an oversimplification of mutation rate. For any given gene, the mutation rate is based on a probability that an event will occur. Therefore, when we consider a particular population of bacteria, we should be aware that the actual rate of new mutation would vary. Even though the rate may be 10^{-5} , we would not be surprised if a population of 1 million bacteria had 9 or 11 new mutations instead of the expected number of 10. We would be surprised if it had 5,000 new mutations, since this value would deviate much too far from our expected number.

S3. In the Ames test, there are several *Salmonella* strains that contain different types of mutations within the gene that encodes an enzyme necessary for histidine biosynthesis. These mutations include transversions, transitions, and frameshifts. Why do you think it would be informative to test a mutagen with these different types of strains?

Answer: Different types of mutagens have different effects on DNA structure. For example, if a mutagen caused transversions, an experimenter would want to use a *Salmonella* strain in which a transversion would convert a *his*⁻ strain into a *his*⁺ strain. This type of strain would make it possible to detect the effects of the mutagen.

S4. In chapter 14, we discussed how bacterial genes can be arranged in an operon structure in which a polycistronic mRNA contains the coding sequences for two or more genes. For a normal operon, there is a relatively short distance between the stop codon in the first gene and the start codon in the next gene. In addition to the ribosomal-binding sequence at the beginning of the first gene, there is also a ribosomal-binding sequence at the beginning of the second gene, and at the beginning of all subsequent genes. After the ribosome has moved past the stop codon in the first gene, it quickly encounters a ribosomal-binding site in the next gene. This prevents the complete disassembly of the ribosome as would normally occur after a stop codon and leads to the efficient translation of the second gene.

Jacob and Monod, who studied mutations in the *lac* operon, discovered a mutation in the *lacZ* gene that had an unusual effect. This mutation resulted in a shorter version of the β -galactosidase protein, and it also prevented the expression of the *lacY* gene, even though the *lacY* gene was perfectly normal. Explain this mutation.

Answer: This mutation introduced a stop codon much earlier in the *lacZ* gene sequence. This type of mutation is referred to as a **polar mutation** and the phenomenon is called **polarity**. The explanation is that the early nonsense codon causes the ribosome to disassemble before it has a chance to reach the next gene sequence in the operon. The ribosomal-binding sequence that precedes the second gene is efficiently recognized only by a ribosome that has traveled to the normal stop codon in the first gene.

S5. A reverse mutation or a reversion is a mutation that returns a mutant codon back to a codon that gives a wild-type phenotype. At the DNA level, this can be an exact reversion or an equivalent reversion.

GAG (glutamic acid)	Forward → mutation	GTG (valine)	Exact → reversion	GAG (glutamic acid)
GAG (glutamic acid)	Forward → mutation	GTG (valine)	Equivalent → reversion	GAA (glutamic acid)
GAG (glutamic acid)	Forward → mutation	GTG (valine)	Equivalent → reversion	GAT (aspartic acid)

An equivalent reversion produces a protein that is equivalent to the wild type with regard to structure and function. This can occur in two ways. In some cases, the reversion produces the wild-type amino acid (in this case, glutamic acid), but it uses a different codon than the wild-type gene. Alternatively, an equivalent reversion may substitute an amino acid that is structurally similar to the wild-type amino acid. In our example, an equivalent reversion has changed valine to an aspartic acid. Since aspartic and glutamic acids are structurally similar, this type of reversion can restore the wild-type structure and function.

Now here is the question. The template strand within the coding sequence of a gene has the following sequence:

3'–TACCCCTTCGACCCCGGA–5'

This template produces an mRNA, 5'–AUGGGGAAGCUGGGGCCA–3', that encodes a polypeptide with the sequence methionine–glycine–lysine–leucine–glycine–proline.

A forward mutation changes the template strand to 3'–TACCCCTACGACCCCGGA–5'.

After the forward mutation, another mutation occurs to change this sequence again. Would the following second mutations be an exact reversion, an equivalent reversion, or neither?

- A. 3'–TACCCCTCCGACCCCGGA–5'
- B. 3'–TACCCCTTCGACCCCGGA–5'
- C. 3'–TACCCCGACGACCCCGGA–5'

Answer:

- A. This is probably an equivalent reversion. The third codon, which encodes a lysine in the normal gene, is now an arginine codon. Arginine and lysine are both basic amino acids, so the polypeptide would probably function normally.
- B. This is an exact reversion.
- C. The third codon, which is a lysine in the normal gene, has been changed to a leucine codon. It is difficult to say if this would be an equivalent reversion or not. Lysine is a basic amino acid and leucine is a nonpolar amino acid. The protein may still function normally with a leucine at the third codon, or it may function abnormally. You would need to test the function of the protein to determine if this was an equivalent reversion or not.