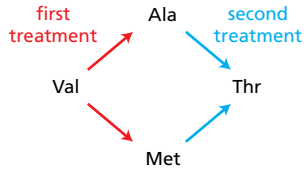
Thinking questions

After treating cells with a chemical mutagen, you isolate two mutants. One carries alanine and the other carries methionine at a site in the protein that normally contains valine. After treating these two mutants again with the mutagen, you isolate mutants from each that now carry threonine at the site of the original valine. Assuming that all mutations involve single-nucleotide changes, deduce the codons that are used for valine, methionine, threonine, and alanine at the affected site. Would you expect to be able to isolate valine- to-threonine mutants in one step?



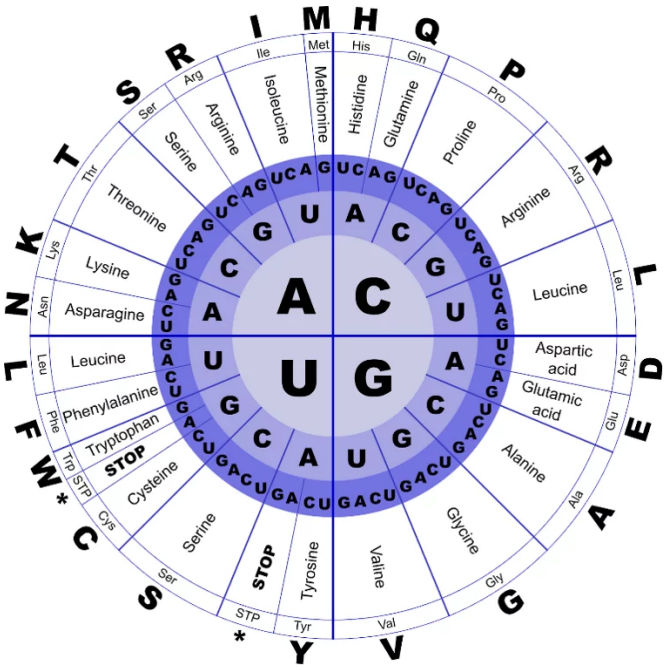
Valine GUG
Alanine GCG
Methionine AUG
Threonine ACG

Which of the following mutational changes would you predict to be the most deleterious to gene function? Explain your answers.

1. Insertion of a single nucleotide near the end of the coding sequence.
2. Removal of a single nucleotide near the beginning of the coding sequence.
3. Deletion of three consecutive nucleotides in the middle of the coding sequence.
4. Substitution of one nucleotide for another in the middle of the coding sequence.

The answer number two would be the most deleterious as it would completely shift the reading frame, for the whole protein. Answer number 1 might only affect a small portion of the protein towards its end. Answer number 3 would add one amino acid but not shift the reading frame and answer 4 would change one amino acid but not shift the reading frame.

Multiple Choice Questions



1. Which statements are <u>not true</u> for the RNA splicing process:
    a. The splicing machinery recognises three sequences located within the introns of the pre-mRNA.
    b. The removed part of the pre-mRNA forms a secondary structure.
    c. Each snRNP binds to specific part of pre-mRNA and all snRNPs do not bind at the same time.
    d. RNA splicing process depends only on the function of snRNPs and not on proteins.

A is false, the splice sites the junctions between the introns and exons. B and C are true. D is false, it depends also on proteins, such as BBP or U2AF.

2. Which statements are true for tRNA:
    a. tRNAs form a secondary structure resembling cloverleaf and consists only of four major ribonucleosides.
    b. Specific parts of tRNA molecule perform specific function.
    c. In the synthesis of tRNA, the aminoacyl-tRNA synthetase only needs to recognize the anticodon of tRNA to ensure the proper specificity.
    d. When isolating tRNA carrying amino acid Leu at its 3' end, one can immediately know what is the sequence of anticodon region in this tRNA.

The answer B is true. A is false as tRNAs typically also contain modified nucleotides; C is false as the tRNA also needs to fit in the catalytic site of the enzyme; D is false as the genetic code is redundant.

3. Which statements are true:
    a. During translation, the binding of tRNA anticodon to all mRNA codons is equally accurate.

b. There are 64 possible combinations for codons (4 x 4 x 4), but only 20 different types of amino acids, which means that most of the nucleotide triplets are never used.
c. The following mRNA 5'-UUGGAUGCGCCAUAAUUUGCUAUAA-3' translates to Met-Arg-His-Asn-Leu-Leu-STOP
d. Simple tripeptide segment Leu-Met-Tyr can be coded with 12 possible mRNA sequences.
e. Base sequence of mRNA can be predicted from the amino acid sequence of the polypeptide product.

C is true as well as D (6x1x2). A is false as some tRNA allow for mismatch at 3rd position of the codon (wobble); B is false as the genetic code is redundant and some amino acids are encoded by more than one triplet; E is false as nearly all amino acids have more than one codon, any given polypeptide can be encoded by several different base sequences.

4. What is true for the following mRNA sequence: 5' – AUGUACUGA – 3'
    a. Corresponding anticodons when the reading frame is 5'- AUG-UAC-UGA-3 are the following:
    5'-CAU-3', 5'-GUA-3', 5'-UCA-3'
    b. Amino-acid sequence for this part is (reading frame is AUG-UAC-UGA): His – Val – Ser
    c. When the translation starts the codon 5'-AUG-3' is located at the P site and the 5'-UAC-3' on the A site.
    d. The last codon will bind release factors at the A site instead of tRNA.
    e. The different tRNAs needed to synthesise this particular amino acid chain are bound by the same factors during protein synthesis.

A is true. B is false as aminoacid sequence is determined by the codons on mRNA, not the complementary sequences. This part of mRNA codes for Met-Try-STOP. C and D are true. E is false, the first one is bound by initiation factors, the second one with elongation factors, for the last one there is no tRNA, release factors bind to the codon.

5. Which of the following statements are true:
    a. Proteins with exposed hydrophobic surfaces are always eliminated with the protein degradation.
    b. Refolding with the help of chaperones is a process that consumes energy.
    c. Hsp60 detects hydrophobic amino acids on a growing peptide chain.
    d. The information for a protein to reach its 3D structure is encoded in its amino acid sequence.

A is false: Chaperones can help folding the proteins with exposed hydrophobic surfaces. B is true, it uses ATP. C is false this is true for Hsp70. Hsp60 on the other hand acts after a protein has been fully synthesised. D is true.

6. Which of the following statements are true:

a. The protein activity solely depends on its amino acids sequence.
b. Proteasome only acts on incompletely folded proteins.
c. Ubiquitin marks are also used for many other purposes in cells.
d. Failure to execute refolding by chaperons and degradation by proteasome could result in nonspecific aggregation of unfolded proteins

A is false because it also depends on the binding of cofactors, post-translational modification, binding of other protein subunits. B is false because it can also degrade normal proteins to ensure short lifetime of certain proteins. C is true: only a certain ubiquitin linkage result in "destruction mark" and proteasome degradation. D is also true.

7. What is true for the following mRNA:
5' – AUGGGUCGUGAGUCAUCGUUAAUUGUAGCUGGAGGGGAGGAGUGA – 3'
   a. Only one peptide can be made from the given mRNA.
   b. One of the amino acid sequences is: Met-Gly-Arg-Glu-Ser-Ser-Leu-Ile-Val-Ala-Gly-Gly-Glu-Glu-STOP.
   c. 15 tRNAs are needed to assemble this peptide.
   d. This mRNA is polycistronic.

The answers are A and B. C is false as it only requires 14 tRNAs (the stop codon does not require a tRNA. D is false as there is only one AUG, so only one start of protein sequence.

<u>TRUE or FALSE</u>

1. A single pre-mRNA can be processed to produce two or more different mRNA molecules

   TRUE or FALSE

The process is called Alternative Splicing (95 % of human pre-RNAs); different combinations of exons in a pre-mRNA are incorporated in different mRNA. A result is a diverse collection of proteins originating from a single gene.

2. Amino acids and proteins are the only macromolecules used as building blocks for ribosomes

   TRUE or **FALSE**

Ribosomes are composed of ribosomal proteins and RNA molecules, the ribosomal RNAs (rRNAs)

3. Anticodons UUA, CUA, UCA do not have a corresponding tRNA.

   TRUE or FALSE

The corresponding codons are UAA, UAG and UGA which are stop codons

4. During the protein synthesis the tRNA linked to the newly synthesized amino acid chain moves from A site to the P site

   TRUE or **FALSE**

The small and large subunits translocate during the protein synthesis. The tRNA stays "fixed" to mRNA.

5. Nonsense-mediated mRNA decay eliminates broken and damaged mRNAs.

   TRUE or **FALSE**

It eliminates the mRNA that are improperly spliced.

6. Side chains of phenylalanine and tryptophan are usually found buried in the hydrophobic code.

   TRUE or FALSE

Those are hydrophobic amino acids.

7. Misfolded proteins enter proteasome in a 3D structure.

   TRUE or **FALSE**

No, they are unfolded as the more through the cap.