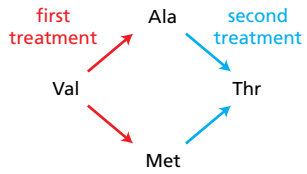
Think about the following problems and discuss them in pairs

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?



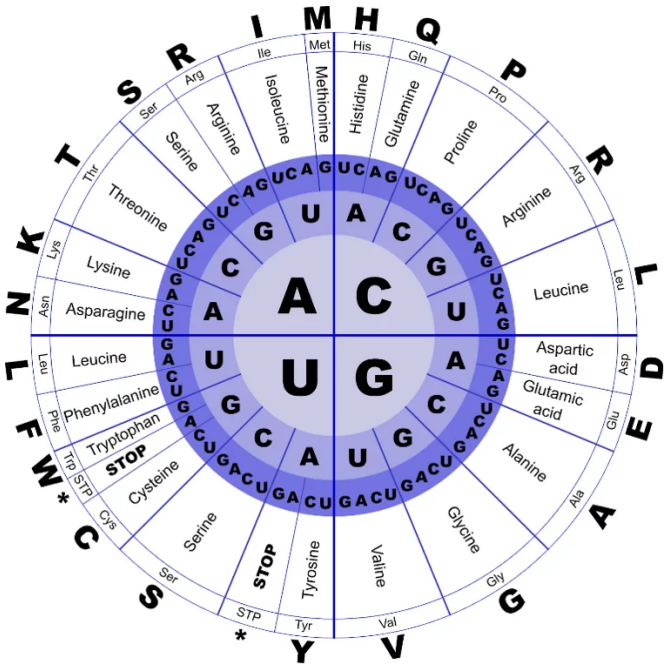
The only codon assignments consistent with the observed changes, and with the assumption that single-nucleotide changes were involved, are GUG for valine, GCG for alanine, AUG for methionine, and ACG for threonine. It is unlikely that you would be able to isolate a valine-to-threonine mutant in one step because that would require two nucleotide changes. Typically, two changes would be expected to occur at a frequency equal to the product of the frequencies for each of the single changes; hence, the double mutant would be very rare.

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 2 is the more deleterious, it would shift the reading frame and the whole sequence of the protein would be changed.

Multiple Choice Questions



1. Which statements are not true 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 not all snRNPs 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 are 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.

A is false, tRNAs also contain modified nucleotides. B is true. C is false, the tRNA also needs to fit in the catalytic site of the enzyme. D is false, there are 6 possibilities of the anticodons – because of the redundant genetic code

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' can translate 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.

A is false, some tRNA allow for mismatch at 3rd position of the codon (wobble)). B is false, the genetic code is redundant and some amino acids are specified by more than one triplet. C is true. D is true: 6x1x2. E is false, 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 tRNA anticodons when the reading frame is AUG-UAC-UGA 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: aminoacid sequence is determined by the codons on mRNA. 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 by the help of chaperones is a process that consumes energy.
    c. Hsp60 detects hydrophobic amino acids on a growing peptide chain.
    d. Proper folding of the protein does not only depend on its amino acid sequence

A is false: Chaperones 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, it can also depend on chaperones.

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 hydrophobic regions.

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' – AUGGGUCGUGAGUCAUCGUUAAUUGUAGCUGGAGGGGAGGAAUGA – 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.

TRUE or FALSE

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

   TRUE

The process is called Alternative Splicing (95 % of human pre-RNAs) goes through this form of splicing; 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

   FALSE

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

3. Anticodons UUA, CUA, UCA cannot be found in tRNA.

   TRUE

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

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.

   FALSE

It eliminates the mRNA that are improperly spliced.

6. Side chains of Phenylalanine, Tryptophan and Tyrosine are usually found buried in the hydrophobic code.

   TRUE

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

   FALSE

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