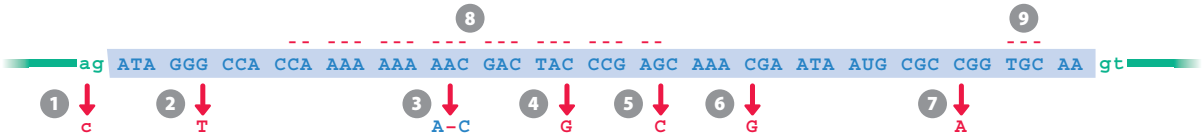
Questions and Answers for Genetics and Genomics in Medicine Chapter 7

Question 1

In the sequence below, the blue nucleotides represent an exon containing coding DNA near the beginning of a large gene, and green lines and letters are the flanking intron sequence. Nine mutations are shown: single nucleotide changes below (1–7) and two large-scale mutations above (8, 9). Red dashes indicate deleted nucleotides. Which mutation class does each belong to? Comment on the likely effect of each.



Answer

The mutations shown are:

1. A splice site mutation, inactivating a splice acceptor site. Often a cause of exon skipping. If this occurs, it will produce a frameshift because the exon has 56 nucleotides, a number not divisible by three.
2. A synonymous mutation. Likely to have no effect (unless it changes an exonic splice enhancer or suppressor).
3. A single nucleotide deletion, causing a frameshift that introduces a premature termination codon after six amino acid-specifying codons. Expected to inactivate gene expression.
4. A nonsense mutation. Expected to inactivate gene expression.
5. A conservative amino acid substitution that replaces a serine codon by a threonine codon. May have minimal effect.
6. A non-conservative amino acid substitution replacing arginine by glycine. Might be pathogenic because of the change from a positively charged amino acid to a neutral amino acid with the smallest possible side chain.
7. Although the first base of the codon position has been substituted, this is a synonymous mutation. Likely to have no effect.
8. A frameshifting deletion (22 nucleotides long). Expected to inactivate gene expression.
9. Deletion of a single amino acid. In this case a cysteine is deleted, which may cause major protein structure difficulties if this *particular* cysteine is involved in disulphide bonding.

Question 2

What is meant by the term dynamic mutations?

Answer

Unstable oligonucleotide expansions of certain types of oligonucleotide repeat are considered to be dynamic mutations, because they can change progressively with time in a directional manner. Specifically, the number of tandem repeats can increase in size both in mitosis and also in meiosis; this

can result in a progressive increase in severity of the phenotype and earlier ages of onset of the disease as we move down from older generations to newer generations.

Question 3

What are the different characteristics of pathogenic unstable expansion of oligonucleotide repeats in coding DNA and noncoding DNA?

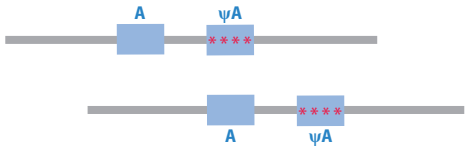
Answer

The pattern of unstable expansion of oligonucleotide repeats differs in coding DNA and noncoding DNA. In coding DNA there is the constraint that the repeat unit length must be divisible by three (otherwise there would be a frameshift) and cannot be too large (otherwise the protein would be inactivated for structural reasons). In practice, the disorders that are seen involve the expansion of CAG repeats that encode glutamine. In the protein the resulting long tracts of polyglutamine and possibly also the expanded repeats at the RNA level seem to be toxic to cells and over a long period have a damaging effect on neurons (which are very long-lived), causing various neurodegenerative diseases.

In noncoding DNA, repeat unit length does not need to be divisible by three and there is not the same constraint on the number of repeats; very large expansions can therefore be seen, as in myotonic dystrophy and fragile X syndrome. Disease is caused by one of two mechanisms: either the very long expanded repeat interferes with the expression of a neighboring gene, as in Friedreich's ataxia and fragile X syndrome. Alternatively, transcripts from a long expanded repeat are toxic to cells and/or have a transdominant effect, as in myotonoic dystrophy (see Box 7.2).

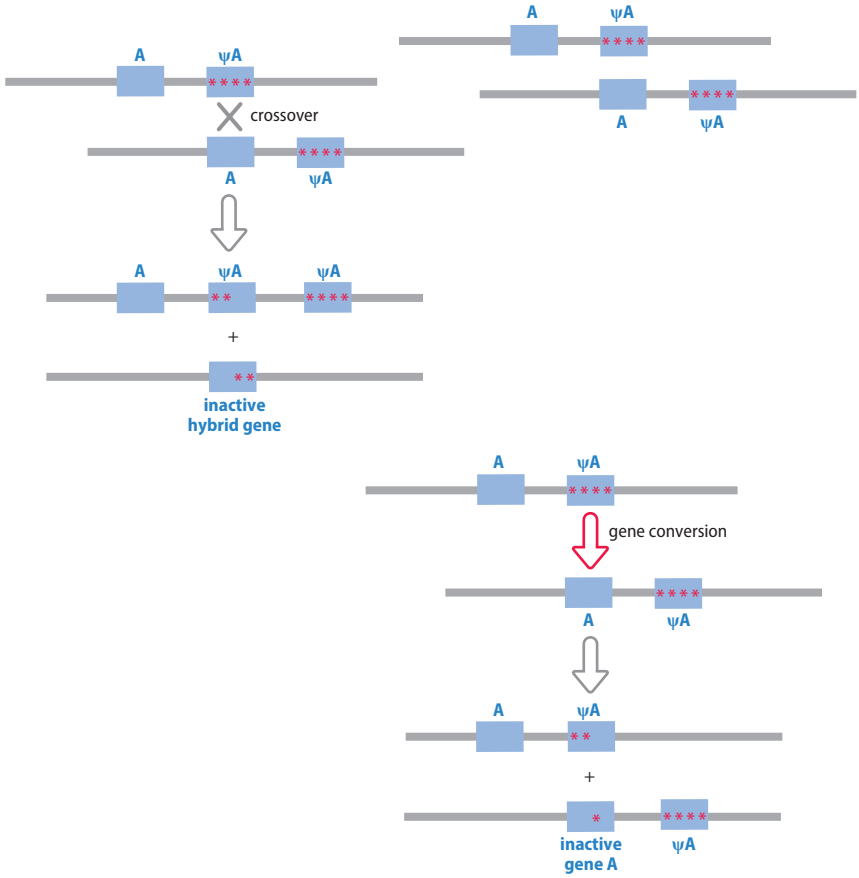
Question 4

In the example below, there has been mispairing of chromatids during meiosis so that a single-copy protein-coding gene, A, and a closely homologous pseudogene, ψA, with multiple inactivating mutations (red asterisks) pair up. Illustrate two types of sequence exchange between these mispaired repeats that can give rise to aberrant sequences causing disease.



Answer

As shown on the left below, crossover between the mismatched repeats produces chromatids with three genes or one gene. The latter is a hybrid gene, part pseudogene and part normal gene, and will be nonfunctional. Alternatively, as shown on the right below, the pseudogene can act as a donor gene in gene conversion whereby a copy of the pseudogene containing one or more inactivating mutations replaces the original sequence on the functional gene. The bottom chromatid will therefore lack a functional gene A.

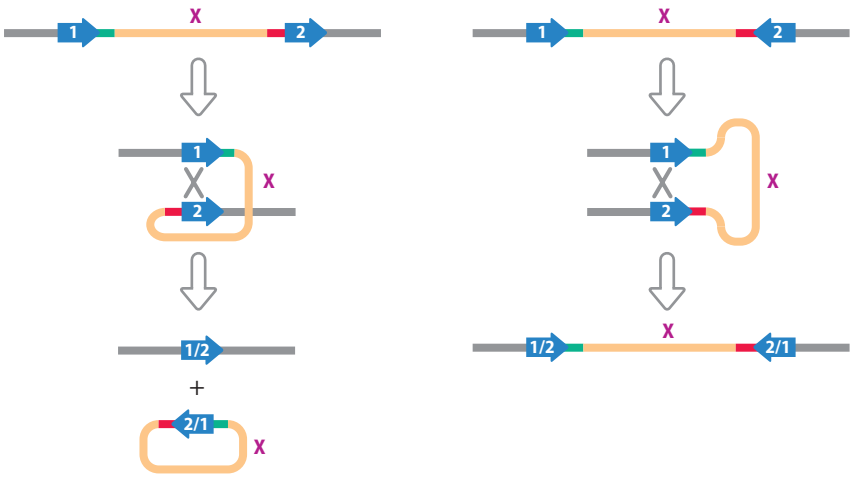


Question 5

In the figure below, two very highly homologous repeats, 1 and 2, occur on one chromatid in the same orientation (direct repeats, shown on the left) or in opposite orientations (inverted repeats, shown on the right). The intervening DNA, shown as an X with a green start sequence and a red end sequence, can loop out, allowing the repeats to align in the same orientation and to engage in intrachromatid recombination. Illustrate how this happens and explain the consequences for each case.

Answer

For direct repeats, the intervening sequence X will be deleted from the chromatid in the form of a circular DNA that cannot propagate through mitosis (as shown on the left). For inverted repeats, the consequence is an inversion (as shown on the right).



Question 6

What is meant by a loss-of-function mutation and a gain-of-function mutation? To what extent do they give rise, respectively, to recessive and dominant phenotypes?

Answer

In practice, a loss-of-function mutation is a mutation in a gene that results in failure to produce a product, or leads to a product that simply cannot carry out its usual function. A gain-of-function mutation means that as a result of a mutation a different gene product is produced with different properties from the usual gene product.

Gain-of-function mutations are expressed in the heterozygote and are associated with dominantly inherited disorders. Loss-of-function mutations may also sometimes be expressed in heterozygotes in the case of phenotypes in which the amount of gene product that is made is crucially important; such mutations underlie some dominantly inherited phenotypes (haploinsufficiency). However, loss-of-function mutations are more frequently associated with recessive disorders.

Question 7

Abnormal protein structure is a key cause of disease in certain single-gene disorders. Give three examples of diseases like this, and a brief outline of how the abnormal protein structure causes disease.

Answer

- Sickle-cell disease. The disease is caused by a specific missense mutation at position 6 in the β-globin chain to a hydrophobic valine residue. The resulting mutant hemoglobin S (HbS) has a strong tendency to aggregate when deoxygenated, resulting in fibers that cause red blood cells to become sickle-shaped. The abnormal sickle cells have a much shorter lifespan than normal red blood cells; the body therefore cannot replace dead red blood cells fast enough, and anemia results. The HbS fibers also block small blood vessels, causing hypoxic tissue damage.
- α_1-Antitrypsin deficiency. The enzyme α_1-antitrysin is synthesized by the liver and secreted, but mutant α_1-antitrypsins carrying an E264V or an E342K substitution fail to undergo processing. As a

result they are retained inside hepatocytes, where they can aggregate to form inclusion bodies that cause the cells to die and eventually result in cirrhosis of the liver.
- Cystic fibrosis. The p.Phe508del mutation is by some distance the most common mutation in cystic fibrosis. Deletion of this phenylalanine causes aberrant protein folding that cannot be rectified by protein chaperones, and so the mutant protein is subject to intracellular degradation.

Question 8

Genotype–phenotype correlations can be poor for many monogenic disorders. That may be due to different genetic and nongenetic contributing factors. For each of the contributing factors listed below, give an example of a disorder (or class of disorders) in which they have a prominent effect and illustrate how the effect causes phenotype variability:

- modifier genes
- cellular mosaicism
- epigenetic effects
- environmental factors.

Answer

- Modifier genes: β-thalassemia. The phenotype is modified by genetic variants in some other globin genes, notably those making α- and γ-globins. Overexpression of α-globin as a result of increased gene copy number accentuates the β-thalassemia phenotype, but overexpression of γ-globins compensates for reduced β-globin production and so reduces the severity of the β-thalassemia phenotype.
- Cellular mosaicism: X-linked disorders. Women are mosaics for X-inactivation patterns: in some cells the paternal X chromosome is randomly silenced; in others the maternal X is silenced. The expression of a mutant X-linked allele therefore depends on the proportion of cells expressing the allele, which can vary between individuals and family members.
- Epigenetic effects: imprinting disorders. Depending on the gene, the paternal or maternal allele can be silenced in at least some cells, causing lack of penetrance in some family members.
- Environmental factors: phenylketonuria. Disease expression is modified by the quantity of phenylalanine in the dietary protein intake.