Questions and Answers for Genetics and Genomics in Medicine Chapter 6

Question 1
In gene regulation, what are the similarities and essential differences between a promoter and an enhancer?

Answer
Both promoters and enhancers are *cis*-acting DNA regulatory elements (consisting of a cluster of several short sequence elements) that are bound by regulatory proteins (including certain transcription factors that bind to the DNA itself and other proteins that bind to the bound transcription factors). A promoter–regulatory protein complex is sufficient to switch on expression of a gene, but an enhancer–regulatory protein complex can interact with the promoter–protein complex to ramp up transcription.

Promoters and enhancers differ in two major ways. First, promoters are located very close to the transcription start site of the gene that they regulate, whereas enhancers are more variable in their location and may be sited some distance from the transcription start site, even hundreds of kilobases away and sometimes within a different neighboring gene. When distantly located, DNA looping is needed to bring the enhancer–protein complex into contact with the promoter complex (see Figure 6.2). Second, the orientation of promoters is important, but that of enhancers is not: it can be reversed without affecting their function.

Question 2
Three classes of *cis*-acting regulatory RNA element are important in the basic RNA splicing reaction carried out by spliceosomes. Where are they located, and what are their characteristics?

Answer
Two of the three regulatory RNA classes are located at exon–intron junctions that define the splice donor site (at the 5' end of an intron sequence within the RNA transcript) and the splice acceptor site (at the 3' end of an intron sequence within the RNA transcript). The third regulatory sequence is the branch site that is located a short distance (tens of nucleotides) before the splice acceptor site.

The splice donor site consensus sequence is defined by an invariant GU sequence at the beginning of the intron (GT at the DNA level) and is embedded within the consensus sequence AG**GU**PuAGU (Pu = purine). It is bound by the U1 snRNA of the spliceosome. The branch site has the consensus sequence PyNPyPyPu**A**Py (Py = pyrimidine; Pu = purine; **A** = invariant adenine) and is bound by the U2 snRNA of the spliceosome. The splice acceptor site consensus sequence is defined by an invariant AG sequence at the end of the intron and is embedded within a pyrimidine-rich consensus sequence $(Py)_{12}$NPy**AG**Pu (N = any nucleotide).

Question 3

Alternative splicing of RNA transcripts must occasionally happen by accident. Quite often, however, the pattern of alternative splicing is thought to be functionally significant. What types of evidence suggest that alternative splicing can be functionally important?

Answer

If alternative splicing were haphazard, we would not expect to see certain nonrandom patterns, including the following:

- Tissue-specific alternative splicing. A frequent finding is that one pattern of splicing is found in one type of tissue and a different pattern in another tissue, leading to different protein isoforms in brain versus liver, for example.
- Functional diversity. The different patterns of alternative splicing can result in different protein isoforms being expressed, not just in different tissues and in different cell compartments but also in ways in which the isoforms can have different functions. Sometimes one isoform is membrane-bound and another one is secreted, or one isoform can bind different proteins than another isoform, as in the +KTS and –KTS isoforms of the Wilms tumor WT1 protein. An extreme example is the production of two quite different proteins by the same gene through a change in translational reading frame, as demonstrated in for the *CDKN2A* gene (see Figure 6.6B).
- Evolutionary conservation. If some sequence variation has been strongly conserved in evolution, it is very likely to be functionally important. The classic example is the huge evolutionary conservation of the +KTS and –KTS isoforms of the Wilms tumor gene *WT1*, as shown in Figure 6.6A. This pattern of WT1 isoforms is found in many animal species, and it appears to have been evolutionarily conserved over hundreds of millions of years.

Question 4

The production of miRNAs uses many components of the cell's RNA interference machinery. What is the natural role of RNA interference in cells?

Answer

RNA interference is a natural cellular defense against viruses and the excess activity of transposons, notably retrotransposons, some of which actively transpose in our cells, especially in neurons. It relies on the observation that double-stranded RNA is a rarity in our cells. It can occur when viruses infect cells and when transcripts are expressed from both sense and antisense strands of transposon repeats (that are in inverted orientations, for example). To limit damage by viruses and to limit excess activity of transposons, our cells use RNA interference to cleave double-stranded RNA into small nonfunctional pieces of RNA that can be degraded.

Question 5

Give brief descriptions of the three principal molecular mechanisms that permit changes in chromatin structure required for epigenetic regulation.

Answer

- DNA methylation. The methylation is confined to cytosines only, and only certain cytosines are methylated. The resulting 5-methylcytosines behave as normal cytosines by continuing to base pair with guanine, and so the base sequence is effectively unchanged.
- Histone modification. Includes a variety of post-translational modifications, notably the methylation and acetylation of specific lysine residues close to the N-terminus of histone proteins, and the occasional use of variant histone proteins.
- Nucleosome repositioning. Various chromatin remodeling mechanisms allow nucleosomes to move laterally and open up nucleosome-free areas or induce nucleosome packing in some chromatin regions.

Question 6

A popular theory holds that gene imprinting evolved in mammals because of a conflict of evolutionary interest between fathers and mothers. What are the essential points of this theory, and why does it not explain all observations of imprinted genes in mammals?

Answer

Propagation of paternal alleles would be favored if the offspring were all very robust, even at the expense of the mother (potentially, a man can father children by very many different mothers). Enhanced propagation of maternal alleles, however, depends on the mother's being healthy enough to go on to have multiple pregnancies.

Paternal alleles might be expected to have a vested interest in supporting the development of the extra-embryonic membranes and placenta (to promote the growth and robustness of the fetus by maximizing the nutrients it can extract from the mother via the placenta). Maternal alleles, by contrast, might seek to limit the nutrient transfer so that it does not compromise the mother's health and future reproductive success.

The theory explains why many known imprinted genes have a role in embryonic and placental growth and development, and is supported by observations on uniparental diploidy (Figure 6.17). But it does not explain why some genes are imprinted that do not have a role in intrauterine growth, and why some genes are imprinted in the opposite direction to the one expected. And it does not explain why imprinting can often be tissue-specific. Perhaps the imprinting mechanism was subsequently co-opted for different purposes.

Question 7

Although patterns of DNA methylation can be stably inherited after cells divide, patterns of DNA methylation on a gamete transmitted by a parent to a child are altered in the child. Explain how this happens.

Answer

The DNA of gametes is extensively methylated (especially so in sperm), but in the zygote the inherited pattern of DNA methylation in the parental genomes is erased by a global demethylation in very early embryonic development. Then, as cells in the early embryo begin to differentiate to give rise to the different cell lineages, there is a wave of re-methylation of the DNA, resetting imprinting patterns according to the sex of the individual.

Question 8

Rett syndrome is a classic example of a Mendelian chromatin disease in which pathogenesis occurs as a result of altered chromatin states at gene loci that are distinct from the one that is mutated. Explain how this happens. Why are individuals with Rett syndrome almost exclusively female?

Answer

Methylation of DNA in our cells occurs at certain CG (= CpG) dinucleotides, whereupon the methylated CG (meCG) is recognized and bound by *trans*-acting regulatory proteins. Rett syndrome usually results from inactivating mutations in the *MECP2* gene, which makes a critically important meCG-binding protein that can bind to meCG at numerous sites across the genome. Failure to produce this protein can affect how a variety of target genes work, including genes that are important in neuron development, and has such a serious effect that inactivating mutations in the single *MECP2* allele in boys are lethal.

Because of X-inactivation, affected females are partly protected (because in some cells an X chromosome containing the mutant allele is inactivated and the normal allele is expressed; however, inactivating the normal X in a relatively high proportion of neurons would be expected to result in a particularly severe phenotype).