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FUNDAMENTAL MOLECULAR GENETIC MECHANISMS

REVIEW THE CONCEPTS

1. Watson-Crick base pairs are interactions between a larger purine and a smaller pyrimidine base in DNA. These interactions result in primarily G-C and A-T base pairing in DNA and A-U base pairs in double-stranded regions of RNA. They are important because they allow one strand to function as the template for synthesis of a complementary, antiparallel strand of DNA or RNA.

2. At 90°C, the double-stranded DNA template will denature and the strands will separate. As the temperature slowly drops below the Tm of the plasmid DNA, the single-stranded oligonucleotide primer present at higher concentration than the plasmid DNA strands hybridizes to its complementary sequence on the plasmid template. The resulting molecules contain a short double-stranded stretch the length of the primer with a free 3′ OH that can be used by DNA polymerase enzyme in sequencing reactions.

3. RNA is less stable chemically than DNA because of the presence of a hydroxyl group on C-2 in the ribose moieties in the backbone. Additionally, cytosine (found in both RNA and DNA) may be deaminated to give uracil. If this occurs in DNA, which does not normally contain uracil, the incorrect base is recognized and repaired by cellular enzymes. In contrast, if this deamination occurs in RNA, which normally contains uracil, the base substitution is not corrected. Thus, the presence of deoxyribose and thymine make DNA more stable and less subject to spontaneous changes in nucleotide sequence than RNA. These properties might explain the use of DNA as a long-term information-storage molecule.

4. In prokaryotes, many protein-coding genes are clustered in operons where transcription proceeds from a single promoter that gives rise to one mRNA encoding multiple proteins with related functions. In contrast, eukaryotes do not have operons but do transcribe intron sequences that must be spliced out of mature mRNAs. Eukaryotic mRNAs also differ from their prokaryote counterparts in that they contain a 5′ cap and 3′ poly(A) tail. Also, ribosomes have immediate access to nascent mRNAs in bacteria so that translation begins as the mRNA is being synthesized. In contrast, in eukaryotes, mRNA synthesis occurs in the nucleus, whereas translation by ribosomes occurs in the cytoplasm. Consequently, only fully synthesized and processed mRNAs are translated in eukaryotes.

5. A simple explanation is that the larger, membrane-spanning, domain-containing protein and the small, secreted protein are encoded by the same gene that is differentially spliced. Specifically, the final exon of the gene could contain the information for the membrane-spanning domain, and in the smaller, secreted protein, this exon could be omitted during splicing.

6. An operon is an arrangement of genes in a functional group that are devoted to a single metabolic purpose. In the case of tryptophan synthesis, the DNA for five genes is arranged in a contiguous array that gets transcribed from a single promoter into a continuous strand of mRNA encoding five proteins. In this manner, the cell simply has to induce one promoter, which transcribes all the necessary genes encoding the proteins (enzymes) to make the amino acid tryptophan. Splicing out intronic sequences or transcribing multiple mRNAs from genes on different chromosomes, as seen in eukaryotic systems, is unnecessary; thus, operons are a logical way to economize on the amount of DNA needed by genes to encode a number of proteins. In addition, this arrangement allows all the genes in an operon to be coordinately regulated by controlling transcription initiation from a single promoter.

7. Since poly(A)-binding protein is involved in increasing the efficiency of translation, a mutation in poly(A)-binding protein would cause less efficient translation. Polyribosomes in a cell with such a mutation would not contain circular structures of mRNAs during translation because lack of the poly(A)-binding protein would eliminate the 3′ binding site for eIF4G.

8. DNA synthesis is discontinuous because the double helix consists of two antiparallel strands and DNA polymerase can synthesize DNA only in the 5′ to 3′ direction. Thus, one strand is synthesized continuously at the growing fork, but the other strand is synthesized utilizing Okazaki fragments that are joined by DNA ligase.

9. Base excision repair is responsible for repairing guanine-thymine mismatches caused by the chemical conversion of cytosine to uracil or by deamination of 5-methyl cytosine to thymine. Mismatch excision repair eliminates base pair mismatches and small insertions or deletions of nucleotides generated accidentally during DNA replication. Nucleotide excision-repair fixes DNA strands that contain chemically modified bases, which ensures that thymine-thymine dimers are repaired in the case of UV light damage.

10. UV irradiation causes thymine-thymine dimers. These are usually repaired by the nucleotide excision-repair system, which utilizes XP complexes and the transcriptional helicase TFIIH to unwind and excise the damaged DNA. The gap is then filled in by DNA polymerase. Ionizing radiation causes double-stranded breaks in DNA. Double-stranded breaks are repaired either by homologous recombination or nonhomologous DNA end-joining. Homologous recombination requires the BRCA1, BRCA2, and Rad51 proteins to use the sister chromatid as template for error-free repair. Nonhomologous DNA end-joining is error-prone because nonhomologous ends are joined together. Since formation of a malignant tumor requires multiple mutations, cells that have lost DNA-repair function are more likely to sustain cancer-promoting mutations. Examples are xeroderma pigmentosum due to mutations in *XP* genes that prevent repair of thymine dimers and a genetic predisposition to breast cancer in individuals with germ-line mutations in the *BRCA1* or *BRCA2* genes.

11. Homologous recombination is the process that can repair DNA damage and also generate genetic diversity during meiosis. In both cases, repair is to double-strand breaks, RecA/Rad51-like proteins play key roles in the recombination process, and Holliday structures form, followed by cleavage and ligation to form two recombinant chromosomes. During DNA repair by homologous recombination, the damaged sequence is copied from an undamaged copy of the homologous DNA sequence on the homologous chromosome or sister chromatid. During meiosis, however, genetic diversity is generated by homologous recombination where large regions of chromosomes are exchanged between the maternal and paternal pair of homologous chromosomes. Also, in meiosis an exchange called crossing over is required for the proper segregation of the chromosomes during the first meiotic cell division.

12. The gene encoding the reverse transcriptase enzyme is unique in retroviruses and closely related retrotrasposons. These viruses contain RNA as their genetic material; a DNA copy of the viral RNA is made during infection and reverse transcriptase catalyzes this reaction. The human T-cell lymphotrophic virus, which causes T-cell leukemia, and human immunodeficiency virus, which causes AIDS, can infect only specific cell types because these cells possess receptors that interact specifically with viral envelope proteins of the progeny virus.

13. a. bottom strand
    b. 5′ACGGACUGUACCGCUGAAGUCAUGGACGCUCGA 3′

14.

Prokaryotes	Eukaryotes
Very little non-coding DNA	Non-coding DNA (introns) interspersed between coding regions (exons)
Genes that carry out similar/complementary functions are in tandem on the chromosome (operon).	Genes with similar/complementary functions are interspersed throughout the chromosome; some are on different chromosomes.

(cont.)

Direct production of mRNA (no processing)	mRNA produced after processing of primary RNA to remove introns, 5′-methyl capping and 3′-polyadenylation
Ribosomes have immediate access to mRNA to initiate protein synthesis (no nucleus).	mRNA has to be translocated from the nucleus to cytoplasm before protein synthesis can begin.
One mRNA ⟶ many polypeptides	One mRNA ⟶ one polypeptide

15. a. Double-stranded DNA won't be unwound long enough to allow for the replication of the DNA.
    b. Translation will not occur because the initiation complex will be unable to bind the AUG start site.
    c. Translation will not occur because $tRNA_i^{met}$ is the only tRNA able to initiate translation.

16. RNA sequence: 5′ UUC UAC AUG AAG CAU CAG AGC CAG UGA 3′

    Protein sequence: ~~Phe~~ ~~Tyr~~ Met Lys His Gly Ser Gly Stop codon

    Not produced because they are before the start codon

17. a. Synthesis is from 5′ ⟶ 3′; as the DNA strand separates, one strand serves as an anti-parallel template whereby more and more of the template for the 3′ end of the newly synthesized strand is revealed as the replication fork advances making the synthesis from 5′ ⟶ 3′ a continuous process. At the same time, the template for the 5′ end of the other strand is continuously revealed as the fork advances, therefore small 5′ ⟶ 3′ fragments need to be synthesized on this strand.
    b. If the replication fork were moving in the opposite direction (left to right) to the example shown above.

18. The newly synthesized strand is the one with the error—if the original strand were targeted, the mutation would be allowed to persist and would be transmitted to all subsequent cells.

19. a. nonsense (TCA ⟶ TGA)
    b. missense AUG ⟶ AUA (methionine ⟶ isoleucine)

20. a.

Lytic	Non-lytic
Infected cell ultimately dies.	Infected cell does not die.
Viral genome does not integrate into host genome; host cell DNA destroyed.	Viral genome integrates into host genome.

b. ii. Viral mRNAs are transcribed by the host-cell translation machinery.