MOLECULAR CELL PHYSIOLOGY - DNA to Protein: Part I

- I. DNA - The Watson - Crick Double Helix
  - A. Two helical polynucleotide chains coiled around a common axis
    - 1. 3' to 5' and 5' to 3'
  - B. Purines & pyrimidines on the inside; phosphate & deoxyribose outside
  - C. Two chains held together by hydrogen bonds
  - D. The precise sequence of bases carries the genetic information
- II. DNA replication
  - A. Semiconservative replication
    - 1. Produces two DNA molecules that each have one "half-old (mother)" and one "half-new (daughter)" strands
  - B. Where does replication begin?
    - 1. Viruses and bacteria - one site
    - 2. Eucaryotes - several sites
  - C. Process
    - 1. DNA polymerases
      - a) Catalyze step-by-step addition of deoxyribonucleotide units to DNA chain
      - b) Proofread newly synthesized strands
    - 2. A primer is required
    - 3. DNA template is essential
    - 4. DNA must be unwound (helicase) and positive supercoiling (gyrase) must be removed
    - 5. Elongation proceeds in the 5' to 3' direction
    - 6. The "other" strand is synthesized with help of Okazaki fragments
  - D. Problems - mutations
    - 1. Substitution (most common)
    - 2. Deletion
    - 3. Insertion
- III. What DNA encodes
  - A. Ultimately (through transcription & translation) - protein
  - B. DNA is like instructions in a book
  - C. The alphabet A, T, G, & C
- IV. Transcription
  - A. DNA to RNA
  - B. Types of RNA
    - 1. Ribosomal RNA (rRNA) - combines with proteins to form ribosomes
    - 2. Messenger RNA (mRNA) - the "blueprint" delivered to the ribosome which is translated into protein
    - 3. Transfer RNA (tRNA) - matches proteins with triplets encoded by mRNA
  - C. How transcription differs from DNA replication
    - 1. RNA polymerases assemble transcripts
    - 2. Several strands can be synthesized at one time
    - 3. Only ONE of the two unwound DNA strands is transcribed
- V. Transcription - synthesis of RNA
  - A. Process of transcription
    - 1. Promotion (promoter) - specific base sequence at beginning of gene
      - a) RNA polymerase initiates correct binding to DNA
      - b) Usually in the vicinity of a TATA box
    - 2. Transcription
      - a) Synthesized 5' to 3' (from 3' to 5' DNA strand)
      - b) RNA strand is complementary
      - c) Uracil replaces Thymine in the complementary RNA strand
      - d) Uracil enables RNA to be distinguished from DNA

MOLECULAR CELL PHYSIOLOGY - DNA to Protein: Part I (continued)

- **V. Transcription - synthesis of RNA (continued)**
  - **A. Process of transcription (continued)**
    - **3. Release of transcript**
    - **4. Transcript modification (eucaryotes)**
      - **a) Intron removal**
        - **1) Exons are the portion that are read**
      - **b) Cap at one end and a poly-A tail on the other**
- **VI. Translation - synthesis of protein**
  - **A. The genetic code**
    - **1. Every three nucleotides (base triplets) specify an amino acid**
      - **a) Nucleotide triplets are referred to as codons**
    - **2. Sets of nucleotides make sets of amino acids**
    - **3. Proteins are made of amino acids**
  - **B. Where it happens**
    - **1. On the surface of ribosomes - cluster referred to as polysome**
  - **C. How it happens**
    - **1. Initiation**
      - **a) The small ribosomal subunit attaches to the mRNA in the vicinity of the start codon, AUG**
      - **b) An initiator tRNA with the anticodon UAC pairs with the AUG codon and then the large ribosomal subunit joins with the small subunit**
      - **c) Initiator tRNA occupies the P site on the large ribosomal subunit**
    - **2. Chain elongation**
      - **a) Another tRNA (with its anticodon) comes along to bind on the adjacent (A) site**
      - **b) Adjacent amino acids become aligned**
      - **c) The tRNA on the P site leaves and a peptide bond is formed between amino acids - energy (GTP) is used**
      - **d) The amino acid occupying the A site moves to the P site**
      - **e) Ribosome moves to align the third codon to the newly opened A site**
      - **f) New amino acid joins the chain**
    - **3. Chain termination**
      - **a) A stop codon (UAG, UAA, or UGA) is encountered**
      - **b) Release factors are invoked**
      - **c) Protein is released**
- **VII. Changes that can occur in DNA leading to variation of species**
  - **A. Gene mutation (molecular level) - base pair replaced, added, or deleted**
  - **B. Crossing over & recombination - section of DNA recombined - expression of alleles**
  - **C. Chromosome aberration - section of DNA**

(THIS LECTURE IS CONTINUED ON THE NEXT PAGE)

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MOLECULAR CELL PHYSIOLOGY - DNA to Protein: Part II

I. **Aminoacyl-tRNA synthetase**
   - A. Specific aminoacyl-tRNA synthetases catalyze attachment of AAs to tRNAs
   - B. There are 20 different aminoacyl-tRNA synthetases, one for each of the 20 different AAs
   - C. Once the tRNA is bound to an amino acid, it is referred to as "activated" because it contains a high-energy bond; the energy can be used later to drive formation of peptide bonds
   - D. The reaction is: AA + ATP + tRNA ? aminoacyl-tRNA + AMP + 2Pi
   - E. Since some amino acids have similar structures, the enzyme can make mistakes, but also has error-checking functions

II. **Transfer RNA (tRNA)**
   - A. Functions of tRNA
     1. Chemical linkage to specific amino acids
     2. Base-pairing with codon in mRNA
   - B. Structure includes 70 to 80 nucleotides with precise 3-dimensional structure
   - C. The tRNA folds into a stem-loop structure that resembles a clover leaf, the stems of which are short double helicies stabilized by base-pairing
   - D. Structure includes an acceptor stem, a T? CG loop, variable loop, anticodon loop, and D loop
     1. Some of the nucleotides are modified (e.g., T? CG loop includes a characteristic pseudouridylate)
     2. Modified nucleotides such as inosine (deaminated product of adenine) can function in the "wobble" position of the anticodon loop (position #1) to enable binding of several mRNA codons

III. **Ribosomal RNA (rRNA)**
   - A. Ribosomes (ribonucleoprotein)
     1. Most abundant RNA-protein complex in the cell
     2. Direct elongation of polypeptides at a rate of 3-5 amino acids per second
     3. Contain several rRNAs and more than 50 proteins, organized into small and large subunits
     4. In procaryotes, ribosomes consist of 50s and 30s subunits that assemble into a 70s unit; in eucaryotes, ribosomes consist of 60s and 40s subunits that assemble into an 80s unit
     5. The ribosome moves along the mRNA during translation
   - B. Ribosome and rRNA structure
     1. Small and large rRNAs exist as stem-loop structures and assemble in ribosomal subunits
     2. Complex structure determined, to some extent, by cryoelectron microscopy

IV. **Initiation, elongation, and termination factors**
   - A. Mechanisms for initiation factors differ in procaryotes and eucaryotes, although the mechanism in eucaryotes is a little more complex and involves a preinitiation complex
     1. The general mechanism involves binding of initiation factors with the small ribosomal subunit prior to subunit assembly
     2. The initiation complex, together with Met-tRNA_i^{Met} binds to mRNA at a specific site, the sequence of which on the mRNA is somewhat "conserved" in procaryotes and eucaryotes
   - B. Elongation factors are required to enable the stepwise addition of amino acids
     1. These factors participate, among other things, in the binding of aminoacyl-tRNAs to the A site on the ribosome
   - C. Peptidyltransferase is an enzyme that participates during elongation and catalyzes transfer of the growing peptide chain on the peptidyl-tRNA at the P site to the activated amino acid on the incoming aminoacyl-tRNA
   - D. Release factors participate in termination of protein synthesis when a stop codon is reached

V. **Polysomes (complete translation of a typical protein in eucaryotes takes about 30 to 60 seconds)**
   - A. Simultaneous translation of mRNA can take place on multiple ribosomes called polysomes