MOLECULAR BIOLOGY OF THE GENE

12.2 DNA REPLICATION

- **Semiconservative:** each strand of original double helix (*parental* molecule) serves as template for new strand in *daughter* molecule.

- **Unwinding:** old strands of parent DNA molecule are unwound as weak hydrogen bonds between paired bases are "unzipped" & broken by enzyme *helicase.*
- **Complementary base pairing:** free nucleotides in nucleus bind with complementary bases on unzipped portions of 2 strands of DNA; catalyzed by *DNA polymerase*.
- **Joining:** complementary nucleotides bond to form new strands; each daughter DNA molecule contains old strand & new strand; catalyzed by DNA polymerase.

- In each species, amount of A = T & amount of G = C; (A +G = T +C).
- One strand of DNA is 5' at top & other strand is 3' at top of strand.
- During replication, DNA polymerase can only join to free 3' end of previous nucleotide.
- 1 strand copied in direction of replication fork & other strand copied in direction away from fork.

Prokaryotic Versus Eukaryotic Replication

- **Prokaryotic DNA Replication**
  - Bacteria have single loop of DNA that must replicate before cell divides.

- Replication may be bidirectional from one point of origin or in only 1 direction.
- Replication only proceeds from 5' to
- Replication begins at special site on bacterial chromosome *(origin of replication)*.
- Bacterial cells can complete DNA replication in 40 minutes; eukaryotes take hours.

- **Eukaryotic DNA Replication**
  - Replication starts at many points of origin & spreads with many replication bubbles – places where DNA strands are separating & replication is occurring.
  - **Replication forks:** V-shape ends of replication bubbles; sites of DNA replication.
  - Eukaryotes replicate their DNA at a slower rate – 500 to 5,000 base pairs per minute.

Accuracy of Replication

- Mismatched nucleotide may occur once per 100,000 base pairs; pause in replication.
- **Proofreading:** removal of mismatched nucleotide by **DNA repair enzymes**.

12.3 GENETIC CODE OF LIFE

RNA Carries the Information

- **Messenger RNA** (**mRNA**) takes message from DNA in nucleus to ribosomes in cytoplasm.
- **Ribosomal RNA** (**rRNA**) & proteins make up ribosomes where proteins are synthesized.
- **Transfer RNA** (**tRNA**) transfers particular amino acid to ribosome.

Genetic Code

- DNA undergoes **transcription** to mRNA, which is **translated** to a protein.

- DNA is a template for RNA formation during transcription.
- **Transcription**: DNA strand serves as a template for formation of mRNA.
- **Translation**: mRNA transcript directs sequence of amino acids in polypeptide.
- **Genetic code:** *triplet code*, comprised of three-base code words (e.g. AUG).
- **Codon**: 3 nucleotide bases of DNA.
- **Degenerate**: 64 triplets to code for 20 naturally occurring amino acids; protects against potentially harmful mutations.
- **Unambiguous**: each triplet codon specifies 1 amino acid.

12.4 TRANSCRIPTION

mRNA is Formed

- Segment of DNA helix unwinds & unzips.
- Transcription begins when **RNA polymerase** attaches to a **promoter** on DNA.
- **Promoter**: region of DNA which defines start of gene, direction of transcription, & strand to be transcribed.
- As **RNA polymerase** moves along *template* strand of DNA, complementary RNA nucleotides are paired with DNA nucleotides of the *coding strand*.
- RNA polymerase adds nucleotides to 3'-end of polymer under construction.
- RNA synthesis is in 5’-to-3’ direction.
- **Elongation** of mRNA continues until RNA polymerase comes to *stop sequence*.
- **Stop sequence** causes RNA polymerase to stop transcribing DNA & to release mRNA transcript.

- Many RNA polymerase molecules work to produce mRNA from the same DNA region at the same time.
- Cells produce thousands of copies of same mRNA molecule & many copies of same protein in shorter period of time than if single copy of RNA used.

12.5 TRANSLATION

- One language (nucleic acids) is translated into another language (protein).

Role of Transfer RNA

- **tRNA** molecules transfer amino acids to ribosomes.
- Single-stranded ribonucleic acid that doubles back on itself to create regions where complementary bases are hydrogen-bonded to one another.
- **Amino acid** binds to the 3' end; opposite end of molecule contains **anticodon** that binds to mRNA codon in complementary fashion.

Role of Ribosomal RNA

- rRNA is produced from DNA template in nucleolus of nucleus.
- rRNA is packaged with variety of proteins into (large & small) ribosomal subunits.
- Subunits move separately through nuclear envelope pores into cytoplasm where they combine when translation begins.
- Ribosomes can float free in cytosol or attach to ER.
    - Ribosomes have binding site for mRNA & binding sites for 2 tRNA molecules.
    - Facilitate complementary base pairing between tRNA anticodons & mRNA codons.
    - rRNA acts as an enzyme (*ribozyme*) that joins amino acids together by peptide bond.

- Ribosome moves down mRNA molecule, new tRNAs arrive, amino acids join, & polypeptide forms.
- Translation terminates when polypeptide formed; ribosome dissociates into 2 subunits.
- **Polyribosomes:** clusters of several ribosomes synthesizing same protein.

Translation Requires 3 Steps

- mRNA codons base-pair with tRNA anticodons carrying specific amino acids.
- Codon order determines order of tRNA molecules & sequence of a.a in polypeptides.
- Enzymes required for all 3 steps; energy (ATP) is needed for first 2 steps.

Initiation:

- Small ribosomal subunit attaches to mRNA in vicinity of *start codon* (AUG).
- First/ initiator tRNA pairs with this codon; then large ribosomal subunit joins to small subunit.
- Each ribosome contains 3 binding sites: the **P** (for peptide) **site**, the **A** (for amino acid) **site**, & the **E** (for exit) **site**.
- Initiator tRNA binds to P site although it carries 1 amino acid, **methionine**.
- A site is for next tRNA carrying next amino acid.
- E site is to discharge tRNAs from ribosome.

Elongation

- tRNA with attached polypeptide is at P site; tRNA-amino acid complex arrives at A site.
- Proteins (*elongation factors*) facilitate complementary base pairing between tRNA anticodon & mRNA codon.

- Polypeptide is transferred & attached by peptide bond to newly arrived a.a in A site; catalyzed by *ribozyme*, which is part of larger subunit.
- tRNA molecule in P site is now empty.
- **Translocation** occurs with mRNA, along peptide-bearing tRNA, moving to P site & spent tRNA moves from P site to E site & exits ribosome.
- As ribosome moves forward 3 nucleotides; new codon now located at empty A site.
- Ribosomes reaches stop codon, termination occurs & peptide is released.

Termination

- Occurs at stop codon that does not code for amino acid.
- Polypeptide is **enzymatically** cleaved from last tRNA by release factor.
- tRNA & polypeptide leave ribosome, which dissociates into its 2 subunits.