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Chapter 2

Molecular Science
Basic Molecular Theory

Stephanie A. Hamilton, EdD, SCT, MB(ASCP)CM
MD Anderson Cancer Center
Houston, Texas

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Cell Cycle

- Cell cycle is guided by set of controls
  - Cyclin proteins and cyclin-dependent kinases promote entry into cell cycle
  - Several proteins (eg., p53) inhibit entry
- 4 phases:
  - G1 (gap 1)
  - S (synthesis, in which DNA is replicated)
  - G2 (gap 2)
  - M (mitosis)
- Interphase: collectively, G1, S, and G2

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Cell Cycle

- "Entry into cell cycle" refers to transition from G1 to S phase
- Cells in G1 have diploid (2N) quantity of DNA
- Cells in late S, G1 and early M have tetraploid (4N) quantity of DNA

Click here

http://www.cellsalive.com/cell_cycle.htm

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Cell Cycle: Mitosis

- Prophase:
  - Follows G2 phase of interphase
  - Centrioles move to opposite poles of nucleus
  - Microtubules begin to assemble to ultimately attach chromosomes, via kinetochores, to the centrioles
- Prometaphase:
  - Nuclear envelope disappears
- Metaphase:
  - Chromosomes align along middle of nucleus
- Anaphase:
  - Chromosomes are pulled apart
- Telephase:
  - Chromatids are located at opposite poles
  - New nuclear envelopes form and cell divides (cytokinesis)

http://www.cellsalive.com/mitosis.htm ← Click here

- ***Mitosis: production of 2 genetically identical daughter cells from 1cell***

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Meiosis

- Occurs only in germ cells
- Consists of meiosis I and meiosis II
- DNA replication precedes meiosis, so process begins with 4N
  - Each chromosome is has 4 chromatids,
    - 2 exact copies of the paternal chromosome (paternal homolog) linked together at the centromere
    - 2 exact copies of maternal chromosome (maternal homolog) linked together at the centromere

Click here → http://www.cellsalive.com/meiosis.htm

Meiosis: production of 4 nonidentical haploid daughter cells from 1 diploid cell

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Meiosis

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- Prophase I:
  - Homologous chromosomes become attached to one another via synapses, forming bivalents
  - Each bivalent consists of 4 chromatids
  - Chiasmata forms between the homologs due to “crossing over” (genetic recombination)
- Prometaphase I and Metaphase I
  - Bivalents align at center
- Anaphase I:
  - Chromosomes move to opposite poles randomly
  - Random mixture of paternal and maternal homologs goes to each daughter cell
  - Each daughter cell is haploid (each has 2 chromatid copies of either maternal or paternal homolog for each chromosome)
- Meiosis II:
  - Similar to mitosis
  - Results in 4 haploid cells

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DNA Replication

- DNA replication:
  - Begins with splitting of the double strand into 2 single strands
  - Then creation of a new complementary strand for each
  - **Semiconservative**: half of the original DNA is preserved in each new double strand

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DNA Replication

- Strand splitting is mediated by 2 DNA enzymes:
  - Topoisomerase II (gyrase): removes supercoiling of DNA by creating transitory brakes in the sugar-phosphate backbone
  - Helicase: unwinds the DNA helix into single strands, facilitating replication, by breaking hydrogen bonds
- Splitting occurs simultaneously at multiple points along DNA molecule resulting in “replication bubbles”
- At ends of bubble, where split strands converge back into double-stranded DNA are “replication forks”

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DNA Replication

- DNA polymerase III binds to exposed single-stranded DNA and begins to create a complementary sequence for each
  - Where C is on template strand, G is added to new strand (and vice versa)
  - Where T is on template strand, A is added to new strand (and vice versa)
- **DNA polymerase III** catalyzes the addition of a dNTP (deoxynucleoside triphosphate) monomer by creating a phosphodiester bond between the free 3′ OH on the growing strand and the 5′ phosphate group on incoming nucleotide
- Synthesis always proceeds in 5′→ 3′ direction and 2 strands are antiparallel
- Template strand therefore is read in the 3′ →5′ direction
- Helicase continues to open the replication fork exposing a new single stranded DNA

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DNA Replication

- Thus, addition of new bases on one strand:
  - Proceeds towards the replication fork
  - In the 5′→3′ direction
  - In continuous fashion (the leading strand)
- Addition of new bases on other strand:
  - Proceeds away from replication fork
  - Is also in the 5′→3′ direction
  - In discontinuous fashion (the lagging strand)
  - DNA polymerase III creates short stretches of DNA (Okazaki fragments)
  - Fragments later ligated by DNA ligase to form an intact strand

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DNA Replication

- DNA polymerase requires a primer sequence to begin replicating
- Primer is in the form of a short segment of RNA (an RNA primer) laid down by RNA polymerase
- A primer is like the pull tab for a zipper
- Enzyme **primase** polymerizes ribonucleotides to form a short RNA strand which acts as the primer
- RNA primer later removed by **DNA polymerase I** and is replaced with DNA

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DNA Replication

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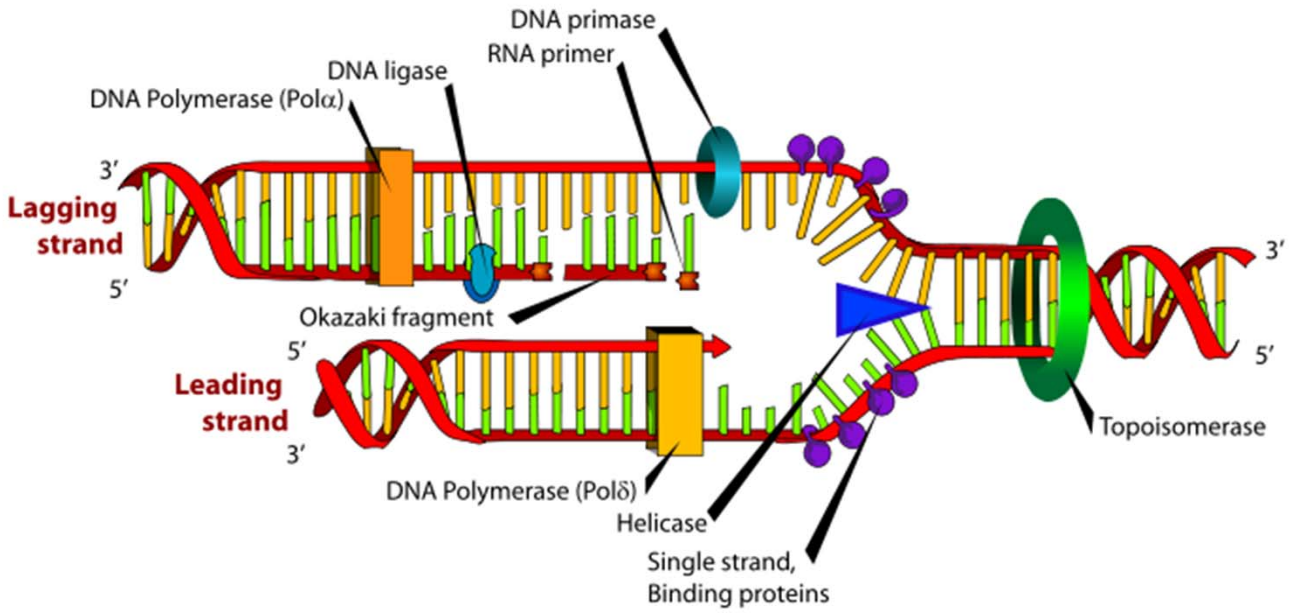

http://cellbiology.med.unsw.edu.au/units/images/DNA_replication_fork.png

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DNA Replication Animation

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http://www.youtube.com/watch?v=teV62zrm2P0

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Gene Structure

- A gene is a segment of DNA that can be transcribed into RNA
- Most RNA transcripts are translated into protein
- Some RNA transcripts remain as RNA (noncoding RNA)

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Gene Structure

- Promoter sequence:
  - A regulatory region of DNA in non-coding sequence
  - First portion of a gene
  - Located farthest “upstream” in 5′ end of gene
  - Is site of RNA polymerase binding to start transcription

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Gene Structure

- Coding region:
  - Portion of gene that is actually transcribed into RNA
    - Genes contain both coding sequences for translation into proteins **(exons)** and untranslated sequences **(introns)**
    - Exon is composed of a specific sequence of 3 nucleotides **(codons)** that encode a specific amino acid
  - Smallest gene (1 exon and 500 nt) codes for a histone
  - Largest gene (70 exons, 70 introns, and 2.5 million nt) codes for the protein dystrophin

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Transcription

- The genetic information carried by DNA is held in the sequence of bases in DNA called the genetic code.
- The DNA sequence is copied into a complementary mRNA sequence by RNA polymerase and the process is called **transcription**.

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Transcription

- DNA sequence is called “sense” if its sequence is same as that of messenger RNA (mRNA) copy produced by these enzymes and then translated into protein.
- The sequence on the opposite strand is complementary to the sense sequence and is therefore called the “antisense” sequence.

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Transcription

- Transcription:
  - Production of mRNA from a DNA template
  - Process begins at a sequence of DNA within promoter region called TATA box
  - TATA box:
    - A 6-nucleotide TATAAA sequence located 25 nt upstream from start of transcription
    - Capable of binding a TATA-binding protein (TBP) which is part of a larger transcription factor complex
    - This complex is capable of binding RNA polymerase

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Transcription

- RNA polymerase:
  - Like DNA polymerase, can elongate a new strand in the 5′→3′ direction
  - Pairs U (uracil) with A (adenine) on the template, instead of T (thymine) as in DNA
  - Ceases following the encoding of an AAUAAA sequence
  - mRNA transcript is produced containing entire coding sequence including exons and introns

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Transcription

- mRNA transcripts must be reduced to portions that will be translated into proteins (exons)
- Splicing: a process by which the introns are removed after transcription
- Spliceosome:
  - RNA-protein complex that can recognize short RNA sequences (eg. GU and AG) that signal start and stop of an intron and thus can remove it
- Exons are joined

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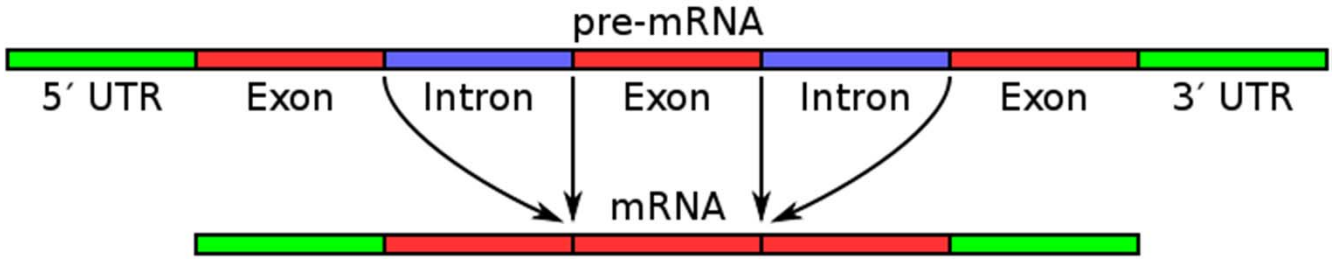
Splicing



http://en.wikipedia.org/wiki/File:Pre-mRNA_to_mRNA.svg. Accessed 07/05/2010.

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Transcription

- Capping: A 7-methyl guanosine cap is added to the 5′ end of the mRNA
  - This will allow binding of mRNA to a ribosome
- Polyadenylation: A poly-A tail sequence is added to the 3′ end (many adenines) which stabilizes mRNA
- Mature mRNA is transported to the cytoplasm for translation

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Translation

- Translation:
  - Production of protein from a mRNA template
  - mRNA becomes bound to a ribosome due to the 7-methyl guanosine cap
  - mRNA strand is passed through ribosome until a start (initiation) codon is recognized

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Translation

- Every amino acid has an amino acid-specific tRNA (transfer RNA)
- tRNA:
  - are small molecules of RNA that have capacity to bind a specific amino acid
  - specific tRNA carry specific amino acids to the ribosomes to be added to the growing chain of protein
  - have a trinucleotide sequence (anticodon) complementary to the mRNA codon
- Anticodon pairs with codon

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Translation

- Process is started with a start codon, AUG, which codes for Methionine
- mRNA strand is then advanced to the next trinucleotide codon and the tRNA bearing the encoded amino acid is brought in next to methionine

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Translation

- A peptide bond is formed between the 2 amino acids. The enzyme peptidyl transferase catalyzes this reaction
- 3 stop (termination) codons:
  - UAA, UAG and UGA
  - Entry of one of these codons into ribosome will release the growing polypeptide chain

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Translation

- Codons:
  - A triplet of RNA nucleotides, which instructs the ribosome to attach a particular amino acid
  - Every possible nucleotide triplet has a corresponding amino acid
  - There are more possible triplets (64) than amino acids
    - Only 21 amino acids are used to make protein
    - Degenerate code: several different codons may denote a single amino acid
      - Ex. UUC, UUG, CUU, CUA all code for leucine

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Protein: A Polypeptide Molecule

- Proteins are synthesized as a linear polymer of amino acids
- Amino acids are held together by peptide bonds, hence called a polypeptide molecule, using mRNA as the template

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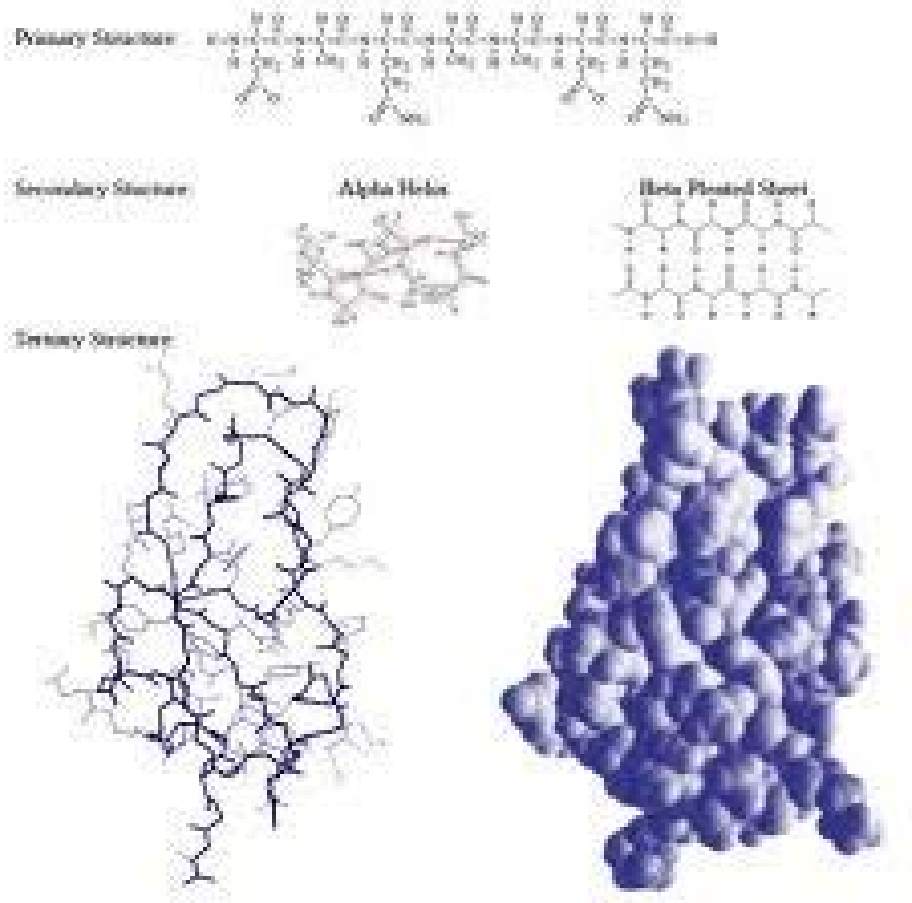
Structure of a Protein



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Control of Gene Expression

- Only small portion of genome encodes proteins
- Most DNA in genome is in noncoding (non-gene) segments
- Much of this DNA is involved in controlling expression of genes
- Selective expression (transcription) of some genes and repression of others, makes it possible to generate various cell types from a single genome

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Control of Gene Expression

- Histones can repressively control gene expression
  - Most of DNA in mature cells cannot be transcribed because of its tight bundling into nucleoprotein complexes (heterochromatin)
  - Enzymes involved in transcription can more easily gain access to DNA that is unbound from histone complexes (euchromatin)

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Control of Gene Expression

- Methylation: another repressive control
  - Most genes are heavily methylated
    - Methyl groups added to the cytosine residues to produce 5-methyl-cytosine, particularly in the promoter sequence
    - Example, X-chromosome inactivation (Barr bodies)
  - These regions of gene contain DNA sequences with high content of cytosine and guanine (CpG islands)
  - DNA methylation is catalyzed by DNA methyltransferases
  - Heavily methylated genes are prevented from transcription

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Control of Gene Expression

- Histones and methylation exert rigid forms of control and are best suited for genes whose expression is to be switched off for the life of the cell
- Dynamic control is desirable for genes that are intermittently expressed
  - Control is mediated by promoters, enhancers, silencers, and transcription factors

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Control of Gene Expression

- Genes are influenced by cis-acting and trans-acting elements:
  - Cis-acting:
    - those that are present on the same DNA strand
    - usually flanking the coding region of a gene
    - exert control only over gene they flank
    - include promoters, enhancers and silencers
  - Trans-acting:
    - encoded elsewhere on DNA strand
    - usually take form of DNA-binding proteins (transcription factors)
    - recognize and bind to cis-acting elements
    - may simultaneously influence several genes

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Control of Gene Expression

- Promoter sequences
  - Found upstream (5') of gene start codon
  - RNA polymerases are sensitive to status of promoter sequence
  - Within promoter region is TATAA sequence (the TATA box)

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Control of Gene Expression

- Enhancer sequences
  - May be found on either side of a gene
  - May be found in coding sequence itself
  - Bind transcription factors
  - Stimulate transcription of the genes they flank
  - Some enhancers influence transcription of entire multigene regions of DNA (locus-activating region)

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Control of Gene Expression

- Silencer sequences: work opposite to that of promoters

- Transcription factors:
  - Proteins that bind to specific DNA sequences
  - Contain certain repetitive structural features
  - Also called *motifs*

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Control of Gene Expression

- Alternative RNA Splicing:
  - A key step in gene expression is mRNA splicing
  - Some mRNA transcripts can be spliced in various ways
  - Results in proteins with variable conformations
  - Thus, from one gene, several different isoforms of a protein can be made simply by splicing
  - Different isoforms have different behaviors

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Techniques for Epigenetic Modification

- DNA methylation: Bisulfate sequencing
- Histone modification:
  - Chromatin immunoprecipitation (ChIP)
  - DNA adenosine methylation identification (DamID)
- siRNA production: Deep sequencing

Go to the following web site to see a PowerPoint on this topic:

*www.plantcell.org/site/.../TTPB4**EPIGENETICS_METHODS**.ppt*

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