GENETICS TEST III

REVIEW
BE ABLE TO DEFINE

• 5’end of DNA/RNA vs. 3’end
  • The fifth carbon of the five carbon sugar is the location of the phosphate group on a nucleotide
  • The third carbon of the five carbon sugar has a hydroxyl group (OH).
  • Nucleotides are linked between the phosphate group at the C-5’ position and the OH group on the C-3’ position.

• Antiparallel
  • two biopolymers are antiparallel if they run parallel to each other but with opposite alignments.
  • An example is the two complementary strands of a DNA double helix, which run in opposite directions to one another.
BE ABLE TO DEFINE

• Retrovirus
  • A type of virus that uses RNA as its genetic material and employs the enzyme reverse transcriptase during its life cycle.
  • Viruses that have an RNA core rather than a DNA core.
  • Retroviruses replicate in an unusual way
    • The RNA serves as a template for synthesis of a complementary DNA by the RNA-dependent DNA polymerase reverse transcriptase.
    • This DNA can be incorporated into the host cell genome; when transcribed, copies of the original retroviral RNA chromosome are also produced

• Reverse Transcriptase
  • A polymerase that uses RNA as a template to transcribe a single-stranded DNA molecule as a product.
BE ABLE TO DEFINE

- **Nucleotide**
  - In nucleic acid chemical nomenclature, a nucleoside covalently linked to one or more phosphate groups. Nucleotides containing a single phosphate linked to the 5' carbon of the ribose or deoxyribose are the building blocks of nucleic acids.
  - The building blocks of DNA
  - They consist of
    - A nitrogenous base
    - A pentose sugar
    - A phosphate group
  - A nucleoside with a phosphate group added

- **Nucleoside**
  - In nucleic acid chemical nomenclature, a purine or pyrimidine base covalently linked to a ribose or deoxyribose sugar molecule.
  - A nucleoside contains the nitrogenous base and the pentose sugar.
BE ABLE TO DEFINE

• mRNA
  • Messenger RNA
  • The template for protein synthesis
  • An RNA molecule transcribed from DNA and translated into the amino acid sequence of polypeptide.

• rRNA
  • Ribosomal RNA
  • Components of ribosomes for protein synthesis
  • The RNA molecules that are the structural components of the ribosomal subunits. In prokaryotes, these are the 16S, 23S, and 5S molecules; in eukaryotes, they are the 18S, 28S, and 5S molecules.

• tRNA
  • Transfer RNA
  • Carry amino acids for protein synthesis
  • A small ribonucleic acid molecule with an essential role in translation. tRNAs contain: (1) a three-base segment (anticodon) that recognizes a codon in mRNA; (2) a binding site for the specific amino acid corresponding to the anticodon; and (3) recognition sites for interaction with ribosomes and with the enzyme that links the tRNA to its specific amino acid.
BE ABLE TO DEFINE

- **Conservative replication**
  - Original helix is conserved and two newly synthesized strands come together.

- **Semi-conservative replication**
  - Each replicated DNA molecule consists of one old strand and one new strand.
  - A mode of DNA replication in which a double-stranded molecule replicates in such a way that the daughter molecules are each composed of one parental (old) and one newly synthesized strand.
  - Meselson-Stahl experiment demonstrated that DNA replication is semi-conservative.

- **Dispersive replication**
  - Parent strands are dispersed into two new double helices.
BE ABLE TO DEFINE

• Pyrimidines
  • One of two types of nitrogenous bases found in nucleotides. Cytosine (C), thymine (T), and uracil (U) are pyrimidines.
  • Six-member single ring pyrimidines.

• Purines
  • One of two types of nitrogenous bases found in nucleotides. Adenine (A) and guanine (G) are purines.
  • Nine-member double ring purines.

![Pyrimidine and Purine structures](image-url)
BE ABLE TO DEFINE

- **Bidirectional replication**
  - A mechanism of DNA replication in which two replication forks move in opposite directions from a common origin.
  - Bacteria DNA replication begins at the origin of replication and is bidirectional rather than unidirectional.

- **Replicon**
  - The length of DNA that is replicated following one initiation event at a single origin is called a replicon.
  - The unit of DNA replication, beginning with DNA sequences necessary for the initiation of DNA replication. In bacteria, the entire chromosome is the replicon.

- **Origin of replication**
  - Sites where DNA replication begins along the length of a chromosome.

- **Reassociation kinetics**
  - Reassociation kinetics provides information about the size and complexity of genomic DNA from an organism.
  - The technique in molecular hybridization procedures where the rate of reassociation of complementary single DNA strands is analyzed.
BE ABLE TO DEFINE

• **Hyperchromic shift**
  - Hyperchromic shift during DNA denaturation is used to determine melting temperature.
  - The increase in UV absorption of heated DNA in solution is called hyperchromic shift.

• **Repetitive DNA**
  - An DNA sequence present in many copies in the haploid genome.

• **Unique DNA**
  - DNA sequences that are present only once per genome.
**BE ABLE TO DEFINE**

- **DNA polymerase**
  - An enzyme that catalyzes the elongation of new DNA at a replication fork by the addition of nucleotides to the existing chain.
  - An enzyme that catalyzes the synthesis of DNA from deoxyribonucleotides utilizing a template DNA molecule.
  - DNA polymerase catalyzes DNA synthesis and requires a DNA template and all four dNTPs.
  - DNA polymerase I, II, and III can elongate an existing DNA strand but cannot initiate DNA synthesis.
  - DNA polymerase I, II, and III possess 3' to 5' exonuclease activity, allowing them to proofread newly synthesized DNA and remove and replace incorrect nucleotides.

- **DNA polymerase I**
  - Only DNA polymerase I demonstrates 5' to 3' exonuclease activity, excising primers and filling in the gaps left behind.
  - Removes RNA primer and aids in DNA gap repair.
  - Responsible for
    - Removing the primer
    - The synthesis that fills gaps produced during synthesis.

- **DNA polymerase II**
  - Aids in DNA repair.
  - Involved in various aspects of repair of DNA damaged by external forces such as UV light.
BE ABLE TO DEFINE

DNA polymerase III
- The enzyme responsible for the 5' to 3' polymerization essential in vivo.
- Its 3' to 5' exonuclease activity allows proofreading.
- Primary synthesis of cells DNA. Responsible for making genome.
- An enzyme that catalyzes the synthesis of DNA from deoxyribonucleotides utilizing a template DNA molecule.

DNA polymerases I, II, IV, and V are involved in various aspects of repair of DNA damaged by external forces such as UV light.

Primary synthesis cells DNA

<table>
<thead>
<tr>
<th>Properties of Bacterial DNA Polymerases I, II, and III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Properties</strong></td>
</tr>
<tr>
<td>Initiation of chain synthesis</td>
</tr>
<tr>
<td>5’–3’ polymerization</td>
</tr>
<tr>
<td>3’–5’ exonuclease activity</td>
</tr>
<tr>
<td>5’–3’ exonuclease activity</td>
</tr>
<tr>
<td>Molecules of polymerase per cell</td>
</tr>
</tbody>
</table>

Removes RNA primer and aids DNA gap repair
Aids DNA repair
BE ABLE TO DEFINE

- **5’ to 3’ exonuclease**

- **3’ to 5’ exonuclease**
  - Allows proofreading behind enzyme. Can only proofread nucleotides that have just been synthesized.

- **Primer**
  - In nucleic acids, a short length of RNA or single-stranded DNA required for initiating synthesis directed by polymerases.
  - A polynucleotide with a free 3’ end bound by complementary base pairing to the template strand, that is elongated during DNA replication.

- **Primase**
  - The enzyme primase synthesizes an RNA primer that provides the free 3’-hydroxyl required by DNA polymerase III
  - An enzyme that joins RNA nucleotides to make the primer.
BE ABLE TO DEFINE

• Single stranded DNA binding proteins
  • In DNA replication, proteins that bind to and stabilize the single-stranded regions of DNA that result from the action of unwinding proteins.
  • (SSBPs)
  • Single-stranded binding proteins stabilize the open conformation.

• Helicase
  • An enzyme that untwists the double helix of DNA at the replication fork.
  • An enzyme that participates in DNA replication by unwinding the double helix near the replication fork.
  • DNA Helicase

• DNA gyrase (topoisomerase)
  • One of a class of enzymes known as topoisomerases that converts closed circular DNA to a negatively supercoiled form prior to replication, transcription, or recombination. The enzyme acts during DNA replication to reduce molecular tension caused by supercoiling.
  • A class of enzymes that converts DNA from one topological form to another. During replication, a topoisomerase, DNA gyrase, facilitates DNA replication by reducing molecular tension caused by supercoiling upstream from the replication fork.
BE ABLE TO DEFINE

- **Leading strand**
  - During DNA replication, the strand synthesized continuously in the direction of the replication fork.

- **Lagging strand**
  - During DNA replication, the strand synthesized in a discontinuous fashion, in the direction opposite of the replication fork.

- **Okazaki fragments**
  - The short, discontinuous strands of DNA produced on the lagging strand during DNA synthesis.

- **DNA Ligase**
  - An enzyme that forms a covalent bond between the 5' end of one polynucleotide chain and the 3' end of another polynucleotide chain. It is also called polynucleotide-joining enzyme.
BE ABLE TO DEFINE

• Telomerase
  • The enzyme that adds short, tandemly repeated DNA sequences to the ends of eukaryotic chromosomes.

• Recombination
  • The process that leads to the formation of new allele combinations on chromosomes.
  • Genetic recombination involves:
    • Endonuclease nicking
    • Strand displacement
    • Ligation
    • Branch migration
    • Duplex separation to generate the characteristic Holliday structure.

• Holliday Structure
  • In DNA recombination, an intermediate seen in transmission electron microscope images as an X-shaped structure showing for single-stranded DNA regions.

• Heteroduplex
  • A double-stranded nucleic acid molecule in which each polynucleotide chain has a different origin. It may be produced as an intermediate in a recombinational event or by the in vitro reannealing of single-stranded, complementary molecules.
  • In transformation, once the extracellular DNA is integrated into the chromosome, the recombinant region contains one host strand and one mutant strand. This region is referred to as a heteroduplex.
GENETIC RECOMBINATION

**Figure 11-10** Model depicting how genetic recombination can occur as a result of the breakage and rejoining of heterologous DNA strands. Each stage is described in the text. The electron micrograph shows DNA in a Y-form structure similar to the diagram in (g); the DNA is an extended Holliday structure, derived from the CoIE1 plasmid of *E. coli*. David Dye, Oxford University, England.
BE ABLE TO DEFINE

- **Gene conversion**
  - The process of nonreciprocal recombination by which one allele in a heterozygote is converted into the corresponding allele.

- **Nucleoid**
  - The DNA-containing region within the cytoplasm in prokaryotic cells.

- **Chromatin**
  - The complex of DNA, RNA, histones, and nonhistone proteins that make up uncoiled chromosomes, characteristic of the eukaryotic interphase nucleus.

- **Histones**
  - Positively charged proteins complex with DNA in the nucleus. They are rich in the basic amino acids arginine and lysine, and function in coiling DNA to form nucleosomes.
  - Chromatin is bound up in nucleosomes with histones H2A, H2B, H3, and H4.
  - The five main types of histones are H1, H2A, H2B, H3, and H4.
BE ABLE TO DEFINE

• Acetylation, methylation, phosphorylation of Histones
  • Used to form histone tails.

• Nucleosomes
  • In eukaryotes, a nuclear complex consisting of four pairs of histone molecules wrapped by two turns of a DNA molecule. The major structure associated with the organization of chromatin in the nucleus.

• Euchromatin
  • Chromatin or chromosomal regions that are lightly staining and are relatively uncoiled during the interphase portion of the cell cycle. Euchromatic regions contain most of the structural genes.
  • Uncoiled and active

• Heterochromatin
  • The heavily staining, late-replicating regions of chromosomes that are prematurely condensed in interphase.
  • Remains condensed and is inactive.
BE ABLE TO DEFINE

• Satellite DNA
  • DNA that forms a minor band when genomic DNA is centrifuged in a cesium salt gradient. This DNA usually consists of short sequences repeated many times in the genome.
  • Is highly repetitive and consists of short repeated sequences
  • Named for fact it isolates at a different density from other DNA in cell.
  • Binds to centromeric DNA.

• Centromere
  • The specialized heterochromatic chromosomal region at which sister chromatids remain attached after replication, and the site to which spindle fibers attach to the chromosome during cell division. Location of the centromere determines the shape of the chromosome during the anaphase portion of cell division. Also know as the primary constriction.
  • Involved in chromosome movement

• Telomere
  • The heterochromatic terminal region of a chromosome.
  • Maintains chromosome integrity.
BE ABLE TO DEFINE

- Moderately repetitive DNA
  - Includes
    - Some genes like rRNA genes
    - Variable number tandem repeats (VNTRs)
    - Minisatellites (repeats of 15–100 bp)
    - Microsatellites (repeats of 2–5 bp)

- Short interspersed elements (SINES) and long interspersed elements (LINES) are dispersed throughout the genome rather than tandemly repeated, and constitute over 1/3 of the human genome.

- These transposable elements are generated via an RNA intermediate and are referred to as retrotransposons.
BE ABLE TO DEFINE

• Sines
  • Repetitive sequences found in the genomes of higher organisms. The 300-bp Alu sequence is a SINE element.

• Lines
  • Long repetitive sequences found interspersed in the genomes of higher organisms.

• VNTR’s
  • Short, repeated DNA sequences (of 2 – 20 nucleotides) present as tandem repeats between two restriction enzyme sites. Variation in the number of repeats creates DNA fragments of differing lengths following restriction enzyme digestion. Used in early versions of DNA fingerprinting.
  • Variable number tandem repeats (VNTRs).

• Retrotransposons
  • Mobile genetic elements that are major components of many eukaryotic genomes, that are copied by means of an RNA intermediate and inserted at a distant chromosomal site.
BE ABLE TO DEFINE

• Induced mutation
  • Induced mutations result from the influence of an extraneous factor, either natural or artificial, such as radiation, UV light, natural or synthetic chemicals.

• Spontaneous mutation
  • Spontaneous mutations happen naturally and randomly and are usually linked to normal biological or chemical processes in the organism.
  • A mutation that is not induced by a mutagenic agent.

• Somatic mutation
  • Somatic mutations occur in any body cell except germ cells and are not heritable.
  • A nonheritable mutation occurring in a somatic cell.

• Germ-line mutation
  • Germ-line mutations occur in cells that make gametes and are inherited.
BE ABLE TO DEFINE

- **Autosomal mutations**
  - Autosomal mutations occur within genes located on the autosomes.

- **X linked mutations**
  - X-linked mutations occur within genes located on the X chromosome.

- **Point mutations**
  - A mutation that can be mapped to a single locus. At the molecular level, a mutation that results in the substitution of one nucleotide for another. Also called a gene mutation.
  - Point mutations are base substitutions in which one base pair is altered.

- **Frameshift mutations**
  - Frameshift mutations result from insertions or deletions of a base pair.
  - A mutational event leading to the insertion of one or more base pairs in a gene, shifting the codon reading frame in all codons that follow the mutational site.
  - A frameshift mutation occurs when any number of bases are added or deleted, except multiples of three, which would reestablish the initial frame of reading.
BE ABLE TO DEFINE

• Missense mutation
  • A mutation that changes a codon to that of another amino acid and thus results in an amino acid substitution in the translated protein. Such changes can make the protein nonfunctional.
  • Missense mutations change a codon resulting in an altered amino acid within a protein-coding portion of a gene.

• Nonsense mutation
  • A mutation that changes a codon specifying an amino acid into a termination codon, leading to premature termination during translation of mRNA.
  • A nonsense mutation changes a codon into a stop codon and results in premature termination of translation

• Silent mutation
  • A silent mutation alters a codon but does not result in a change in the amino acid at that position of the protein.
BE ABLE TO DEFINE

• Lethal mutation
  • Lethal mutations interrupt an essential process and result in death.

• Neutral mutation
  • A mutation with no immediate adaptive significance or phenotypic effect.
  • No change in fitness of the organism.
  • Neutral mutations can occur in a protein coding region, (hydrophilic for hydrophilic) or (hydrophobic for hydrophobic) which does not change function.

• Conditional mutation
  • A mutation expressed only under a certain condition; that is, a wild-type phenotype is expressed under certain (permissive) conditions and a mutant phenotype under other (restrictive) conditions.
  • Expression of conditional mutation depends on the environment in which the organism finds itself.

• Temperature sensitive mutation
  • A conditional mutation that produces a mutant phenotype at one temperature range and a wild-type phenotype at another.
  • Gene products functions at one temperature but not another.
  • Example: temperature sensitive coat color variations in Siamese cat and Himalayan rabbits.
BE ABLE TO DEFINE

• Transition mutation
  • A mutational event in which one purine is replaced by another or one pyrimidine is replaced by another.

• Transversion mutation
  • A mutational event in which a purine is replaced by a pyrimidine or a pyrimidine is replaced by a purine.

• Gain function mutation

• Loss of function mutation

Mutations can be classified according to their phenotypic effects as:

- loss-of-function (of enzyme or protein)
  Dominant loss of function can occur if mutant protein binds to normal protein or inhibits normal protein function

- gain-of-function (from mutant to wild type... or up regulates normal gene...etc.)
BE ABLE TO DEFINE

• Tautomerism shift
  • A reversible isomerization in a molecule, brought about by a shift in the location of a hydrogen atom. In nucleic acids, tautomerism shifts in the bases of nucleotides can cause changes in other bases at replication and are a source of mutations.

• Depurination
  • Loss of one of the nitrogenous bases (usually a purine) in an intact double-helical DNA molecule.

• Deamination
  • By removal of amino group, cytosine is converted to uracil, or adenine is converted to hypoxanthine.
  • DNA base damage by depurination and deamination is the most common cause of spontaneous mutation.
BE ABLE TO DEFINE

• Transposons
  • Transposons are transposable genetic elements that can move within or between genomes.
  • Integrations of transposons into new genomic locations can act as naturally occurring mutagens.

• Mutagens
  • Any agent that causes an increase in the spontaneous rate of mutation.
  • Mutagens are natural or artificial agents that induce mutations
    • Fungal toxins
    • Cosmic rays
    • Ultraviolet light
    • Industrial pollutants
    • Medical x-rays
    • Chemicals in tobacco smoke

• UV thymine dimers
  • In a polynucleotide strand, a lesion consisting of two adjacent thymine bases that become joined by a covalent bond. Usually caused by exposure to ultraviolet light, this lesion inhibits DNA replication
BE ABLE TO DEFINE

• Mismatch repair
  • A form of excision repair of DNA in which the repair mechanism is able to distinguish between the strand with the error and the strand that is correct.

• If proofreading fails, **mismatch repair** becomes activated
• The correct DNA strand is recognized based on DNA methylation of the parental strand.
• Since new DNA is unmethylated (slow enzyme)… and old DNA is methylated and has “survived” a generation, the new DNA is preferentially repaired.
• A strong link between defective mismatch repair and cancer has been documented
BE ABLE TO DEFINE

- Postreplication repair
  - **Postreplication repair** responds *after* damaged DNA has escaped repair and failed to be completely replicated.
  - Through the processes of recombination, the correct complementary sequence is recruited from the parental strand and inserted into the gap opposite the lesion (damaged DNA).
  - The new gap is filled by DNA polymerase and ligase (*Figure 15-11*).

*Figure 15-11* Postreplication repair occurs if DNA replication has skipped over a lesion such as a thymine dimer. Through the process of recombination, the correct complementary sequence is recruited from the parental strand and inserted into the gap opposite the lesion. The new gap is filled by DNA polymerase and DNA ligase.
BE ABLE TO DEFINE

- **SOS repair**
  - SOS repair system is the last resort to minimize DNA damage. DNA synthesis becomes error-prone, inserting random and incorrect nucleotides in places that would normally stall DNA replication.
  - Can't use another system to repair “gaps” and other lethal damage, this system forces DNA polymerase to insert any base (better a small mutation than lose the whole genome).
  - SOS repair can itself become mutagenic but allows cells to survive with DNA damage that would otherwise kill it.
BE ABLE TO DEFINE

- Photoreactivation repair
  - Light-induced repair of damage caused by exposure to ultraviolet light. Associated with an intracellular enzyme system.

**Photoreactivation repair** involves a photoreactivation enzyme (PRE) that cleaves the bonds between thymine dimers (**Figure 15.12**)

- The enzyme must absorb a photon of light to cleave the damaged DNA
- Humans and other organisms lack photoreactivation repair

**Figure 15-12** Damaged DNA repaired by photoreactivation repair. The bond creating the thymine dimer is cleaved by the photoreactivation enzyme (PRE), which must be activated by blue light in the visible spectrum.
BE ABLE TO DEFINE

• Excision repair
  • Removal of damaged DNA segments followed by repair. Excision can include the removal of individual bases (base repair) or of a stretch of damaged nucleotides (nucleotide repair).
  • Excision repair involves three steps
    • Removal of the mutation by a endonuclease
    • Gap filling by DNA polymerase
    • Sealing of the nick by DNA ligase.
  • There are two types of excision repair
    • Base excision repair (BER)
      • Corrects DNA containing a damaged DNA base
    • Nucleotide excision repair (NER)
      • Repairs bulky lesions and involves the uvr genes.
EXCISION REPAIR

**FIGURE 15-13** Base excision repair (BER) accomplished by uracil DNA glycosylase, AP endonuclease, DNA polymerase, and DNA ligase. Uracil is recognized as a noncomplementary base, excised, and replaced with the complementary base (C).

**FIGURE 15-14** Nucleotide excision repair (NER) of a UV-induced thymine dimer. During repair, 13 nucleotides are excised in prokaryotes, and 28 nucleotides are excised in eukaryotes.
BE ABLE TO DEFINE

• Double strand break repair
  • Double-strand break repair (DSB repair) reattaches two broken DNA strands.
  • Homologous recombination repair occurs during the late S or early G2 phase of the cell cycle
  • Nonhomologous end joining is activated in G1 (many genes involved including breast cancer gene BRCA1)
DOUBLE STRAND BREAK REPAIR

**FIGURE 15-16** Steps in homologous recombination repair of double-stranded breaks.

1. **Double-stranded break**
2. **Break detected and 5' ends digested**
3. **3' end invades homologous region of sister chromatid**
4. **Sister chromatids**
5. **DNA synthesis across damaged region**
6. **Heteroduplex resolved and gaps filled by DNA synthesis and ligation**

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BE ABLE TO DEFINE

• **Ames test**
  • A bacterial assay developed by Bruce Ames to detect mutagenic compounds; it assesses reversion to histidine independence in the bacterium *Salmonella typhimurium*.
  • The Ames test uses a number of different strains of *Salmonella typhimurium* selected for their ability to reveal the presence of specific types of mutations.
  • The Ames test is used extensively during the development of industrial and pharmaceutical chemical compounds.
  • Many known carcinogens have been shown by the Ames test to be strong mutagens.
    • More than 60 compounds found in cigarette smoke test positive in the Ames test and cause cancer in animals.
    • Note this is a first step, not proof of carcinogen.
What is the Central Dogma of molecular genetics?

- DNA → RNA → Protein
  - DNA → RNA = transcription
  - RNA (mRNA) → Protein = translation

![Diagram](image-url)
CONCEPT QUESTIONS

• Is the genome of prokaryotes linear or circular, DNA or RNA, and Single or double stranded?
  • Prokaryotes genomes are circular, double stranded DNA.
• Is the genome of eukaryotes linear or circular, DNA or RNA, and Single or double stranded?
  • Eukaryote genomes are linear, double stranded DNA.
• Is the genome of viruses linear or circular, DNA or RNA, and Single or double stranded?
  • Viruses can be any of the above.
What are the differences between RNA and DNA?

<table>
<thead>
<tr>
<th>Comparison</th>
<th>DNA</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>DeoxyriboNucleic Acid</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>Function</td>
<td>Long-term storage of genetic information; transmission of genetic</td>
<td>Used to transfer the genetic code from the nucleus to the ribosomes</td>
</tr>
<tr>
<td></td>
<td>information to make other cells and new organisms.</td>
<td>to make proteins. RNA is used to transmit genetic information in</td>
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<td></td>
<td></td>
<td>some organisms and may have been the molecule used to store</td>
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<tr>
<td></td>
<td></td>
<td>genetic blueprints in primitive organisms.</td>
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<tr>
<td>Structural</td>
<td>B-form double helix. DNA is a double-stranded molecule consisting</td>
<td>A-form helix. RNA usually is a single-strand helix consisting of</td>
</tr>
<tr>
<td>Features</td>
<td>of a long chain of nucleotides.</td>
<td>shorter chains of nucleotides.</td>
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<tr>
<td>Composition of</td>
<td>deoxyribose sugar phosphate backbone adenine, guanine, cytosine,</td>
<td>ribose sugar phosphate backbone adenine, guanine, cytosine, uracil</td>
</tr>
<tr>
<td>Bases and Sugars</td>
<td>thymine bases</td>
<td>bases</td>
</tr>
<tr>
<td>Propagation</td>
<td>DNA is self-replicating.</td>
<td>RNA is synthesized from DNA on an as-needed basis.</td>
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<tr>
<td>Base Pairing</td>
<td>AT (adenine-thymine)</td>
<td>AU (adenine-uracil)</td>
</tr>
<tr>
<td></td>
<td>GC (guanine-cytosine)</td>
<td>GC (guanine-cytosine)</td>
</tr>
<tr>
<td>Reactivity</td>
<td>The C-H bonds in DNA make it fairly stable, plus the body destroys</td>
<td>The O-H bond in the ribose of RNA makes the molecule more reactive,</td>
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<td>enzymes that would attack DNA. The small grooves in the helix also</td>
<td>compared with DNA. RNA is not stable under alkaline conditions, plus</td>
</tr>
<tr>
<td></td>
<td>serve as protection, providing minimal space for enzymes to attach.</td>
<td>the large grooves in the molecule make it susceptible to enzyme</td>
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<tr>
<td></td>
<td></td>
<td>attack. RNA is constantly produced, used, degraded, and recycled.</td>
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<tr>
<td>Ultraviolet</td>
<td>DNA is susceptible to UV damage.</td>
<td>Compared with DNA, RNA is relatively resistant to UV damage.</td>
</tr>
<tr>
<td>Damage</td>
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</table>
What is the tetranucleotide hypothesis?

- An early theory of DNA structure proposing that the molecule was composed of repeating units, each consisting of the four nucleotides represented by adenine, thymine, cytosine, and guanine.
- DNA structure seemed to lack the chemical diversity necessary to store extensive genetic information. Proposed that identical groups of the four nucleotides were repeated over and over.
- Proven incorrect by Chargaff.

Old “tetranucleotide concept”: no information in a tetranucleotide
Why was protein considered to be the hereditary material and not nucleic acids?

- For a long time, protein was favored to be the genetic material.
- It is abundant in cells.
- It was the subject of the most active areas of genetic research.
- DNA was thought to be too simple to be the genetic material, with only four types of nucleotides as compared to the 20 different amino acids of proteins.
What was Griffith’s transformation experiment?

- Griffith showed that avirulent strains of *Diplococcus pneumoniae* could be transformed to virulence.
- He speculated that the transforming principle could be part of the polysaccharide capsule or some compound required for capsule synthesis.
- Something in the heat killed S extract transformed living R to living S.
- Proved there was a transforming principle.
EXPERIMENTS

• Griffith’s transformation experiment
  • Showed that avirulent strains of Diplococcus pneumoniae could be transformed to virulence. Some speculated that the transforming principle could be part of the polysaccharide capsule or some compound required for capsule synthesis and not a hereditary change.
What did Avery, McLeod and McCarty experiment prove?

• They demonstrated that the transforming principle was DNA and not protein.
Experiments

- Avery-Macleod-McCarty experiment
  - This experiment demonstrated that the transforming principle was DNA and not protein in 1944. First a purification procedure was performed, consisting of killing type IIIS bacteria with heat. Next, the protein was removed using chloroform, and the polysaccharide capsules were destroyed with an enzyme. Then, the active portion was precipitated out by alcohol fractionation and removed in the form of fibrous rods. Chemical analysis showed that the proportions of carbon, hydrogen, nitrogen, and phosphorus in this active portion were consistent with the chemical composition of DNA. Next the enzymes trypsin, chymotrypsin and RNase were used to destroy all proteins and remaining RNA. After all this the transforming activity still remained until treated with the DNA destroying enzyme DNase.
What was the Hershey Chase experiment and what did it prove?

- **Hershey and Chase** (1952), using *Escherichia coli* and an infecting virus (bacteriophage T2), demonstrated that DNA, and not protein, is the genetic material.
- Using radioisotope $^{32}$P (labels DNA) and $^{35}$S (labels protein), Hershey and Chase demonstrated that DNA enters the bacterial cell during infection and directs viral reproduction.
• Hershey and Chase
  • This experiment used Escherichia coli and bacteriophage T2. It demonstrated that DNA, and not protein, is the genetic material. Radioisotope $^{32}$P labels DNA and $^{35}$S labels protein. The phage’s $^{32}$P DNA enters the bacteria during infection and not $^{35}$S.
  • Using radioisotope $^{32}$P (labels DNA) and $^{35}$S (labels protein), Hershey and Chase demonstrated that DNA enters the bacterial cell during infection and directs viral reproduction.
What did Chargaff demonstrate in analysis of DNA nucleotides in different species?

- **Chargaff** (1949–1953) showed that the amount of A is proportional to T and the amount of C is proportional to G, but the percentage of C + G does not necessarily equal the percentage of A + T.

- The proportions change from species to species.
If A = 23% what is T, G and C?

- A = T; therefore T = 23%
- A + T + G + C = 100%; therefore 23 + 23 + G + C = 100%
  - G + C = 54%
- G = C; therefore C = 27% and G = 27%

\[ \text{% A} = \text{% T} \]
\[ \text{% G} = \text{% C} \]
\[ \text{% A} + \text{% T} + \text{% G} + \text{% C} = 100\% \]

Calculating %
- If A = 10%, then what is T, G, C?
  - T = 10%
- %G + %C = 80%; so G = 40% and C = 40%.*
EXPERIMENTS

• Chargaff
  • DNA from any cell of all organisms should have a 1:1 ratio (base Pair Rule) of pyrimidine and purine bases and, more specifically, that the amount of guanine is equal to cytosine and the amount of adenine is equal to thymine.
260nm vs. 280nm as part proof that DNA is the hereditary material

- **UV light** is capable of inducing mutations in the genetic material and is most mutagenic at a wavelength of 260 nm.
- **DNA and RNA** absorb UV light most strongly at 260 nm, but protein absorbs most strongly at 280 nm, a wavelength at which no significant mutagenic effects are observed.
- Again, this provides **indirect evidence for DNA as the genetic material.**
What did the Meselson Stahl experiment prove?

• The Meselson-Stahl experiment demonstrated that:
  • DNA replication is semiconservative
  • Each new DNA molecule consists of one old strand and one newly synthesized strand.

Meselson and Stahl (1958), using $^{15}$N-labeled *E. coli* grown in medium containing $^{14}$N, demonstrated that:

- DNA replication is semiconservative in prokaryotes
- each new DNA molecule consists of one old strand and one newly synthesized strand

<table>
<thead>
<tr>
<th>Predictions</th>
<th>Conservative</th>
<th>Semiconservative</th>
<th>Dispersive</th>
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<td><strong>First replication</strong></td>
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<td><strong>Second replication</strong></td>
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What was Taylor-Woods-Hughes experiment on eukaryotic chromosomes?

• The Taylor-Woods-Hughes experiment demonstrated that DNA replication is semiconservative in eukaryotes.
EXPERIMENTS

• Taylor – Woods – Hughes experiment
  • This experiment demonstrated that DNA replication is semiconservative in eukaryotes. The root tips of the Vicia fabia was used because it provided an excellent source of dividing cells. Monitored replication by labeling with H3-thymidine (radioactive)
DNA Replication

DNA Replication (Semiconservative)

- Stabilizer Proteins
- Leading Strand
- DNA Polymerase
- Replication Fork
- RNA Primer
- RNA Polymerase
- Okazaki Fragment
- DNA Ligase
- Parent Strand
- Lagging Strand
- Helicase
- Gyrase

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DNA Replication
DNA Replication

A SUMMARY OF DNA REPLICATION

1. Helicases unwind the parental double helix.
2. Single-strand binding proteins stabilize the unwound parental DNA.
3. The leading strand is synthesized continuously in the 5’ → 3’ direction by DNA polymerase.
4. The lagging strand is synthesized discontinuously. Primase synthesizes a short RNA primer, which is extended by DNA polymerase to form an Okazaki fragment.
5. After the RNA primer is replaced by DNA (by another DNA polymerase, not shown), DNA ligase joins the Okazaki fragment to the growing strand.

DNA polymerase
RNA primer
Okazaki fragment being made
Primase
REPLICATION FORK
Parental DNA
DNA polymerase
DNA ligase
Overall direction of replication
<table>
<thead>
<tr>
<th>Across</th>
</tr>
</thead>
<tbody>
<tr>
<td>4. The RNA that codes for sequence of amino acids is the ___RNA</td>
</tr>
<tr>
<td>6. DNA that is uncoiled and active.</td>
</tr>
<tr>
<td>10. Chemist who showed that %A = %T and %C = %T.</td>
</tr>
<tr>
<td>12. A mutation that changes DNA sequence but does not change amino acid sequence</td>
</tr>
<tr>
<td>18. Meselson-Stahl experiment with N14 and N15 proved that DNA replicates ____-conservatively.</td>
</tr>
<tr>
<td>19. The ___ strand of DNA is made continuously.</td>
</tr>
<tr>
<td>21. At the 3’ end of mRNA in eukaryotes is a _______ A tail</td>
</tr>
<tr>
<td>23. ___ cells make gametes; so mutations in these cells would be passed to the next generation.</td>
</tr>
<tr>
<td>24. ___ mutation changes only one amino acid</td>
</tr>
<tr>
<td>27. ___ mutations happen naturally</td>
</tr>
<tr>
<td>28. ___ mutations result in death of the organism.</td>
</tr>
<tr>
<td>29. If a purine is replaced by a pyrimidine then a ___ has occurred.</td>
</tr>
<tr>
<td>30. DNA ligase joins ________________ fragments together.</td>
</tr>
<tr>
<td>31. ___ mutations are expressed or not expressed depending on environment.</td>
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</tbody>
</table>
CROSSWORD

Down
1. short piece of RNA made by enzyme to provide DNA polymerase with a 3’ hydroxyl
2. Adenines and guanines are ___.
3. ___ is DNA that remains condensed and inactive like telomere and centromere.
4. adds repeated units of DNA to the ends of chromosomes.
5. Nitrogenous base unique to RNA.
6. change in one base of DNA that creates a premature stop codon
7. The __ strand of DNA is made discontinuously
8. The enzyme that makes DNA and RNA both grow in the 5’ to 3’ direction, which means they add nucleotides to the 3’ ___ (not “end” but name of functional group)
9. Cytosines, thymines and uracils are ___.
10. Monomers of DNA made of a nitrogenous base, a pentose sugar and a phosphate group
11. enzyme that unwinds DNA helix during replication.
12. Plus or minus one or two nucleotides.
13. Factory site where proteins are synthesized (singular form of word.)
14. If a pyrimidine replaces a pyrimidine (or purine replaces a purine) then ___ has occurred.
15. releases tortional strain as DNA helix is opened up.
16. Mutations in ___ cells of the body are not inheritable.
17. Hershey chase used radioactive ___ to label proteins.
CONCEPT QUESTIONS

• The RNA that codes for sequence of amino acids is the ___RNA
  • Messenger
• DNA that is uncoiled and active.
  • Euchromatin
• Chemist who showed that %A = %T and %C = %G.
  • Chargaff
• A mutation that changes DNA sequence but does not change amino acid sequence.
  • Silent
CONCEPT QUESTIONS

• Meselson-Stahl experiment with N14 and N15 proved that DNA replicates __________-conservatively.
  • Semi
• The _____ strand of DNA is made continuously.
  • Leading
• At the 3’end of mRNA in eukaryotes is a ______ A tail.
  • Poly
• ___ cells make gametes; so mutations in these cells would be passed to the next generation.
  • Germ
CONCEPT QUESTIONS

• ____ mutation changes only one amino acid.
  • Missense
• ____ mutations happen naturally
  • Spontaneous
• ____ mutations result in the death of the organism.
  • Lethal
• If a purine is replaced by a pyrimidine then a ____ has occurred.
  • Transversion
CONCEPT QUESTIONS

• DNA ligase joins _______ fragments together.
  • Okazaki
• ______ mutations are expressed or not expressed depending on environment.
  • Conditional
• Short piece of RNA made by enzyme to provide DNA polymerase with a 3’ hydroxyl
  • Primer
• Adenines and guanines are _____.
  • Purines
CONCEPT QUESTIONS

• ___ is DNA that remains condensed and inactive like telomere and centromere.
  • Heterochromatin
• Adds repeated units of DNA to the ends of chromosomes.
  • Telomerase
• Nitrogenous base unique to RNA.
  • Uracil
• Change in one base of DNA that creates a premature stop codon.
  • Nonsense (mutation)
CONCEPT QUESTIONS

• The _____ strand of DNA is made discontinuously.
  • Lagging

• A enzyme makes DNA and RNA both read in the 5′ to 3′ direction, which means the enzyme adds nucleotides to the 3′_____.
  • Hydroxyl

• Cystosines, thymines and uracils are ________.
  • Pyrimidines

• Monomers of DNA made up of a nitrogenous base, a pentose sugar and a phosphate group.
  • Nucleotides
CONCEPT QUESTIONS

- Enzyme that unwinds DNA helix during replication.
  - Helicase
- Plus or minus one to two nucleotides.
  - Frameshift (mutation)
- Factory site where proteins are synthesized.
  - Ribosome
- If a pyrimidine replaces a pyrimidine (or purine replaces a purine) then ____ has occurred.
  - Transition
CONCEPT QUESTIONS

• Releases tortional strain as DNA helix is opened up.
  • (DNA) Gyrase
• Mutations in ____ cells of the body are not inheritable.
  • Somatic
• Hersey Chase used radioactive ____ to label proteins.
  • Sulfur \((^{35}\text{S})\)
WORD SEARCH

• Three models of DNA replication
  • Conservative
  • Semi-conservative
  • Dispersive
• DNA ____ elongate an existing DNA strand but cannot initiate DNA synthesis.
  • Polymerase
• DNA ____ is a member of a larger group of enzymes referred to as DNA topoisomerase.
  • Gyrase
• The enzyme ____ synthesizes an RNA primer
  • Primase
• The lagging strand is synthesized as _______ fragments.
  • Okazaki
WORD SEARCH

• DNA _____ covalently connects two adjacent segments of DNA
  • Ligase
• ______ separates DNA strands or opens DNA helix.
  • Helicase
• The length of DNA that is replicated following one initiation event at a single origin is called a _______.
  • Replicon
• Gene _____ is characterized by nonreciprocal genetic exchange between two closely linked genes.
  • Conversion
• In genetic recombination there involves a duplex separation to generate the characteristic known as the ____ structure.
  • Holliday
CONCEPT QUESTIONS

• Watson and Crick proposed DNA is a right-handed double helix in which the two strands are ________ and the bases are stacked on one another.
  • Antiparallel

• _______ at the ends of linear chromosomes consist of long stretches of short repeating sequences and preserve the integrity and stability of chromosomes.
  • Telomeres

• _____ excision repair (NER) repairs bulky lesions and involves the uvr genes
  • Nucleotide
CONCEPT QUESTIONS

• Chromatin is bound up in nucleosomes with positive charged proteins called _________________.
  • Histones
• DNA __________ covalently connects two adjacent segments of DNA pieces.
  • Ligase
• Bacterial chromosomes are double-stranded DNA and compacted into a ______
  • Nucleoid
• ______ remodeling must occur to allow the DNA to be accessed by DNA binding proteins.
  • Chromatin
CONCEPT QUESTIONS

• Amino Acid template for amino sequence
  • Messenger RNA (mRNA)

• RNA component of ribosome
  • Ribosomal RNA (rRNA)

• Carries amino acid for protein synthesis
  • Transfer RNA (tRNA)

• Phosphate group end; DNA polymerase ends; stationary end
  • 5’

• Hydroxyl end; DNA polymerase starts; DNA polymerase starts, growing end
  • 3’
CONCEPT QUESTIONS

• This strand 5’ end next to 3’
  • Antiparallel

• Retroviruses; RNA serves as template for synthesis of DNA by the RNA-dependent DNA polymerase; DNA\(\rightarrow\)RNA
  • Reverse transcriptase

• Building blocks of DNA; made of monomers (nitrogenous base, pentose sugar and phosphate group)
  • Nucleotides

• Hypothesis for DNA replication that; original helix is conserved, 2 newly synthesized strands come together
  • Conservative

• Hypothesis for DNA replication that; 1 old and 1 new strand with each replicated DNA molecule; Meselson-Strahl proved this in their experiment
  • Semi-conservative
CONCEPT QUESTIONS

• Hypothesis for DNA replication that; parental strands are dispersed into 2 new double helixes
  • Dispersive
• Thymine, cytosine, uracil; small bases
  • Pyrimidines
• Adenine, guanine; big bases
  • Purines
• Bacterial DNA and Eukaryotes have what type of replication
  • Bidirectional replication
• Length of DNA that is replicated following one initiation event at a single origin; whole bacterial genome is considered one of these
  • Replicon
CONCEPT QUESTIONS

• DNA synthesis originates at a single point which is called
  • Origin of replication (OriC in E. Coli)
• During DNA denaturation is used to determine the melting temperature
  • Hyperchromic shift
• Provides information about the size and complexity of genomic DNA from an organism
  • Reassociation kinetics
• Possess 3’ to 5’ exonuclease activity, allowing them to proofread newly synthesized DNA and remove and replace incorrect nucleotides; they can not initiate DNA synthesis; 5’-3’ polymerization
  • DNA Polymerases I, II, and III
• Demonstrates 5’-3’ exonuclease activity, excising RNA primers and filling in the gaps left behind
  • DNA Polymerase I
CONCEPT QUESTIONS

• Has only 5’-3’ polymerization and 3’-5’ exonuclease activity (proofreading)
  • DNA polymerase II
• Primary synthesis cells DNA, also has 5’-3’ polymerization and 3’-5’ exonuclease activity
  • DNA polymerase III
• Made by enzyme primase (short RNA) at the 5’end
  • Primer
• Enzyme for primer, synthesizes RNA primer provides the free 3’ hydroxyl
  • Primase
• Stabilize the open conformation; keeps the DNA single stranded
  • Single-stranded binding proteins (SSBPs)
CONCEPT QUESTIONS

• Separates DNA strands or opens DNA helix
  • Helicase

• Relieves torsional strain; supercoiling when DNA opens.
  • DNA gyrase

• This strand of DNA is made continuously
  • Leading

• This strand of DNA is made discontinuously
  • Lagging

• The lagging strand is synthesized, each with an RNA primer
  • Okazaki fragments
CONCEPT QUESTIONS

• Covalently connects two adjacent segments of DNA pieces (Okazaki Fragments)
  • DNA ligase
• Adds repeated units of DNA to the ends of chromosomes
  • Telomerase
• This process involves; endonuclease nicking, strand displacement, ligation, branch migration, duplex separation to generate the characteristic Holiday structure
  • Genetic recombination
• Nonreciprocal genetic exchange between two closely linked genes
  • Gene conversion
• Double stranded molecule of nucleic acid originated through the genetic recombination of single complementary strands derived from different sources.
  • Heteroduplex
CONCEPT QUESTIONS

• Bacterial chromosomes are compacted in an irregularly shaped region within the cell of a prokaryote that contains all or most of the genetic material
  • Nucleoid
• Bound up in nucleosomes with Histones: H2A, H2B, H3, and H4; located in the nucleus of Eukaryotic cells
  • Chromatin
• (+) charged proteins complexed with DNA in the nucleus; function in coiling DNA to form nucleosomes
  • Histones
• Acetylation, methylation, phosphorylation are important for histone modification
  • Histone tails
• Any of the repeating subunits of chromatin occurring at intervals along a strand of DNA, consisting of DNA coiled around a histone
  • Nucleosomes
CONCEPT QUESTIONS

- Uncoiled and active in gene expression
  - Euchromatin
- Condensed and is inactive in gene expression; centromeric and telomeric DNA
  - Heterochromatin
- Highly repetitive and consists of short repeated sequences. Isolates at different density from other DNA in cell; binds to centromeric DNA
  - Satellite DNA
- Primary constrictions along Eukaryotic chromosomes; mediate chromosomal migration during mitosis and meiosis; kinetochore proteins are the region that bind to the spindle fibers during cell division
  - Centromere
- Sequences short tandem repeats, stability, and integrity of the chromosome
  - Telomere
CONCEPT QUESTIONS

- Includes some genes like rRNA genes; variable number tandem repeats (VNTRs); mini and micro satellites
  - Moderately repetitive DNA
- Results from influence of an extraneous factor, either natural or artificial; radiation, UV light, natural and synthetic chemicals
  - Induced mutations
- Happen naturally and randomly and are usually linked to normal biological or chemical processes in the organism
  - Spontaneous mutations
- Occur in any body cell except germ cells and not heritable
  - Somatic mutations
- Occur in cells that make gametes and are inherited
  - Germ-line mutations
CONCEPT QUESTIONS

- Occur within genes located on the autosomes; recessive: you won't see a detectable phenotype, dominate: mutations will be expressed phenotypically in the first generation.
  - Autosomal mutations

- Occur within genes located on the X chromosome; recessive: arising in the gametes of a homogametic female may be expressed in hemizygous male offspring
  - X-linked mutations

- Base substitutions in which ONE base pair is altered
  - Point mutations

- Results from insertions or deletions of a base pair; +/- one or two nucleotides
  - Frameshift mutation

- Changes only one amino acid
  - Missense mutation
CONCEPT QUESTIONS

• Change in one base of DNA that creates a premature stop codon
  • Nonsense mutation
• Changes DNA sequence but does not change amino acid sequence
  • Silent mutation
• Interrupt an essential process and result in death of organism; example Tay-Sachs, Huntington chorea disease
  • Lethal mutation
• No change in fitness of the organism
  • Neutral mutation
• Depends on the environment in which the organism finds itself; example temperature sensitive mutation, Himalayan rabbits.
  • Conditional mutation
CONCEPT QUESTIONS

- Pyrimidine replaces pyrimidine or purine replace purine
  - Transition mutation
- Purine interchanges with a pyrimidine or vice versa
  - Transversion mutation
- Mutation that produces a phenotype different from that of the normal allele; mutant to wild type
  - Gain of function mutation
- Mutation that produces alleles with reduced or no function; mutant protein binds to normal protein
  - Loss of function mutation
- Alternate chemical forms that differ by a single proton shift in the base; temporary shift, can change the bonding structure allowing for non-complementary base pairing
  - Tautomeric shift
CONCEPT QUESTIONS

• Removes a purine
  • Depurination
• Takes an amino acid off the base; cytosine forms uracil
  • Deamination
• Can disrupt the reading frame; transposable genetic elements that can move within or between genomes
  • Transposons
• Natural or artificial agents that induce mutations; fungal toxins, x-rays, cosmic rays
  • Mutagens
• A lesion consisting of two adjacent thymine bases that become joined by a covalent bond. Caused by exposure to UV light, inhibits DNA replication
  • UV thymine dimers
CONCEPT QUESTIONS / DEFINITIONS

- Uses a number of different strains of *Salmonella typhimurium* selected for their ability to reveal the presence of specific types of mutations
  - Ames Test
- Homologous recombination
  - Genetic exchange at equivalent positions along two chromosomes with substantial DNA sequence homology.
- Pol α
  - Functions in synthesis of the RNA primer during initiation on the leading and lagging strands in eukaryotes.
  - Possesses low processivity
- Pol δ
  - Synthesizes the lagging strand in eukaryotes.
- Pol ε
  - Synthesizes the leading strand in eukaryotes.
CONCEPT QUESTIONS / DEFINITIONS

• C banding and G banding

  • Mitotic chromosomes have a characteristic banding pattern.
  • In C-banding, only the centromeres are stained (Figure 12.11).
  • G-banding is due to differential staining along the length of each chromosome (Figure 12.12).
  • The differential staining reactions reflect the heterogeneity and complexity of the chromosome.
  • The unique banding pattern allows the distinction of identical-sized chromosomes and centromere placement.
  • This precision allows homologs to be distinguished from one another, including translocated segments.
Questions

Prepared and Compiled from various sources by
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The Academic Support Center @ Daytona State College
http://www.daytonastate.edu/asc/ascsciencehandouts.html