

CYTOGENETICS

The Nucleus

- The nucleus is separated from the cytoplasm by the nuclear envelope, a membrane system that consists of two concentric membranes, one layered just inside the other.
- The outer membrane faces the cytoplasm, and the inner membrane, separated from the outer membrane by a narrow space, faces the nuclear interior.
- The nuclear envelop is perforated by pores that form openings some 70 to 90 nm wide through both membranes.

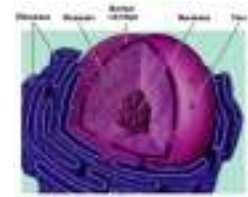
- The pores are filled by a ring like mass of proteins, the **annulus**, which controls movement of larger molecules such as DNA and Proteins through the nuclear envelope.
- Most of the space inside the nucleus is occupied by masses of very fine, irregularly folded chromatin fibers, about 10 to 30 nm in thickness, which contain the nuclear DNA.

Monday, October 28, 2013

3

Nucleolus (Nucleoli)

- The nucleolus is a subpart of the chromatin specialized for assembly of ribosomal subunits.
- The RNA of ribosomes is synthesized from genes in the nucleolus; ribosomal proteins are synthesized in the cytoplasm and assembled with ribosomal RNA into ribosomes in the nucleolus.
- No membranes separate nucleoli from the surrounding chromatin in the nucleus.



Control Center

- The nucleus is the ultimate control center for cell activities.
- Within the chromatin the information required to synthesize cellular proteins is coded into the DNA.
- Each DNA segment containing the information in a protein constitutes a gene.
- The information in a Protein-encoding gene is copied into a messenger RNA (mRNA) molecules that moves to the cytoplasm through the pores of the nuclear envelop.

- In the cytoplasm, mRNA molecules are used by ribosomes as directions for the assembly of proteins.
- Other DNA regions store the information for making additional RNA types (rRNA and tRNA) that carry out accessory roles in protein synthesis and other functions in the nucleus and cytoplasm.

Monday, October 28, 2013

6

Duplication of Chromatin

- A second major function of the nucleus involves duplication of the chromatin as part of cell reproduction.
- Just before cell division, all the components of chromatin, including both DNA and chromosomal proteins, are precisely doubled.

- The duplication of DNA in chromatin is known as replication.
- During cell division the two copies of each duplicated chromosome are precisely separate so that the two cells resulting from the division each receive a complete set of chromosomes and genes.

Monday, October 28, 2013

8

Cell Division

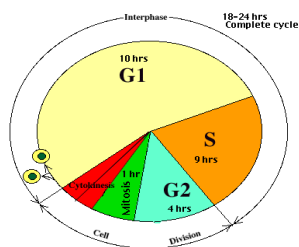
- Cells can divide to produce identical copies by packaging a complete set of genetic information into each new cell.
- This information is contained in the chromosomes.
- A eukaryotic cell contains a nucleus so the process of cell division involves nuclear division, called mitosis, and division of the cytoplasm, called cytokinesis.
- In mitosis, the daughter (or progeny) cells contain the same genetic information as the parent cell.

Sister Chromatids

- Sister chromatids are genetically identical. Each chromosome has replicated and folded before cell division begins.
- In eukaryotic cell division each sister chromatid of a replicated chromosome separates from its partner.
- This assures each daughter cell of receiving a complete set of chromosomes.

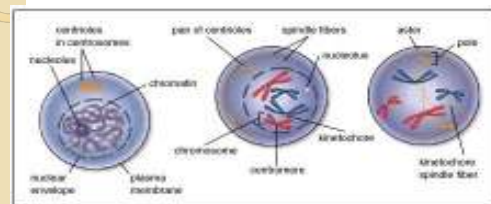
Mitosis/Cell Cycle

- Mitosis is one of the stages in the life cycle of a cell. It refers to the division of the nucleus.



Early Stages of Mitosis

- In the early stages of mitosis, the spindle forms and kinetochores of chromosomes attach to spindle fibers.



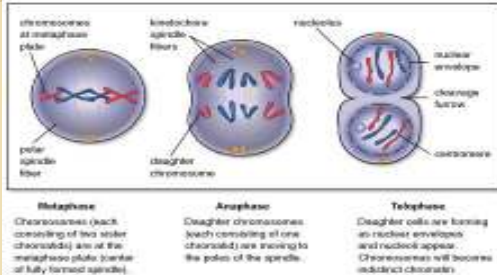
G₂ of interphase
Chromosomes and centrioles have duplicated in preparation for mitosis.

Prophase
Chromosomes are now distinct; centrosomes begin moving apart and nuclear envelope is fragmenting.

Prometaphase
Spindle is in process of forming and kinetochores of chromosomes are attaching to kinetochore spindle fibers.

Separation of Sister Chromatids

- Once chromosomes are in the center of the spindle, the sister chromatids separate and move in opposite directions.
- At the end of mitosis there are 2 nuclei, each with a



Process of Mitosis

- Mitosis is the process by which the contents of the eukaryotic nucleus are separated into 2 genetically identical packages.
- Chromosomes replicate prior to the beginning of mitosis.
- As mitosis begins they condense and become visible they appear as sister chromatids joined at the centromere. Mitosis is divided into 4 stages.
- During prophase, the nuclear envelope disintegrates and a spindle of microtubules forms.
- Centrioles may help organize the spindle as in this animal cell.

- The chromosomes begin to move toward the midplane of the spindle with centromeres attached to spindle fibers, and metaphase has been reached.
- Metaphase yields to anaphase as the centromeres separate and the sister chromatids, now termed chromosomes, are pulled toward opposite poles of the spindle.
- During the final stage, telophase, a nuclear envelope forms around each set of chromosomes, the spindle disappears and the chromosomes decondense.
- The result is 2 nuclei, each with an identical set of chromosomes.

Monday, October 28, 2013

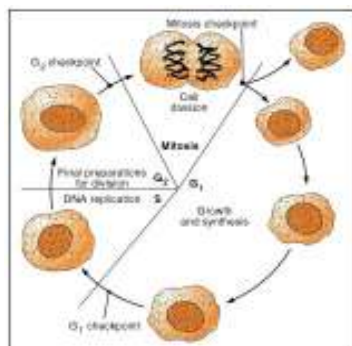
15

Cytokinesis

- Cytokinesis is the division of the cell contents outside of the nucleus. It occurs with both mitosis and meiosis.
- In cells without walls, it is accomplished by pinching of the cell.
- In plant cells, the wall prevents pinching; instead vesicles line up along the middle of the cell.
- As they fuse they form the separation between daughter cells.

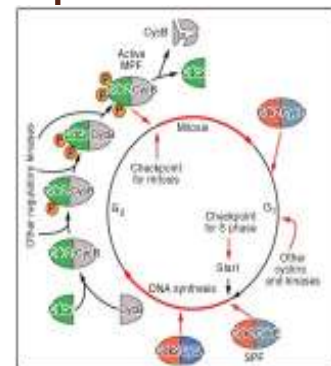
G₁ Phase of Interphase

- Once mitosis is complete, the cell cycle enters G₁.
- The transition from one stage to the next is controlled by checkpoints at which conditions are assessed before the cell proceeds to the next stage.



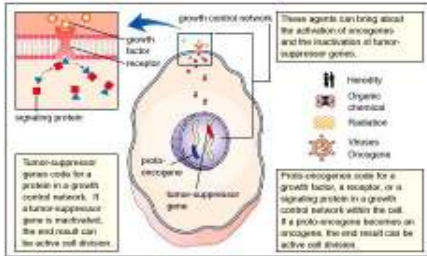
Cell Cycle Checkpoints

- The G₁ and G₂ checkpoints involve a protein called Cdk.
- When bound to another protein called cyclin, Cdk acts as an enzyme.
- During G₂, for example, cyclin accumulates and binds to a specific Cdk protein called Cdc2 to form MPF, mitosis promoting factor. When a threshold level of MPF is reached, mitosis begins.



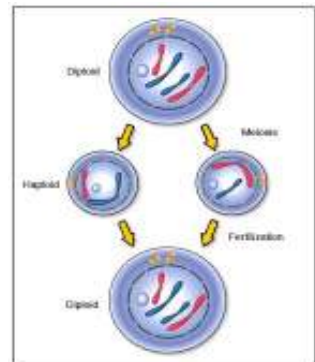
Oncogenes

- The genes that code for growth factor receptors, or regulatory proteins, are called proto-oncogenes.
- A mutation in a proto-oncogene can result in an oncogene.
- Oncogenes are cancer genes because they lead to uncontrolled cell division.
- Tumor suppressor genes code for regulatory proteins that limit reactions leading to cell division. Inactivation of tumor suppressor genes can also lead to uncontrolled cell division.



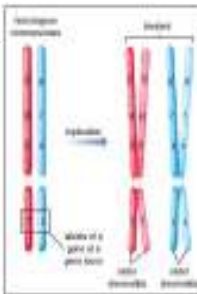
Meiosis

- Sexually reproducing organisms are diploid; they have two of every type of chromosome.
- Prior to sexual reproduction meiosis reduces the number of chromosomes by half and fertilization restores the original number.



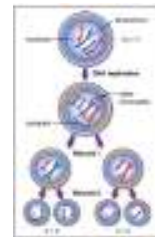
Homologous Chromosomes

- Chromosomes come in homologous pairs in diploid organisms.
- Homologues carry the same genes, but not necessarily the same forms or alleles of those genes.
- Unless the cell is dividing they are found in an unduplicated state.
- One homologue is inherited from each parent.
- The one from the father is termed the paternal chromosome.
- The one from the mother is the maternal chromosome.
- At the beginning of cell division the DNA replicates and each chromosome is now a pair of identical sister chromatids.



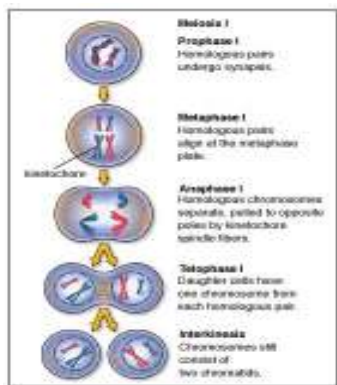
Ultimate Goal of Meiosis

- The ultimate goal of the process of meiosis is to reduce the number of chromosomes by half.
- This must occur prior to sexual reproduction.
- The cell at the top contains two homologous pairs of chromosomes, for a total of four chromosomes.
- The final products of meiosis, four daughter cells, each contain one chromatid from each original homologous pair, for a total of two chromosomes.

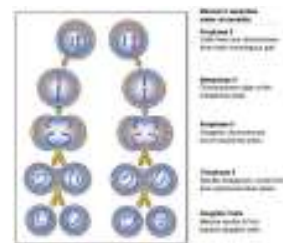


Overview of Meiosis I

- Meiosis I reduces the number of chromosomes by half, but each chromosome still contains two sister chromatids.



Overview of Meiosis II



Process of Meiosis

- Meiosis is the process by which a diploid nucleus divides twice to produce 4 haploid nuclei., the divisions are called meiosis I and meiosis II.
- In the life cycles of diploid organisms meiosis precedes sexual reproduction.
- Among animals, the products of meiosis are gametes—eggs or sperm. DNA is replicated prior to the start of meiosis. The identical sister chromatids are joined at the centromere as in mitosis.
- Unlike in mitosis, homologous chromosomes pair with one another.
- These pairs intertwine during early prophase of the first meiotic division and may exchange segments.

- This exchange is called crossing over.
- During prophase I, the nuclear envelope disappears and the spindle forms.
- The homologous pairs lie side by side as they reach the midplane of the spindle and attach to spindle fibers in Metaphase I.
- Metaphase ends and Anaphase I begins as the partners in each pair of homologous chromosomes separate as they are pulled toward opposite poles of the spindle.
- These chromosomes still consist of sister chromatids joined at their centromeres.

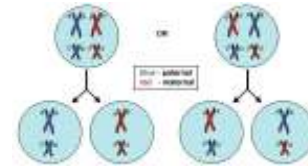
Monday, October 28, 2013

26

- During Telophase I the spindle disappears, nuclear membranes may re-form and the 2 nuclei, each containing a haploid set of chromosomes, are separated as cytokinesis divides the cytoplasm.
- Prophase II begins with the formation of a spindle and the still duplicated chromosomes move toward its mid-plane.
- At Metaphase II they are lined up and attached to spindle fibers. Anaphase II begins when centromeres separate and sister chromatids, now considered chromosomes, begin moving in opposite directions.
- During Telophase II the nuclear membrane re-forms, the spindle disappears and cytokinesis divides the cytoplasm.
- The result is 4 haploid cells.

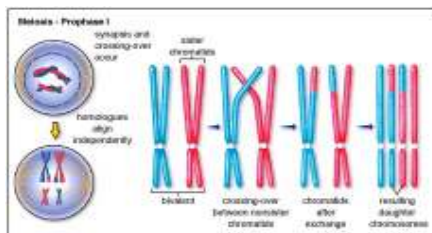
Recombination

- Recombination is the formation of new allele combinations in a gamete. It results from two events in meiosis, independent assortment and crossing over. Independent assortment occurs in meiosis I when each pair of homologous chromosomes lines up on the metaphase plate.
- Each pair lines up independently of other pairs. In each pair the paternal chromosome may be on the left or right.
- The number of possible combinations of maternal and paternal chromosomes in the nuclei produced by meiosis equals 2 raised to the power of n, where n is the number of pairs of chromosomes.
- For the 23 pairs of human chromosomes this amounts to over 8 million combinations.



Synapsis

- When homologous chromosomes undergo synapsis during prophase I, equivalent sections may be exchanged between nonsister chromatids.
- This process, called crossing-over, further adds to variability among the products of meiosis.



Alleles

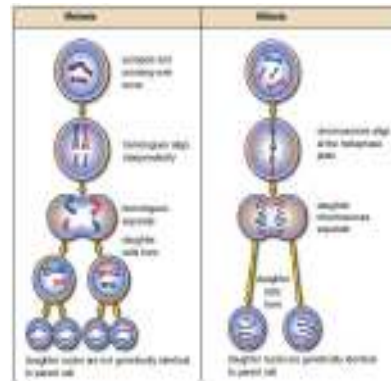
- Consider two homologous chromosomes, one with dominant alleles at all gene loci and the other with recessive alleles at those loci. In prophase I of meiosis, homologous chromosomes line up side by side in a process called synapsis.
- The alignment is very precise and places the genes on one homologue opposite the equivalent genes on the other homologue.
- While together, the chromatids of different homologues overlap in one or more places.

- At the point of overlap an exchange of chromosomal pieces takes place. This is called crossing-over.
- This exchange is reciprocal; equivalent pieces of chromosomes are exchanged.
- Although the exchanged pieces have the same genes, they may not have the same alleles.
- After crossing-over, 2 of the 4 chromatids have dominant alleles at some loci and recessive alleles at others.
- This recombination of alleles is a major source of genetic variability in populations.

Monday, October 28, 2013

31

Comparison of Cell Division



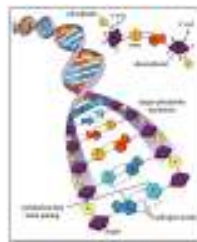
Evolution of Sex

- Most protists and many multicellular organisms have means of reproducing without sex.
- In other words, they skip meiosis and/or fertilization.
- They fragment, send out runners, or their unfertilized eggs are capable of cell division.
- Evolutionary biologists are interested in the question of why sex evolved.
- Meiosis and fertilization introduce genetic variability.
- In a changing environment, this increases chances that some offspring will survive.
- Genetic information is reshuffled during meiosis, producing genetic diversity in populations.
- A diploid cell contains two sets of chromosomes.
- The maternal set was contributed by the mother and the paternal set was contributed by the father.
- A pair of homologous chromosomes consists of one maternal and one paternal chromosome.
- Homologous chromosomes carry the same genes but may have different forms or alleles of the genes.

- During prophase of meiosis, homologous chromosomes pair and non-sister chromatids exchange sections of DNA through the process known as crossing-over or recombination.
- The resulting chromosomes may now contain different combinations of alleles than were found in the chromosomes inherited from the parents.
- During metaphase I of meiosis, the maternal and paternal chromosomes of one homologous pair align independently of the maternal and paternal chromosomes of the other homologous pairs.
- The orientation of homologs in two different cells undergoing meiosis is shown.
- Genes that are located on different chromosomes undergo independent assortment because of the random alignment of the maternal and paternal chromosomes.
- Gametes produced by meiosis have different combinations of alleles as a result of both recombination and independent assortment.

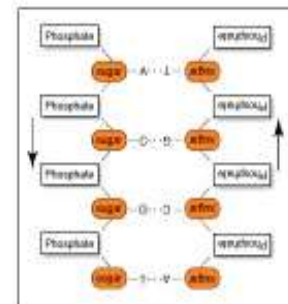
DNA Structure

- DNA, the genetic material, is a macromolecule made of monomers called nucleotides.
- A DNA molecule resembles a twisted ladder.
- A sugar-phosphate backbone forms the sides of the ladder.
- The phosphate group of one nucleotide is covalently bonded to a hydroxyl group on the sugar of the next.
- Hydrogen bonding between bases creates the rungs of the ladder. Each strand has a free phosphate group at the 5' end and a free hydroxyl group at the 3' end.
- The numbers refer to the carbons in the sugar part of each nucleotide.



Complementary Base Pairings

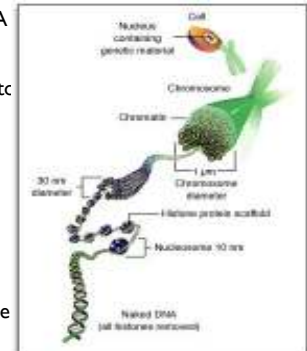
- The two strands of the DNA molecule run in opposite, or anti-parallel, directions. Complementary base pairing dictates that A always bonds with T and G always bonds with C.



- DNA is composed of 2 strands of nucleotides wound around one another in a double helix.
- The outer longitudinal structure consists of alternating sugars and phosphate groups.
- Paired nitrogenous bases are in the interior of the molecule. If the molecule unwinds, its structure resembles a ladder with phosphates and sugars forming the sides.
- The rungs are the nitrogenous bases of nucleotides on opposite sides of the ladder.
- There are 4 different bases and only certain pairs are possible in forming the rungs.
- Adenine is always opposite thymine and guanine is always opposite cytosine.
- These complementary pairs of nitrogenous bases lie next to one another because their sugar-phosphate chains run in opposite directions.
- This anti-parallel orientation of the two nucleotide strands brings the bases into position for hydrogen bond formation.
- The sides of the ladder are twisted in a spiral fashion and make a complete turn every 10 base pairs.

Chromosomes

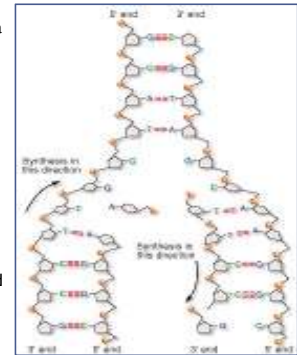
- In order for the long DNA molecules to fit into the **nucleus** of the cell it must be coiled and packaged into **chromosomes**.
- The DNA double helix wraps around proteins called histones.
- These DNA/protein complexes are called nucleosomes. The nucleosomes coil and loop into the chromosome structure shown here.



- DNA molecules are extremely long, thin and delicate.
- They are packaged so that they can fit in the nucleus and be protected from damage.
- DNA is attracted to clusters of histone proteins.
- A portion of the DNA wraps around a cluster twice. This occurs at regular intervals along the molecule.
- This shortens the DNA so it can fit within the nucleus. The chromosomes are still fairly long and their movement during cell division is facilitated by condensation.
- Following replication they loop and fold back on themselves forming tight packages of DNA and histone.
- This shortens and thickens the chromosomes so they are visible under a microscope at the beginning of prophase.

Template

- During replication of the DNA molecule, each strand serves as a template for the assembly of a new strand.
- The DNA strands separate, and free, unattached nucleotides associate with their complementary bases through hydrogen bonding.
- DNA polymerase, the enzyme that adds nucleotides to the newly forming strand, can add them only at the 3' end. Ultimately, two DNA duplexes are formed, each containing one old strand and one newly formed strand.
- This is called semiconservative replication.



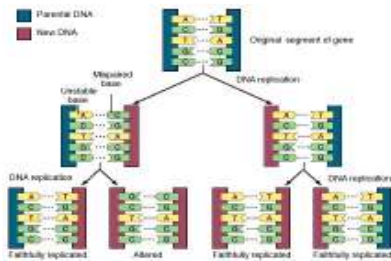
The Rule of Base Pairing

- The rule of base pairing helps explain how **DNA** is replicated prior to cell division. Enzymes unzip the DNA by breaking the hydrogen bonds between the base pairs. The unpaired bases are now free to bind with other nucleotides with the appropriate complementary bases. The enzyme Primase begins the process by synthesizing short primers of **RNA** nucleotides complementary to the unpaired DNA. DNA polymerase now attaches DNA nucleotides to one end of the growing complementary strand of nucleotides.

- Replication proceeds continuously along one strand, called the leading strand, which is shown here on the left.
- The process occurs in separate short segments called Okazaki fragments next to the other, or lagging, strand on the right.
- This difference is due to the fact that DNA polymerase can only add new nucleotides to the 3 prime end of a nucleotide strand.
- A primer begins any new strand, including each Okazaki fragment.
- An enzyme replaces the RNA primer with DNA nucleotides. Then an enzyme called DNA ligase binds the fragments to one another.
- There are now 2 DNA molecules. Each consists of an original nucleotide strand next to a new complementary strand.
- The two molecules are identical to each other.

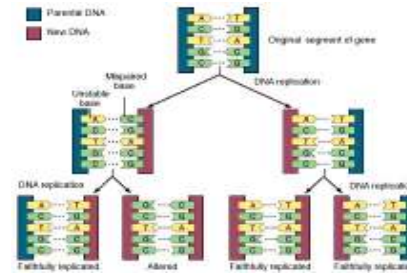
Nucleotide Mispairings

- Sometimes during DNA replication the wrong nucleotide may be inserted.
- In the case illustrated, a nucleotide with a C base has been incorrectly paired with a nucleotide on the original strand with an A base.



DNA Repair Mechanisms

- There are DNA repair mechanisms that catch most of these mispairings, but some get by.
- The result is some copies of the DNA with an altered genetic message called a mutation.



Recombination

- These four double-stranded DNA molecules represent replicated copies of two homologous chromosomes carrying different alleles for genes A and B.
- Recombination between two of these molecules begins when an endonuclease nicks one strand of a double helix and unwinds the DNA.
- The nicked strand invades the neighboring homologous chromosome. In bacteria, RecA protein coats the invading strand and facilitates pairing.
- This creates a hybrid region called heteroduplex DNA.
- The displaced strand binds to the single stranded region of the other chromosome.
- A second nick is made and ends are resealed.
- The cross-strand structure generated is called a Holliday intermediate.

- The position of the crossed strands can move or migrate along the DNA molecule, increasing the length of the heteroduplex region.
- To visualize resolution, the structure is rotated or isomerized.
- A resolving enzyme can cut the structure in two different ways as indicated by the arrows.
- If horizontal strands are cut, as on the top, the resulting chromosomes are recombinant for genes A and B.
- If vertical strands are cut, as on the bottom, the resulting chromosomes are not recombinant for genes A and B.
- Looking at all four strands, the resolution shown on the top created two chromosomes recombinant for genes A and B, while no recombinant chromosomes were generated by the resolution on the bottom.

Gene Conversion

- The early events in recombination generate a hybrid or heteroduplex region of DNA.
- Sequence differences in the strands making up the heteroduplex DNA result in noncomplementary bases within the helix.
- The mismatch repair system corrects mismatches, converting one allele to another.
- Some combinations of mismatch correction alter the expected 2:2 proportion of alleles in the resulting chromosomes.
- Conversion can occur within the heteroduplex region whether recombinant chromosomes are generated during resolution or not.

DNA Mutation

- A point mutation is a change in the nucleotide sequence of a gene.
- This can result from an error during replication that is not corrected by the usual repair mechanisms.
- Under normal circumstances adenines and thymines should bind to one another as should cytosines and guanines.
- In a base substitution mutation, a non-complementary nucleotide is incorporated into the new strand.
- The resulting altered triplet may code for a different amino acid or even a stop codon.

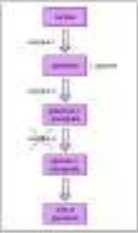
- Here we see 4 codons and their corresponding amino acids.
- A change within the second triplet results in a change in the amino acid.
- None of the other amino acids are affected. Insertions and deletions result in frame shift mutations.
- The insertion of an extra nucleotide not only affects the triplet it is part of, but all subsequent triplets, thus drastically altering the protein product.
- The result is similar if a nucleotide is deleted.

DNA Repair

- Mistakes in base pairing occur during replication of DNA. DNA can be damaged by environmental influences such as radiation or exposure to certain chemicals.
- Such damage can result in alteration of the genetic information.
- The repair mechanism recognizes and removes incorrect or damaged nucleotides.
- The undamaged strand serves as a template for replacement with appropriate intact nucleotides.

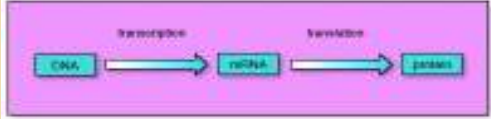
Protein Synthesis :Gene Activity

- Genetic diseases in humans are caused by mutations.
- Often these diseases are the result of a problem in metabolism, in particular, the lack of an enzyme used in one step of a metabolic pathway.
- For example, galactosemia is a genetic disease that results from the inability to completely break down the sugar lactose. Individuals with the disease lack a functional enzyme 3 in this metabolic pathway.
- On the basis of this type of information, and other evidence, the one gene - one protein hypothesis has been proposed.
- It states that the function of a gene is to provide the information to make a particular protein, a polypeptide, that will be used as an enzyme or a structural protein.



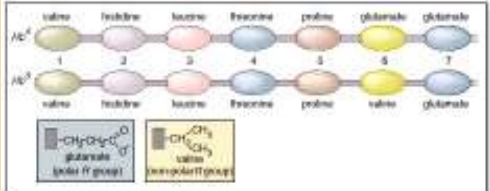
Central Dogma

- The central dogma of molecular biology explains how the information, or message, contained in a gene produces a protein.
- The diagram shows that two steps are involved. In the first step, called transcription, the DNA serves as a template to make an RNA molecule which is a copy of the message.
- In the second step, called translation, the RNA message is translated into protein language.
- This directs the construction of a protein with a particular sequence of amino acids.



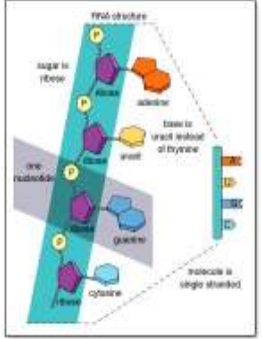
Amino Acid Sequence

- The amino acid sequence of a protein is the message coded by DNA.
- Even if that message is faulty, it can still be transcribed and translated.
- In the case of sickle cell anemia, one nucleotide is different from that in the normal gene.
- This causes a change of one amino acid (see #6 in the illustration) and results in alteration of protein (hemoglobin) function.



Structure of RNA

- The structure of RNA is similar to that of DNA in that it is a polymer of nucleotides.
- However, RNA differs from DNA in that it is a single stranded molecule, it contains uracil (U) instead of thymine as A's complementary base, and the sugar is ribose instead of deoxyribose.



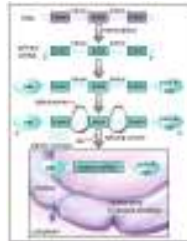
Transcription

- Transcription is the process by which RNA is assembled from a DNA template.
- The 2 strands of the DNA double helix are held together by bonds between complementary base pairs. DNA encodes genetic information in the sequence of bases along one strand.
- The portion of the DNA molecule that is a single gene, or coding region, is bounded by termination and promoter sites.

- A molecule of RNA polymerase binds to the promoter site.
- It moves along the DNA separating the 2 strands of the double helix.
- Each now unpaired base will bind to a nucleotide in the vicinity which has the appropriate complementary base.
- In the synthesis of RNA, uracil is the nucleotide complementary to adenine.
- This process ceases when RNA polymerase reaches the termination site.
- The DNA strands bind to one another once again as the new RNA molecule moves away.
- This RNA is a copy, or transcript, of the message contained in the gene.

mRNA Modification

- The mRNA molecule that is produced by transcription must undergo some modifications before it leaves the nucleus to direct protein synthesis.
- One modification involves the removal of introns. Introns are short sections of DNA that do not contain information on the protein to be built.
- One or more introns may be embedded in the DNA strand. The adjacent portions of the strand that do contain information on protein structure are called exons.



Genetic Code

- Translating the RNA message, written as a sequence of nucleotides, into protein language, written as a sequence of amino acids, requires the use of a decoder, called the genetic code.
- Nucleotides in the RNA message are read 3 at a time. Each nucleotide triplet, called a codon, is translated, using the genetic code, into a particular amino acid.
- The genetic code is universal; it is used by nearly all living things, from bacteria to humans.

The table shows the genetic code, mapping 64 possible codons (4 nucleotides per position) to their corresponding amino acids. The first column lists the first nucleotide (G, C, A, U), the second column lists the second nucleotide (G, C, A, U), and the third column lists the third nucleotide (G, C, A, U). The right side of the table lists the amino acids, such as Glycine, Alanine, Valine, Leucine, Isoleucine, Methionine, Phenylalanine, Tyrosine, Serine, Proline, Threonine, Asparagine, Aspartic acid, Glutamine, Glutamic acid, Lysine, and Stop.

Start Codon

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- The genetic code is universal; it is used by nearly all living things, from bacteria to humans.
- Start codon is AUG

Messenger RNA

- Messenger RNA, mRNA, carries the genetic message from the DNA.
- Two other types of RNA molecules are also involved in protein synthesis.
- Transfer RNA, tRNA, and ribosomal RNA, rRNA, help in the decoding process.

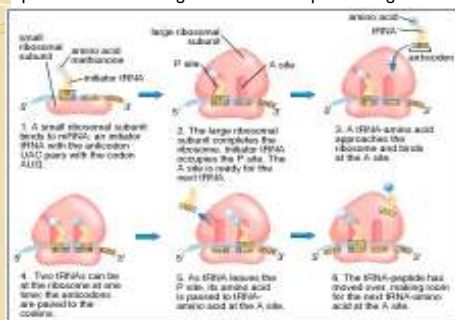
Translation

- Translation is the process by which the information contained in messenger RNA is used to direct the synthesis of a polypeptide.
- This information is carried in the sequence of bases on the messenger RNA molecule.
- The polypeptide can be assembled once RNA binds to a ribosome. Ribosomes consist of large and small subunits. The large subunit has 2 binding sites for transfer RNA, the P and A sites.
- Transfer RNA carries an amino acid, which will be incorporated into the polypeptide. Its anticodon is a triplet complementary to the codon on messenger RNA that specifies that particular amino acid. A ribosome assembles on the start codon, AUG.

- Transfer RNA with the anticodon UAC and carrying the amino acid methionine, binds to the codon.
- The transfer RNA is in the P site of the ribosome.
- The A site is available for a second transfer RNA with an anticodon complementary to the second messenger RNA triplet.
- The amino acid carried by the second transfer RNA binds to the methionine.
- The first transfer RNA leaves the P site and the second transfer RNA moves there, still bound to messenger RNA.
- This brings the third messenger RNA codon to the now-empty A site and the appropriate transfer RNA can bind to it.
- The third amino acid is added to the chain and translocation occurs once again.
- This process of polypeptide chain elongation continues until a stop codon is reached.
- A release factor binds to the A site. It carries no amino acid, but facilitates release of the polypeptide and the messenger RNA from the ribosome.

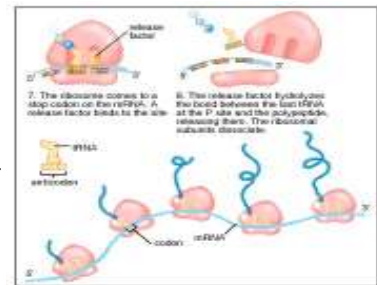
Stages of Protein Synthesis

- Translation consists of three stages: initiation, elongation, and termination. The first two stages are shown here. Initiation involves steps 1 and 2 while elongation involves steps 3 through 6.



Termination

- The last stage occurs when the stop codon is reached. Several ribosomes, collectively called **polyribosomes**, move along an mRNA at one time. This allows several proteins to be made at the same time using the same mRNA molecule.



Process of Translation

- A single messenger RNA molecule can be translated a number of times and produce many molecules of polypeptide.
- As a ribosome moves along the messenger RNA, additional ribosomes will follow.
- The result is an RNA with several ribosomes along its length.
- These ribosomes have growing polypeptide chains of increasing length as they move from initiation to termination site.
- The group of ribosomes is called a polyribosome or polysome.

Mutations

- When an incorrect base is inserted during **DNA replication**, the resulting point mutation can affect the **gene** product, the protein, in various ways.
- In the case illustrated, we are looking at the mRNA that has been made from the DNA.
- The 3rd base in the codon shown is supposed to be C.
- If a mutation occurred which caused it to be a U, the codon would still code for the amino acid tyrosine.
- This is a silent mutation because the protein is unchanged.



- If the 3rd base changed to a G, this would result in a stop codon which would halt protein production at that point.
- If the first base was changed to a C, the codon would code for a different amino acid, histidine.
- The protein would have one amino acid wrong which might, or might not, affect its function.
- A point mutation is a change in the nucleotide sequence of a gene.
- This can result from an error during replication that is not corrected by the usual repair mechanisms.
- Under normal circumstances adenines and thymines should bind to one another as should cytosines and guanines.
- In a base substitution mutation, a non-complementary nucleotide is incorporated into the new strand.

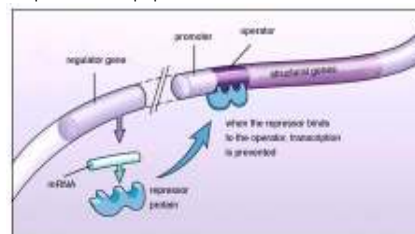
- The resulting altered triplet may code for a different amino acid or even a stop codon. Here we see 4 codons and their corresponding amino acids. A change within the second triplet results in a change in the amino acid. None of the other amino acids are affected. Insertions and deletions result in frame shift mutations. The insertion of an extra nucleotide not only affects the triplet it is part of, but all subsequent triplets, thus drastically altering the protein product. The result is similar if a nucleotide is deleted.

Gene Regulation

- Regulating gene activity can be accomplished by controlling the transcription/translation process.
- Prokaryotes, the bacteria, and eukaryotes, all other organisms, regulate gene expression in different ways.
 - **Operons**
 - **Inducible Enzymes**
 - **Tryptophan Attenuation**
 - **Tryptophan Synthesis**

Operons

- In prokaryotes, genes are turned on and off using operons. An operon includes a series of structural genes along a segment of DNA and two other portions of DNA, a promoter and an operator.
- When the structural gene products (proteins) are required by the cell, the operator area is open which allows the RNA polymerase to attach at the promoter and transcription occurs.
- If the proteins are not needed by the cell, the operator area is blocked by the attachment of a repressor protein and transcription can not occur.
- The lac operon and the trp operon are described in the next two screens.



Inducible Enzymes

- Some enzymes are termed inducible. This means that they are produced only in the presence of the substances they act upon. E. coli has three genes for the metabolism of lactose.
- These genes are clustered together along with their operator and promoter in an operon.
- In the absence of lactose a repressor molecule binds to the operator and blocks transcription of the 3 genes because RNA polymerase cannot attach to the DNA.
- When lactose is present it binds to the repressor and removes it.
- Now transcription can proceed.
- In this way, E. coli avoids producing the enzymes when it doesn't need them.

Tryptophan Attenuation

- The five genes needed for the biosynthesis of the amino acid tryptophan are expressed together in the trp operon in E. coli.
- Initiation of transcription is controlled by a repressor. When tryptophan is present, it binds to the trp repressor and together they bind to the operator.
- RNA polymerase cannot move past the repressor molecule and no transcription occurs.
- If tryptophan is lacking in the medium, repressor does not bind to the operator and transcription begins.
- An additional control called attenuation occurs early in transcription of the leader region of the trp operon.

- Transcription stops if a terminating stem loop is formed in the RNA by complementary base pairing between sequences designated 3 and 4.
- An alternative secondary structure can form between regions 2 and 3. If this structure forms, sequence 3 is not available to form the 3-4 termination stem loop, so transcription continues.
- The stem loop that forms is determined by the speed with which the ribosome moves through the region.
- The ribosome follows RNA polymerase, translating the mRNA into amino acids in this leader portion of the transcript.
- If tryptophan is lacking, the ribosome stalls when it encounters two trp codons in the mRNA, because tRNAs charged with tryptophan are in short supply.
- The stalled ribosome allows the 2-3 stem loop to form.
- The 3-4 terminating stem loop will not form and transcription continues.

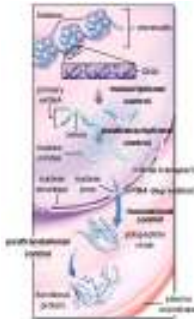
Tryptophan Synthesis

- The genes for the enzymes which catalyze synthesis of the amino acid tryptophan are clustered together on a chromosome.
- Along with their promoter and operator they form the trp operon.
- As the genes are transcribed and then translated, tryptophan levels increase.
- Tryptophan, acting as a corepressor, binds to a repressor molecule.
- This complex binds to the chromosome, blocking RNA polymerase and thus preventing further tryptophan production.
- These are called repressible enzymes because the presence of their end product, tryptophan, inhibits, or represses, their production.

Eukaryotic Gene Regulation

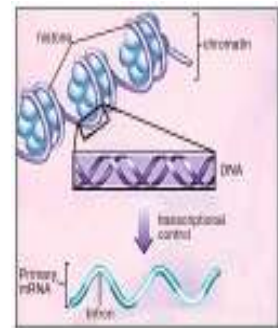
Eukaryotes use 6 primary ways to control the expression of genes.

1. Transcriptional control affects the selection of genes to be transcribed and the rate of transcription.
2. Posttranscriptional control affects the processing of mRNA.
3. mRNA transport affects the rate at which mRNA leaves the nucleus.
4. mRNA degradation occurs because some mRNA transcripts contain sequences that cause them to be degraded prior to translation.
5. Translational control affects how long mRNA remains in the cytoplasm and the ability of mRNA to bind to ribosomes.
6. Posttranslational control occurs by regulating the additional processing that proteins require to be functional or through feedback inhibition.



Transcriptional Control

- Transcriptional control is the most common means of gene regulation. Protein binds to a regulatory DNA sequence; this inhibits or facilitates binding of RNA polymerase to the promoter.
- Regulatory proteins are the subjects of intensive research.



Eukaryotic Transcription Factors

- In eukaryotes, RNA polymerase II transcribes protein coding genes.
- A basal level of transcription requires a set of basal transcription factors that bind to DNA or other proteins.
- First, a protein recognizes and binds to the TATA sequence at the promoter.
- TATA binding protein (TBP) bends the DNA. Auxiliary factors bind to the TATA binding protein.
- This protein complex promotes binding of RNA polymerase and its associated proteins.
- The complete preinitiation complex denatures the nearby DNA helix.

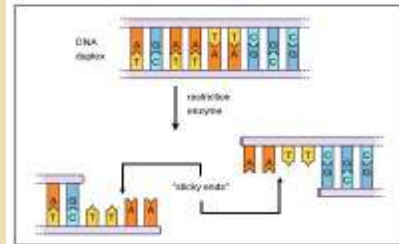
- RNA polymerase II and its associated proteins move along the unwound DNA and produce a messenger RNA complementary to the DNA template.
- The level of transcription is increased above the low basal levels by activators bound to specific enhancer sequences.
- Activators can act to stabilize the preinitiation complex and make it easier for the TATA binding protein or RNA polymerase to bind to the DNA.
- Stabilization of the complex by activator proteins may also allow binding of subsequent RNA polymerase molecules.
- Activator proteins increase transcription, while inhibitor proteins decrease transcription.
- Together these regulatory proteins modulate transcription by determining where, when and how much transcription occurs in a particular type of cell.

Signal Transduction

- A molecular signal sent by one cell is processed and converted into a response by a series of steps known as signal transduction.
- Signaling molecules bind to specific receptors within the membrane.
- The signaling molecule bound to the receptor causes the release of a second messenger molecule into the cytoplasm.
- The signal is relayed through a series of molecules by chemical or conformational changes.
- In one type of signal transduction, the transfer of a phosphate molecule relays the signal.
- A cascade of phosphorylations can amplify the original signal.
- Eventually, the signal is converted into a response such as activation of an enzyme within the cytoplasm or activation of a transcription factor.
- If a transcription factor is activated, it enters the nucleus and binds to DNA. A change in gene expression is one possible outcome of signal transduction.

Recombinant DNA Technology

- Recombinant DNA is DNA taken from two different sources and fused into a single DNA molecule.
- Special DNA cutting enzymes, called restriction enzymes, cut the DNA at specific sites.
- Each restriction enzyme recognizes a different nucleotide sequence.
- DNA that is cut with a restriction enzyme will have single-stranded ends, called "sticky ends".
- Two molecules of DNA cut with the same restriction enzyme will have the same exposed nucleotides and will undergo complementary base pairing.



- Recombinant DNA technology has extensive applications in developing pharmaceuticals. The first drug created using recombinant DNA was human insulin.
- To make the recombinant DNA, the insulin gene is cut from human DNA with restriction enzymes.
- The DNA is then placed in a vector, such as a plasmid, and another enzyme, DNA ligase, seals the plasmid containing the insulin gene.
- The plasmid is placed into another bacterial cell and this new cell produces multiple copies of the gene, called clones, when it divides.
- The host bacterial cell also expresses the gene product, in this case insulin.
- This technology is possible because the genetic code is universal. DNA functions in the same way, whether in a human cell or a bacterial cell.

Plasmids

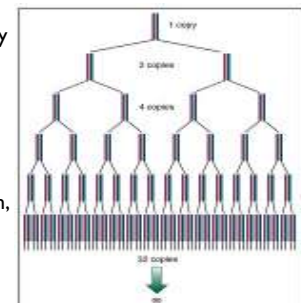
- Bacteria have small loops of DNA called plasmids. In genetic engineering, plasmids can be cut open with restriction enzymes, and DNA from other organisms can be inserted.
- Restriction enzymes recognize specific nucleotide sequences at which they make cuts in the DNA molecule.
- They cut in such a way as to leave jagged ends with unpaired bases.
- These are called sticky ends because they will hydrogen bond to complementary nucleotides.
- A section of foreign DNA with the appropriate bases on its own sticky ends can bind to the plasmid and DNA ligase helps join the ends.
- The result is recombinant DNA.

Probe

- When plasmids containing a foreign gene are introduced into a bacterial culture, not all the bacterial cells will pick up the plasmids.
- To find the cells that have the foreign gene, a probe can be used.
- A probe is a single-stranded nucleotide sequence that will hybridize (pair) with the introduced gene.
- The probe is radioactive or fluorescent so that cells with the probe, and thus the gene of interest, can be recognized.

Polymerase Chain Reaction (PCR)

- In order for scientists to study DNA, it is necessary that they have large enough quantities, much larger than most samples of DNA that are usually obtained.
- A technique called polymerase chain reaction, PCR, allows for the amplification of DNA molecules.



- A segment of DNA can be rapidly copied, or amplified, thousands of times with a laboratory technique called PCR or polymerase chain reaction.
- The DNA of interest is mixed with a heat-resistant form of DNA polymerase, the four types of nucleotides, and primers.
- The primers are synthetic strands of nucleotides which are designed to base pair with the ends of the DNA.
- The mixture is heated to separate the DNA strands and then cooled so that the primer binds to one end of the DNA.
- Now DNA polymerase, beginning at the primer, adds complementary nucleotides along the single-stranded DNA.
- The DNA sequence has now been doubled.
- The process is repeated as the mixture is alternately heated and cooled and the quantity of DNA doubles again and again.
- Each cycle of heating, cooling, and DNA doubling takes about 5 minutes. After 20 cycles there will be 1,048,576 copies of the DNA molecule.
- This technique has revolutionized molecular biology in such areas as diagnosis of disease, study of genetic disorders and forensic medicine.

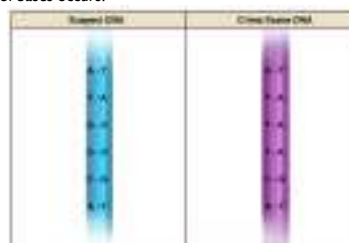
DNA Sequencing

- DNA sequencing using the enzymatic extension reaction makes use of the chemical difference between normal deoxyribonucleotides and dideoxynucleotides.
- Deoxyribonucleotides contain a hydroxyl group in position 3 of the sugar ring and DNA polymerase can join the phosphate of the next nucleotide to that hydroxyl end.
- A dideoxynucleotide can be added to a growing chain but does not contain a hydroxyl group at position 3, so no further nucleotides can be added.
- Addition of dideoxynucleotide stops DNA synthesis.
- The DNA template to be sequenced is denatured in a tube.
- The reaction mix contains a primer complementary to the template, DNA polymerase, the four deoxyribonucleotides and one labeled dideoxynucleotide, in this case dideoxyA.

- The dideoxyA is present at lower concentration than the analogous deoxyA.
- The primer base pairs with a complementary sequence and DNA synthesis begins.
- When the dideoxyribonucleotide is inserted, DNA synthesis on that molecule stops.
- With the optimum concentrations for the deoxy and dideoxyribonucleotides, there will be molecules synthesized that stop at each of the A's in the new sequence.
- The fragments generated are separated by size using gel electrophoresis and the tag on the dideoxyribonucleotide allows the fragments to be visualized.
- DNA sequencing reactions are done using each of the four dideoxyribonucleotides and if each dideoxy has a unique fluorescent label, all four reactions can be carried out in the same tube.
- When the reaction products are separated, there should be labeled fragments corresponding to each base along the template, with the four bases represented by different recognizable tags.

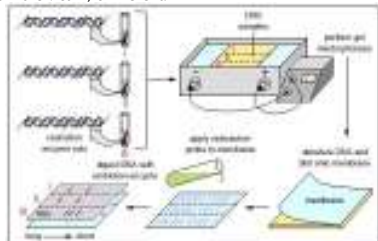
Recombinant DNA Applications Genetic Fingerprinting

- How can we tell if the DNA in hair found at a crime scene came from the crime suspect? Since the DNA of every individual is unique, it should be possible to do genetic fingerprinting.
- It is too costly and time consuming to compare two DNA samples by looking at the sequence of bases (A, G, T and C) for all 3 billion nucleotide positions. Instead, restriction enzymes are used that cut the DNA whenever a certain sequence of bases occurs.



Restriction Fragment Length Polymorphisms (RFLPs)

- Cutting DNA with restriction enzymes produces DNA fragments of varying length.
- These can be sorted using gel electrophoresis. Every individual will have a unique assortment of fragment sizes (RFLPs - restriction fragment length polymorphisms).
- In the figure you can see that sample I, which came from the crime scene, and sample II, which came from the suspect, are the same, whereas sample III, which came from the victim, is different.



Electrophoresis

- Large charged molecules can be separated on the basis of their size using the technique of gel electrophoresis.
- Charged molecules will move through a thin layer of gelatinous material when an electric current is applied.
- Samples of material are placed at one end of the gel in individual lanes.
- Electrodes are attached to each end and current is applied.
- Molecules move toward the electrode whose charge is opposite their own.
- The lighter molecules move more rapidly and thus move farther while the current is on.
- If a dye is applied, the location of the molecules can be observed.

Biotechnology Products

- One important application of recombinant DNA technology is the mass production of proteins.
- Insulin, for example, can be mass produced by placing the gene for insulin into a bacterium.
- Large cultures of bacteria make large quantities of insulin which is then harvested.
- Vaccines can also be made this way.
- Vaccines are forms of a disease agent that triggers an immune response without producing the disease.
- For a bacterial disease, modified bacterial cells have traditionally been used as a vaccine, but this sometimes actually causes the illness.
- The immune response is often triggered by the recognition of a surface protein on the bacterial cell, not the bacteria itself.
- The surface protein can be made using recombinant DNA, thus creating a safe vaccine.

Plants and Animals

- Domestic plants and animals can be improved or made more useful to humans by the addition of specific genes.
- Traditionally, genetic improvements have come about by selective cross breeding, a time consuming process. Now, genes can be inserted directly into the DNA, even genes from other species.
- For example, corn plants have been made resistant to attacks by larvae of the European corn borer by inserting a gene from a bacterium into the corn genome.
- Genes for human milk proteins can be inserted into cows so that cows will produce human milk.
- This milk can be used instead of formula by non-breast-feeding babies.
- Many other applications are under development and the potential is enormous.

Gene Therapy

- A new, and potentially very beneficial, application of DNA technology is gene therapy.
- The goal in gene therapy is to correct genetic diseases caused by mutant genes that do not produce functional enzymes.
- In gene therapy, the normal gene is inserted into cells to restore proper enzyme production.
- In some cases (ex vivo), cells are removed from a patient's body, the proper DNA is inserted, and the cells are then returned to the patient's body.
- In other cases (in vivo) carrier molecules or agents, such as viruses, are used to take the normal gene into cells in the patient's body.