Lesson 1:
The Mechanics of Cells and DNA

Cells are the fundamental units of life. Biotechnology research is usually done on the cellular or subcellular level. Knowing the cellular components and how the parts of a cell work is therefore necessary. Of particular importance is the core component of biotechnology research, DNA.

The Parts of Cells

All cells contain DNA. However, the structures found in the cells may vary. The cells found in plants and animals are similar, but they do have some differences. Bacteria cells are very different from plant and animal cells.

An animal cell (Figure 1.1) has a cell membrane, or plasma membrane, that forms the boundary of the cell. The cell membrane is primarily a lipid (fatty substance), carbohydrate, and protein structure. The membrane's primary function is to control the movement of substances into and out of the cell.

Inside the cell membrane is the cytoplasm, which consists of the contents of the cell, excluding the nucleus. The fluid of the cytoplasm helps control the movement of many substances within the cell. The cytoplasm includes many structures, called organelles, that fill different specialized functions in the cell.

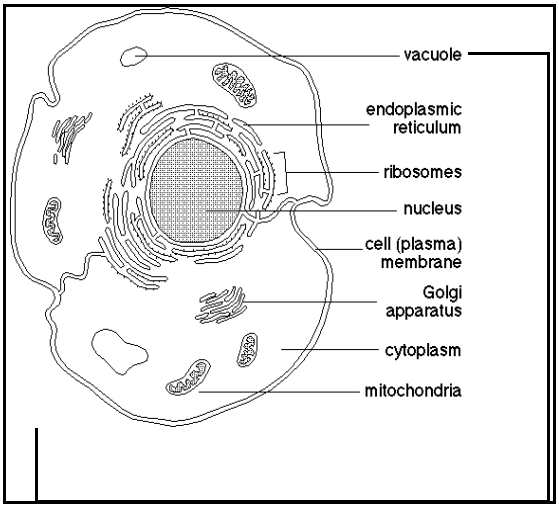
Mitochondria are the powerhouses of the cell. They break down nutrients to provide energy to the cell. Hundreds of mitochondria may be found in a single cell.

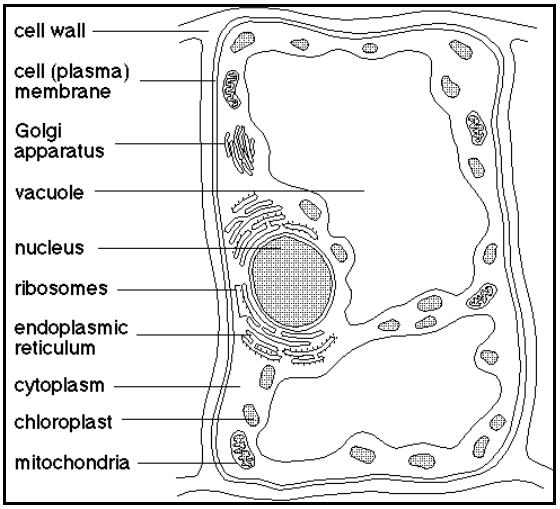
The endoplasmic reticulum is a large network of membranes that transports material within the cell. Ribosomes are found on the endoplasmic reticulum. They are the sites where protein molecules are assembled, or synthesized. These protein molecules are important to the cell and to the organism as a whole because they control the chemical activities of cells.

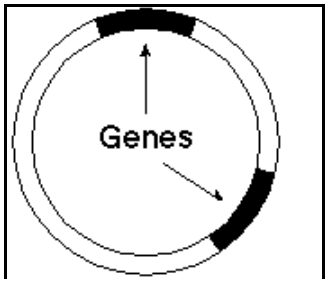
The Golgi apparatus works with the endoplasmic reticulum in transporting proteins. It packages protein molecules for transport within and outside the cell.

Vacuoles are the storage units of the cells. They store water, enzymes, pigments, and other substances.

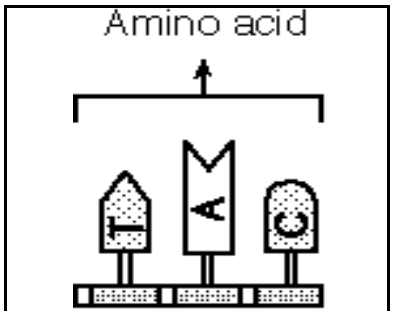
The control center of a cell is called the nucleus, which is defined by a pair of nuclear membranes. Inside the nucleus, chromosomes consisting of DNA can be found. Chromosomes are essentially tightly wrapped pieces of DNA that function as a unit. Genes are segments of DNA on chromosomes that produce a polypeptide (protein). Genes are responsible for the expression of genetic traits because the proteins produced by genes







control the appearance, growth, and functioning of the organism.



Plant cells (Figure 1.2) contain these structures, but they also have a few differences. They have more vacuoles, which can be very large in mature plant cells. Plant cells have chloroplasts; they contain the chlorophyll used in photosynthesis. The cells also have a rigid outside layer called a cell wall that is composed of cellulose. The cell wall provides support for the plant cell and works collectively with the walls of other cells to support the plant. The cell wall has openings that allow substances to pass through it. The cell membrane in plant cells is just inside the cell wall.

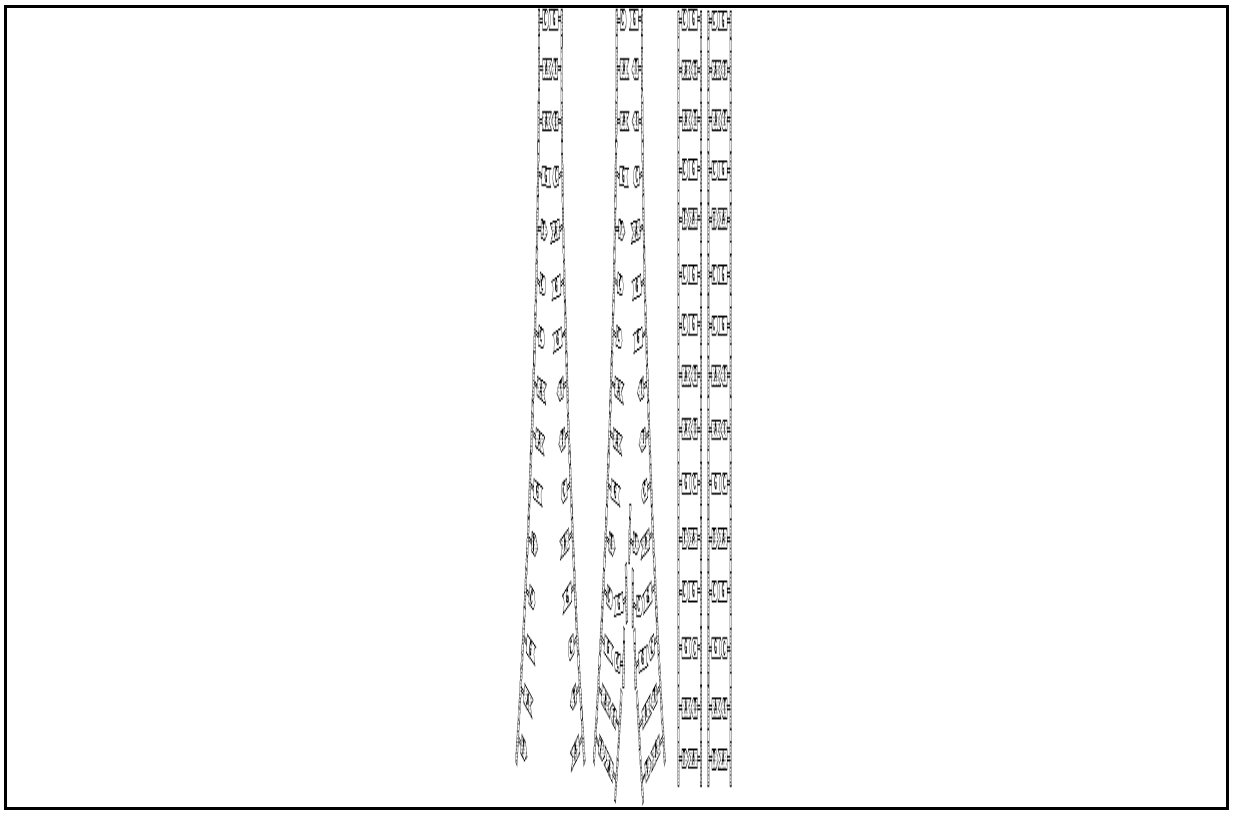
Bacteria have a cell wall and cell membrane. They also have ribosomes that carry out protein synthesis. Unlike animal and plant cells, the chromosomal material is not contained within a nuclear membrane but instead forms a nucleoid region. A unique structure found only in bacteria cells is the plasmid (Figure 1.3). One or more plasmids can be found in a cell. Plasmids are essentially small circular pieces of DNA that code for specific traits and replicate independently of the chromosomal DNA. They normally contain only a few genes. Plasmids play an important role in biotechnology because they can be easily modified to produce pharmaceuticals.

DNA

Genetic modification involves the manipulation of DNA, or deoxyribonucleic acid. DNA is the genetic material of the cell. It is composed of small chemical units called nucleotides. A nucleotide consists of three parts: a phosphate group, a sugar unit (deoxyribose), and a base unit that contains nitrogen. DNA has four different nitrogen bases, creating four different nucleotides. These nucleotides are named after the nitrogen base. The four nitrogen bases are adenine (A), guanine (G), thymine (T), and cytosine (C). The nitrogen base units contain the code used to build proteins. A single strand of DNA may contain more than 100 million base pairs. DNA is not a simple molecule.

The Structure and Function of DNA

James Watson, a biologist, and Francis Crick, a physicist, were the first to discover the structure of DNA. They won the Nobel Prize in 1962 for their work. Watson and Crick found that two strands of nucleic acid are intertwined in a double helix structure. It looks like a twisted or spiraling ladder. The phosphate and sugar units form the sides of the ladder, while the nitrogen base units form the rungs. The nitrogen base adenine will only bond to thymine, and guanine will only bond to cytosine. Hydrogen chemical bonds join the base units.



The DNA in a plant, animal, or bacteria cell is essentially the same except for the sequence of bases it contains. This similarity of DNA makes the manipulation and transfer of DNA between these different life-forms possible. If the structure or function of plant DNA was different from animal DNA, then DNA from a plant could not be spliced into the DNA of an animal.

DNA is the blueprint of a cell. A builder of a house uses a blueprint to see where and how to install the walls, windows, plumbing, electricity, and many other things. In the same way, the cell uses its DNA to determine what types of proteins to build, or synthesize. The proteins produced during protein synthesis by the cells in an organism function as the chemical basis for the development of the organism.

Codons are sections of DNA three nucleotides long (Figure 1.4). Codons code for one of the twenty amino acids that are the building blocks of proteins. The codons are lined up end to end to form the DNA strand. Using this code, amino acids are lined up and linked together to form polypeptides. Two or more polypeptides are then linked together to form proteins. The kind and sequence of amino acids makes the shape of one polypeptide different from another. The shape of a protein is strongly related to how the protein will function.

DNA not only codes for protein production in a cell but also passes this code on to new cells formed by cell division. Essentially, DNA copies itself before a cell divides. This process is called DNA replication.

DNA Replication

Cell division occurs when a cell grows and begins to get too large. When a nonsex cell in a plant or animal divides, the DNA in that cell must first replicate itself so that the two new cells have the same genetic material. Otherwise, each time a cell divided, it would lose half of its DNA. In DNA replication (Figure 1.5), the genetic material copies itself using a strand of DNA as a template. Replication begins when a protein made by a cell undergoing division binds to a section of the DNA called the origin. This event signals an enzyme to begin breaking the hydrogen bonds that hold the two strands of the helix together, causing the double helix structure of the DNA to “unzip.” As the DNA strands come apart, a complex enzyme called DNA polymerase that is

found in the nucleus of cells binds to each DNA strand segment and begins to add a new base unit to the strand. The added base must be compatible with the base on the parent DNA strand. Another enzyme then bonds the new nucleotides together with the parent DNA strand. Each DNA molecule now consists of one parent strand and one newly formed strand. With replication complete, the cell can then divide.

Summary

Cells have many organelles that must function together in order for the cell to survive. Each organelle has one or more functions that help the cell live, grow, and divide. DNA is very important to the cell since the code for building proteins is contained in the cell's DNA. The process of DNA replication allows the genetic code to be passed on to daughter cells.

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Lesson 2:
Cell Reproduction and Genetics

After organisms begin life, they grow and eventually reach maturity and reproduce. Their offspring then begin to grow and develop. A similar life cycle goes on at the cellular level. Young cells grow and mature until they are stimulated to reproduce. Cell reproduction takes place by cell division, in which the material in a cell is divided to produce two new cells. Cell division produces both body tissue cells and sex cells needed to produce offspring.

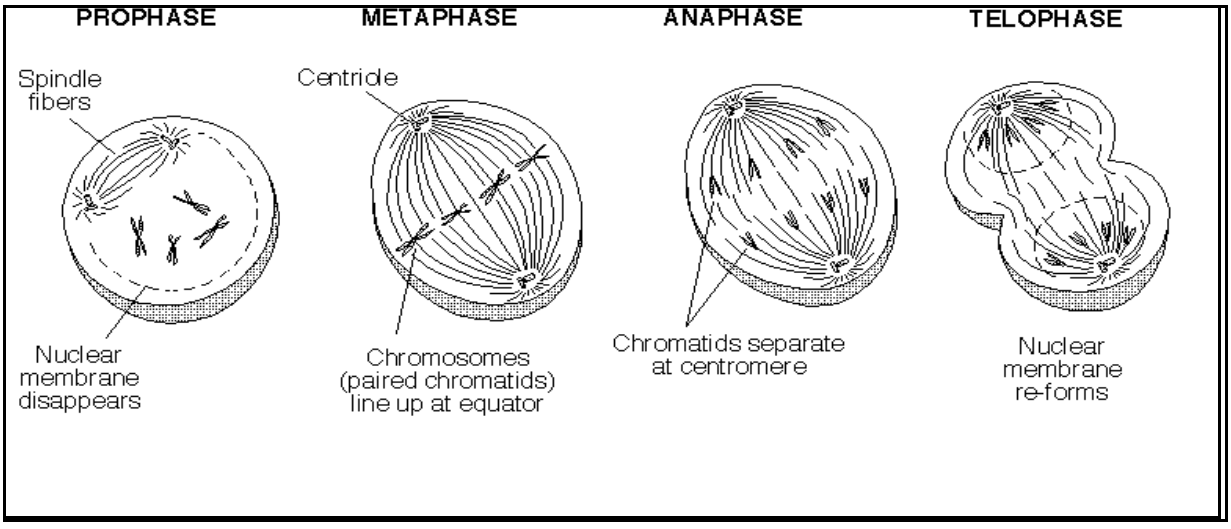
Mitosis

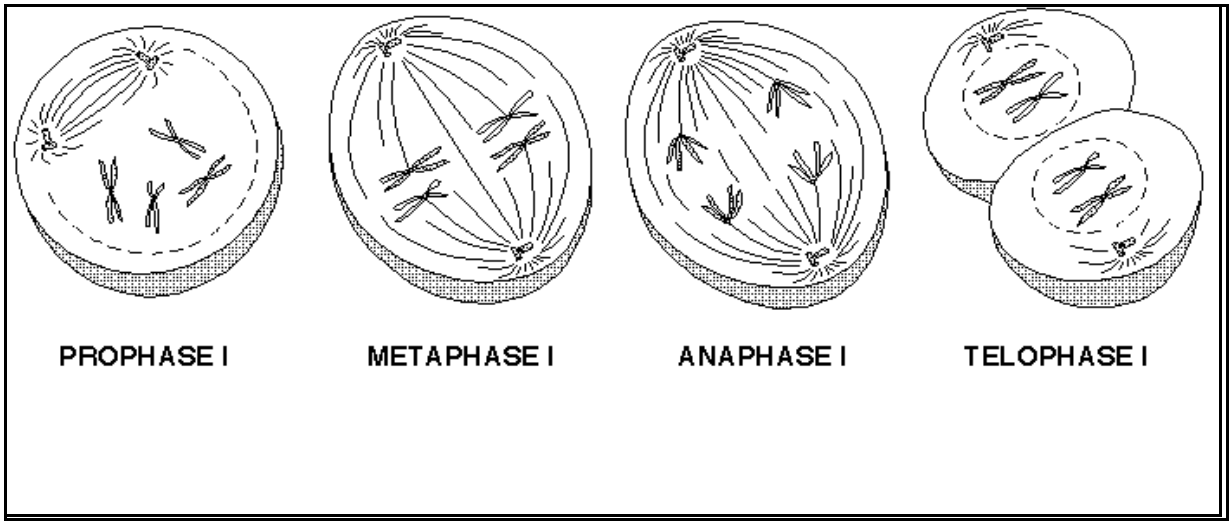
Mitosis is a type of cell division that takes place in somatic cells. Somatic cells include all the cells in an organism except the sex cells, or gametes (ova or sperm). Before mitosis begins, a cell replicates its genetic material. Each of the two new cells created by mitosis will therefore contain the same number of paired chromosomes as the parent cell. They will have two complete sets of chromosomes, or a diploid number of chromosomes. The process of mitosis involves four stages, which are illustrated in Figure 2.1.

The first stage of mitosis is prophase. The chromosomal material coils and condenses, and a double-stranded chromosomal structure becomes visible. This structure consists of two paired chromatids created by the duplication of DNA. Each double-stranded chromosomal unit has a point where the two chromatids connect. This point of connection, which is a body called the centromere, can occur at any point along the chromatids. The nuclear membrane then gradually dissolves. A network made up of complex protein units like hollow tubes, which are known as microtubules, begins forming around structures called centrioles, which start to move to opposite ends of the cell. The centrioles serve as anchors for the network of microtubules. This entire network is called the spindle. Spindle fibers extend from the centrioles toward the center of the cell. At this point, prophase ends.

Metaphase is the next stage of the process. The chromosomal units move to the center of the cell and form a line between the two poles formed by the centrioles. Each spindle fiber attaches to the centromere of one of the chromosomal units.

The third stage is called anaphase. The centromeres break and allow the spindle fibers to pull the two chromatids of the chromosomal unit apart. The chromosomes move toward opposite poles of the cell. The poles move even farther apart, elongating the cell. At the end of anaphase, the two poles of the cell each have a complete set of chromosomes.





The last phase, telophase, begins differently in animal and plant cells. In animal cells, the cell membrane pinches in at the center of the cell until the cell is completely divided into two cells. In plant cells, a structure called a cell plate forms and begins to divide the cell into two cells. A cell membrane forms on both sides of the cell plate, and eventually the cell plate changes into a cell wall. After the cell membrane or cell plate begins to form, a nuclear membrane develops around the two sets of chromosomes. The chromosomes themselves begin to uncoil and lose their distinct outlines. Mitosis ends when this process is complete.

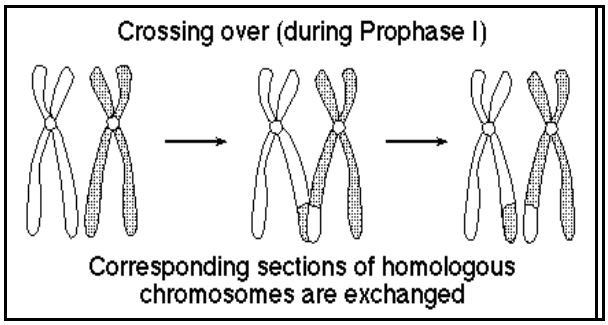
Meiosis

Meiosis is also a type of cell division, but it produces gametes rather than somatic cells. Meiosis produces four gametes, since it consists of two phases of cell division. The gametes produced contain only half the number of chromosomes of the original parent cell, or a single set of chromosomes. It is for this reason that these gametes are referred to as haploid cells.

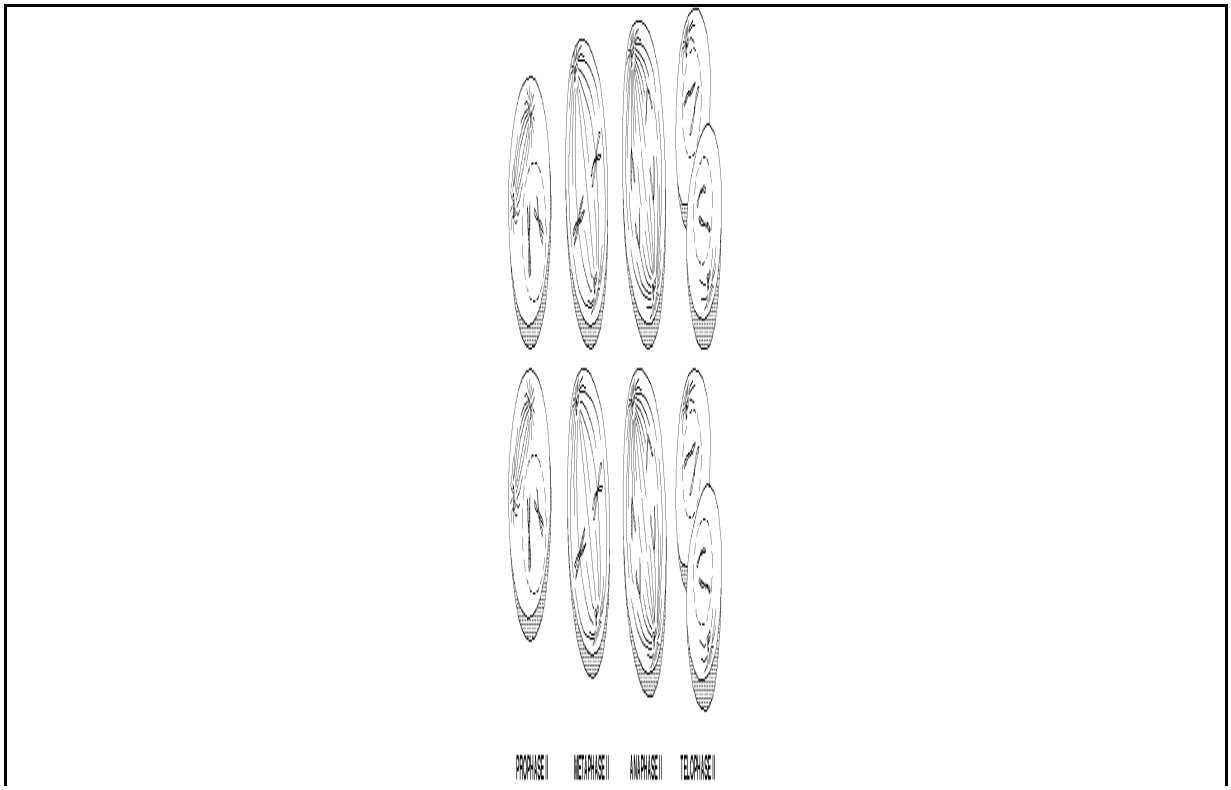
The first cell division process, or meiosis I (Figure 2.2), is somewhat similar to mitosis. The chromosomes replicate before the beginning of meiosis, just as they do before mitosis. During prophase I of meiosis, homologous chromosomes pair together. Homologous chromosomes are paired chromosomes that contain the same set of genes. One of the homologous chromosomes came from one of the organism's parents, and the other came from the other parent. They form a tetrad, a grouping of four chromatids side by side. The nonpaired chromatids may exchange segments through a process called "crossing over," in which a segment from one chromatid breaks off and reattaches to another. The process results in a change in the makeup of the chromosomes (see Figure 2.3). This exchange happens randomly. As in mitosis, the centrioles move apart and the spindle forms. The nuclear membrane dissolves.

Metaphase I is marked by homologous chromosomes lining up in the center of the cell. The spindle fiber ends attach to the centromeres of paired chromatids.

During anaphase I, homologous chromosomes separate and are pulled to different poles of the cell. The two cells formed after telophase will therefore not be genetically identical.



Telophase I can be identified by two distinct events. The first event is the formation of the cell membrane in animal cells or development of the cell plate and cell membrane in plant cells. The second event is the



formation of nuclear membranes around the two new nuclei. The result is two haploid daughter cells.

After meiosis I, the two cells go through a short period of rest and then begin meiosis II, shown in Figure 2.4. Unlike mitosis or meiosis I, the two cells do not undergo DNA replication. Meiosis II begins with the development of the spindle fibers and the movement of paired chromatids to the center of the cell during prophase II. During metaphase II, the chromosomal units line up in a row between the two poles and become attached to the spindle fibers. In anaphase II, the chromatids separate and move toward the opposite poles. In telophase II, the center of each of the two cells closes off with the formation of a cell membrane or cell plate, nuclei form, and the chromosomes uncoil. Meiosis yields four haploid daughter cells that are not identical to the parent cell.

Differences Between Mitosis and Meiosis

Mitosis and meiosis have four major differences. One of the more obvious differences is that mitosis produces two cells from one parent cell, while meiosis produces four cells from one parent cell. Another obvious difference is that mitosis produces diploid somatic cells, while meiosis produces haploid gametes. However, a more subtle difference is that while mitosis produces two identical cells, meiosis produces four nonidentical cells. During mitosis, chromosomes double and contribute an identical chromosome to each daughter cell, while in meiosis homologous chromosomes split and contribute nonidentical chromosomes to each daughter cell. The last major difference between mitosis and meiosis is that in meiosis a tetrad forms and allows "crossing over" of genes to occur between homologous chromosomes.

Dominant and Recessive Genes

Most chromosomes in all species of plants and animals work in pairs. For example, cattle have 60 chromosomes in the nucleus of every somatic cell. These chromosomes function as 30 pairs of chromosomes. Each chromosome has a homologous chromosome that has genes that code for the same information but in a somewhat different way. Each gene in a gene pair is either dominant or recessive.

The interaction between dominant and recessive genes can be seen by looking at coat color in cattle. One section of one of the chromosomes codes for coat color. The gene for coat color is found at the same location on both chromosomes. If one of these genes codes for black and the other codes for white, what will the coat color of the animal be? Since the black gene is a dominant gene in cattle, the animal in question would have a black coat. The dominant gene is expressed, or seen in the animal. A dominant gene masks or covers up the expression of a recessive gene, which will not be apparent as a physical trait of the animal. In this example, the recessive gene is the gene that codes for white.

Homozygous and Heterozygous Gene Pairs

The term allele is used to describe either of the two possible expressions of a gene or multiple genes that code for a specific trait. An allele is usually represented by a letter of the alphabet. If the gene acts as a dominant gene, a capital letter is used to represent it; a lowercase letter is used to represent a recessive gene. If a plant or animal has two dominant alleles or two recessive alleles for a specific trait, it is homozygous for that specific trait. The terms homozygous dominant and homozygous recessive are used to differentiate between the two types of homozygous traits. If, however, a plant or animal has one dominant allele and one recessive allele, it is heterozygous for that trait.

Genotypes and Phenotypes

The genotype of an animal or plant refers to the specific combination of the alleles it possesses for each genetic trait. It is the actual genetic make up of the organism; for the example given above, the genotype would be either BB, Bb, or bb. The phenotype is the expression or appearance of a trait as determined by the genotype.

Mutations

What happens when a base unit is mistakenly inserted, deleted, or miscoded during the replication of DNA? Such a mistake is called a mutation. A mutation is an alteration of the nucleotide sequence found in a DNA molecule. This alteration can happen during replication prior to the beginning of mitosis or meiosis; it affects the organism differently depending on when the mistake occurs. If the mutation occurs just prior to mitosis, the change in the genetic code will be passed on to the daughter cell and any cell descending from the parent cell. Cancer is an example of a somatic cell mutation in which the mutated cell rapidly reproduces. Mutations can also occur just prior to meiosis. In this case, the mutation is passed on to an organism's offspring if the gametes are fertilized. The offspring would have the altered DNA in every one of its cells.

Some mutations can be very beneficial, some can have a negative effect, and others may have no visible effect on an organism. An example of a positive mutation is the mutation that led to the development of the Polled Hereford breed of cattle. This breed was developed in the early 1900s by a rancher who noticed that calves from his Hereford cattle occasionally did not develop horns.

Summary

Cell reproduction is carried out through the processes of mitosis and meiosis. The genetic material passed on through these processes includes dominant and recessive genes and heterozygous and homozygous gene pairs. The genes an organism possesses determine its genotype and phenotype. Sometimes mutations also have an effect on the organism.

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Lesson 3:
Genetic Modification

Genetic modification is a very complicated process performed by highly trained researchers. This lesson will cover the basic procedures used in genetic engineering.

Gene Mapping and Gene Sequencing

Before the driver of a car makes a change in the planned route, he or she should check a road map to find out which roads to add to the route and which roads to avoid. Making changes in the genetic code of a cell is much the same, except that in this case the driver or researcher is driving blind because DNA cannot be seen except under extremely powerful microscopes. The researcher needs a map of the chromosomes of an organism to be able to select specific genes for modification. Gene mapping is the process of finding the location of genes for specific traits on the chromosomes of an organism. An example is a genome map of a corn plant, which shows the parts of the chromosomes that are responsible for plant height.

Gene sequencing is a related process that shows the order of all the base units (A, T, C, G) as they line up on a particular gene. A gene sequence is really a map of a single gene, which may be comprised of 100,000 or more base pairs. Gene sequencing is important to scientists because it allows them to recognize how to cut out a particular gene or gene fragment so that it can be placed into the DNA of the cell being modified.

DNA Extraction

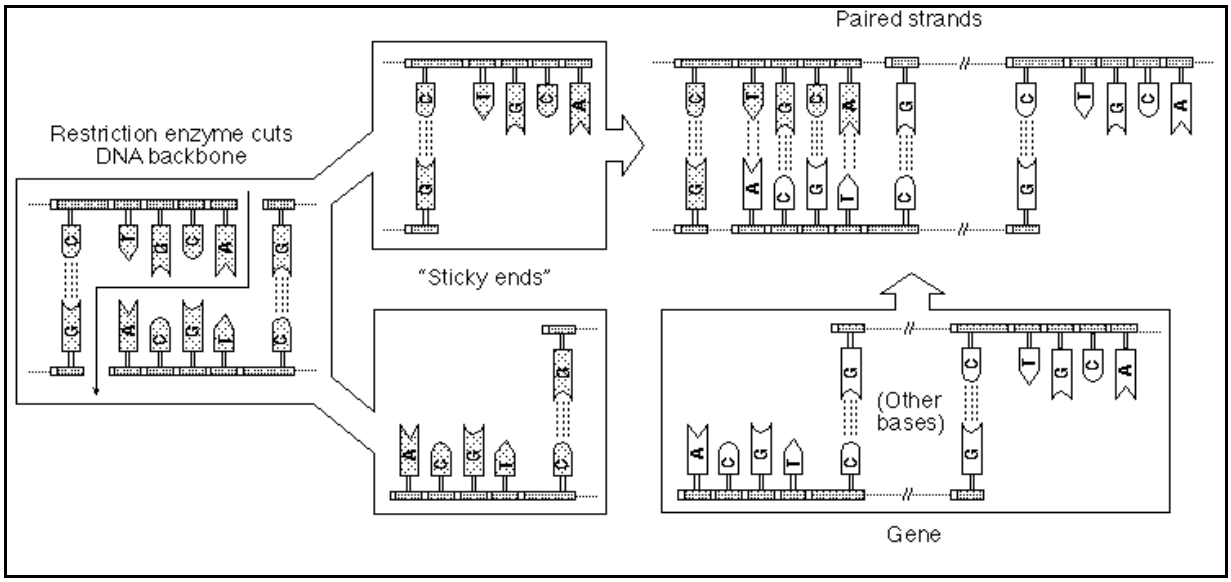
Once a desired gene has been identified, it must be removed from the cell. DNA extraction is a three-step process. The cell membrane or cell wall must first be broken down to release the cytoplasm. The nuclear membrane must also be broken to release the chromosomes. Researchers accomplish this with the use of a surfactant, a fatty acid compound much like a household detergent. These compounds consist of lipids, just like the cell membrane. The surfactant breaks down the cellular membranes at a rate determined by temperature. Heat accelerates this process.

The second step involves the use of a protease, such as the enzyme papain. DNA strands in the chromosome wrap around protein molecules called histones. The protease will split this protein and the other protein contents of the cytoplasm.

The last step involves separating the DNA from the other cell components. Cold alcohol is added to the cellular solution. The DNA strands will clump together and rise to the top of the alcohol, since DNA is insoluble in alcohols. The DNA is then collected for later use.

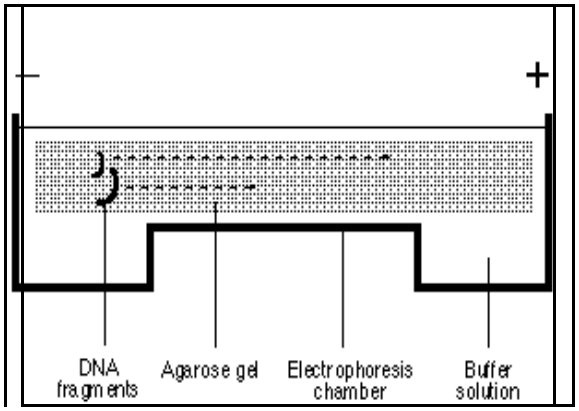
Restriction Digestion

Restriction digestion is the process of cutting DNA into smaller fragments. The DNA is cut by restriction enzymes, which are essentially biochemical scissors. Each restriction enzyme cuts DNA at a specific sequence of nucleotide base pairs, as illustrated in Figure 3.1. The sequence is called a restriction site. All DNA contains natural restriction sites. Researchers use restriction enzymes to cut genes or DNA fragments out of extracted DNA strands. Restriction digestion is useful to researchers in several ways. For example, researchers can identify whether a strand of DNA has a particular gene on it. They can also cut a gene from a strand for gene splicing.



Gel Electrophoresis

Gel electrophoresis is a process in which researchers apply an electric current to a gel to separate different lengths of DNA fragments into groups. The researchers can then recover a desired gene or gene fragment. Performing gel electrophoresis requires an electrophoresis box, a buffer solution, a special power supply, and a gel made from agarose or another agent. One end of the electrophoresis box has a positive pole and the other has a negative pole. The gel rests between them. The researcher places DNA fragments that have been stained with a dye in small wells or pockets at the end of the gel nearest the negative pole and applies an electric current to the gel. The buffer solution keeps the gel moist and facilitates the flow of the electrical current. The current causes the DNA fragments, which are negatively charged, to be repelled away from the negative pole and attracted to the positive pole (Figure 3.2). Short lengths of DNA will move through the gel faster than long lengths. The electric current is removed just before the short fragments reach the end of the gel. Fragments of DNA of the same size will be grouped at one spot on the gel. The markings caused by fragments of different sizes are called bands.



Gene-Splicing

Gene-splicing is the process of inserting a piece of DNA into a chromosome of a cell. It is also called ligation because the enzyme ligase is the biochemical glue that joins the pieces of DNA. Gene-splicing involves several steps. The process begins with the researcher cutting out a piece of DNA with a restriction enzyme. The correct restriction enzyme must be used so that the ends of the DNA will be "sticky," meaning that they contain bases that are complementary to the bases of the fragment to be incorporated. Gel electrophoresis must be performed to separate the DNA fragments by size and isolate the appropriate fragment. The

researcher then joins the ends of the selected fragment to the DNA being transformed through a chemical reaction called a ligase reaction. Ligase chemically joins two DNA fragments by causing a bond to form between the phosphate portion of each fragment. The reaction is often done in a test tube. The result is a cell containing DNA from two different sources that forms a new genetic code, which is therefore called recombinant DNA.

This cell is then grown into an organism. In transgenic animals, the gene is spliced into the chromosomes of a fertilized egg, which is then implanted in the female reproductive tract. In plants, the plant cell with the transferred genetic material (often referred to as a transgene) is stimulated to grow into a plant. In each case, the organism has a copy of the new genetic information in every cell.

Summary

Genetic engineering is accomplished through the use of several processes. Gene mapping is used to locate the desired trait. DNA extraction isolates the DNA containing the desired gene. Restriction digestion then cuts the extracted DNA into specific pieces. Electrophoresis separates the DNA fragments into groups of like size. Gene-splicing joins the isolated piece of DNA to the DNA being modified. These basic genetic engineering technologies are used in research laboratories across the country.

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