DNA Structure & Function, Mitosis

Purpose

This lab is intended to familiarize the student with the structure of DNA and how that structure facilitates the processes of protein synthesis, cell division and gametogenesis. The primary objectives of this lab are for the student to:

- Understand how the base pair rule governs the pairing of the nitrogenous base portions of the nucleotides, which comprise the DNA and RNA molecules.
- Understand the steps of protein synthesis and the molecules which facilitate them.
- Understand the life cycle of the cell and the cellular activities which are going on during each phase (and their sub-phases).
- Understand how the processes of Mitosis and Cytokinesis result in Eukaryotic cell division.
- Understand how the process of Meiosis results in the production of gametes and how it differs from mitosis.
- Observe Mitosis in a typical plant cell and be able to identify which stage of the cell cycle the cell is in by visual analysis.
- Visualize the chromosomes in a human karyotype.

Background

I. Introduction

All of the information to create and maintain a living organism is contained in the nucleus of the cell in the form of the deoxyribonucleic acid (DNA) molecule. The entire DNA molecule is quite large with a complex “double-helix” shape (approximating a twisted ladder). Organizationally, however, it is made up of repeated segments made of 3 basic subunits. Those 3 subunits are a ring-shaped (deoxyribose) sugar, a phosphate group, and a nitrogenous base. The sugar and phosphate molecules are strung together in a line to form the “sides” of the ladder with the nitrogen bases projecting laterally from the chain to form one half of each “rung.” To form the overall DNA molecule, two of these side pieces line up with the nitrogenous bases binding to each other in the middle to form the rungs of the ladder. There are only 4 different nitrogenous bases, adenine (A), thymine (T), cytosine (C) and guanine (G). A and G are molecules called purines (A.G. Purine), and C and T are molecules called pyrimidines. According to the base pair rule, A always pairs with T and C always pairs with G. Because of the base pair rule, if you have one side of the DNA strand, it is possible to build the other side accurately. This is the basis behind both Replication of DNA as well as production of molecules as in protein synthesis.

II. Cell Division

Recall the theory of heredity, which states that DNA is passed down for parent to offspring during reproduction. A cell’s life cycle, called the cell cycle, has 3 basic stages. The normal growth and development phase of the cycle is called interphase. During this phase, the DNA, which is located in the nucleus, is in a very loose, diffuse, ribbon-like state called chromatin. The DNA must be in this form in order to be read. Towards the end of interphase, the genetic material is duplicated so that there are two copies of the DNA.
The next phase of the cell cycle is mitosis, in which the DNA is condensed, organized and separated to opposite ends of the cell in preparation for cell division. There are 4 separate smaller phases that can be distinguished within this portion of the cell cycle. The first mitotic phase is prophase ("pro" = before), in which the nucleus breaks down, the loose chromatin condenses into very dense, tightly coiled structures called chromosomes ("colored bodies") and the centrioles (which have also been duplicated) migrate to opposite ends of the cell. The basic shape of chromosomes is that of an "puffy X" (imagine two hotdogs connected at their centers). Using this analogy, the individual hotdog like structures are called (sister) chromatids, and the portion where they are joined is called the centromere. During metaphase, the next mitotic phase, the chromosomes line up along the midline of the cell, called the metaphase plate ("meta" = middle), between the centrioles. Small tubules called spindle fibers grow out of the centrioles toward the chromosomes and eventually attach to them at the centromere. The next phase is anaphase ("ana" = without), in which the spindle fibers pull apart the sister chromatids which then migrate to the opposite ends of the cell (at this point the sister chromatids are once again referred to as chromosomes). When the chromosomes reach the centrioles at the opposite ends of the cell, this signifies the end of anaphase and the beginning of telophase, the last mitotic phase. Telophase can be thought of as the opposite of prophase. The chromosomes loosen up and return to the chromatin state and the nuclear membrane reforms.

The last primary phase of the cell cycle is cytokinesis. Cytokinesis is the physical separation of the one starting cell into two daughter cells. Cytokinesis begins during telophase and happens differently in plant and animal cells. In animal cells, cytokinesis is a pinching in of the cell membrane in the middle of the now elongate cell into two new, identical daughter cells. In plant cells, a cell membrane begins to form in pieces in the middle of the cell, which eventually walls off the two new daughter cells. Each new cell resulting from cell division has the normal somatic (diploid) chromosome number (2n; 2 copies of each chromosome).

The micrograph below displays examples of the different stages of the cell cycle in onion root tip cells.
Meiosis is the process that results in the gametes (sex cells). Meiosis is a process involving two stages of division that ultimately results in 4 cells, which have only one copy of each chromosome, or the haploid number (1n).

III. Protein synthesis

The genetic instructions are contained in the sequence of the bases in the DNA molecule. Every set of three bases in sequence is called a codon. Each codon in the DNA molecule codes for a specific amino acid in the production of a protein. The challenge is getting the instructions from inside the nucleus to metabolic machinery into the cytoplasm without altering the original template. Gene expression is the term that refers to the process of directing the synthesis of a specific protein using the information stored in the DNA sequence. This is done with the use of an intermediate “temporary copy” of a specific portion of the DNA strand. The intermediate molecule is called ribonucleic acid (RNA), and there are 2 types. The first step protein synthesis, generating a messenger RNA (mRNA) copy of the DNA sequence, is called transcription. The molecule that does the transcribing is an enzyme called RNA polymerase. This complex protein moves down the DNA strand matching each nitrogenous base with a complimentary mRNA version. This produces a growing “ribbon” of mRNA complimentary to the DNA template. Besides the difference in the sugar molecules of DNA and RNA (ribose sugar as opposed to the deoxyribose sugar of DNA), there is also a base difference. RNA replaces the base thymine (T) with the base uracil (U) so that in RNA, the matching base for A is U.

The second part of protein synthesis, making a protein from the working mRNA copy, is called translation. Translation is initiated when the newly made strand of mRNA is transported out of the nucleus into the cytoplasm where it attaches to a ribosome. The ribosome has three small pockets, binding sites in it, the A-site, the P-site and the E-site. The first codon of the mRNA strand attaches to the A-site. The mRNA strand then begins to move along the ribosome one codon at a time. The second type of RNA, transfer RNA (tRNA) is matches up to the mRNA strand at these sites on the ribosome. Unlike the mRNA molecule, the tRNA is not a long strand. The function of tRNA is to ferry amino acids to the ribosome so that they can be strung together in sequence to create the primary protein structure. The tRNA molecules are smaller, only containing only 3 base pairs, called an anticodon because they correspond to the codons on the mRNA strand. Also attached to each tRNA strand is a specific amino acid.

After the first tRNA molecule attaches to the codon in the A-site, the mRNA strand moves along the ribosome so that the tRNA which was in the A-site moves over to the P-site. This opens up the A-site up where the second mRNA codon is now located. A tRNA molecule with the second complimentary anticodon then attaches to the A-site which brings the two attached amino acids together and they form a peptide bond between them. With the next shift down the mRNA strand, the original tRNA ends up in the E-site where it then detaches from both the mRNA and the amino acid and returns to the cytoplasm where it picks up another amino acid and is recycled. Meanwhile, the second codon is now in the P-site, and a new tRNA molecule (with the anticodon that is complimentary to the third codon in the sequence) attaches at the A-site. The new amino acid attached to this new tRNA molecule binds to the second amino acid and the chain has grown 1 link longer. This process continues until the ribosome reaches the end of the mRNA strand and the protein is complete.
Laboratory Exercises
I. Karyotype slide
   Researchers will often construct a map of all of a person’s chromosomes (particularly in the case of inherited genetic abnormalities like Down’s syndrome, etc.) This is called a karyotype. In this exercise you will examine a prepared microscope slide of a karyotype. Draw the karyotype on your results sheet at 1000x.

II. Observation of mitosis in plant cells
   In this exercise you will be observing a slide of an onion root tip. Cells in this area are rapidly dividing in order to facilitate root growth. Find a representative cell in each of the stages of cell division (interphase, prophase, metaphase, anaphase & telophase) and make a drawing of each on your results sheet. Remember to return your slide to the tray and put away your microscope properly when you are finished.

You DO NOT need to do a formal lab write-up for this lab. You need only fill out the following results sheet (3 pages).
Results Sheet
DNA Structure & Function

I. Karyotype Slide

What are the two primary differences between DNA and RNA?
__________________________________________________________
__________________________________________________________

What are the two major steps in protein synthesis? _____________ & _____________ .

Which molecule is used as the working template that carries the genetic information from the nucleus out to the ribosomes? ________________

Which molecule is used to “ferry” amino acids to the ribosome during protein synthesis? ________________
Each sequence of 3 nitrogenous bases on the DNA molecule, called a ____________, codes for a specific ________________ molecule in the primary structure of a protein.

II. Observation of mitosis in plant cells.

During prophase, the ________________ migrate to opposite ends of the cell.

The stages ________________ and ________________ are almost exact opposites of each other.

_______________ is the process that results in the “physical” separation of the original cell into two new ________________ cells

Red blood cells have no nucleus. What does this mean in terms of the cells’ life span?

___________________________________________

Name one area in your body in which cells are actively dividing all the time (hint, most cancer treatments target rapidly dividing cells). ________________

_______________ is the process that results in the production of the gametes.
Draw your phases of the cell cycle below and label the metaphase drawing:

[Blank drawing with phases labeled]