DNA and Replication

DNA: For the basics- go to: http://teacherweb.com/TN/MEMPHIS/KRISHNAN/apt12.aspx

The fluid material of the nucleus is surrounded by the familiar double membrane called the nuclear envelope.

DNA, chromatin and chromosomes can be found inside the nucleus.

Chromatin is DNA that is combined with proteins, including histone and non-histone proteins.

Chromatin is coiled into larger, highly visible bodies that are called chromosomes.

Chromatin occurs in one or two forms: euchromatin or heterochromatin. Euchromatin contains nearly all the functional genes; this region uncoils to do transcription and translation. Heterochromatin contains the DNA that hasn’t been identified as genes and normally does not undergo transcription.

1. DNA STRUCTURE

DNA stands for deoxyribonucleic acid. All cells need a set of instructions in order to survive.

The instructions are provided in the form of DNA.

The “deoxy” implies that there is one less oxygen atom in each unit of DNA than RNA. The DNA molecule can be broken down into three parts.

1) Nitrogen Bases 2) Sugar backbone 3) Phosphate groups

The nitrogen bases are broken down into two groups.

* 1. Pyrimadine: Single ring bases: cytosine and thymine
  2. Purines: Double ring bases: guanine and adenine

The backbone of the chain consists of alternating sugar and phosphates. Linked by shared oxygen atomsa phosphodiester bond.

The nucleotide chain has two ends. The end with the phosphate attached to the #5 carbon of the sugar molecule is known as the 5’ end. The end with the OH group attached to the #3 carbon of the sugar molecule is called the 3’ end. Synthesis of the nucleic acid chain always proceeds from the 5 to the 3 end.

1. DOUBLE HELIX

DNA is almost always found in a double strand, while RNA is almost always a single strand.

The backbone is made of strong covalent bonds

The two strands are weakly attracted to each other by H bonds forming a double helix. Adenine will always form two hydrogen bonds with Thymine

Guanine will always form three hydrogen bonds with Cytosine.

The strands are antiparellel with sugar and phosphate backbones going in opposite directions.

One strand runs in the 5’ to 3’ direction and is paired with the second strand which runs 3’ to 5’. The hydrogen bonds may be broken or disrupted by raising the pH to 12 and by raising the heat to boiling

1. DNA REPLICATION AND SYNTHESIS

DNA replication animation

A. DIRECTION

DNA synthesis occurs in a 5’ to 3’ direction starting at the origin of replication.

Remember- \*\* There are two strands to DNA- The strand that is synthesized in the 5' to 3' direction is called the leading strand and is described below:

First the enzyme, helicase unwinds the two strands of DNA.

Once the strands are separated, a replication fork is established.

As the helicase unwinds a portion of the DNA, the other portion of the molecule is stressed, due to the increased winding of the circular molecule. In order to relieve this stress, an enzyme called topoisomerase breaks and reattaches the DNA molecule.

An enzyme, DNA polymerase, begins to match the free-floating deoxynucleotides found in the cell fluid to the bases presented by the opening up of the DNA strand.

As nucleotides of the unwound segment finds new partners, helicase unwinds more of the DNA strand creating a moving replication fork.

LAGGING STRAND SYNTHESIS

The second strand, called the lagging strand is synthesized as small pieces called okazaki fragments.

Okazaki fragments which are 1,000 to 2,000 base pairs on length and synthesized in a 5’ to 3’ direction. Lagging strand DNA synthesis starts at the RNA Primer which is a sequence of approximately 10 nucleotides of RNA. The RNA primer is placed down an enzyme call Primase.

The DNA polymerase continues the strand in a 5’ to 3’ direction

Then DNA Ligase seals up the newly laid DNA bases and Gyrase winds the DNA molecule up.

DNA polymerase will replace the RNA nucleotides in the primer with DNA bases

Review of enzymes of DNA synthesis: 1) Helicase 2) DNA Polymerase

3) Ligase 4) Gyrase 5) Primase 6) Topoisomerase

DNA synthesis is semi-conservative. Each new double helix is made up of one new strand and one old strand. DNA synthesis is very accurate. DNA polymerase makes very few errors. But what happens if there is a mistake in DNA synthesis? How are mistakes corrected?

D. DNA REPAIR

1. DNA POLYMERASE (3’ TO 5’)

DNA polymerase not only puts bases down in order, but it also proof reads what it has laid down. If the nucleotide that is laid down is the wrong one, polymerase will undo what it has done by going back and removing the wrong base. In a 5’ to 3’ direction DNA polymerase lays down the base, but in the 3’ to 5’ direction it proof reads.

1. PHOTOREACTIVATING ENZYME

U.V. light is a mutagen that causes thymine-thymine dimmer. The T-T dimmer can be fixed by the photoreactivating enzyme.

This enzyme is activated by the sunlight and breaks the thymine to thymine dimers

1. EXCISION REPAIR

Excision repair is responsible for repairing a broad spectrum of DNA damage from U.V. damage to bulky lesions. The damages are all repaired by the excision repair system.

1. Abilities

Repair compounds of different structures.

Make repairs when compounds bind in different places.

Detect damage in DNA.

2. Mechanisms for Repair

Some sort of recognition of damage is made by the enzyme.

Two nicks are made on either side of the lesion.

Lesion is removed and DNA is resynthesized by DNA polymerase The DNA is sealed by DNA ligase

Excision repair requires the presence of the complimentary strand of double helix during repair. Repair is done by complimenting the bases.

MUTATIONS: Inheritable change in DNA molecule. Occur by:

errors in replication from mutagenic agents, mutagens such as radiation (X-ray and UV light).

Certain chemicals can cause a change in DNA.

\*\*\* Genetic variation are the raw material for evolution.

E. TYPES OF MUTATIONS or changes in the DNA

* + - 1. Point mutation: Change of one nucleotide to another.

These are:

substitution - can be silent, missence or nonsence; frameshift - deletion or insertion of a single nucleotide Inversion - exchange of two nucleotides in the sequence

* + - 1. Larges changes in the DNA:

Breakage and loss of a fragment of DNA

Extra copies of DNA

Transformation- bacteria only

Transduction- viral exchange of host DNA

Translocation- eukaryotes only

Conjugation - in bacteria only

* + - 1. Changes in inherited DNA can also occur by meiosis ( know the three ways)

1. Similarities and differences between prokaryote and euakaryote DNA replication:

prokaryotes have a circular DNA- means no problem with ends

eukaryotes have linear DNA - problem with replicating ends (telemers); ends have repeat sequencesenzyme telemerase can extend ends up to an early age; after that every time DNA replicates, it is shortened. prokaryote - one origin of replication; eukaryote- many origins of replication (more than one chromosome) similarities - the actual process is same (lagging and leading strand) - all enzymes same.

1. Early experiments in DNA
2. In 1928, Fred Griffith performed the first experiment in which he first injected mice with a live strain ofvirulent (deadly) bacteria, S strain, and not to anyone's surprise, all of those mice died. Then, he killed the virulent bacteria cells by heating them. Mice injected with these heat-killed virulent bacteria did not die. In another set of mice, Griffith injected a live non-virulent strain of bacteria, R strain, and these mice did not die, the result which Griffith expected.

The surprise came when Griffith injected a group of mice with both live non-virulent bacteria and heat-killed virulent bacteria. In that group, some of the mice died. When Griffith examined those mice, he found live virulent bacteria in their blood. Griffith drew the conclusion that the genetic information in the heat-killed virulent bacteria was TRANSFORMED into the non-virulent strain to cause them to become virulent.

1. Oswald Avery followed up on Griffith's experiment in the following decade. Like Griffith, Avery first usedheat to kill virulent bacteria. He treated one sample with DNAse and another with protease and added each sample separately to a culture of live non-virulent bacteria to determine which was responsible for changing them into virulent bacteria. Only the non-virulent cells that were given DNA (protease treated sample) from the dead virulent strain became virulent, so Avery concluded that DNA must be the transforming factor.
2. Griffith - analyzed the components of DNA - made of phosphate sugar and base; Chargoff rule: A = T andC = G in DNA of all organisms
3. Stahl: http://www.sumanasinc.com/webcontent/animations/content/meselson.htmldone to determine if DNA replication is conservative, semi-conservative or dispersive 5. Hershey and Chase: done to determine if DNA or protein is the genetic material

http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi? it=swf::535::535::/sites/dl/free/0072437316/120076/bio21.swf::Hershey%20and%20Chase%20Experiment Practice questions for DNA: DNA