NUCLEIC ACIDS

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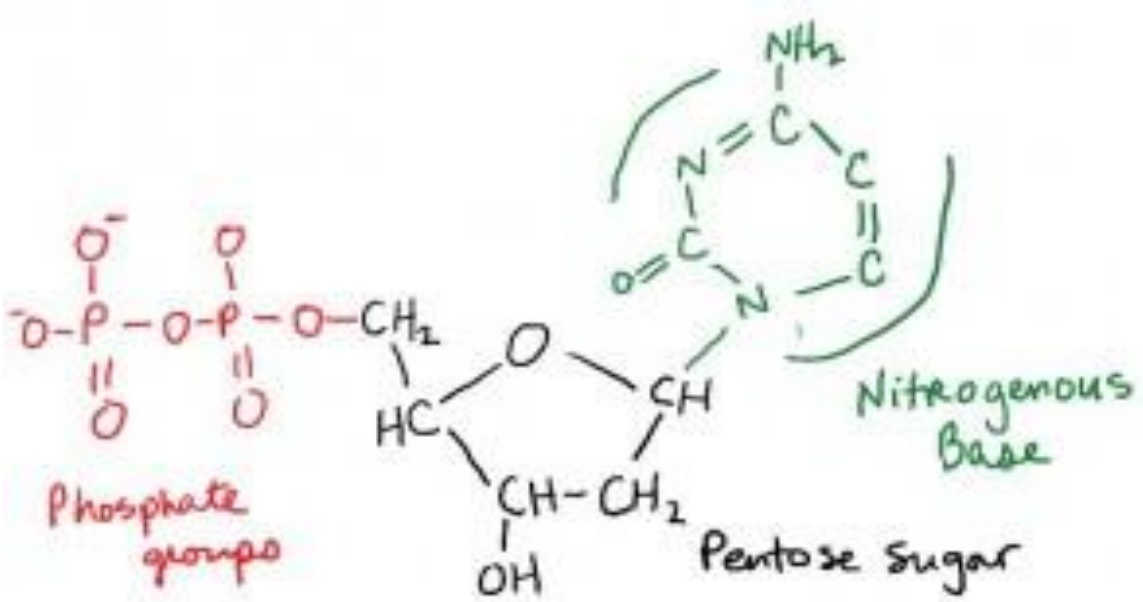
- Not used primarily for either cellular energy or structural integrity of the cell
- Storage and expression of genetic information

- Deoxyribonucleic acid-DNA
- Ribonucleic acid-RNA

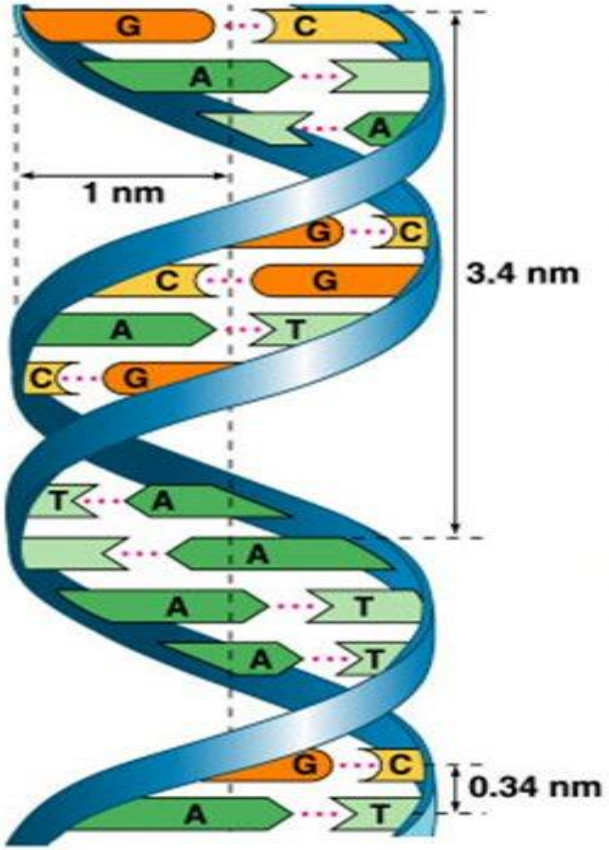
- DNA replicates precisely
- Selectively expresses information

Building blocks-nucleotides

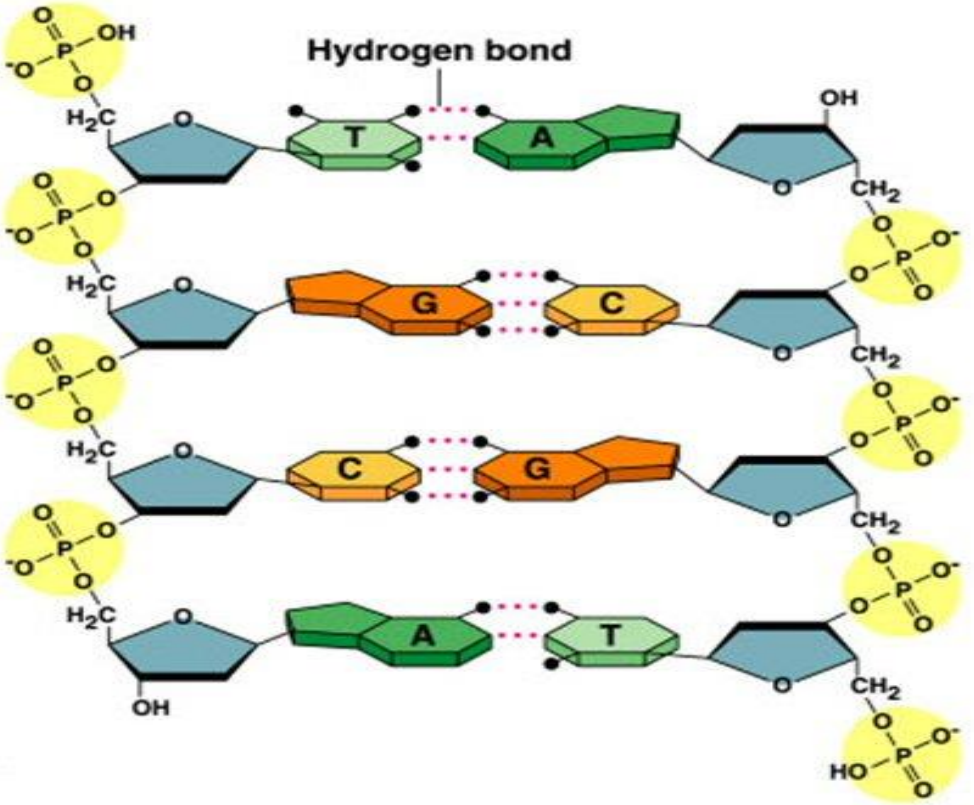
Cytosine



- DNA- a polynucleotide containing many mononucleotides covalently linked to each other by 3‘, 5' phospho-diester bonds
  - Double stranded molecule- 2 strands wind around each other forming a double helix



(a) Key features of DNA structure



(b) Partial chemical structure

- Paired in anti-parallel manner- 5' end paired with 3' end of the other strand

- Given the sequence of bases on one strand –determine sequences of bases on the complementary strand

- Base pairs held by hydrogen bonds
- Hydrophobic interactions between stacked bases stabilise the structure of the double helix

REPLICATION

Process by which a replica or identical copy of DNA is made

Occurs every time a cell divides so that information can be preserved and handed down to the offspring

Similar to making a copy of a file onto a disk so you can take that file to a different computer

Begins with a partial unwinding of the double helix at an area known as the replication fork. Unwinding is accomplished by an enzyme known as DNA helicase

- Under an electron microscope, the unwound section appears as a bubble and thus known as a replication bubble

As the two strands separate and the bases are exposed, the enzyme, DNA polymerase moves into position where synthesis will begin

How does the DNA polymerase enzyme know where to begin synthesis?

Is there some sort of marker, a start point?

– The start point for DNA polymerase is a short segment of RNA known as an RNA primer. “Primer” is indicative of its role which is to prime or start DNA synthesis at certain points. The primer is laid down complementary to the DNA template by an enzyme known as RNA polymerase or primase.

- The DNA polymerase (once it has reached its starting point as indicated by the primer) then adds nucleotides one by one in an exactly complementary manner, A to T and G to C

How does the polymerase know the base to add?

- DNA polymerase is described as being template “dependent” in that it will read the sequence of bases on the template strand and then synthesize the complementary strand. The template strand is always read in the 3´ to 5´ direction (i.e starting from the 3´end of the template and reading the nucleotides in order towards 5´end of the template).

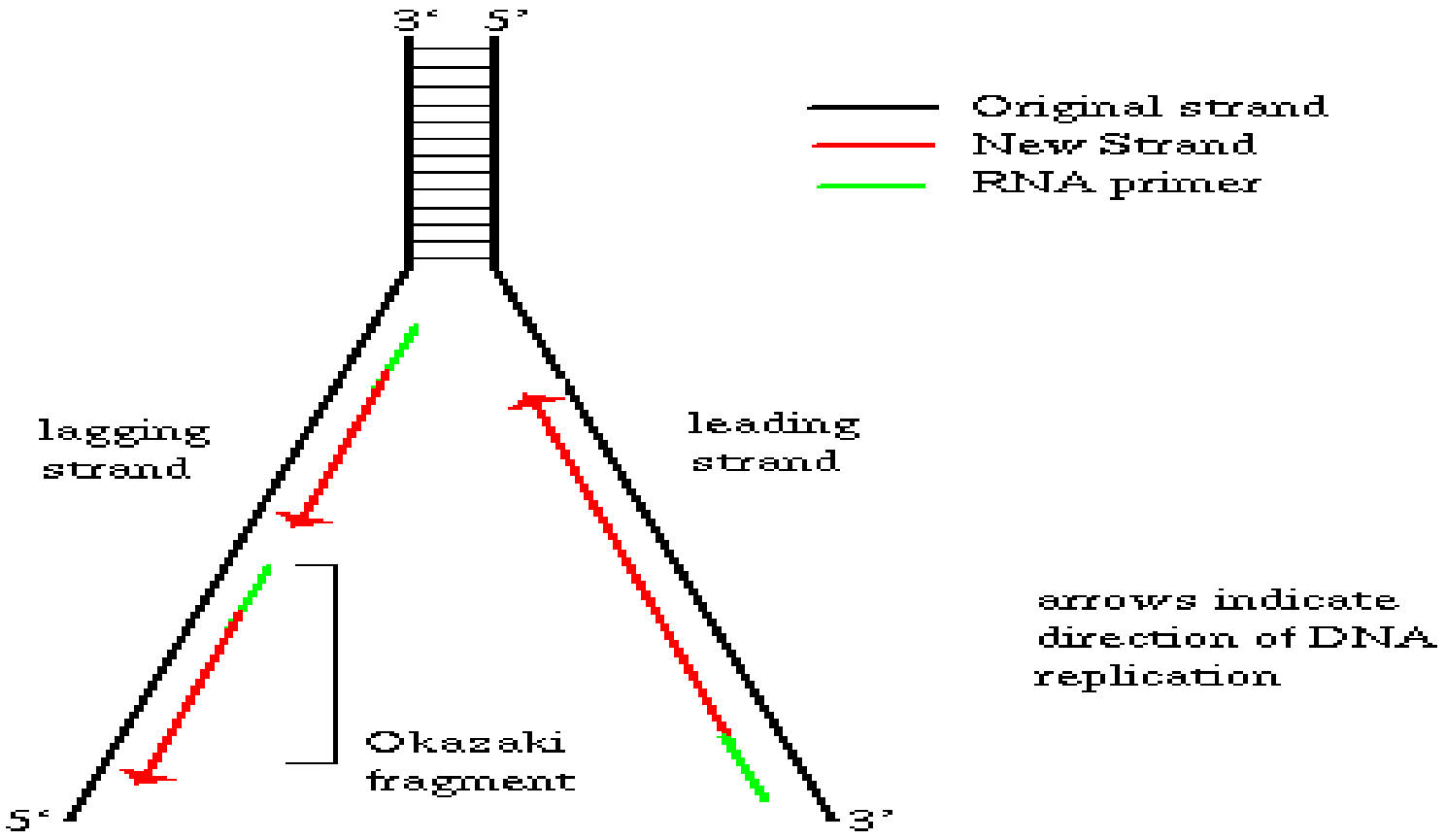
The new DNA strand (since it is complementary) must be synthesized in the 5´ to 3´ direction (remember that both strands of a DNA molecule are described as being anti-parallel). DNA polymerase catalyses the formation of the hydrogen bonds between each arriving nucleotide and the nucleotides on the template strand.

In addition to catalyzing the formation of hydrogen bonds between complementary bases on the template and the newly synthesized strands, DNA polymerase also catalyzes the reaction between the 5´phosphate on an incoming nucleotide and the free 3´ OH on the growing polynucleotide (phosphodiester bond).

As a result, the new DNA strands can grow only in the 5´to 3´direction, and strand growth must begin at the 3´ end of the template. Note that a phosphodiester bond is formed between the OH group of the sugar and the 5´ phosphate group of the incoming nucleotide

Recall that the original DNA strands are complementary and run anti parallel. Only one new strand can begin at the 3´end of the template DNA and grow continuously as the point of replication (the replication fork) moves along the template DNA. The other strand must grow in the opposite direction because it is complementary, not identical to the template strand. The result of this side's discontiguous replication is the production of a series of short sections of new DNA called Okazaki fragments. To make sure that this new strand of short segments is made into a continous strand, the sections are joined by the action of an enzyme called DNA ligase which ligates the pieces together by forming the missing phosphodiester bonds.

Fig 1: Showing DNA replication



The last step is for an enzyme to come along and remove the existing RNA primers. This is the job of yet another type of DNA polymerase which has the ability to dismantle the primers and replace them with the deoxynucleotides that make up DNA.

Fig 2: DNA REPLICATION OF OKAZAKI FRAGMENTS

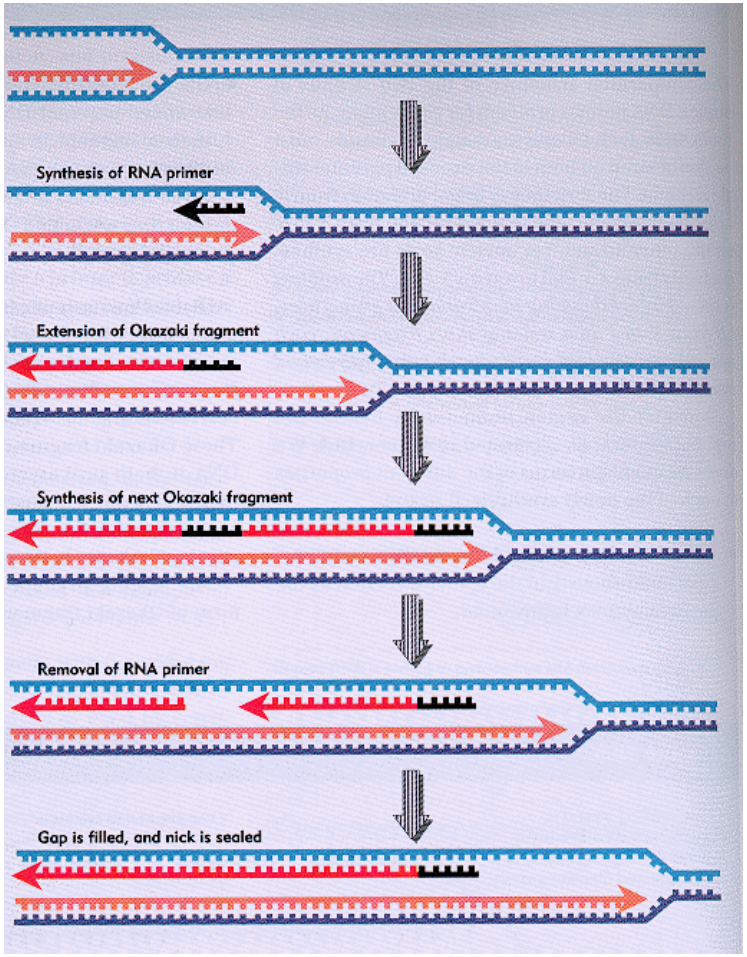
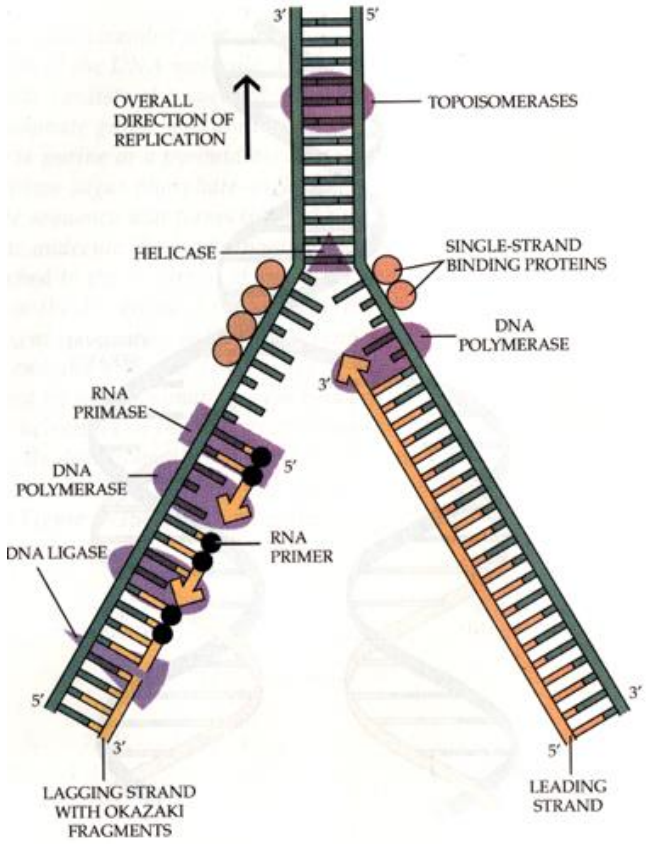


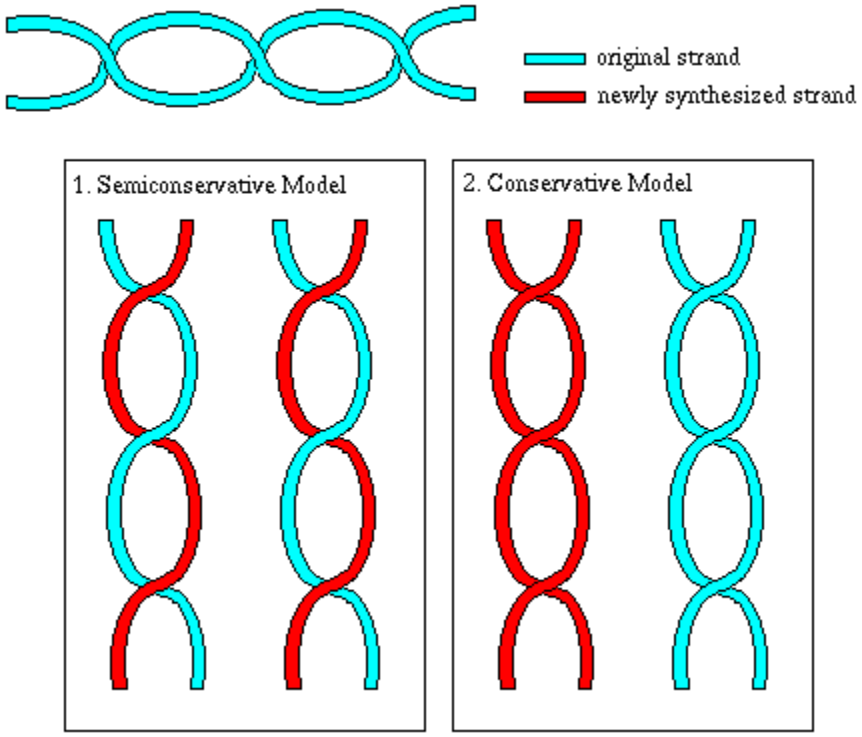
Fig 3: DNA REPLICATION



Since each strand is complementary to its old template strand, two identical new copies of the DNA double helix are produced during replication. In each new helix, one strand is the old template and the other is newly synthesized, result described as replication being semiconservative.

Francis Crick described the DNA replication process and the fitting together of two strands as being like a hand in a glove. The hand and glove separate, a new hand forms inside the old glove, and a new glove forms around the old hand. As a result, two identical copies now exist

Fig 4: Semi conservative nature of replication

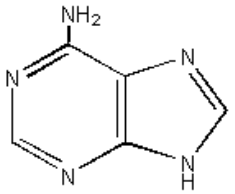


The process of DNA replication in all organisms is amazing, but in humans it seems particularly difficult to conceive. The sum of all genes in a human cell-the human genome- is estimated to be approximately 3 billion base pairs, and a single DNA chain might contain up to 250 million pairs of bases. What is even more incredible is how few mistakes are made in this process despite the immense size of human DNA

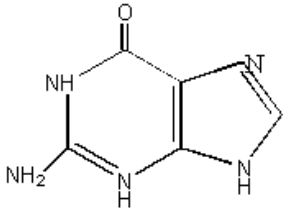
- An error occurs only about once in each 10-100 billion bases. As you would probably expect, the complete process of DNA replication in human cells takes several hours. To replicate such huge molecules of human DNA at this speed requires not one, but many replication forks, forming replication bubbles and producing many segments of DNA strands that eventually meet up together and are joined to form the newly synthesized double helix.

DNA polymerases proofread the nascent product; their 3´to 5´exonuclease activity examines the outcome of each polymerisation step.

The Four Bases of DNA (Plus One in RNA)!

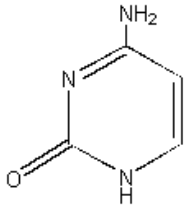


Adenine

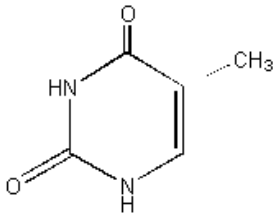


Guanine

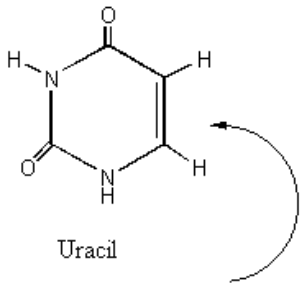
Purine (double ring) bases



Cytosine



Thymine



Uracil

This one's in RNA!

Pyrimidine (single ring bases)

TRANSCRIPTION

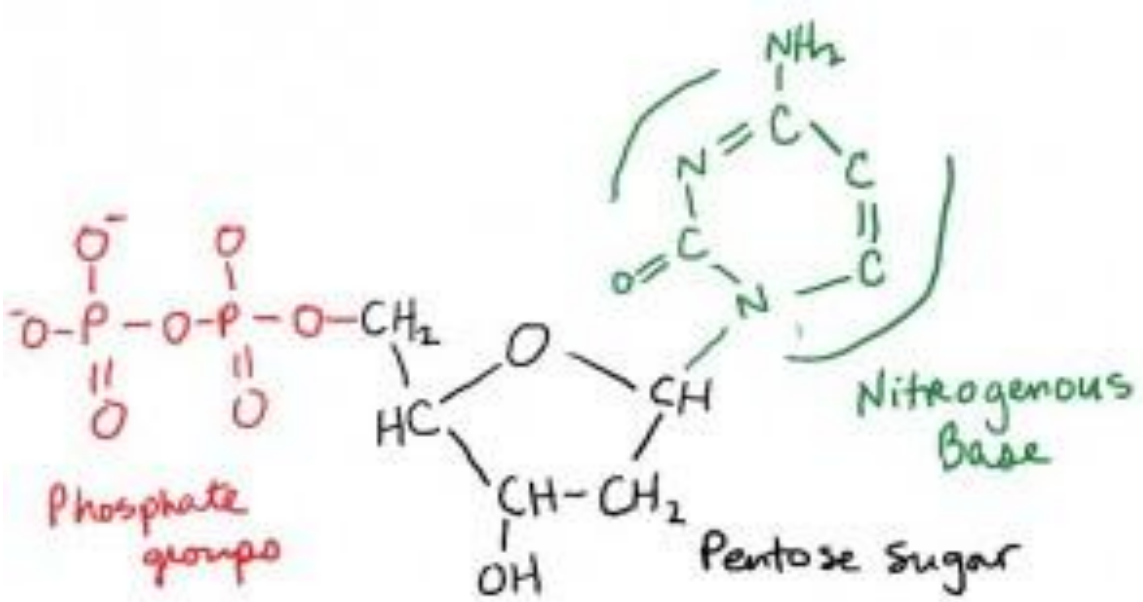
Objectives:

- Identify major types of cellular RNA
- Describe major steps in transcription
- Describe the different processing and splicing events that occur during mRNA synthesis

The process of converting the information contained in a DNA segment into mRNA.

Building blocks-nucleotides

Cytosine



This process of converting the information contained in a DNA segment into proteins begins with the synthesis of mRNA molecules containing anywhere from several hundred to several thousand ribonucleotides depending on the size of the protein to be made.

Each of the 100,000 or so proteins in the human body is synthesized from a different mRNA that has been transcribed from a specific gene on DNA

Why do we need mRNA if DNA holds all the genetic information, the instructions for the proteins the cell is supposed to produce?

- For eucaryotic cell, there is need to protect DNA because once it is damaged in any way, then the coding sequence is changed and a mutation which could greatly affect the cell or even the whole organism could result

If DNA was to venture out into the cytoplasm where the ribosomes are in order to give instructions for which proteins were to be made, then it would be more vulnerable to damage from chemicals and other agents.

- How is the DNA supposed to get the information it codes out to the ribosomes which carry out the instructions in the cytoplasm?
  - There must be a messenger and this messenger is mRNA

Messenger RNA (mRNA) is synthesized in the cell nucleus by transcription of DNA, process similar to DNA replication.

- As in replication, a small section of the DNA double helix unwinds, and the bases on the two strands are exposed . RNA nucleotides (ribonucleotides) line up in the proper order by hydrogen- bonding to their complementary bases on DNA, the nucleotides are joined together by a DNA dependent RNA polymerase enzyme and mRNA results.

- Unlike what happens in DNA replication where both strands are copied, only one of the two strands is transcribed into mRNA (remember that RNA is a single-stranded molecule). The DNA strand that is transcribed is called the template strand (also known as the antisense strand), while its complement is called informational strand (also called the coding or sense strand)

Since the template strand and the informational strand are complementary, it follows that the mRNA molecule produced during transcription is a copy of the DNA informational strand.

How do the polymerase and helicase enzymes know where to begin? In other words where does one gene start and stop and the next one begin?

- The starting point of a gene is marked by a certain base sequence which is called a promoter site. These sites are recognised by a factor called “sigma”. It is sigma’s job to recognise the promoter sites and “tell” the DNA dependent RNA polymerase where to begin transcription.

Once the RNA polymerase has been directed to the start point of the gene by sigma, the sigma factor is released and the RNA polymerase carries out the process of transcription.

- The promoter sites act as a start signs

Similarly, there are base sequences at the end of a gene that signal a stop to mRNA synthesis. Just as there is a sigma factor to help signal the beginning of a gene, another factor called “Rho” aids in terminating the process of transcription.

When the end of the gene is near, the Rho factor binds to the mRNA and interacts with the RNA polymerase. The interaction of Rho with the RNA polymerase causes the enzyme to “fall off” the DNA template strand, thus stopping transcription.

STRUCTURE AND FUNCTION OF RNA

- RNA is structurally similar to DNA
  - Both nucleic acids are sugar-phosphate polymers and both have nitrogen bases attached to the sugars of the backbone- but there are several important differences.

- They differ in composition:
  - The sugar in RNA is ribose, not the deoxyribose in DNA
  - The base uracil is present in RNA instead of thymine

- They also differ in size and structure:
  - RNA molecules are smaller (shorter) than DNA molecules
  - RNA is single stranded, not double stranded like DNA

Another difference between RNA and DNA is in function. DNA has only one function- storing genetic information in its sequence of nucleotide bases. But there three main kinds of ribonucleic acid, each of which has a specific job to do.

Ribosomal RNAs- exist outside the nucleus in the cytoplasm of a cell in structures called ribosomes. Ribosomes are small, granular structures where protein synthesis takes place. Each ribosome is a complex consisting of about 60% ribosomal RNA (rRNA) and 40% protein.

Messenger RNAs-are nucleic acids that record information from DNA in the cell nucleus and carry it to the ribosomes

Transfer RNAs- the function of transfer RNAs (tRNA) is to deliver amino acids one by one to protein chains growing at ribosomes

Please note that DNA transcription is a highly selective process. In most mammalian cells, for example, only about 1% of the DNA nucleotide sequence is copied into functional RNA sequences (mature messenger RNA or structural RNA).

The selectivity occurs at two levels:-

- Only part of the DNA sequence is transcribed to produce nuclear RNAs.
- Only a minor proportion of the nucleotide sequences in nuclear RNAs survives the RNA processing steps that precede the export of RNA molecules to the cytoplasm

RNA polymerases catalyse all DNA transcription.

- There are three RNA polymerases known:
  - RNA polymerase I-18S, 5.8S and 28S rRNA
  - RNA polymerase II-mRNA precursors snRNA
  - RNA polymerase III-tRNA and 5S rRNA

In eucaryotic cells, RNA molecules undergo extensive processing before they are translated into protein. In procaryotic cells, RNA synthesis (transcription) and protein synthesis (translation) occur concurrently i.e ribosomes translate the 5’ end of an RNA molecule into protein while the 3’end of the RNA molecule is still being synthesized

Consequently, there is relatively little opportunity to alter the RNA transcripts before they are translated into protein.

In eucaryotes, by contrast, transcription (in the nucleus) is separated both temporarily and spatially from translation (in the cytoplasm)

The RNA transcripts in the nucleus are immediately packaged into ribonucleo-protein complexes and subjected to RNA splicing, in which certain portions of the nucleotide sequence are removed.

Only when splicing is complete are the packaging proteins removed and the RNA molecules transported out of the nucleus to the cytosol, where ribosomes begin translating the RNA into protein.

- RNA splicing is an important intermediate step in the transfer of genetic material in eucaryotes. It provides a number of advantages for the cell, including the potential for a single gene to make several different proteins. This may explain why eucaryotic cells have a nucleus where splicing can occur without interference from ribosomes

ANTIBIOTIC INHIBITORS OF TRANSCRIPTION

- Antibiotics are interesting molecules because many of them are highly specific inhibitors of biological processes. *Rifampicin* and *Actinomycin* are two antibiotics that inhibit transcription in quite different ways.

- Rifampicin specifically inhibits the initiation of RNA synthesis by interfering with the formation of the first phosphodiester bond in the RNA chain.

Actinomycin D inhibits transcription by an entirely different mechanism. It binds tightly and specifically to double-helical DNA and thereby preventing it from being an effective template for RNA synthesis (refer to its use in treatment of cancers)

More than a hundred deaths result each year in the world from the ingestion of poisonous mushrooms, particularly *Amanita phalloides*

One of the toxins in this mushroom is α-amanitin, a cyclic octapeptide that contains several unusual amino acids. α-amanitin binds very tightly to RNA polymerase II and thereby blocks the formation of precursors of mRNA.

Polymerase III is inhibited by higher concentrations of α-amanitin (1μM), whereas polymerase I is insensitive to this toxin. α-amanitin blocks the elongation phase of RNA synthesis