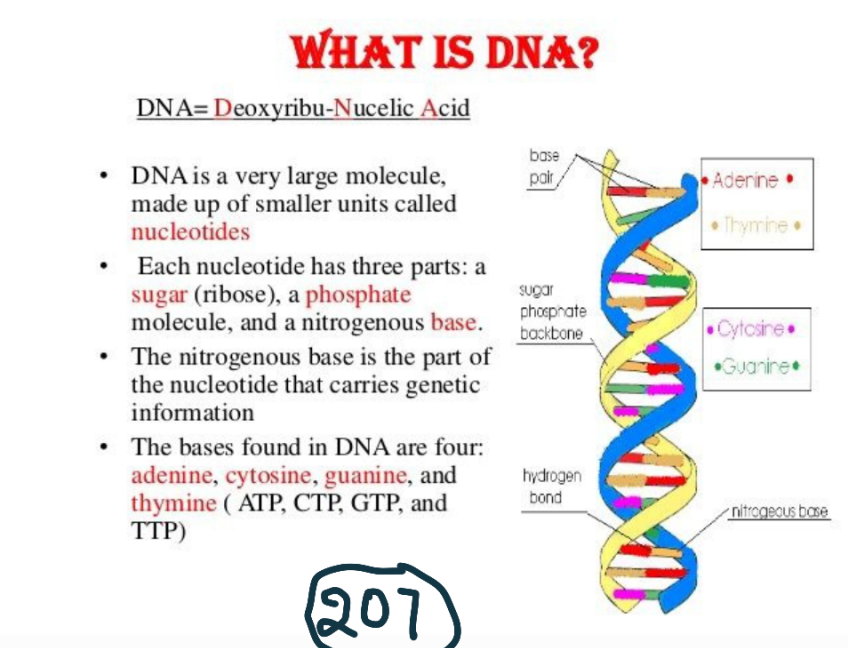
Tuberculosis- part 1 (220209_180815)
Tuberculosis - part 2. Tuberculosis continued- (220217_105048)
Tuberculosis - part 3. TB disease (220224_130739)
Tuberculosis - part 4. Treatment of TB disease- (220328_193900)
Tuberculosis-part 5. Elimination (220328_193619)

Tuberculosis - part 6. Tb treatment- DNA replication and protein synthesis

12.2. DNA replication



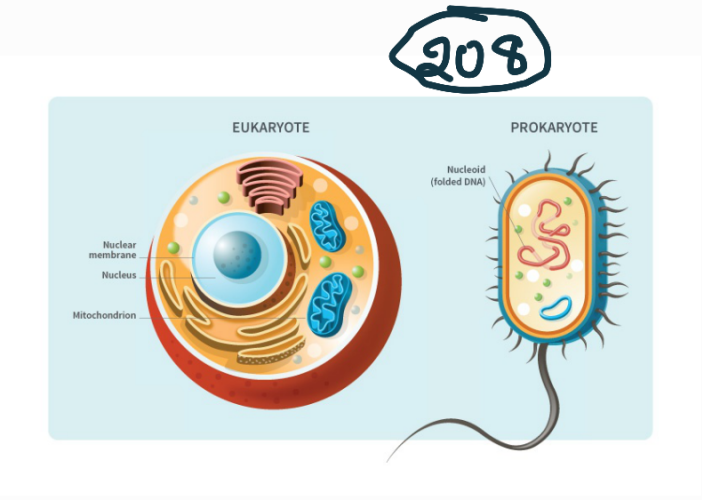
The second focus of anti tb drug action is on DNA replication. These are a few tb drugs which inhibit DNA replication. Let us try to understand DNA replication.

12.2.1. What is DNA?

Deoxyribonucleic acid or DNA (207)is the fundamental unit of all life: man, fruit or bacteria. It contains instructions for life or directions for traits as diverse as the color of a person's eyes, the scent of a rose, and the way in which TB bacteria infects a lung cell. There is no

life without DNA.

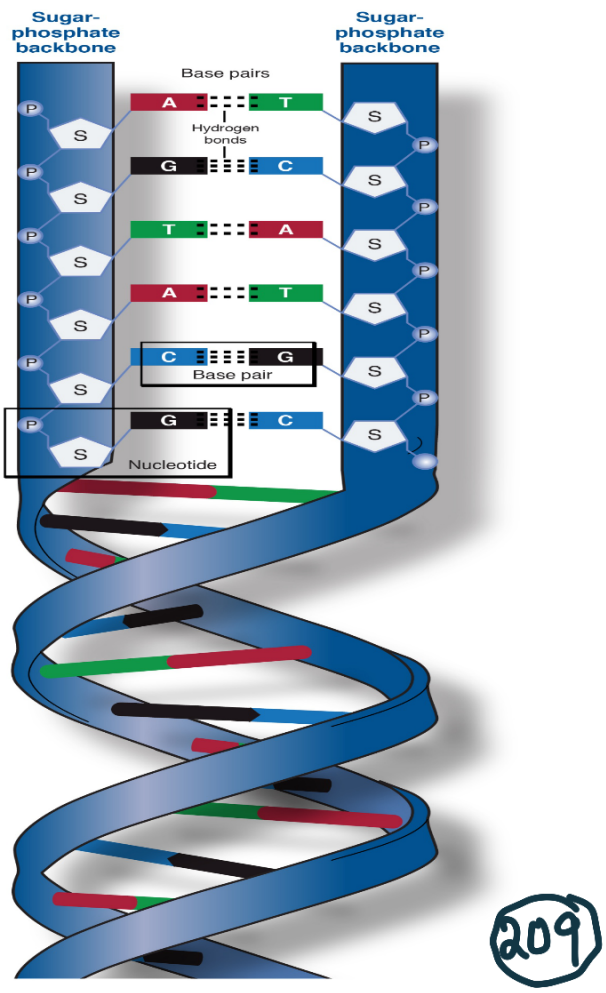
Where exactly do you find DNA? It depends on whether the organism has membrane-bound nucleus or not. Organisms composed of cells that contain nuclei are classified as *eukaryotes*, whereas organisms composed of cells that lack nuclei are classified as *prokaryotes (208)*. In *eukaryotes*, DNA is housed within the nucleus, but in *prokaryotes*, DNA is located directly within the cellular cytoplasm, as there is no nucleus available. A bacterial cell has cell capsule, cell wall and membrane, cytoplasm with chromosome, and ribosome. It does not have other organelles like mitochondria, endoplasmic reticulum, or nucleus.



12.2.2. What components make up DNA?

All DNA is composed of a series of smaller molecules called *nucleotides*. In turn, each nucleotide is itself made up of three primary components: a nitrogen-containing region known as a *nitrogenous base,* a carbon-based sugar molecule (all macromolecules like

carbohydrates, proteins and fats have carbon in their molecule. How do we know if a molecule has carbon? A carbon based molecule like sugar burns and chars when lighted, a molecule without carbon like salt does not) called *deoxyribose*, and a phosphorus-containing region known as a *phosphate group* attached to the sugar molecule (209). The phosphate and sugar form the backbone like the poles of a ladder.The ladder poles are not straight, however. They are twisted several times.



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The arrangement of nucleotides and the phosphate sugar backbone is unique. It is the same in every double stranded DNA.

There are four different DNA nucleotides, each defined by a specific nitrogenous base: adenine (often abbreviated "A" in writing), thymine (abbreviated "T"), guanine (abbreviated "G"), and cytosine (abbreviated 'C') (209) . The nucleotides on each string face each other and are linked by hydrogen bond. Nucleotide A faces nucleotide base T on the opposite

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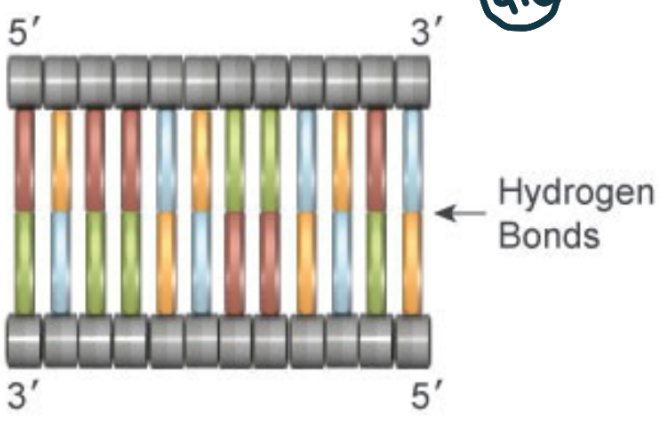
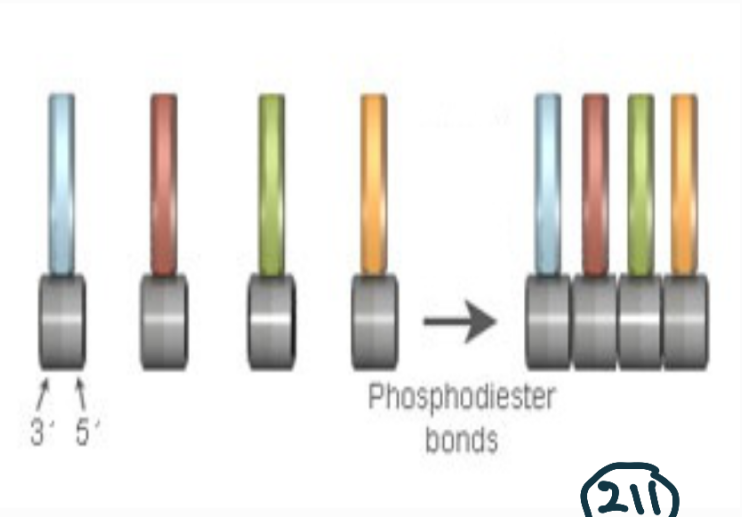


string and G faces C on the opposite strings. They form the rungs of a ladder. While nucleotides opposite each other are linked by *hydrogen bonds (210)*, nucleotides adjacent to each other on the same string are attached to each other at the sugar-phosphate backbone by a chemical (*phosphodiester) bond* (211,212,213)-the phosphate group of one nucleotide bonds like Lego bricks with the sugar molecule of the next nucleotide, and so on. They connect only one way round. The phosphate of one nucleotide slides into the sugar of the next. It is this alternating sugar-phosphate arrangement that forms the "backbone" of a DNA molecule. To cut DNA molecules, you need to break these phosphodiester bonds, which is accomplished by the enzyme *phosphodiesterase*. The sugar-phosphate backbone is described as extending, or growing, in the 5' to 3' direction The carbon atoms of the five-carbon sugar are numbered clockwise from the oxygen as 1', 2', 3', 4', and 5' (1' is read as "one prime") (214). The phosphate group is attached to the 5' carbon of one nucleotide and the 3' carbon of the next nucleotide. The sugar and the



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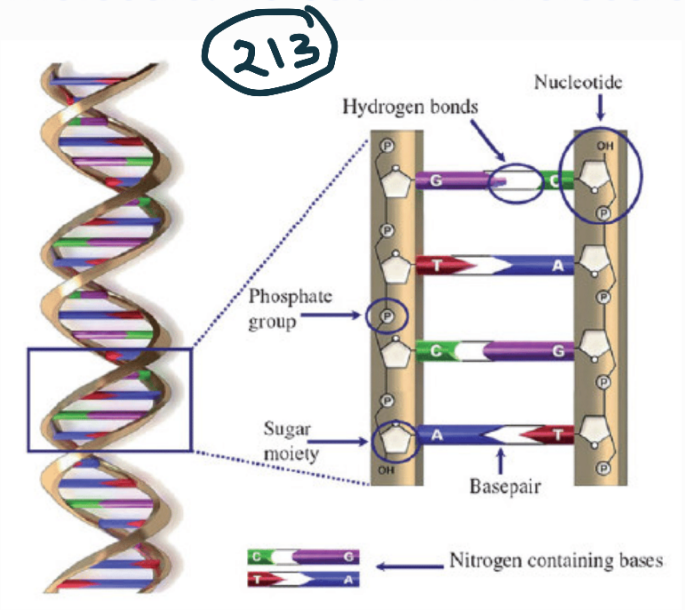

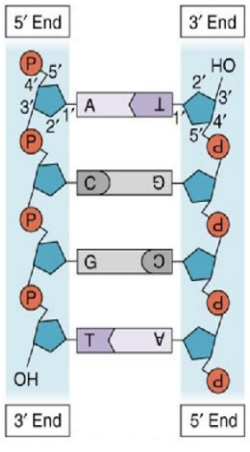

During DNA replication, DNA polymerase READS the parent molecule in the 3' → 5' direction.

New DNA is synthesized in the 5' → 3' direction (opposite).

(How to Remember? When you READ a book you would read chapters 3 to 5)

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phosphate group are the same for every nucleotide.

When the new DNA US made there is no right or left ot top or down direction in DNA. It is indicated by the 5' to 3'. All enzymes involved in replication or transcription follow this direction.

Since the deoxyribose sugar is negatively charged it allows several proteins and enzymes to attach themselves to it during replication and transcription processes. The DNA binding proteins perform important functions like copying the DNA (replication), controlling when, where and how much a gene gets read (transcription regulation), and much more.

12.2.3. How is the DNA strand organized?

DNA is double stranded- it has two strands, with each strand made of nucleotides (A, T, G, C) with phosphate sugar (P-S) backbone. Double-stranded DNA consists of two polynucleotides that are arranged such that the nitrogenous bases within one polynucleotide are attached to the nitrogenous bases within another polynucleotide by way of special chemical bonds called *hydrogen bonds.* This base-to-base bonding is not random; rather, each A in one strand always pairs with a T in the other strand, and each C always pairs with a G. This means that if one strand carries the sequence ATGC, the other has to have the sequence TACG. The nucleotides in a base pair are complementary which means their shape allows them

to bond together with hydrogen bonds. The A-T pair forms two hydrogen bonds. The C-G pair forms three hydrogen bonds and therefore the affinity between them is stronger. The hydrogen bonding between complementary bases holds the two strands of DNA together. Hydrogen bonds are not chemical bonds. They can be easily disrupted. This permits the DNA strands to separate for transcription (copying DNA to RNA) and replication (copying DNA to DNA). The nucleotides look like rungs of a ladder, the two strands are like the poles of the ladder made of sugar and phosphate, and the poles are twisted. When the cell divides the DNA strands are copied resulting in two old or parental strands and two new or daughter strands, giving rise to two double stranded DNA. The double-stranded DNA that results from this pattern of bonding looks much like a ladder with sugar-phosphate side supports and base-pair rungs.

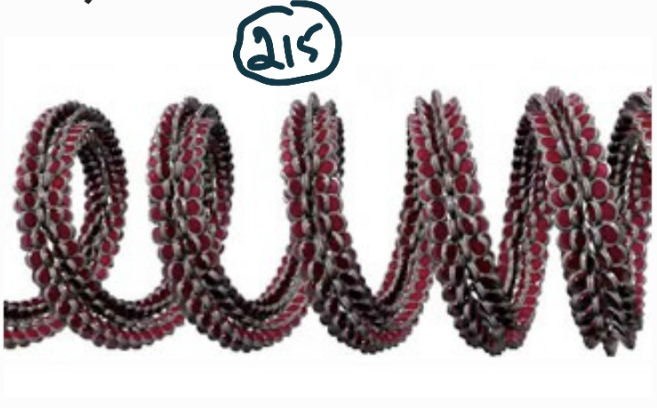
Note that because the two polynucleotides that make up double-stranded DNA are "upside down" relative to each other, their sugar-phosphate ends are anti-parallel, or arranged in opposite orientations. This means that one strand's sugar-phosphate chain runs in the 5' to 3' direction, whereas the other's runs in the 3' to 5' direction (214). It's also critical to understand that the specific sequence of A, T, C, and G nucleotides within an

organism's DNA is unique to that individual, and it is this sequence that controls not only the operations within a particular cell, but within the organism as a whole.

Beyond the ladder-like structure described above, another key characteristic of double-stranded DNA is its unique three-dimensional shape -a spiral shape called a helix. DNA actually takes the form of a double helix, a ladder-like structure that is twisted along its entire length.

12.2.4. How is DNA packaged inside cells?

Most cells are incredibly small. For instance, one human alone consists of approximately 100 trillion cells. Yet, if all of the DNA within just one of these cells were arranged into a single straight piece, that DNA would be nearly two meters long! So, how can this much DNA be made to fit within a cell? The answer to this question lies in the process known as DNA packaging, which is the phenomenon of fitting DNA into dense compact forms (215). During DNA packaging, long pieces of double-stranded DNA are tightly looped, coiled, and folded so that they fit easily within the cell. *Eukaryotes (eg. Humans)* accomplish this feat by

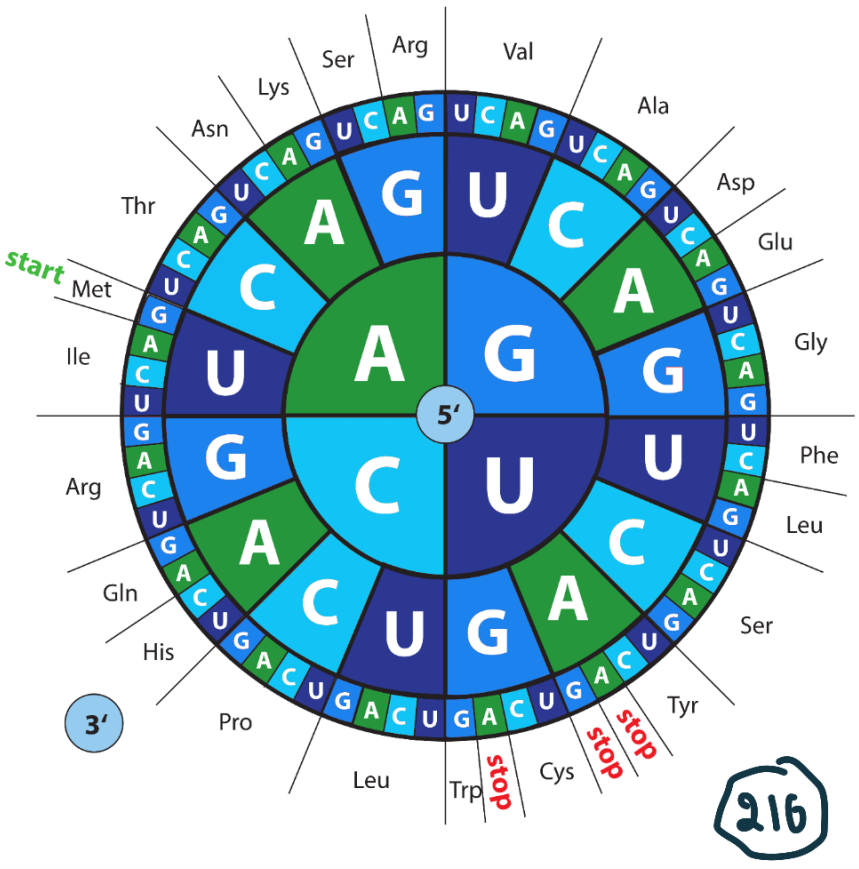


wrapping their DNA around special proteins called histones, thereby compacting it enough to fit inside the nucleus . Together, eukaryotic DNA and the histone proteins that hold it together in a coiled form is called chromatin.

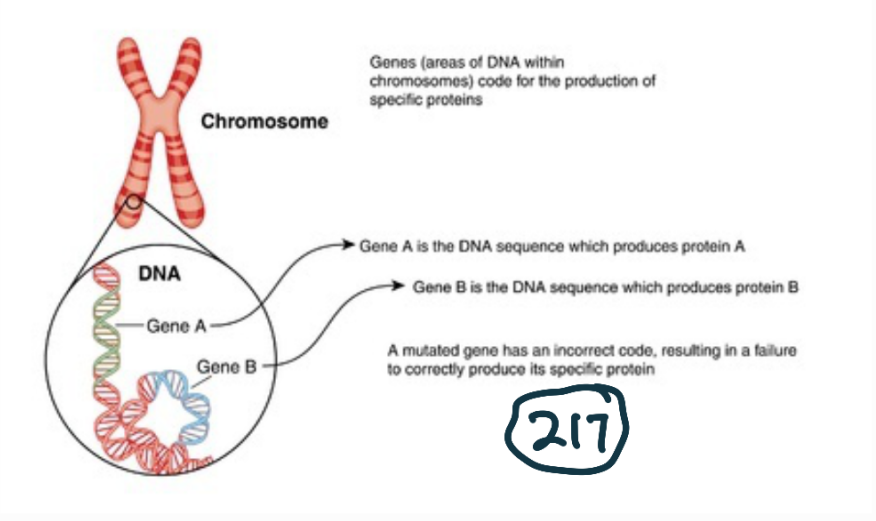
12.2. 5. What is genetic code?

DNA can be further compressed through a twisting process called supercoiling. Most *prokaryotes* (bacteria) lack histones, but they do have supercoiled forms of their DNA held together by special proteins. In both eukaryotes and prokaryotes, this highly compacted DNA is then arranged into structures called *chromosomes* which take different shapes in different types of organisms. For instance, most *prokaryotes (bacteria)* have a single *circular chromosome*, whereas most *eukaryotes* have one or more *linear (straight, line like) chromosomes,* which often appear as X-shaped structures .

The most important role for DNA is to carry the information from one generation to the next. This information is written in code using the four bases as chemical letters. The codes that make up the genes have been cracked. It is called *genetic code* (216)which is common for all living beings containing the DNA.



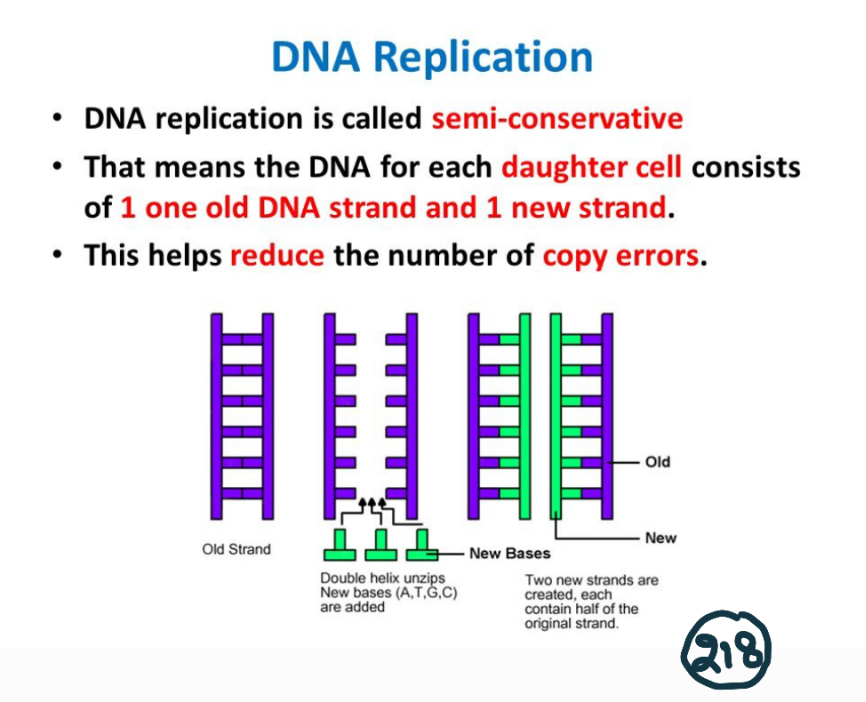
Genes (217) are parts of DNA that carry the instructions for making aminoacids and chains of aminoacids called proteins which build the living beings and are responsible for all the metabolic functions and survival. The building blocks of proteins are aminoacids and there are 20 amino acids. The sequence in which the nucleotides are arranged and the sequence in which aminoacids are arranged and the 3D shape of the aminoacid chain determines the function of the protein. The instructions are written in genes as three letter codons. Each three letter codon finally becomes translated into an aminoacid. With four letters and 20 aminoacids it is possible to make 64 three letter words.



In M.tuberculosis there is only one chromosome and 4,385,518 nucleotide base pairs, with 65.59% of base pairs being G-C. It has 4194 genes. Compare this with human genome which has 3 billion base pairs and about 30000 genes.

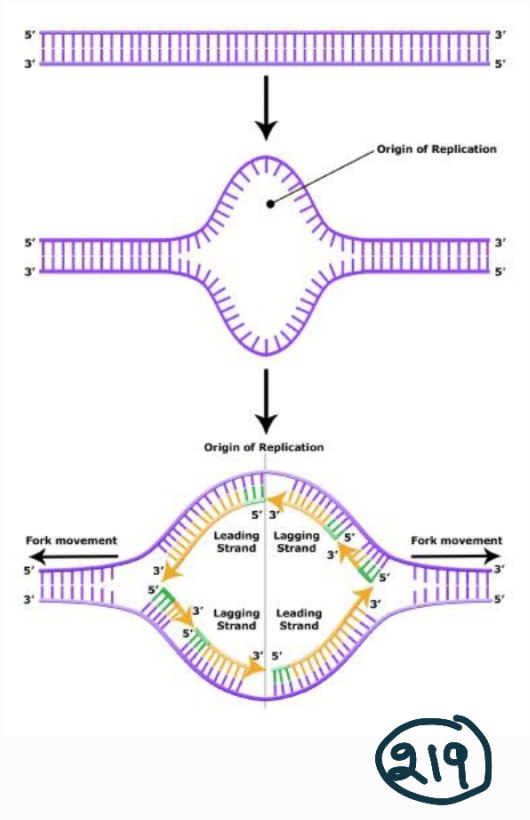
12.2.6. What is DNA replication?

The purpose of DNA replication is to make an exact copy of DNA. The instructions carried by the parental DNA should be transferred to the daughter DNAs when the cell divides. Since there are two strands what we get out of replication are four strands, two old and two new resulting in two double stranded DNA molecules. That's why replication is called semiconservative because it preserves one parental strand in each of the two daughter strands (218).



Replication- there are three steps in DNA replication- initiation, elongation and termination.

Please remember that the two strands are attached to each other, twisted several times. The strands are attached to each other by hydrogen bonds between the nitrogenous bases. For replication to happen, the sequence on each strand has to be read and complementary or mirror image strands with nucleotides have to be made. If the strands are attached and twisted this cannot be done. The strands need to be opened up and uncoiled. The first step is to *separate the two strands.* This has to happen at a particular point. This point is called the *origin of replication (219).* How's the point chosen? We know there are two base pairs on the two strands- A bonds with T and G bonds with C. There are two hydrogen bonds between the nucleotides A and T on the opposite strands compared to 3 between nucleotides between C and G. For separating the strands you need to break this hydrogen bond and keep the strands separate till the replication is completed. This needs energy. You cannot waste precious energy. It is easier to break two hydrogen bonds between A and T and it require less energy as compared to C and G which has 3 hydrogen bonds. The point that's usually chosen is the one where



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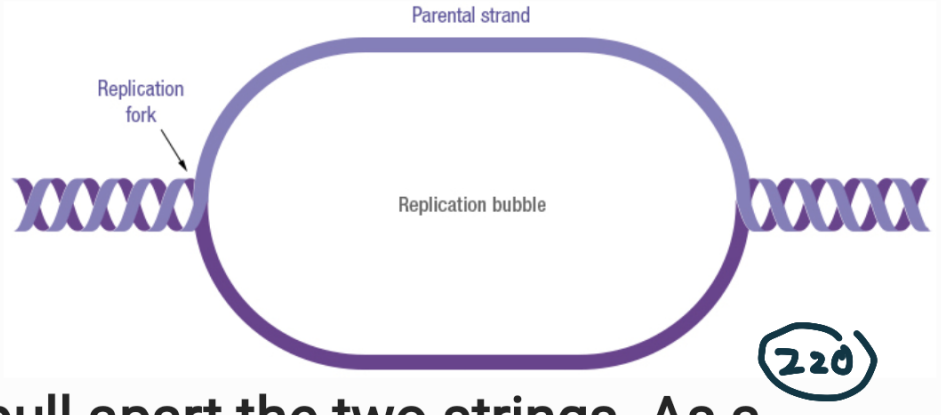
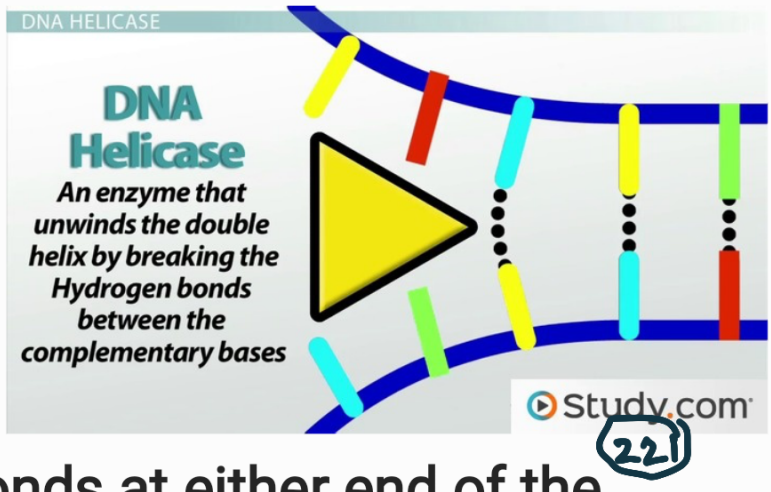
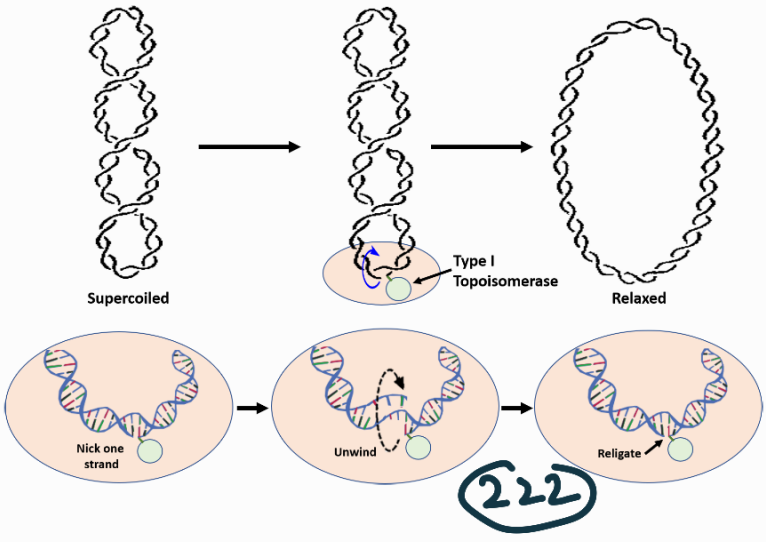
there is a heavy *concentration of nucleotides A and T*. In human cell there are multiple origins of replication but in bacteria there is only one.

The next step is separation of A and T on opposite strands. Certain proteins called *initiator* bind to the nucleotide sequence at origin of replication and act to pull apart the two strings. As a result a gap appears between the two strands and this is called *replication bubble (219,220),* like when you separate two strands of a twisted string. They were connected and now they are separated. The laying of nucleotides can start now. An enzyme called *helicase* (221) whose task is to separate the strands by removing the hydrogen bonds at either end of the bubble, comes into play. There are two *replication forks* on either end of the replication bubble. At each fork the enzyme helicase tries to separate the two strands like unzipping. It requires a lot of energy in the form of ATP. There are proteins which prevent the strands from snapping back or reannealing or reconnecting.

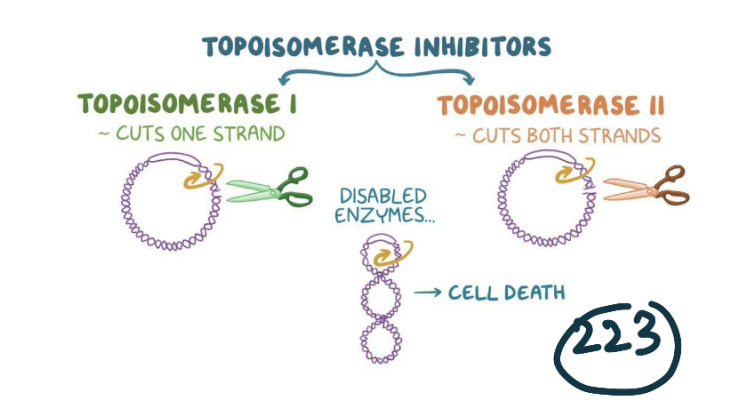




As helicase tries to unwind the strands of DNA in front, the DNA further down becomes more coiled, bunched up and develops supercoil. Try to untwist a twisted two strand circular string. Beyond the point where the



untwisting happens, the strings get tensed and bunched up. Helicase cannot move forward and do its job unless this supercoil is removed. Here comes the enzyme *topoisomerase* (222,223)which removes the supercoil. There are three types of topoisomerase- Type 1, 2 and 4. Topoisomerase looks like T with two arms. How does it remove the coiling? Make a cut in one of the strands, open the strands and reattach them in the other direction. This is what topoisomerase does. One arm of the enzyme acts like scissors cutting the strand at a particular place to release tension and supercoiling. This enzyme is called *nuclease domain* of topoisomerase. Nuclease domain cuts a strand and unwinds. It will continue to cut. This may result in fragmentation of DNA downstream. To prevent this the

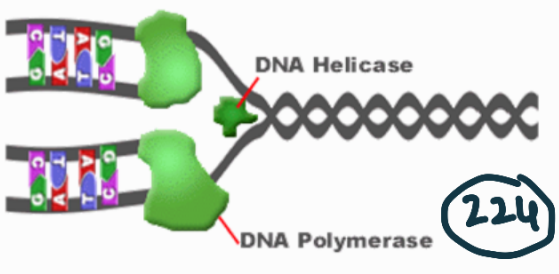


second arm or *lipase domain* of topoisomerase restitches the strand back together. Type 1 and 2 isomerases are found in human cells and type 2 and 4 in bacteria. Type 1 does not require energy or ATP. Type 2 and 4 require energy or ATP.

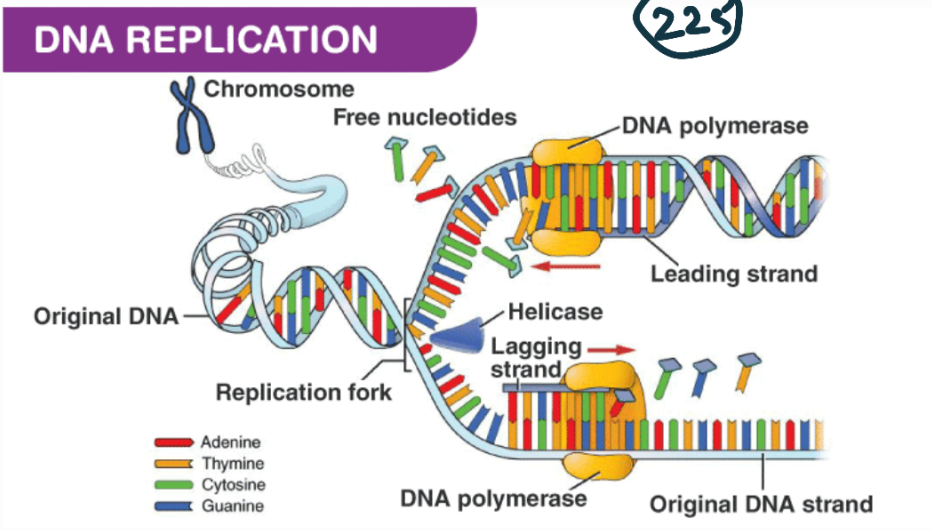
In human cells anticancer drugs that act on topoisomerase 1 (irinotikan - colon cancer, and topotekan- ovarian and lung cancer) and topoisomerase 2 (use it etoposide- ovarian, lung cancer and teniposide- leukemia, lymphoma). In bacteria - drugs used to inhibit type 2 topoisomerase are called fluoroquinolones. *These drugs increase the activity of nuclease domain but inhibit the lipase domain-cuts the DNA but not restitch thereby stopping the replication process.*

The two strands with their nucleotides are separate now. The two enzymes that have helped are *helicase* and *topoisomerase*. *Remember, drugs which inhibit these enzymes will stop the replication process.*

The strand which is used to make copy is called the *leading strand* and the other strand is called the *lagging strand*. This is important because the process of copying DNA on lagging strand is different from that in the leading strand. Helicase has opened the strands, topoisomerase has uncoiled the strands. The next enzyme is DNA polymerase (224,225) (polymerase because it helps in



forming several nitrogenous bases or polymer). It is responsible for arranging the nucleotides in the right sequence



using the nucleotides on the parental strand. There are free nucleotides available. The enzyme picks up nucleotides from the free floating nucleotide letter box(!) and arranges them complementary to the nucleotides on the leading strand. If the letter on parental strand is A the complementary base pair is T, if it is C the complementary base pair is G. If the sequence is AAATTGCG then the DNA polymerase lays down nucleotides - TTTAACGC. But this enzyme can only add deoxyribonucleotides to the 3'-OH group of an existing chain , and since the starting point is 5' end or phosphate end it cannot begin synthesis *de novo. In other words, it can* only attach new DNA nucleotides to an existing strand of nucleotides. Wait, there is an enzyme called primase (primase produces RNA molecules, the enzyme is a type of RNA polymerase) which synthesizes short RNA nucleotide sequences (10 to 12 bases in length) called primers from the 5' end. It lays down a short chain of 4 to 10 complementary RNA nucleotides, creating a starting point with 5' end so that

DNA polymerase can continue the process. These RNA sequences are complementary to DNA nucleotides, which serves as its template. The primers serve as a starting point and lay a foundation for DNA synthesis. Remember we are making DNA copy of the parental DNA. But these primers are RNA nucleotides, not DNA. Don't worry, later, after elongation is complete, the primer of 4 to 10 RNA nucleotides is removed and replaced with DNA nucleotides by DNA polymerase.

Elongation of the nucleotides on the DNA strand--the addition of nucleotides to the new DNA strand--begins after the primer has been added. Nucleotides are added , one by one, by the DNA polymerase 3, in the exact order specified by the original (template) strand - adenine is always paired with thymine and cytosine is always paired with guanine. So, for example, if the original strand reads A-G-C-T, the new strand will read T-C-G-A.

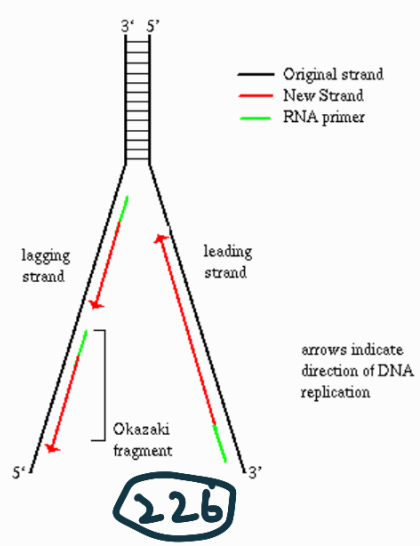
DNA is always synthesized in the 5'-to-3' direction, meaning that nucleotides are added only to the 3' end of the growing strand.

Leading strand

DNA polymerase type 3 reads the nucleotides on the leading strand of DNA and synthesises DNA nucleotides. On the leading strand the formation of nucleotide is continuous. One strand, which is complementary to the parental DNA strand, is synthesized continuously toward the replication fork so the polymerase can add nucleotides in this direction. This continuously synthesized strand is known as the leading strand.

Lagging strand

In the other or lagging strand, *primase* starts the process, reads and syntheses a couple of nucleotide (4 to 10 maximum). *DNA polymerase type 3 , then starts laying nucleotides in the 5 ' to 3' direction*. Remember helicase is still active. As *helicase* continues to unwind DNA on lagging strand it exposes longer length of nucleotide strand on the other direction. (as the replication fork moves in the lagging strand, the replication fork on the leading strand moves in the opposite direction leaving a gap on the lagging strand with no nucleotides). Primase again lays down a couple of RNA nucleotides in the gap in the lagging DNA strand. This gets repeated several times. So, on the lagging DNA



strand unlike in the leading strand *you find stretches of DNA nucleotides and stretches of RNA nucleotides or primers (226).* The outcome expected is *an all DNA nucleotide strand, not mixed one with RNA primers and DNA nucleotides.* Primers are no longer needed. So, *DNA polymerase 1* snips off RNA primers, using its *nuclease*, reads the sequence and replaces the RNA primers with DNA nucleotides and the gaps between fragments are sealed by its *ligase*. DNA polymerase III does most of the elongation work, adding nucleotides one by one to the 3' end of the new and growing single strand. Other enzymes, including DNA polymerase I, are responsible for removing the RNA primer after DNA polymerase III has begun its work, replacing it with DNA nucleotides and sealing the gaps.

The process of DNA replication can be summarized as follows:

- DNA unwinds at the origin of replication.
- New nucleotide bases are added to the complementary parental strands. One new strand is made continuously, while the other strand is made in pieces.
- RNA Primers are laid on both the strand. In the lagging strand multiple stretches of RNA primer

whereas in leading strand only one primer. These primers are removed, new DNA nucleotides are put in place of the primers and the backbone is sealed.

Now, nucleotides have been laid on both the strands. DNA polymerase 3 proof reads the nucleotides, on both the strands and checks if all the nucleotides are correct. Any incorrect nucleotide is replaced with correct one.

Replication process comes to an end when two helicase enzymes moving in different directions come close to each other. They recognise there is no more strand to be separated and the activity of DNA polymerase 3 comes to a stop. All enzymes are removed.

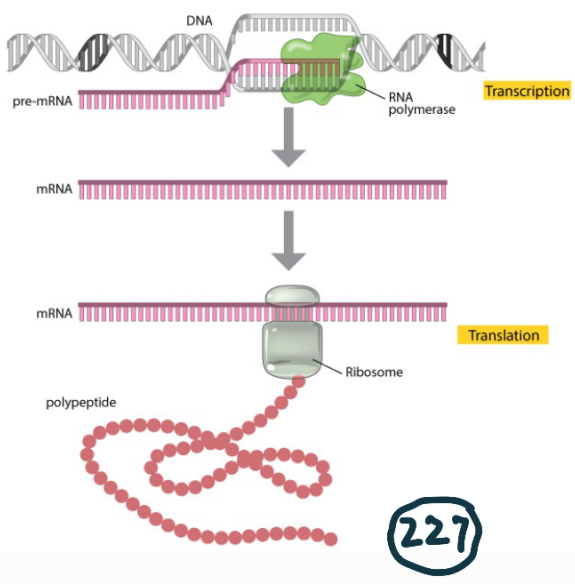
Points of attack

The points in the process of replication which can be targeted for suppression by drugs include:

Enzyme helicase
Enzyme topoisomerase
Enzyme DNA polymerase 3

12.3. Protein Synthesis

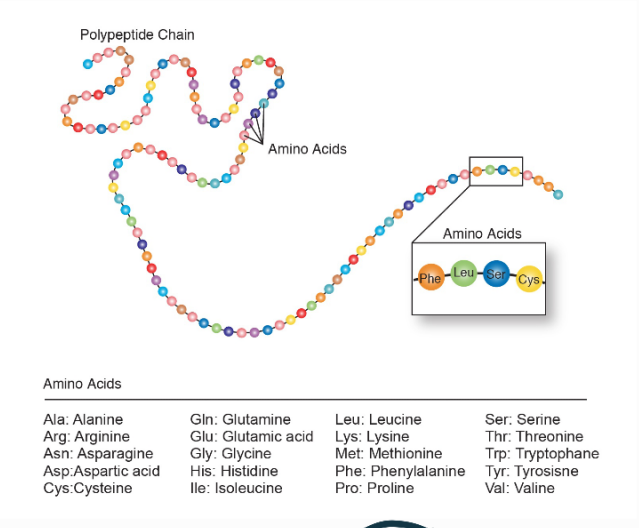
There are various processes involved in synthesis of proteins. Drugs whose mechanism of action is directed at different points in the protein synthesis process are used to kill or inhibit the pathogens.



Proteins are synthesised following instructions from DNA. Proteins are one of the three essential macromolecules; the other two being carbohydrates and lipids. While carbohydrates and lipids are used mainly for energy, proteins are used as building blocks to make molecules which control growth and survival of all living cells. They are produced in a two step process called protein synthesis both in bacteria and humans: the instructions carried by DNA are first transcribed onto RNA, then RNA is translated into protein (227). Sequences of DNA determine what proteins are to be produced and by what amount. DNA----RNA---Protein.

12.4.1. What is a protein?

Proteins are everything providing structural support to executing different functions from catalysing reactions, transporting oxygen and other molecules, and defending the cells from infection (antibodies). They are made of



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large number of aminoacids joined end to end (228). The chain of aminoacids fold into different complex 3D shapes and the precise shape along with the aminoacid content determines what the protein does.

12.4.2. Proteins are expressed from genes

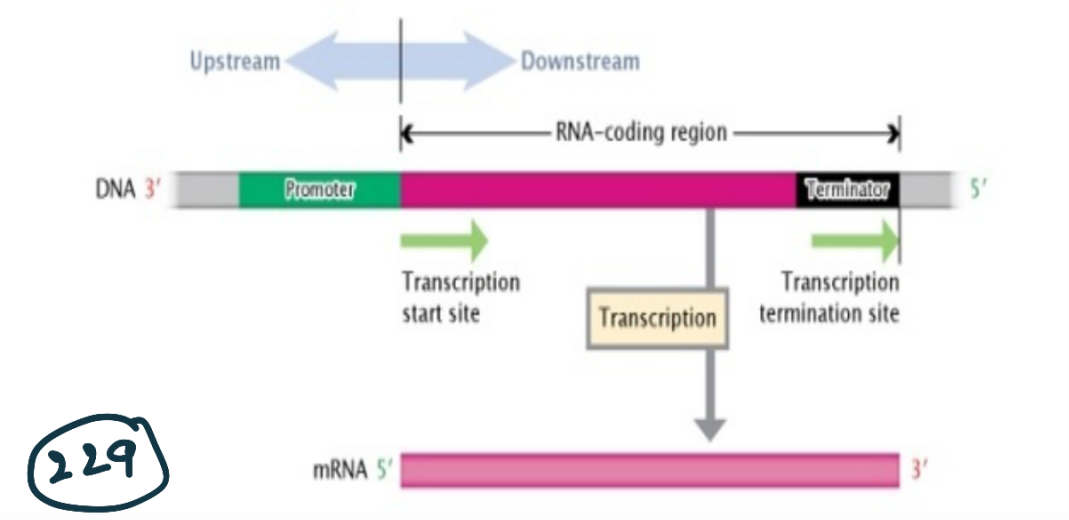
There are 20 aminoacids. Protein synthesis starts with instructions in sequences of DNA called genes. Each gene carries instruction for making a protein with specific chain of aminoacids. Protein Synthesis is also called gene expression because they are made with instructions from genes. In the simplest sense, expressing a gene means manufacturing its corresponding protein, and this multilayered process has two major steps- transcription and translation.

12.4.3. Transcription and translation

Transcription

Structures in the cell identify the start and end of a gene and read the DNA sequence between them

(the order of A, C, G and T bases within the gene). A molecular message (an mRNA molecule) is produced that reflectss the sequence of the gene itself. In most respects, mRNA looks similar to a single-stranded piece of DNA except that in RNA the nucleotide *Thymine* is replaced by nucleotide *Uracil*. The process of transcription involves three stages: initiation, elongation and termination.



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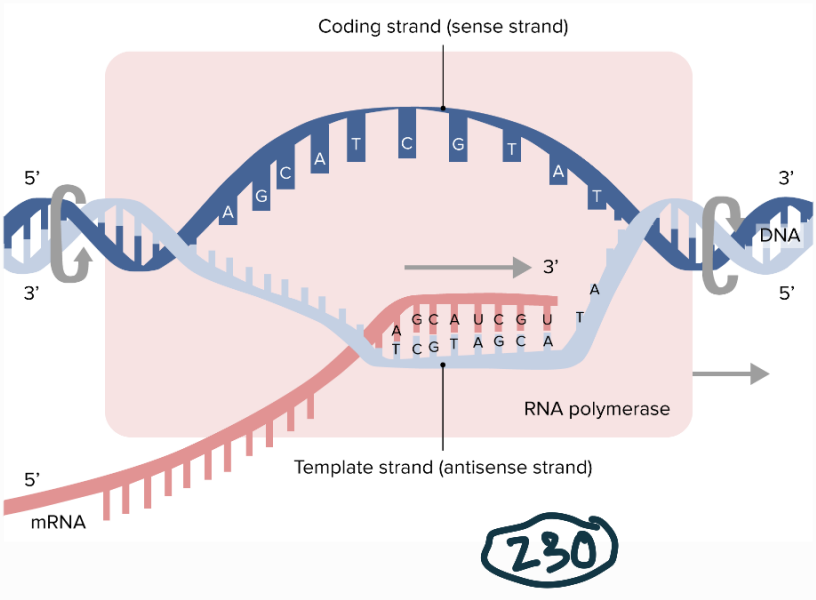
A DNA transcription unit (229)is composed, from its 3' to 5' end, of an RNA-coding region (pink rectangle) flanked by a promoter region (green rectangle) and a terminator region (black rectangle). Both the promotor region and terminator region are composed of a sequence of nucleotides with some other proteins complexing with them (promotor protein for promotor region, rho protein for terminator region).

Which protein is made when is decided by a promotor protein. Within any cell, only a proportion of proteins are produced at any one time. Proteins that perform essential roles are produced constantly, while others are

expressed only when they are needed. Cells also need large amounts of some proteins (such as the enzymes involved in continuous processes like transcription and translation) and smaller amounts of others (such as hormones). But how does a cell decide which genes to express and how much to make?



Promoters are the sequences of DNA that determine when a gene is expressed. These sections of DNA provide a 'landing site' for *RNA polymerase (230)*(the protein that reads DNA and makes an mRNA copy). Different promoter sequences have different strengths, and genes with 'strong' promoters are expressed at a higher level than those with 'weak' promoters.

The process begins with attachment of *RNA polymerase (230)* enzyme to promotor region on DNA. Remember, in DNA replication complementary copies of DNA are made by DNA polymerase (it makes multiple segments of DNA and therefore a 'polymer'). In transcription, RNA polymerase is making a polymer of RNA from DNA. Why RNA copy, why not DNA copy? Because if it is DNA copy

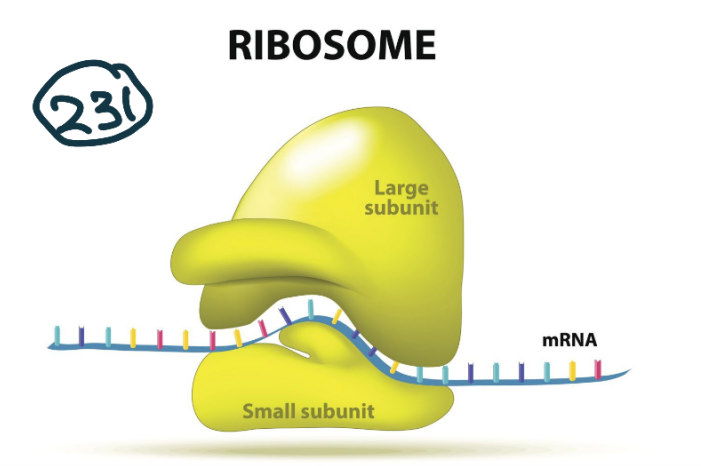
it is not translated into aminoacid chain at ribosome. It translates only message in the form of RNA. The enzyme *RNA polymerase in bacteria is RNA polymerase holoenzyme.* It is only one with multiple functions- it makes all the three types of RNA, mRNA, rRNA and tRNA. . In human cell there are seperate RNA polymerases 1,2,3 which make mRNA, rRNA and tRNA and it needs a transcription factor to attach to the promotor region. Both RNA and DNA are made up of a chain of building blocks called nucleotides, but they have slightly different chemical properties. While DNA is usually double stranded, RNA is single stranded; the sugar in RNA is ribose as against the sugar in DNA which is deoxyribose; RNA like DNA has four nucleotide bases with phosphate sugar backbone, but the nucleotides are different: the thymine is replaced by Uracil in RNA. The nucleotides in RNA are Adenine, cytosine, Guanine and Uracil. The type of RNA that contains the information for making a protein is called messenger RNA (mRNA) because it carries the information, or message, from the DNA to the protein making factory, the ribosome. There are two other RNAs - ribosomal RNA (rRNA) and transfer RNA (tRNA) which we will learn during translation.

Once the point of initiation is decided by the promotor region, separation of the strands of DNA, breaking the

hydrogen bonds between the nucleotides, laying of the complementary RNA nucleotides- everything is done by RNA polymerase holoenzyme. It does all these functions. Only thing we do not know is if it can proof read like DNA polymerase. In bacteria, transcription and translation may happen simultaneously because there is no nucleus. The enzyme moves forward making a RNA copy of the selected DNA sequence. The elongation process is the same in both bacteria and humans.

The process gets terminated when the polymerase holoenzyme reaches the terminator region on DNA. The m RNA breaks off.

Translation

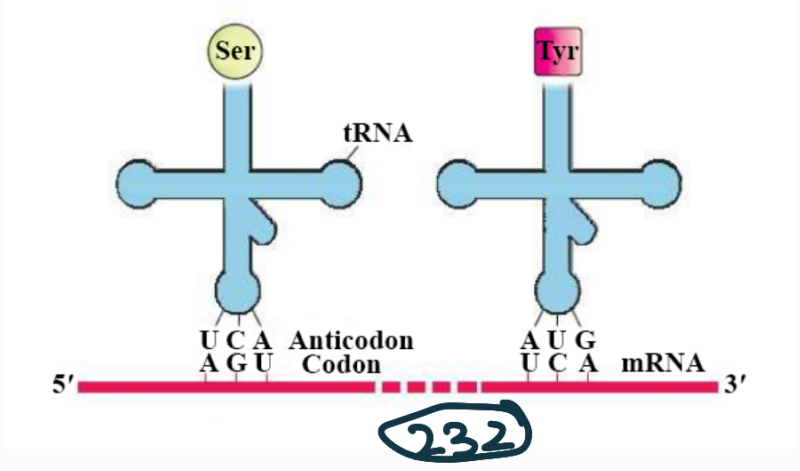


Translation, the second step in getting from a gene to a protein, takes place at the protein making machinery called *ribosome* (231) which is in the cytoplasm in both bacteria and humans. In eukaryotes or humans, mature mRNA molecules must leave the nucleus and travel to the cytoplasm, where

the ribosomes are located. On the

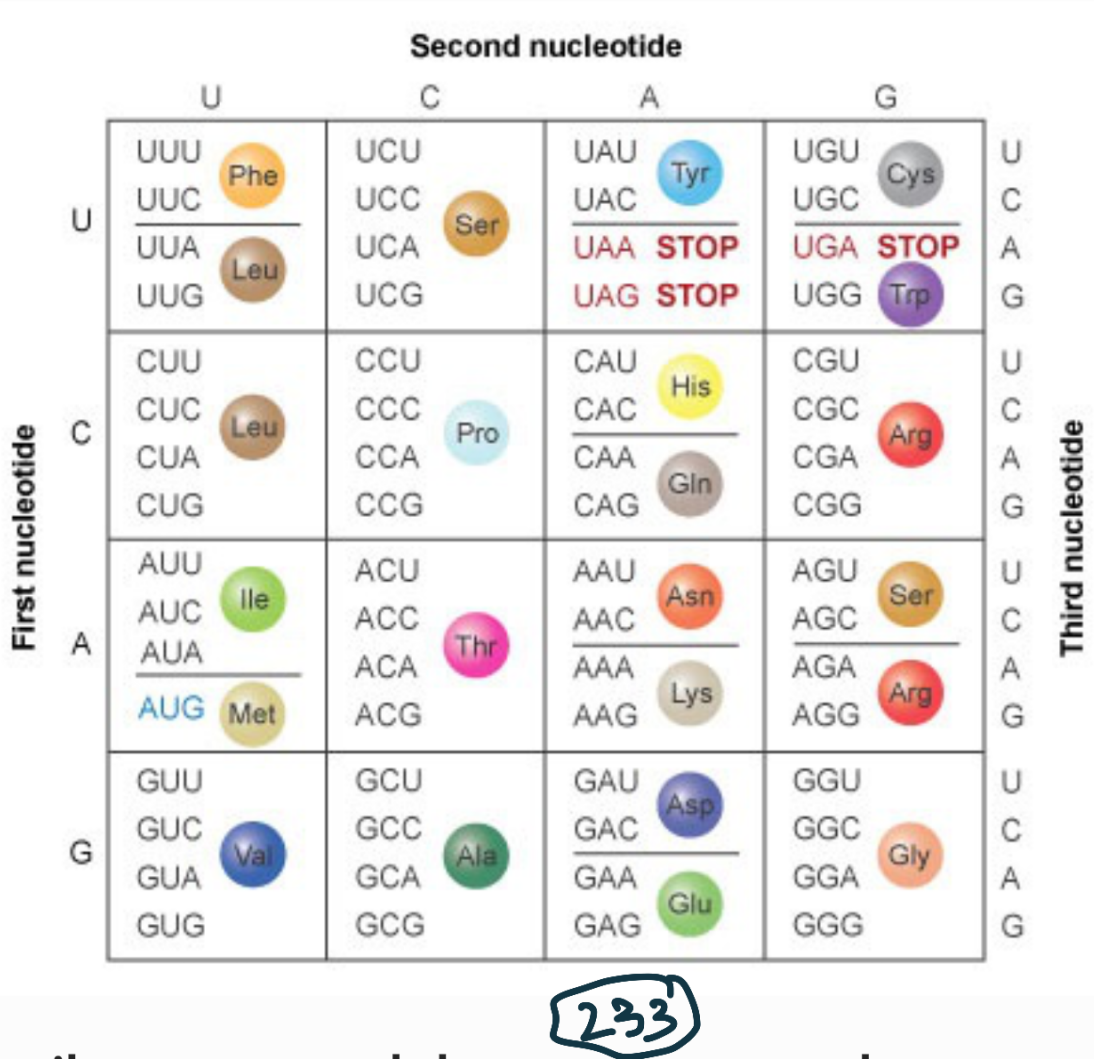
other hand, in prokaryotic organisms or bacteria , which do not have nucleus, ribosomes can attach to mRNA while it is still being transcribed. In this situation, translation begins at one end (the 5' end) of the mRNA while the other end (3' end) is still attached to DNA.

Ribosome (231) is composed of *two subunits*: the large (50S) subunit and the small (30S) subunit (S, for svedberg unit, is a measure of sedimentation velocity and, therefore, mass). The subunit mass is different in humans and bacteria . It is bigger and heavier in humans(60 and 40 S). Each subunit exists separately in the cytoplasm, but the two join together on the mRNA molecule at the beginning of translation. The ribosomal subunits contain *proteins and specialized RNA* molecules—specifically, ribosomal RNA (rRNA) and transfer RNA (tRNA). Both the RNAs like mRNA as already mentioned are made by RNA polymerase holoenzyme. The tRNA molecules (232) are adaptor molecules—they have one end that can read the triplet code in the mRNA through



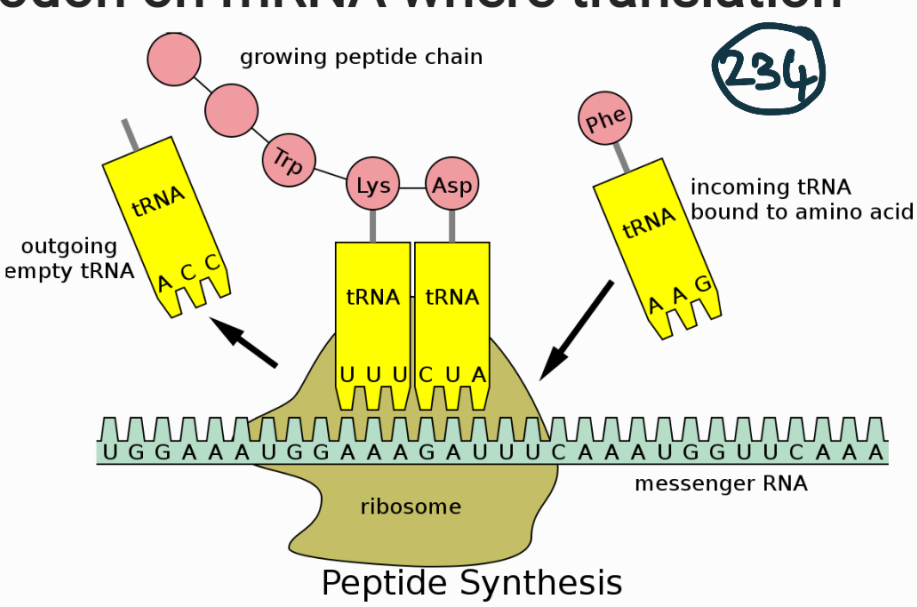
complementary base-pairing, and another end that attaches to a specific amino acid.

A ribosome receives the mRNA molecule and starts to build a chain of amino acids (a protein) that exactly matches the instructions within the mRNA. The ribosome 'reads' the mRNA sequence as a series of three-base codons. Each codon t ells the protein-making machinery which amino acid to add next. How does ribosome read the sequence and interpret? The genetic code (233) is the cell's 'instruction manual' for producing a protein from an mRNA sequence. The same genetic code manual is used by all living beings with DNA. Each codon has the same 'meaning' in any given cell (with a few minor exceptions). For instance, the codon AGA is an



instruction to add the amino acid arginine to a growing protein – whether that protein is growing within bacterial cells or human cells. In other words, every cell follows the same rules to make a new protein.Three-base-long sections of mRNA (codons) are 'read' in sequence at the ribosome. Each codon corresponds to a particular amino acid, which is added to the growing protein chain. Multiple codons can code for the same amino acid. The codons are written 5' to 3', as they appear in the mRNA. AUG is an initiation codon; UAA, UAG, and UGA are termination (stop) codons. When the ribosome reaches UGA on mRNA it stops reading because the codon UGA means stop.

The region on mRNA to which ribosome binds is called the *ribosome binding region*. Once the binding occurs, the large subunit of ribosome joins the small subunit. The small subunit has the sites for reading codons on the mRNA and the large subunit has the sites for adding anticodons with corresponding aminoacids by tRNA. Next to it is the start codon on mRNA where translation begins. The start codon is usually methionine (AUG). Using the mRNA as a template, the ribosome traverses



Peptide Synthesis

each codon (3 nucleotides) of the mRNA, pairing it with the appropriate amino acid provided by a tRNA . tRNA (234) contains a complementary anticodon on one end and the appropriate amino acid on the other. It catalyzes the attachment of each new amino acid to the growing chain and assembles finally the protein, one amino acid at a time. As and when a new aminoacid is formed it is attached to the preceding aminoacid by peptide bond and moves back and its initial place is taken by a new tRNA with new amonoacid (234). As the new aminoacids are added to the chain through peptide bond the chain grows (234). Peptide bond is formed by *peptidyl transferase* enzyme. The aminoacid is released from tRNA and gets attached to the growing chain. With each reading and addition of aminoacid the ribosome moves forward. The tRNA is then released to the cytoplasm to pick up another amino acid. This process is repeated until all the codons in the mRNA have been read by tRNA molecules, and the amino acids attached to the tRNAs have been linked together in the growing polypeptide chain in the appropriate order (234) . The process stops when the ribosome encounters stop codon. At this point, translation must be terminated, and the new protein must be released from the mRNA and ribosome. This also results in release of the mRNA from the ribosome and subsequent dissociation of the ribosome. For example the following is the codon

message carried by mRNA and it is being read by ribosome and forms a chain of aminoscids using tRNA.

AUGUGGAAAUGGAAAGAUUUCAAAUGGUUCAAAUGA

The triplet codons for this will be
AUG UGG AAA UGG AAA GAU UUC AAA UGG UUC AAA UGA

Refer to the genetic code (Figure 233)

AUG= start codon
UGG= Trp tryptophan
AAA= Lys lysine
UGG=Trp tryptophan
AAA=Lys lysine
GAU= Asp aspartame
UUC= Phe phenylalanine
AAA= Lys lysine
UGG= Trp tryptophan
UUC= Phe phenylalanine
AAA= Lys lysine
UGA= stop codon

stop codon
The protein with aminoacids formed will be:

Tryptophan---lysine---tryptophan---lysine---aspartame---phenylalanine---lysine---tryptophan---phenylalanine---lysine

The tRNA that are picked up

UAC anticodon with 'start'
ACC anticodon with tryptophan
UUU anticodon with lysine
ACC anticodon with tryptophan
UUU anticodon with lysine
CUA anticodon with aspartame
AAG anticodon with phenylalanine
UUU anticodon with lysine
ACC anticodon with tryptophan
AAG anticodon with phenylalanine
UUU anticodon with lysine
ACU anticodon with 'stop'

Points of attack :

Points where TB bacilli can be attacked to kill.
RNA polymerase
Ribosomal subunit
Formation of polypeptide chain