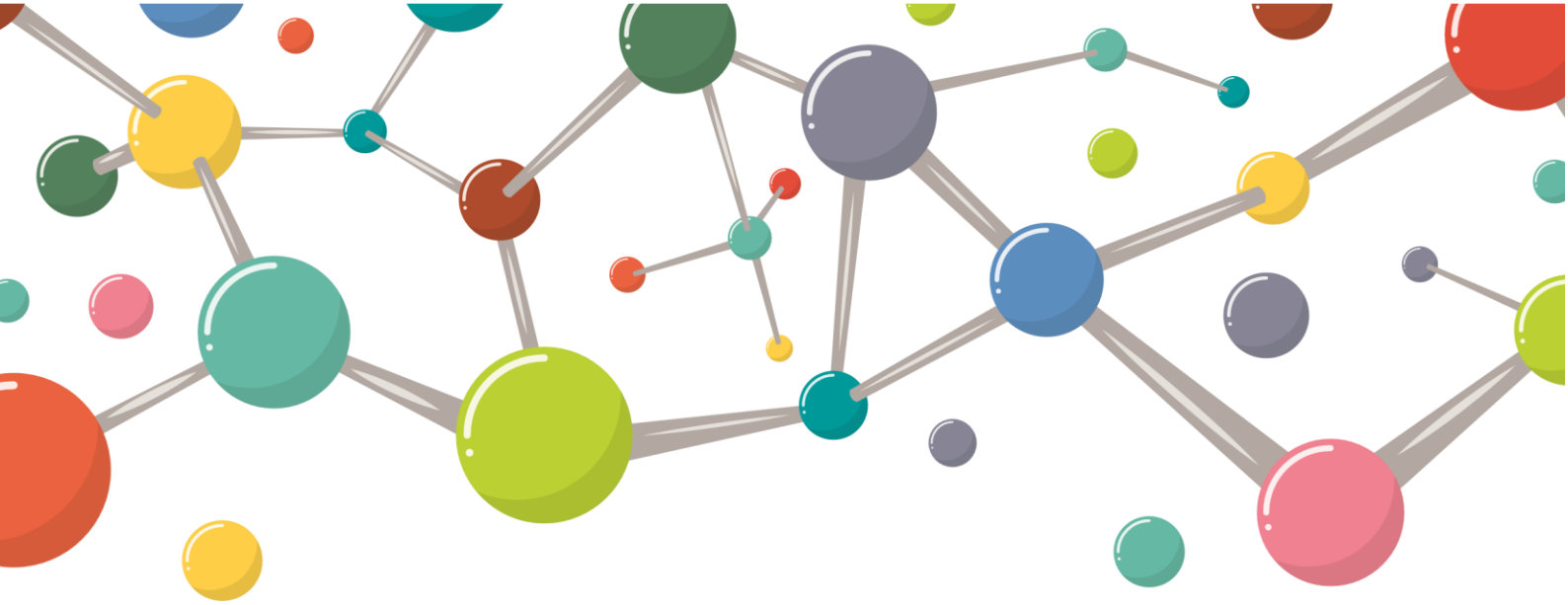

MOLECULAR BIOLOGY

SHEET (4)

WRITER: MOLECULAR BIOLOGY TEAM
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DOCTOR: DR. NABIL, DR. KHALDON

Black color: doctor's explanation, Navy color: slides, Blue color: extra information, Red color: Dr. Khaldun's notes, highlighted sentence: modified, purple color: book notes

How DNA is packaged in both prokaryotic and eukaryotic systems?

-In order to compact DNA molecules to fit inside a cell, specific proteins interact with DNA—> Forms a condensed nucleoprotein (RNA or DNA associated with protein) complex called Chromatin.

What's the definition of chromosome ?

-simply chromosome is defined as nucleic acid or (DNA + protein) in eukaryotic or in prokaryotic cells

- Chromosome: the molecule that gets transported from cells during meiosis to form gametes.

-In a non-dividing nucleus, DNA is found in the form of chromatin, during the interphase of the cell cycle, more specifically during S phase, DNA gets replicated in the nucleus while It is still in the form of loose and spread out chromatin. After the completion of the interphase, the cell enters meioses where the DNA in the form of chromatic condensed and takes up to form chromosome.

-Note: chromatin varies greatly between the three domain of living organisms

-In prokaryotic cells it is called nucleoid and in eukaryotic cells it is packaged as chromosome

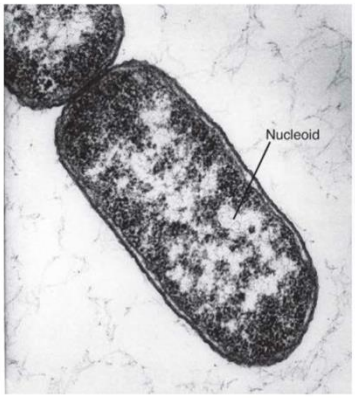


Figure 05.01: An electron micrograph of a thin-section of Escherichia coli. The nucleoid is the light region.

About Figure 05.01 :

***This is a bacterial cell and the white material called nucleoid, which is composed of DNA, RNA and proteins**

Bacterial chromatin can be released from the cell by lysis.

-lysis: gentle cell technique that avoids DNA breakage or protein denaturation

*Remember that prokaryotic cells don't have nucleus to have DNA inside it, it is composed of a large molecule of double stranded helical closed DNA molecule

E.coli DNA is a closed circle

- Length approximately 1600 µm
- Fits into a cell 0.5 µm in diameter and 1 µm long

-DNA forms a condensed nucleoprotein complex called the nucleoid

So the length of DNA is much greater to fit into the cell

So it must be some type of packaging and compacting to the DNA to fit in this limited space of bacterial cell

* Remember, chromosome in prokaryotic cells is composed of one DNA molecule, and in eukaryotic cells we have 46 chromosomes—> each chromosome is carrying one double stranded DNA , so in somatic cells we have 46 double stranded DNA molecules composed of billions of base pairs

Note: Even prokaryotic cells have associated proteins but these proteins aren't histones, these proteins play an important role in "gene regulation", Where they are deciding factor in how much of the DNA transcribed and when the transcription occurs.

How is this huge number of molecules in DNA packaged in very limited space in bacterial cells or in the nucleus of eukaryotic cells?

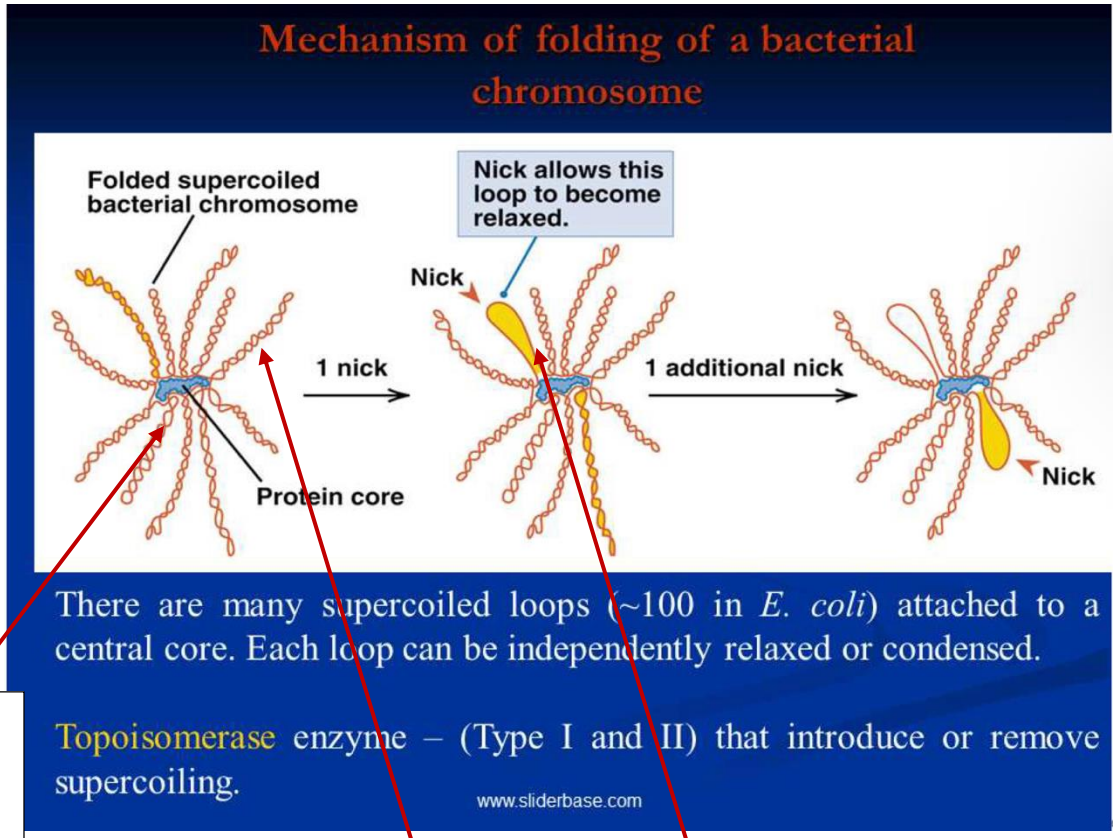
1-Bacterial chromatin

- Electron micrograph of released *E.coli* DNA reveals chromatin loops
  -Mostly supercoiled DNA loops with some relaxed loops (cause by nicks)

  -Each loop appear to be insulated from the others

  -Current model propose supercoiled loops attached to a protein core
- *E.coli* estimated to have 400 loops
- Each loop is topologically independent

The bacterial chromosomes are compacted in super helical form after some loops are formed



This is how it looks inside the cytoplasm:
The DNA is folded into supercoiled and attached to a protein or scaffold protein which is called the core protein

-These supercoiled could be nicked by some enzymes called topoisomerase

It converts each supercoiled into loop like structure

The stages of how supercoiled form into prokaryotic cells in order to be packaged:

-This is a circular chromosomal DNA (first structure)—> <u>it will fold into loops</u> as it attached to core protein (scaffold protein) —>so there are number of loops (second structure) these number varies from one species to another

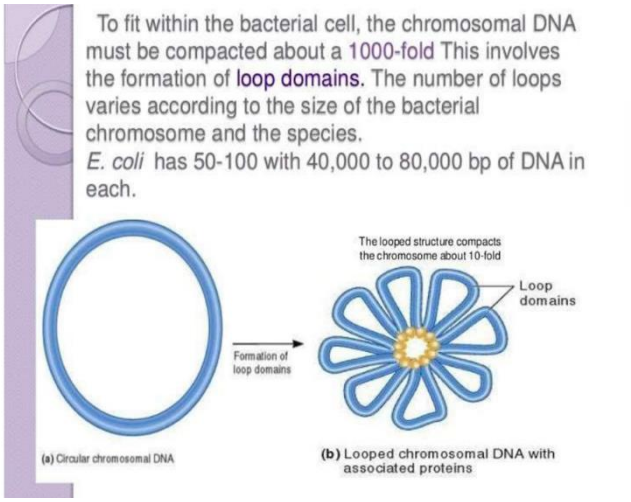
-This protein core is what's responsible for bacterial DNA bending and forming these loops.

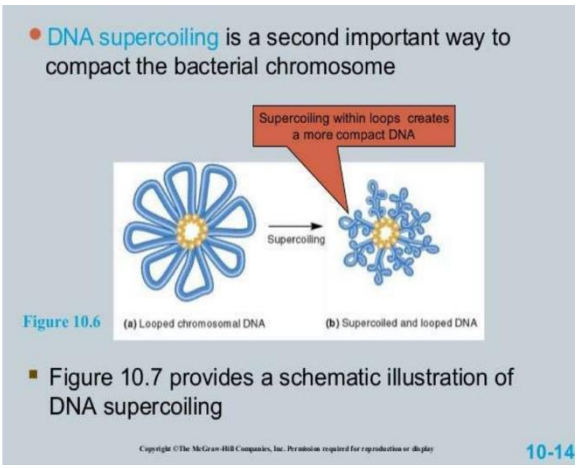
-Each loop contains specific number of Base pairs, so by doing this looping mechanism on the circular DNA—> that will cause a compact and packaging of this big molecule into smaller volume to fit in .

-After these loops are formed they are converted into supercoiled by specific topoisomerase (third structure)

- These supercoiled have less volume than the previous stage

- DNA in prokaryotic is packaged into supercoiled and loop structure within the cytoplasm of the bacterial cell and this will reduce the volume to more than 1000 folds .





-Each loop carries about 40000-80000 base pairs as in the second structure

-This is the last stage in which loops are converted to supercooled by a specific topoisomerase

-some proteins that help to determine bacterial chromatin architecture

-MuKB protein—> help in organise and compact DNA

- H-NS (histone like nucleoid structuring): appear to be homodimer or oligomer—> forming bridges

-DNA bind proteins like:

-FIs: inversion stimulation

-IHF

-Hu

To sum up

Circle ————> loops————->supercoiled

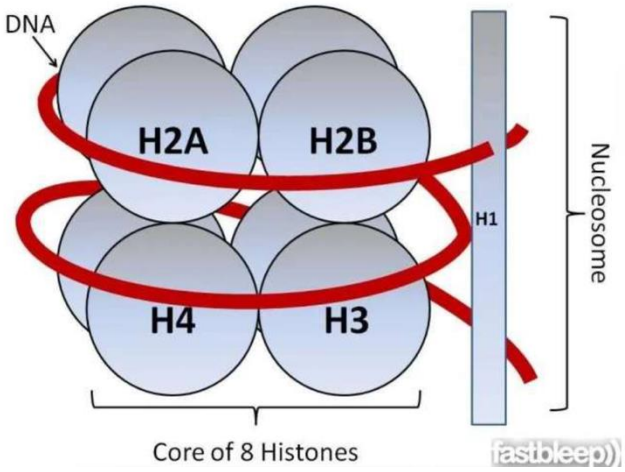
Folding By topoisomerase

2-Eukaryotic Chromatin

-Highly condense structures called chromosomes
-Germ cells (reproductive cells) have a characteristic number of chromosomes (n) also called haploid number
-somatic cells are diploid (2n)
-A certain organism has a different number of chromosomes in their somatic cells, an octaploid (8n) or tetraploid (4n), their gametes would always have half that number.
-Remember: somatic cells in human is composed of 46 chromosome, each chromosome is carrying one DNA molecule and the size of DNA molecule varies from one chromosome to another

- The collective length of these DNA on these chromosome is very big in comparable to the size of the nucleus in human cells
… So there must be a mechanism in which this big size of DNA molecule to be packaged and compacted in the nucleus of the human cells.

-Multiple levels of chromatin packing



- **First level of organisation : interaction between DNA and histones**
  -Chromatin contains 5 major classes of histones

- Histones are octamer proteins and they are 4 types (H2A,H2B,H4,H3) and two from each other—> so it is 4 dimers ,they have very specific nature of composition

-The DNA which is wrapped around them is about 146 base Pairs

-The binding between DNA and histones is electrostatic in nature, why?

Because DNA is negatively charged (because of the backbone which is composed of sugar phosphate and the phosphate is negative charge)

TABLE 5.1 Histone Composition

Histone	Molecular Mass (kDa)	% Lysine	% Arginine	% Lysine + Arginine
H1	~21.0	29	1.5	30.5
H2A	14.5	11	9.5	20.5
H2B	13.7	16	6.5	22.5
H3	15.3	10	13.5	23.5
H4	11.3	11	14.0	25.0

- these proteins which are called histone are basic protein

lysine and arginine which are basic amino acid so it is (positively charged)

-Each histone has a high percentage of the basic amino acids Lysine and Arginine

-What is the name of amino group in the R group of lysine?

It depends on the type of carbon in which the amino group attached to (alpha or beta or gamma)

If the amino acid attached to alpha carbon, this name is (alpha carbon)

If the amino acid attached to beta carbon, this name is (beta carbon)

If the amino acid attached to epsilon carbon, this name is (epsilon amino)

-So because of epsilon amino in Lysine R group is positively charge at physiological PH it will give the positive charge to the protein.

- The same thing for Arginine, the positively charge of R group of the Arginine is called guanidino group and it's positively charge at physiological PH

- So that why the proteins in histone have positive charge at physiological PH

What will happen to these charges above the physiological ph?

These positively charge will be disassociated and lose their positive charges and histones will be unable to bind to the DNA at PH above the physiological PH because their pKa is higher than physiological PH so as you reach they pKa by raising the PH above the

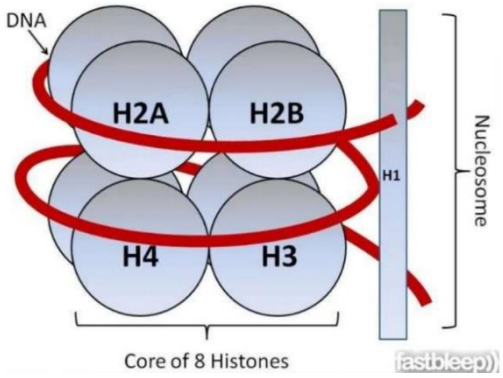
physiological PH that will cause dissociation of these positively charge and converted to uncharged

-So that's why histone has high percentages of (Lysine and Arginine)

What is the pKa of epsilon amino of Lysine? It is about 10
-So when you start raising the PH above 10 the lysine will lose it's positive charge
-Note: histones have conserved amino acid sequence

- So if you compare histones in any eukaryotic system you will see high homology of amino acid sequence in any type of these histones

-And you will see high percentage of homology in amino acid sequence if you compare between different type of histones, that indicates the importance of the structure of these histones that will give an important function and a vital function to the cell (organism). If these histones are not of that nature of structure (rich in amino acids) and not having homologous structure the cell will not be able to live.
Homologous: The amino acid sequence is the same in 70% of the sequence
Example: if I compare 100 amino acids in H2A and H2B, we will see 70 amino acids in both types of histones are same.
Another example: if I compare amino acid sequence in H2A in human with amino acid in H2A in mouse I will see 70% having the same amino acid sequence.



- Attraction can be broken by high salt concentration (this occurs as the positive charge of the salt's cation form electrostatic interactions with DNA, "shielding" the negative charge of the phosphate group and replacing the positively charged groups in the histones.
- Purified DNA and purified histone mixed together will reconstitute chromatin
- Extremely well conserved proteins

As we see in the structure above about histones and DNA. The DNA wrapped around it, it is about one wrapping and 3 quarters of DNA strands which composed of 146 base pairs.

-DNA will continue to the right direction in this structure (toward H1 histones) and wrapping on another unite like this (the proteins of histones) to give another nucleosome

-Between nucleosome and another there will be <u>double stranded DNA connecting them that is called the linker which are connecting them together</u>

-These structure of 8 histones and H1 histones wrapped with DNA called the whole nucleosome or chromatosome.

-Treatment with micrococcal nuclease cleaves DNA in the linker to release free nucleosome. (It can't cleave the DNA attached to histone protein)

-146 bp DNA wrapped around octameric histone core held in place by H1

-Without H1 it is called the core nucleosome

-Nature of H1 interaction and the core particle is not precisely known.

X-ray crystallography reveals the atomic structure of nucleosome core particles

-Histone octamer has two-fold symmetry

- A-T rich minor grooves tend to contact the histone octamer.

يُجاهِدُ المرءُ والآمالُ تدفعُهُ

وليس يَظفَرُ إلا بالذي قُدِرا!

DNase degrade DNA in this region

These are beads that are composed of nucleosome

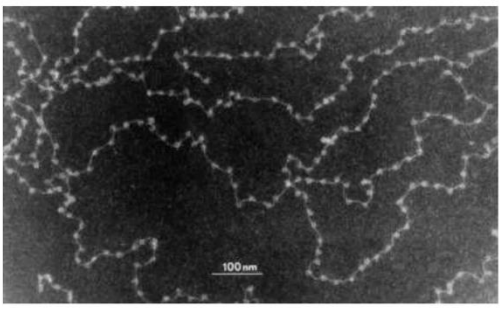


Figure 05.12: Electron micrograph of chromatin. The beadlike nucleosome particles have diameters of approximately 11 nm.

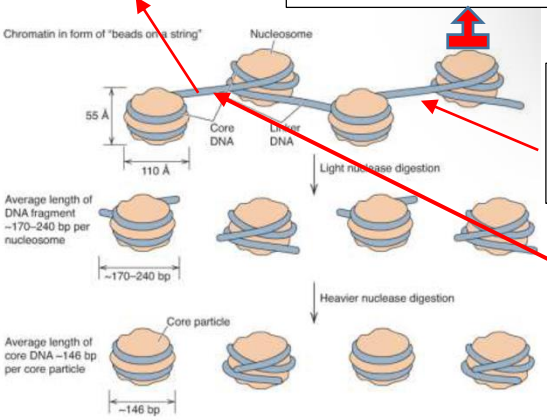


Figure 05.13: "Beads on a string" structure of chromatin.

These threads are the DNA double stranded attached to H1

H1 will bind here

This electron micrograph is showing us threads and beads.

—How did we know this?

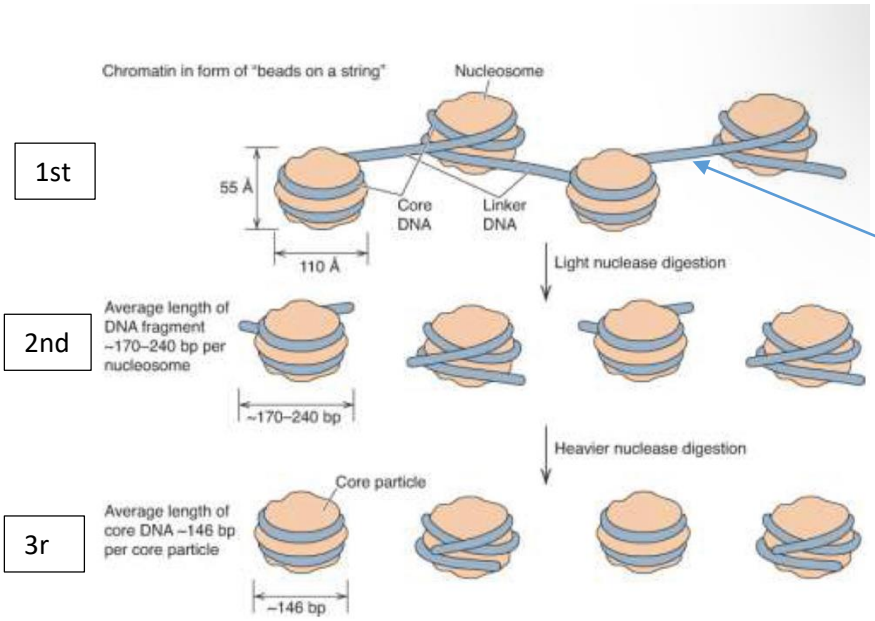
It was done by an experiment, for example if you take the chromatin, which is thread like structure at this stage of formation of chromosome, and you subject it to be digested by <u>nucleases</u>

Nucleases: they are enzymes that will degrade the nucleic acid

Note : it is different from DNase.

Note : the nuclease doesn't able to digest the DNA that binds directly to the histones.
why?
Because DNA is protected by histones, so it's not exposed to nucleases attack.

<u>Remember:</u> the function of DNA linker is different from function of H.



It is composed of 200 bp

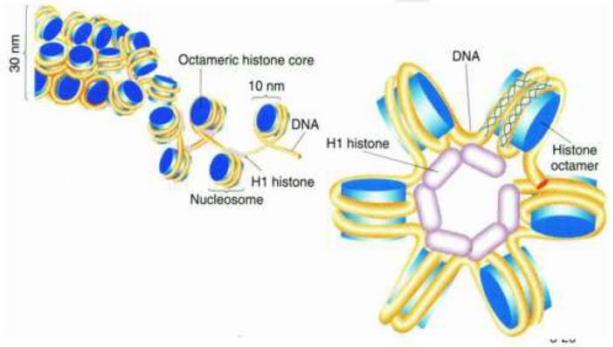
From 1st to 2nd :If you subject your chromatin in this stage to <u>light digestion of nuclease</u>, which is very low concentration of nuclease, it will be able to cut thread like structure.

From 2nd to 3rd:prolonged nuclease digestion gradually cleave additional nucleotides until the DNA is about 146 bp long.

In first to second structure: if you subject the 1st substrate to light nuclease digestion and you subject the 2nd to proteases and subject them to electrophoreses —> you will find a ladder of DNA fragments composed of 200 base pairs spaces, this means that you will see (200,400,600,800) of partial digestion of nuclease that will tell you that the distance between each nucleosome and the other is about 200 base pairs
This number of 146 base pairs around it and the rest will be the linker.
-The experiment above to investigate more about the structure of nucleosome phase in the formation of chromatid.

Now let's talk about the 2nd phase of DNA packaging after the nuclei Somali phase formation.

30nm fiber



-The next level of chromatin organization in not yet resolved 30nm fiber

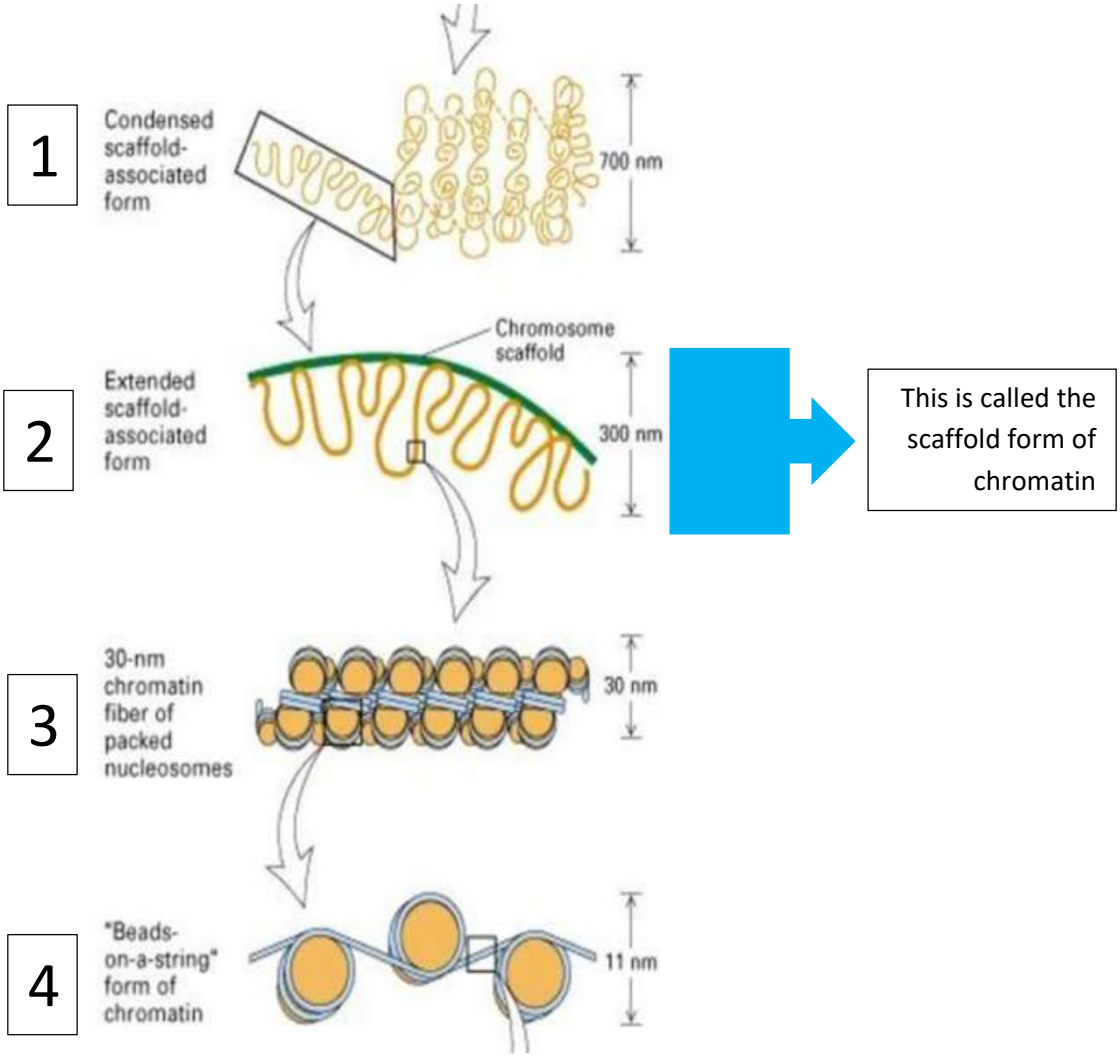
-May be an artifact of conventional electron microscope

- **Remember: nucleosomes are not found in prokaryotic system**
  We have some loops and supercoiled called nucleoid and those nucleoid in prokaryotic cells is composed of DNA, RNA and proteins.

  1-The first phase: histone proteins and DNA at the first phase of packaging
  2-The second phase of packaging is the folding of these nucleosomal structures on top of each other to form 30 nm diameter which is called the 30 nm filament
  The diameter of naked DNA without nucleosome structure is 2 nm
  So——>there is an increase in diameter after nucleosomal structure formation and that will increase in the packaging of DNA

  -So if the 10 nm structure fold in each other they will form 30 nm structure, the packaging in 30 nm is more efficient than others
  - to sum up :
  We started with diameter of 2 nm then 10 nm and finally we reached 30 nm, this will help more to compact our DNA into more compacted structures as you will see…

-Scaffold model :



-Predicts that non-histone proteins form a central scaffold along the long axis.

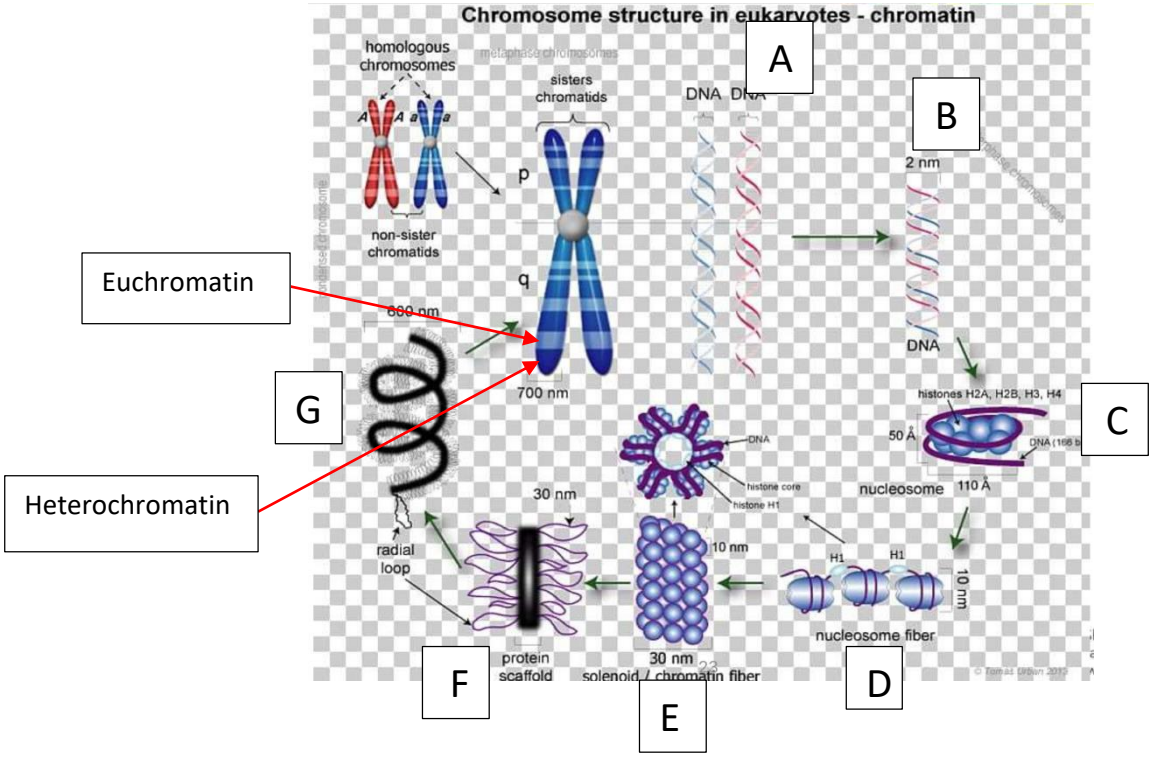
-Histone depleted chromosomes have DNA loops attached to a protein scaffold.

The next phase in compactness of our DNA is: that these 30 nm chromatin fiber (3) will fold on each other as well as it will be attached to a protein non-histone protein called scaffold protein (2)

➔ **This will increase the diameter of the structure from 30nm to 300 nm after linking to scaffold protein so more compactness is happening (2).**

***Scaffold form of chromatin also it will fold on each other to form condensed scaffolded form (1), which has a diameter of 700 nm and these are the starting formation of chromatin**

-<u>Chromatin or chromosomes</u>: DNA + Histones + non-histone proteins (like scaffold structure)



-To form a structure like this sister chromatid we started from double stranded DNA molecule (A), it will be 2 nm diameter (B)

-In the formation of nucleosome (B to C) we increase the diameter from 2 nm to 10 nm

-Folding of these nucleosomal fibers(D) into solenoid fibers of 30 nm(E) then the attachment of solenoid filament with scaffold protein(G)

That will form the loop structure (G)

And the loop structures will fold to each other to form the chromosome.

-So this started from 2nm to 700 nm diameter this is a good progress of compactness of our DNA into chromatin or chromosome

-Each chromosome has many genes and the genes inside the chromosome are masked (covered) by scaffold and histone proteins and they will be unable to replicate or transcript to happen, unless these histones and

proteins are released from the DNA and exposed back to double helical structure (B) in order to DNA replication and genes transcription to happen.

-So active genes when they transcribed, they must be exposed All the histones and other protein must be removed from that region(B) in which the gene is going to be transcribed in order to transcription factors and other proteins to come and read the information here(B) and transcribed this information into RNA.

NOTE: heterochromatin: inactive vs Euchromatin: active (go back to the image)

-Euchromatin: Active or ready for transcription because the histones and other proteins are removed.

-Heterochromatin: not active or not ready for transcription because it's masked with histones and other proteins.

- For a gene to be transcribed, histones and other proteins must be removed from that region, and because of this histones are subjected to modification such as (block their positive charges by chemical modification could be acetylation, and those they will lose their charges and they will be unable to bind to that specific region in the DNA, so DNA will be exposed to transcriptions factors and the transcription will start.

The telomere:

An important structure in chromosome , located at the end of it, they contain repetitive DNA sequence that distinguish them from non-telomeric regions in the chromosome

telomere : are small sequences (short tandem) repeated hundreds or thousands of times at the end of each chromosome

Muller, who coined the term Telomere

Short tandem like (TTAGGG) repeated hundreds or thousands of

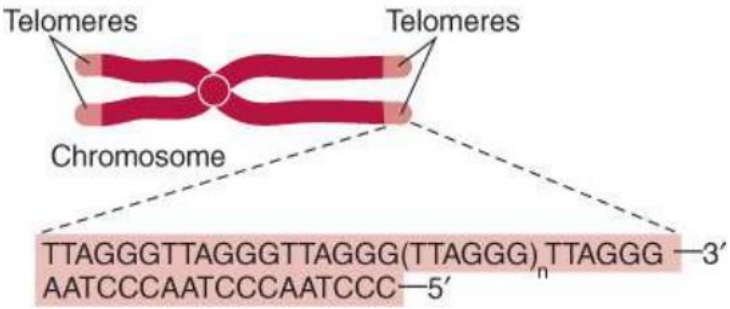


Figure 05.23: 3′-Telomere overhang. Telomeres, shown in pink, are located at the ends of the sister chromatids. Each telomere has a G-rich 3′-overhang.

times at the end of each chromosome

They are extremely important for the stability of the entire chromosome

Hermann J.Muller and Barbara McClintock , performed in the late 1930s revealed that the end of eukaryotic chromosomes are essential for the chromosome's stability McClintock reached the similar conclusion when observed that broken chromosomes in maize fused together

This was proven by experiments that deleted certain internal and end sequences in chromosomes

The experiments showed that internal deletions did not affect chromosomal stability and the chromosomes remained stable , while deleting sections found in the end of the chromosome never resulted in producing stable chromosomes

These sequences coding for nothing (non-coding sequences) , they are present there to protect the edges of the chromosomes

3' overhang(sticky end) is composed of short tandem repeated many times at each end , it is a result of the fact that the 3' end of one of the strands in the DNA of the chromosome is longer than its complementary strand

The strand with G-rich repeated sequence (TTAGGG in human telomeres) is the strand with the overhang , and this sequence is repeated "n" times

- Chromosome end has 3' overhang
- Single strand ranging from 100's bp in yeast to 1,000's bp in vertebrates
- Folds back to form a loop

Functions (importance) of telomere

- " how telomeres protect the edges of the chromosome? " Chromosome could stick to each other and cause disturbance, translocation and mixing of chromosome with each other (this is an important function of telomere to prevent the stick or attachment of chromosome with each other or disturbance of karyotyping
- Protect the genes within the chromosome from shortening as the cell is replicating (as the replication process or division process is going on the edges of the chromosome will be shortened , that shortening will happen in this case to the telomere so will protect our DNA sequence in chromosome (genes) from eating out by the replication process (shortening will take place to telomeres) protecting the actual coding sequence from shortening or degradation until the telomers become very short and the edges of the coding sequences of our

chromosomes will be shortened and thus our genes will be destroyed and the cell will (maybe) die

Telomeres are very important, they are also related to aging process so somatic cells or aged cells that have many divisions and DNA replication their telomeres is very much shorter than in embryonic cells (newborn)

*****As much the cells replicated the telomere becomes shorter***

****the length of the telemeters is related to the age of the cell***

cancers cell have very long telomeres and this is why the cancer cells are not die and the division continues because their telomers are long and they are protecting the coding sequence so they are immortal and continue to divide

 # how the adult and cancer cells keep their telomere long though it is divided and replicated while the somatic cells after multi-replication can't keep there telomers long ?

An enzyme that is responsible to build up the telomeres and that enzyme is called telomerase , telomerase have a very interesting mechanism of action will discussed later ,this telomerase enzyme it was found that is very active in embryonic cells and cancer cells but with age it will lose its activity and will be unable to rebuilt telomeres at the end of chromosome

telomerase responsible for replication in that region (end of chromosome)

cause of shortening is DNA replication

**** in medical aspect there is some food and vitamins that sold to increased the length of the telomeres by activating telomerase (but no experiment has been done on human being overhang there life longer)**

****Telomerase activity in cancer cells higher than in human cells***

****there is no bad effect of very long telomeres***

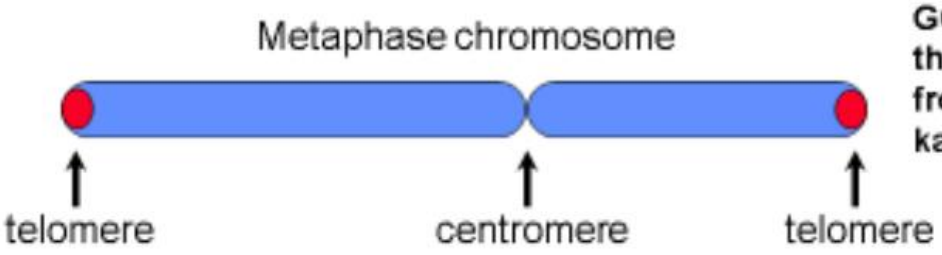
****the good effect of telomerase I) grow of embryonic cells II) increase living time of cells***

و من رامَ العَلا من غير كدٍ

أضاعَ العمر في طلبِ المحالِ

Telomeres and aging

Telomeres are protective "caps" on chromosome ends consisting of short 5-8 bp tandemly repeated GC-rich DNA sequences, that prevent chromosomes from fusing and causing karyotypic rearrangements.



- **telomerase (an enzyme) is required to maintain telomere length in germline cells**
- **most differentiated somatic cells have decreased levels of telomerase and therefore their chromosomes shorten with each cell division**

The 3' is longer than 5' This is the nature how god create these end of chromosomes and this is a positive credit of chromosomes because when I will take about telomerase action that will build telomeres it will use this piece of 3' overhang
Another benefit of 3' overhang is the formation of telomeres t- and D-loops (check the pic.) at the end of the chromosome for protection of chromosome and preventing chromosomes to link to each other and destroying the karyotyping

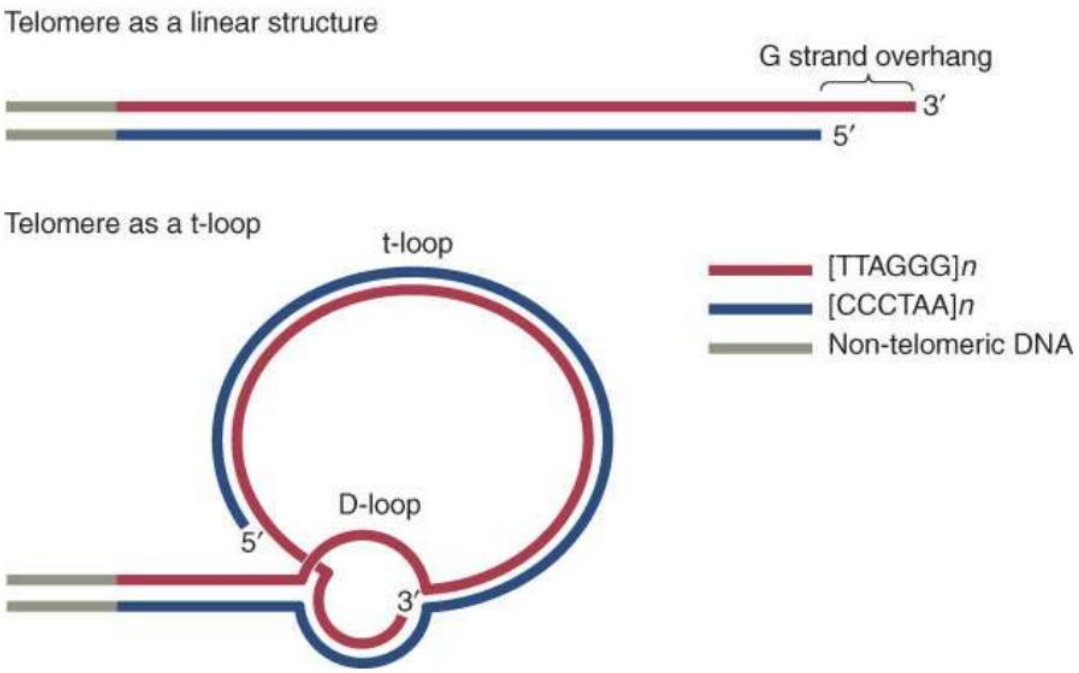
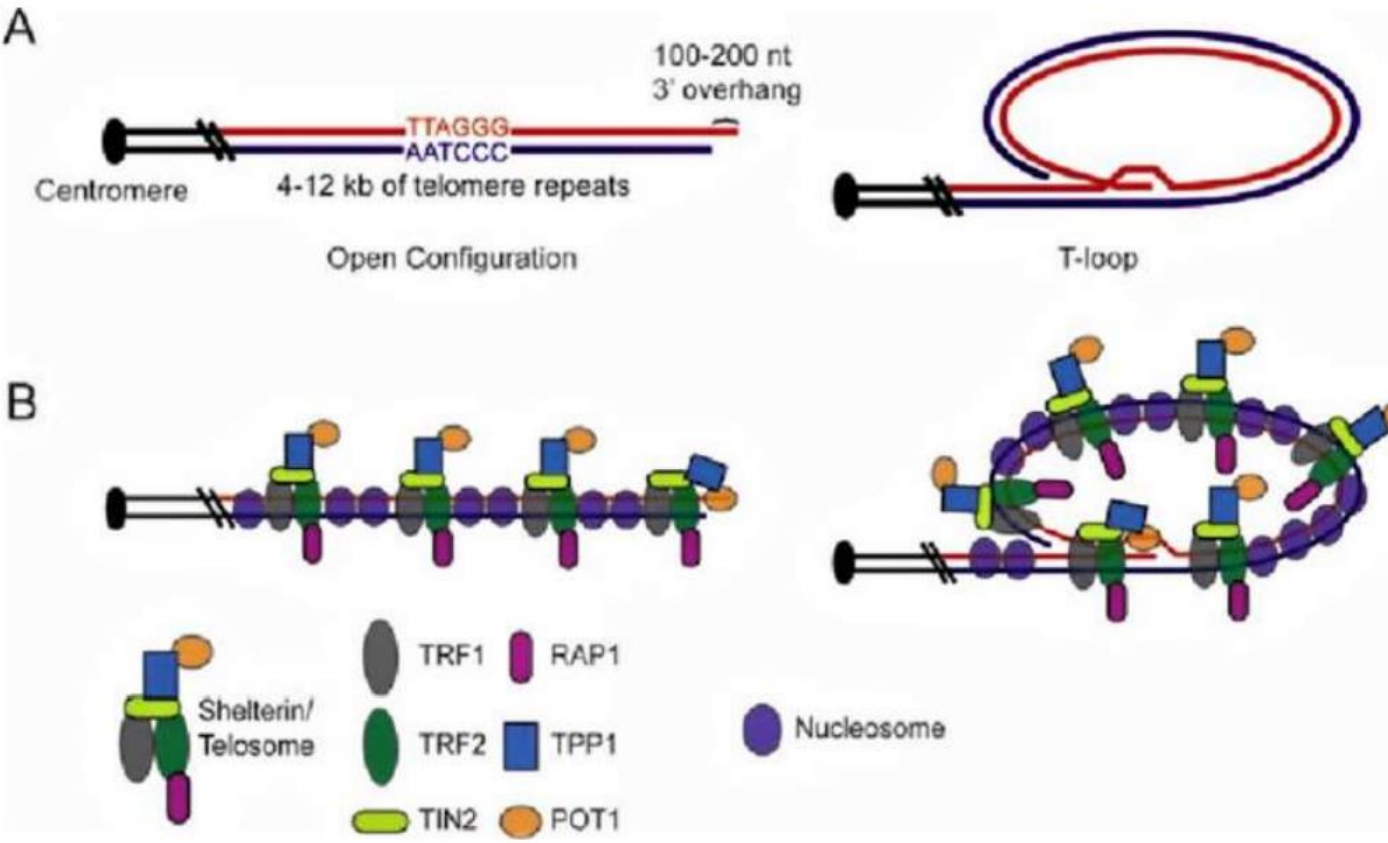


Figure 05.24: Structure of telomere t- and D-loops.

There are proteins that are important for the formation of this loop (-t or -d) for protection and the collection of these proteins are called (shelterin proteins or telosome)that will participate in the formation of the loop to hide this 3' overhang and prevent them from sticking to another 5'-3' sticking end of another chromosome and thus there will be a disturbance of chromosomal structure and arrangement in the cell

The telomere contains two loops, one larger 't-loop' which consists of both the G rich strands and its complementary c-rich strand , and the smaller ' D-loop' which result from the overhang of the 3' G-rich strand
The overhang of the G-rich strand binds with previous section in the telomere where localized denaturation of the G-rich and C-rich strands forms a bulging in the telomere . the overhang region then binds to its complementary C-rich single strand in the section forming the D-LOOP
These loops at the end of the telomere help stabilize the entire chromosome like a knot



** doctor mentioned the shelterin without any more explanation

every time DNA is replicated during cell division, parts of the telomere are lost

#the enzyme telomerase is required to replace the telomeres lost during chromosomal replication

Importance of looping: in cell division to keep the chromosomes separate **and after DNA replication in order to prevent chromosome from attachment to each other**

Effect of attachment of chromosomes to each other: link between different chromosomes, translocation, inversions and a lot of chromosomal disarrangement that will disturbe our genome

** telomeres prevent attachment of chromosomes but does not affect crossing over

Telomeres involved in DNA replication (primer link to it) and this RNA primer must be removed and when it is removed it will have a gap that can't be filled with normal replication it will require a telomerase enzyme in special mechanism

Werner syndrome helicase:

- **Protein involved in telomerase formation**
- **Defective in Werner syndrome**

Syndromes that result in disorder of telomerase formation such as: the genetic disease Werner Syndrome may lead to premature aging.
Werner Syndrome Helicase (WRH): is a protein involved in telomerase formation this protein becomes defective in Werner Syndrome

A genetic disease associated with premature aging
• Eukaryotic chromosomes lose DNA from their ends during replication
• Require the enzyme telomerase
• Failure to replace telomeres is potentially responsible for finite number of divisions of cells in culture
Because telomeres are important for chromosomal stability, the loss of telomeres (due to the cell undergoing multiple replications) without replacement is the reason why healthy (non cancerous) somatic eukaryotic cells have a finite and limited number of replications in culture
While eukaryotic somatic cells have a limited number of replications they can go through before they apoptosis because they can't replace their telomeres , germ cells contain telomerase and can therefore divide indefinitely (without any limit)
• Most cancer cells have telomerase

Most cells that undergo infinite unlimited divisions such as prokaryotic bacteria, cancer cells, and germ cells have telomerase to replace the lost telomeres during cell replication .

أدري بأنك لا تخاف الطفل حياً

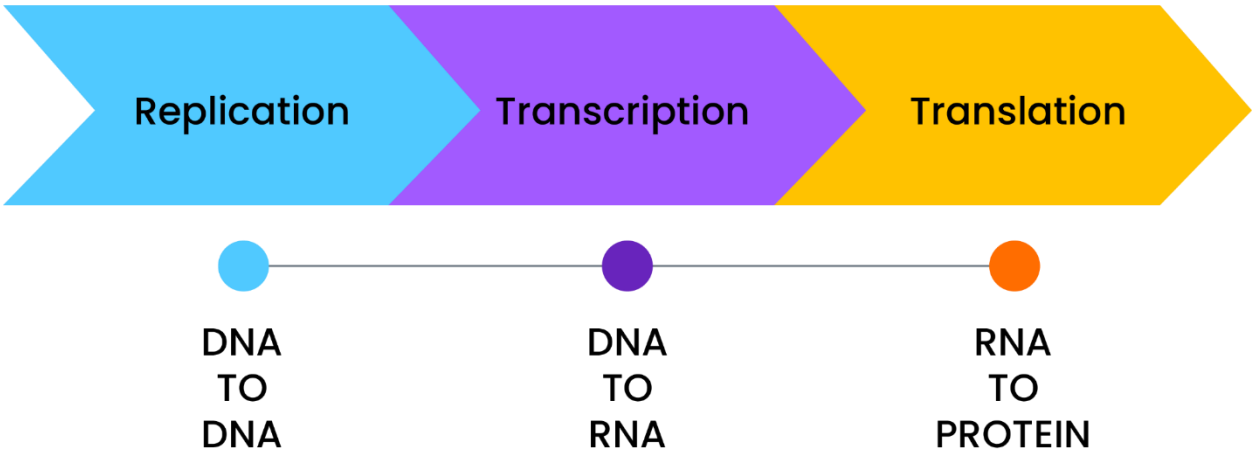
إنما أدعوك صدقاً، أن تخاف من الصغار الميتين

دعواتكم لاهلنا فيه غزة

General features of DNA replication:

To start we have to review this information:

- Central dogma of molecular biology: explains the flow of genetic information to make a functional product, a protein.
  It consists of three main types, as you can see in the diagram:



And in some organisms such as viruses, there is a fourth type, which is called “Reverse transcription” (RNA to DNA).

Let's start with the first type which is DNA replication. During DNA replication, various molecules, enzymes, and substrates are required to successfully replicate DNA.

1- DNA polymerase:

It's an enzyme whose function is Binding or adding nucleotides to the growing DNA strand, using an existing DNA template.

Substrates of DNA polymerase:

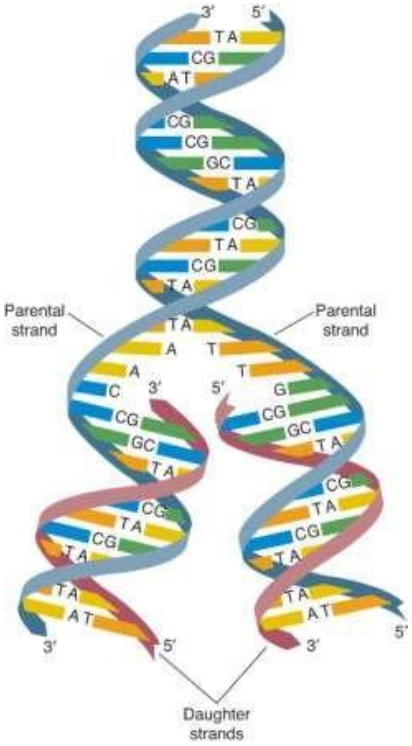
- Deoxynucleotides
- DNA template strand: from the parental strands, each one of them will act as template for DNA polymerase.
  But how are templates provided by parental strands?
  By partial denaturation.

2- DNA helicase:
DNA helicase unwinds the double-stranded DNA helix, separating the two strands.
If the strands unwind in opposite directions, it forms supercoils, halting DNA replication.
Topoisomerases are enzymes needed to relieve supercoiling and allow replication to continue.

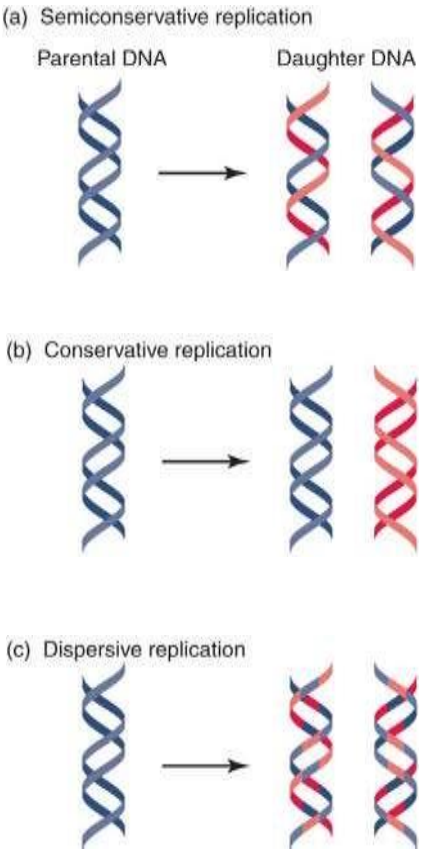
3- primer:
It is required to initiate the synthesis process. In the context of DNA replication, the primer consists of small RNA fragments (around 15-20 ribonucleotides) that have a complementary sequence at the initiation site of replication.
The primer provides a 3' hydroxyl group which can attach to the 5' phosphate group of the coming nucleotide during DNA synthesis.
It helps initiate the addition of new nucleotides to the growing DNA strand.
However, it's important to note that the primer used in PCR (Polymerase Chain Reaction) or DNA sequencing may differ in composition or method of generation with the primer in DNA replication.

4- deoxyribonucleotide triphosphates (dNTPs)
- dATP, dCTP, dGTP, and dTTP - are necessary for DNA synthesis.
However, dUTP is not utilized to maintain the fidelity of DNA replication.
—----------

models of DNA replication.
1- semi-conservative model (the true model)
It's the model that fulfills the structure and function in transmitting genetic information from one generation to another.
It states that a parental DNA strand acts as a template for the synthesis of a new complementary strand.
Following replication, each resulting DNA molecule contains one original parental strand and one newly synthesized strand that is complementary and antiparallel to the parental strand. (each daughter molecule has one parental strand and one newly synthesized strand).



Watson and crick model of DNA replication



2- conservative replication model (wrong model):

Parental strands unwind at replication site to allow the sequence to be read by the DNA polymerase and then rewinds.

It suggests that during the replication process, the parental double-stranded DNA remains intact, and a completely new double-stranded DNA molecule is formed.

It doesn't fulfill the function of genetic information from one generation to the next generation.

كيف ممكن تصير ؟

"GOD KNOWS"

3- Dispersive replication model (wrong model):

It states that the parental DNA strands are fragmented, and the resulting DNA molecules contain a mix of parental and newly synthesized segments.

How was the semi-conservative model discovered?

By “Meselson-Stahl experiment”. How?

1-Bacteria were grown overnight in a medium containing heavy nitrogen isotope (N15) to label their DNA (so all DNA will have N15 in it).

-The bacteria rapidly replicate every 20 minutes.

2-The bacteria were then transferred to a medium containing light nitrogen isotope (N14).

3-DNA samples were collected at different time intervals after the transfer.

4-Extraction of DNA by Homogenization (as we learned in the previous lecture):

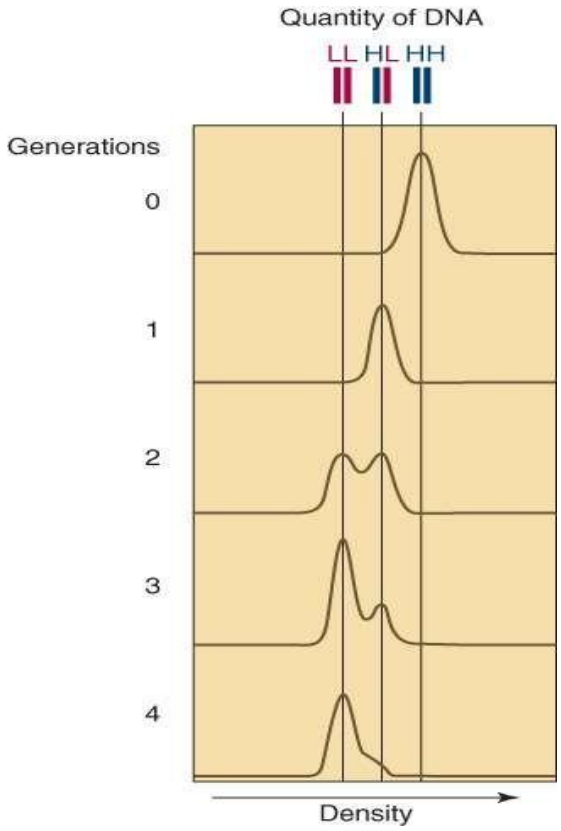
The bacterial cells will be harvested from the culture medium, and lysed using lysozymes to dissolve cell wall and then we use SDS in order to denature the proteins and membranes, then they will centrifuge and then undergo a series of chemical treatments such as proteinase digestion and phenol extraction to remove cellular proteins and other contaminants. The extracted DNA will then be purified using techniques such as ethanol precipitation and then the resulting purified DNA samples will centrifuge, taking the pellet and wash it from ethanol by dissolving it in an aqueous buffer and now we have the prepared DNA.

5-separated molecules using CsCl equilibrium gradient centrifugation:
During this stage, a CsCl solution is used to create a density gradient in a centrifuge tube. The DNA sample, typically in the form of a solution or dissolved in a buffer, is carefully layered on top of the CsCl gradient.

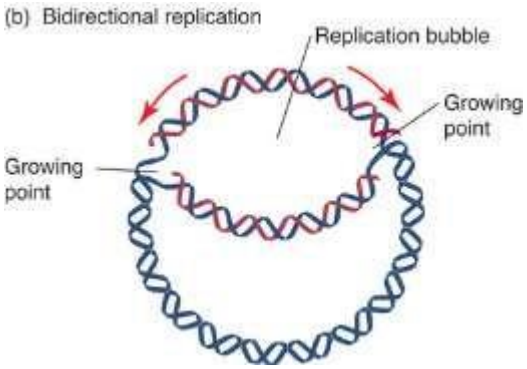
ولستُ أخشى رجوعَ الكفِّ خائبةً

إن كُنتَ -يا مالكَ المُلكِ- الذي يُعْطِي

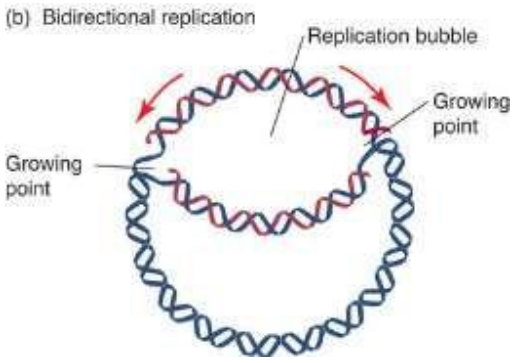
When the centrifuge is spun at high speeds, the DNA molecules move through the CsCl gradient based on their density. The centrifugal force causes the DNA to migrate to a position in the gradient where the density of the CsCl matches its own density, resulting in equilibrium. This process separates the DNA molecules based on their densities, allowing for purification and isolation of specific DNA fragments. (heavy DNAs will sediment in CsCl that has an equal density to them, and light DNAs has the same condition).



Meselson-Stahl experiment showing semi-conservative DNA replication



In unidirectional replication a single growing point moves in the direction. shown by the red arrow.



In bidirectional replication two growing point move in opposite directions as shown by the red arrow

Semi-conservative replication model is the only one consistent with results.

—----------

In DNA replication (in bacterial and eukaryotic DNA), bidirectional replication (means that one chain grows from 5'to3' but other chain grows from 3'to5') occurs where DNA synthesis proceeds in opposite directions.

- θ-structure and be explained by unidirectional or bidirectional replication.
-Labeling expt. confirmed that it is bidirectional

The DNA strand that grows in 3’ to 5’ direction is formed by joining short fragments together.

This process starts from a replication bubble, which forms after the partial denaturation of both DNA strands. At the replication fork, each fork moves in an opposite direction until they meet at a specific point. At this meeting point, two newly circularized DNA molecules are formed and separated from each other through a specific mechanism.

This phenomenon is observed in prokaryotic systems, where the DNA is circular. In eukaryotes, the process is similar, but the DNA is linear instead of circular. In eukaryotes, there are hundreds to thousands of replication forks simultaneously replicating the DNA. In contrast, prokaryotes typically have a single replication fork due to the smaller genome size. For example, the human genome contains around 6 billion base pairs, while the genome of E. coli consists of around 6 million bases. This difference in genome size allows E. coli to replicate its DNA within approximately 20 minutes, whereas humans require approximately 20 hours for DNA replication to complete.

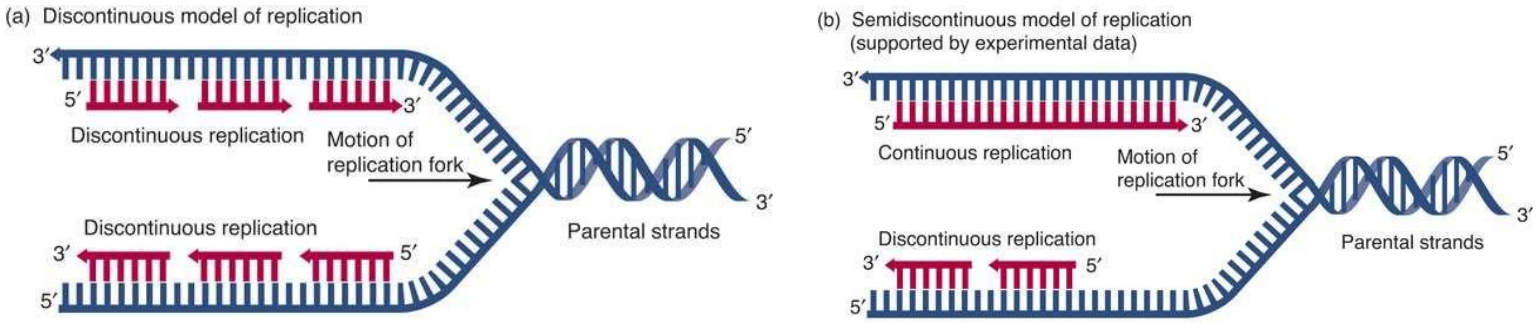
The formation of replication bubbles occurs at specific sites in the genome called origins of replication. In cases where only one replication fork is present there is one origin of replication. while in eukaryotes there are also hundreds or thousands of origins of replication in which at each the replication bubbles and replication forks will be formed and move in opposite directions until they finish the whole genome.

Replication involves the duplication of the entire genome, whereas transcription occurs selectively for specific genes or sets of genes based on the cellular requirements or environmental conditions.
Replication happens to the whole genome
Transcription happens to some genes (not all)

During DNA replication, an important characteristic is that it proceeds only in the 5' to 3' direction in both prokaryotes and eukaryotes. This is because DNA polymerases in these organisms specifically possess the activity to polymerize DNA only in the 5' to 3' direction. They lack polymerase activity in the 3' to 5' direction.For instance, eukaryotic DNA polymerases exhibit 5' to 3' polymerase

activity as well as 3' to 5' exonuclease activity, which enables proofreading and editing of newly synthesized DNA.
It is crucial to note that every single-stranded newly synthesized DNA molecule, whether in prokaryotes or eukaryotes, must be synthesized in the 5' to 3' direction.
This is because the parental DNA strands are antiparallel, and thus the newly synthesized strands must also be antiparallel to them.

to fulfill this requirement (synthesis in 5’ to 3’ direction). The mechanism of synthesis differs between the leading and lagging strands. The leading strand is synthesized continuously, while the lagging strand (because it lags behind the continuously replicating strand) is synthesized discontinuously in the form of small fragments known as Okazaki fragments. These fragments are later joined together by DNA ligase. The leading strand requires only one primer for synthesis in 5’ to 3’ direction, while the lagging strand requires multiple primers. The primers, consisting of RNA, provide the 3' hydroxyl group necessary to initiate synthesis with the first complementary deoxynucleotide. This ensures the formation of phosphodiester bonds and enables the progression of replication.



Models proposed by Reiji Okazaki to explain in vivo DNA replication. Parental strands are shown in blue and newly replicated strands are shown in red.

لا يعشقُ الصيف إلا من به خللٌ

أمّا الذي يهوى الشتاء فهيمُ

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