Genetics & molecular biology

◉ Sheet ○ Slide

Number:		3
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>>Forms Of **Supercoiling**

1. **Plectonemic**: **the twisted thread**:

> (DNA) بكون زي الخيط او الحبل الي لافين علي بعض

(الفكرة إنو البلازميد عندو قابلية بأن يلف علي بعضو)

(**tendency intrinsic property**)

من دراستهم اكتشفو انو المادة الوراثية المفصولة من بدائية النوى زي البلازميد المفصول من البكتيريا بكون على شكل (Plectonemic).

حقيقية النوى بكون الها شكل تاني ما بتكون طويلة، بتكون مضغوط أكتر زي الزنبرك فبكون مضغوط أكتر بالتالي الحيز في النواة بكون أقل .

2. **Solenoidal** (left handed)

> DNA packaging (solenoidal) increases in nuclear division because of DNA replication —> DNA must transfer to daughter cells.

This coiling is not just for DNA replication but also for transcription. In transcription we need specific part of gene or set of genes so the DNA should be accessible in certain region and for short time to have m-RNA.

>Histones

لما نعمل DNA كان دايما انو العينة ما بتكون نقية مية بالمية بيطلع معه نسبة معينة من البروتين فاستنتجو انه DNAفصل بكون مخزون في النواة مع البروتين

>Nucleosome: structure of DNA bounded around protein core.

Allows fitting of DNA in small space regulations of transcription (for gene or a set of genes)

بلف حوالي 1.65 لفة

This proteins called Histones (H1,H2a,H2b,H3,H4) , - octamer (8 of proteins,, 2 of each)- and its rich with positively charged a.a like Arg & Lys. Histones have tails or extensions which important on interconnections between two nucleosomes and it's important for regulation of genes .

انو بكون لكل خلية او نسيج تخصص شو تصنع ومتى تصنع، , بمرحلة معينة(الطفولة،المراهقة...) بتحكم بمتى يترجم الجين وبأي نسيج.

>لما يفحصوا المناطق القريبة بين المادة الوراثية والبروتين بلاقوا انو هاي المناطق بتكون مضغوطة أكتر يعني انو ال (DNA)لافف أكتر وبقوة أكبر.... فهاي المنطقة بتكون أقرب على البروتين وبتكون عند :

>Minor groove (rich in A.T, because it's easier to make compression)

- DNAالي شحنتو سالبة (بسبب الفوسفات) رابط بقوة مع البروتين الي شحنتو موجبة

(Electrostatic interaction)

Linker DNA: links between two nucleosomes

164 bp

Linker DNA = 54 bp

المقطع كلو بكون

200 bp

Zig-zag model (30nm fiber):

Nucleosomes, linker DNA & H1 (not a part of protein core, link the nucleosome with linker DNA to maintain the structure of the chromosome)

Post-translationally modification:

Adding of methyl or acetyl group to Lys, or adding of phosphate group to serine

إضافة هاي المجموعات الي شحنتها سالبة على البروتين الي شحنتو موجبة بعمل تعادل للشحنة فبالتالي

التجاذب الي كان موجود بين ال DNA والبروتين بقل فبصير أسهل أعمل ترجمة ولما أخلص من الترجمة بتنفصل هاي المجموعات عن البروتين وبرجع الترابط زي أول

>Lys rich in N- terminus (tails of histones)

وعليها بترتبط المجموعات

when the level of acetylate on the extensions of histones increase _the chromatin condensation decreases so if I add nuclease the nucleic acid will break down

Nuclease: breaking of phosphodiester bond

Acetylation & deacetylation:

lys+ acetyl group = all the chromatid become acetylated

الانزيم المسؤول عن إضافة مجموعة الاستيل:

Acetyl transferase (HAT)

والانزيم المسؤول عن حذف هاي المجموعة اسمو:

Histone deacetylase (HDAC)

-مجموعة الاستيل بنحصل عليها من مصدرين

-Krebs cycle

-f.a degardation

Condensed chromatin :(Heterochromatin)

Relaxed chromatin: (Euchromatin).

CoA (co-enzyme A): group that joined to another group like Acetyl, between them SH group (sulfhydryl) linking by very strong bond.

Acetyl CoA: source of Acetyl in acetylation.

We can see it in citric acid cycle and in degradation of fatty acid.

Acetyl group link to histone (H2A, H2B, H3, and H4) and decreases the interaction between histone and DNA, so the condensed structure transformed to relaxed one which is associated with gene transcription. This process is part of gene regulation.

Gene regulation: very important process, why?

Because it's not enough to have genes, we should know where they have to express and the amount that our body needs it. So we have activators and reducers for expression.

مثلا في أشخاص النظام الغذائي تبعهم غني بالبروتين، بالتالي بكون عندهم ضغط كبير على الأنزيمات إلي بتكسر البروتينات وبتحولها ليوريا ، فبحتاج الجسم انو تزيد عدد هاي الانزيمات والي بتحكم بهاي العملية هو

(DNA wrapped around nucleosome give level of compaction about 7folds).

Overall compaction in chromosome is>10000 folds, so nucleosome also round 30nm fiber (zigzag model) but it's still provide 100 folds.

Fiber expanded to make a loop (75000 bp).

6 loops —> rosette.

30 rosettes —>coil.

10 coils —> chromatid.

Chromatid —> chromosome.

We have higher levels of compaction: **Nuclear scaffold:** groups of protein interact with DNA includes: histone (H1), SMC, topoisomerase.

Nuclear scaffold coiling the loops together to make rosette.

SMC (structural Maintenance of Chromosome):

Family of Atlases (make hydrolysis to ATP and convert it to ADP) important to maintain the structure of chromosomes.

- In eukaryotes there are 6 SMC proteins work as dimmers (hetero dimmers).

DNA عاملين زي المشابك وبثبتو ال.

-E.coli's cell division occurred each 15-20min so it's DNA duplicating each 15-20 min.

وهون بنستنتج انو حتى على مستوى البكتيريا وهي غير حقيقة النواة في

مستوى عالي من الضغط والتنظيم.

-DNA Replication Follows a Set of fundamental rules.

1. DNA replication is Semi-conservative according to Meselson- Stahl experiment, he proved that in each generation there's a parental strand and a new strand.
2. Replication begins at an origin and proceeds bidirectionally.

We're studying the E. coli a type of prokaryotic that has a circular DNA, there's a special region in this circular DNA to start the replication from it, which called OriC. (Origin of replication)

*when the 2 strands of DNA open here in this origin it forms a replication bubble.

*Replication of the 2 strands starts at the same time so its bidirectional, (in the 2 directions), not unidirectional.

*Replication forks: are dynamic points where parent DNA is begin unwound and the separated strands quickly replicated.

3. DNA synthesis proceeds from 5' to 3' direction and is semicontinous.

*Semicontinous: means that one of the strands is continuous in replication and the other one is discontinuous (Okazaki Fragments).

 the continuous strand called: leading strand (from 5' to 3').

 the discontinuous strand called: lagging strand (from 3' to 5').

*In leading strand the direction is the same of the replication.

*the lagging strand, discontinuous in the opposite direction. *Called Okazaki relative to the scientist.

كل ما بتفتح قطعة من السلسلة يتم نسخها حتى نهاية السلسلة.

* So we need an enzyme to join these fragments and an enzyme to remove the primer.

(Conclusion)

*The leading strand is synthesized as single strand from the point of origin toward the opining replication fork.

*the lagging strand is synthesized discontinuous against overall direction of replication from the replication fork toward the origin.

DNA is Synthesized by DNApolymerase.

*DNApolymerase is the enzyme needed for DNA replication.

*DNApolymerase needs: template, Primer and nucleotides.

***Polymerization**: joining monomers, nucleotides in this situation.

* **Polymerization rate** (nucleotides added per second).

*DNApolymerase doesn't start from nothing, it needs hydroxyl group (3' end) to start from it.

*DNApolymerase joins 2 nucleotides together by phosphodiester bond.

*Primer= starter, where DNApolymerase starts from, it is an RNA sequence and we need it for providing hydroxyl group.

*what's the point from adding a primer? To add a free hydroxyl group to the DNApolymerase to start synthesis.

*when DNApoly. Join 2 nucleotides together, Inorganic pyrophosphates release.

* Growing DNA strand (primer) + Incoming deoxynucleoside 5’ tri phosphate
Inorganic pyrophosphate + new phosphodiester bond between 2 nucleotides.

(Conclusion)

*DNA polymerization reaction is guided by:

 DNApoly. Activity requires a single unpaired strand to act as a template and a primer strand (RNA segment complementary to the template) to provide a free hydroxyl group at the 3’ end, to which a new nucleotide unit id add.

Each incoming nucleotide is selected in part by base pairing to the appropriate nucleotide in the template strand. The reaction product has a new free 3 hydroxyl, allowing the addition of another nucleotide.

Replication is very accurate:

*Replication proceeds with high fidelity.

* In E.coli a mistake occurs/10^9-10^10 nucleotides 4.6*10^6 Bp.

*Discrimination between correct and incorrect nucleotide:

1- Hydrogen bonding (base selection)

2- Common geometry of the strand A-T and G-C, (active site of DNApoly. accommodate correct geometry). Incorrect bases rejected before phosphodiester bond formed. However, this doesn’t account for high fidelity of polymerase in replication.

3- Proofreading activity. (Reading from 3’ to 5’). After phosphodiester bond was formed.

(Before forming it the mistake is usually repaired by rejecting the phosphodiester bond forming, but if it was formed it’s repaired by proofreading.)

☒ An incorrect nucleotide may be able to hydrogen-bond with a base in the template, but it generally will not fit into the active site. Incorrect bases can be rejected before the phosphodiester bond is formed.

☒ Proofreading: the 3’ to 5’ exonuclease activity of DNApoly. Removes the mispaired nucleotide (double check), and the polymerase begins again.

☒ **Exonuclease**: يتم التصحيح من الطرف فقط.

Why mistakes happen?

For each nitrogen base (A, T, G, C) there's 2 forms called tautomers one form of them is rare and the other is common, for example C is a rare tautomeric form of Cytosine (C*) that pairs with A instead of T because it has almost the same way of hydrogen attachments.

<u>E.coli has at least five DNApolymerases.</u>

* 1,2,3,4 and 5. (4 and 5 will talk about it later.)

*there's differences between them in the structural gene that's responsible for polymerization activity.

*In their molecular weight (3>2>1)

* All of them do proofreading. (Exonuclease activity from 3' to 5').

*DNApoly. 1 (ONLY) has a 5' to 3' exonuclease activity, (for removing the primer). DNApoly.1 responsible for repair and removing of primes and replacing them.

 DNApoly. 3 have the largest polymerization rate so it's the responsible for DNA replication in E.coli. *DNApoly.3 has the largest processivity. DNApoly.3 responsible for replication.

*Processivity: number of nucleotides added before this DNApoly. Dissociates and another one bends.

(لا تتم عملية النسخ ب دي ان ايه بوليميريز واحد فقط)

<u>Klenow fragments</u>

- In DNApoly.1

كلينو هو عالم قام بإجراء تجربة على دي ان اي بوليميرز 1 حيث قام بمعالجته ب بروتين, ووجد أنه يقوم بإلغاء خاصية:

5' to 3' exonuclease activity.

ولكن بقي جزء منه ما زال قادرا على:

5’to 3’ polymerase activity and 3’to 5’ exonuclease activity for proofreading.
وسمي هذا الجزء باسمه.

*Klenow fragment: it retains the:

- 5’ to 3’ polymerase activity.
- ☒ 3’ to 5’ exonuclease activity for proofreading.

But it loses its 5’ to 3’ exonuclease activity.

- ☒ The 5’ to 3’ exonuclease activity of intact DNA (or RNA) paired to the template strand, in process known as nick translation.

Nick translation.

*nick means broken bond= hydroxyl group free and phosphate group free.

*How Poly.1 do that?

1- Recognizing the nick. (For example the nick is because of Okazaki fragments).

2- In this process, an RNA or DNA strand paired to DNA template is simultaneously degraded by the 5’ to 3’ exonuclease activity of DNApoly.1 and replaced by the polymerase activity of the same enzyme.

These activities have a role in both DNA repair and the removal of RNA primers during replication (both described later). The strand of nucleic acid to be removed (either DNA or RNA) is shown in green in the figure in page 79, the replacement strand in red.

DNA synthesis begins at a nick (broken phosphodiester bond, leaving a free 3 hydroxyl and a free 5 phosphate).

DNApoly.1 extends the non template DNA strand and moves the nick along the DNA- a process called nick translation.

A nick remains where DNApoly.1 dissociates, and later sealed by another enzyme (DNA ligases).

DNApoly.3

(Subunits of DNApoly.3, table 25-2, page 82.)

*α subunit: Polymerization activity.

*€ subunit: 3’ to 5’ proofreading exonuclease.

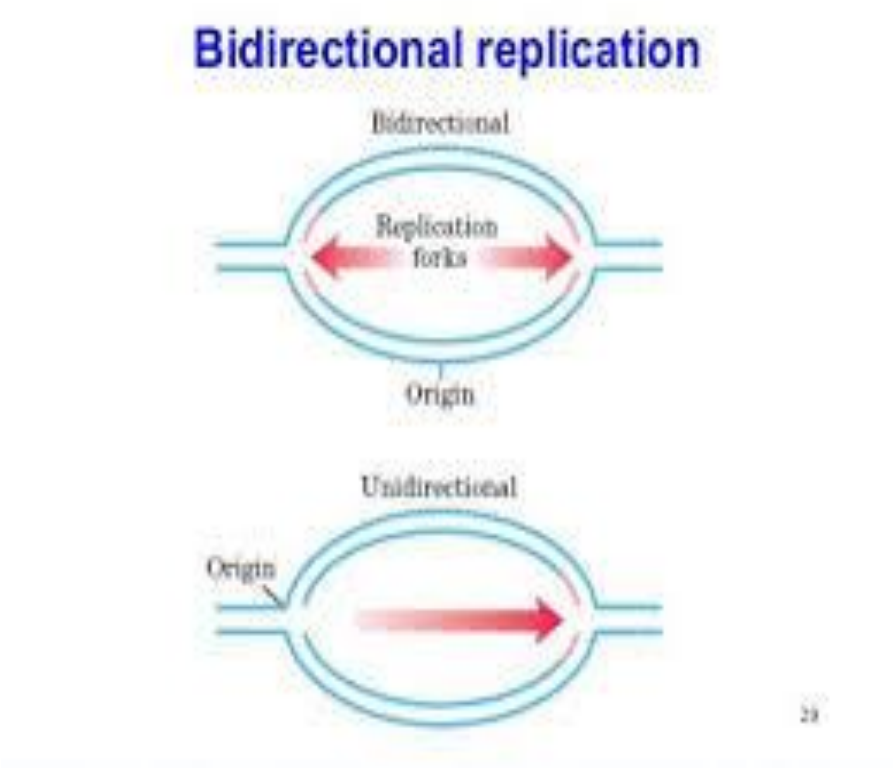
*~~Θ~~, □ : stable template binding core enzyme dimerization (polymerize DNA with minimum processivity).

*Ṿ, Ś, Ṣ: clamp-loading complex that loads ß subunits on lagging strand at each Okazaki fragment.
*ß subunit: DNA clamp required for optimal processivity. (Increase processivity)

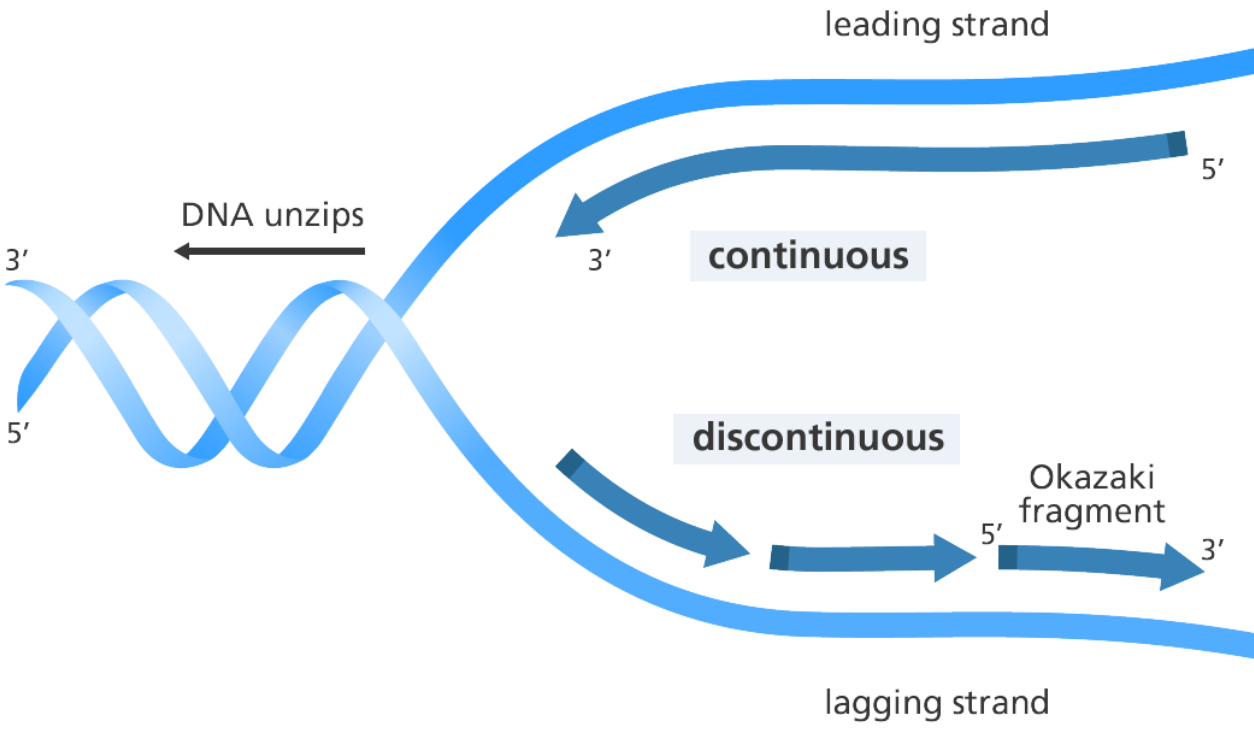
>>DNA Replication requires many enzymes and protein factors:

1-Helicase: move along the DNA and separate the strands, using chemical energy from ATP. (2 strands of DNA Opens)
2- Topoisomerases: release the topological stress in the helical DNA structure created by strand separation.
3- Primase: (adding primer) synthesis segment of RNA.
4- DNA ligases: seal the nick remains in the DNA.
5- DNA polymerase 1: Removal of primer (removes RNA primer and replaces it by DNA sequence).
6-Single strand DNA-binding protein (SSB): stabilize the separated DNA strands.

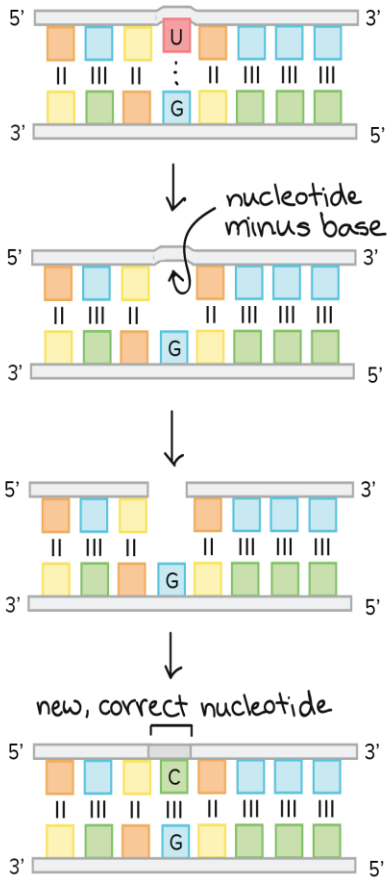
☒ The OriC is a repeated sequence of A and T because there are only 2 hydrogen bonds so it's easier to break them.



DNA replication fork



Proofreading



Deamination converts a cytosine base into a uracil.

The uracil is detected and removed, leaving a base-less nucleotide.

The base-less nucleotide is removed, leaving a small hole in the DNA backbone.

The hole is filled with the right base by a DNA polymerase, and the gap is sealed by a ligase.