Genetics & molecular biology

◉ Sheet ○ Slide

Number:		4
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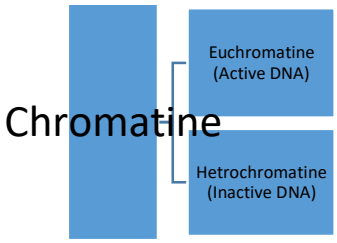
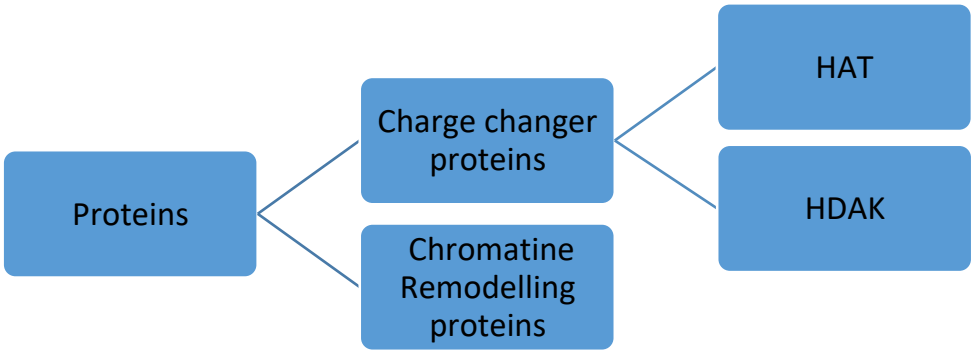
DNA is 2m tall and condensed in 6 micrometer nucleus diameter
DNA + Histone = Nucleosome
Linker DNA links Nucleosomes together
Chromatin is divided into two





Histone tails are rich with lysine; HAT takes acetyl from acetyl co-A to neutralize lysine +ve charge ---- so DNA repulse with Histone

HDAK converts -ve charge from histone to +ve charge by removing acetyl and putting it on Co-A enzyme (Acetyl group is –ve charged)

This process converts Euchromatin to Heterochromatin vice versa

Non-histone proteins: help histone with folding.

Topoisomerase is a non-histone protein, helps in replication process by relaxing DNA helix.

SMC proteins are non-Histone proteins which are ATPases

Bacteria have Histone like protein helps with packaging, it is histone like (functionally not structurally).

Z DNA	A DNA	B DNA	
left	right	Right-handed	Helix sense
12	10	10.5	Mean bp/turn

Z DNA is unique and rare and rich with G C bonds.

Most of our DNA is present in our Nucleus, it can exist in mitochondria and in chloroplasts.

Mitochondria are very similar to bacteria
1- Circular DNA 2- similar Ribosomes 3- genetic codes differ nuclear 4- Mammalian mitochondrial DNA lacks introns and non- coding regions

Gene is not: A segment of genetic material that determines /codes for a protein / enzyme

Gene is: All the DNA that encodes the primary sequence of some final gene product (polypeptide / RNA)

One gene one enzyme-------One gene one polypeptide

Transposons (jumping genes/ selfish DNA/ parasites DNA/Junk DNA…): segments of nontranslated DNA that move around to different positions in the genome of a single cell.

Class I Retrotransposons, move in the genome by being transcribed DNA□RNA then back to DNA by reverse transcriptase to insert in a new location **"Copy and paste"**

Class II Transposons, consisting only of DNA that move directly from one position to another within the genome using a transposase to **"cut and paste"** them within the genome.

Satellite DNA •

Simple sequence DNA ~ 10bp ➢

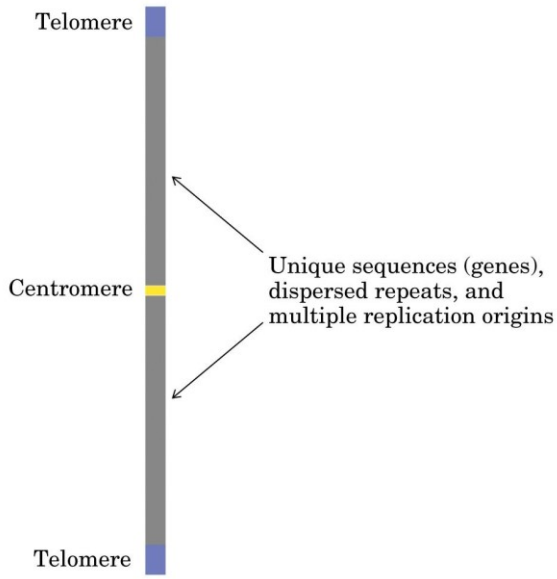
Highly repetitive DNA ➢

Tend to produce a different frequency of nucleotides A, C, G and T ➢

Have a different density from bulk DNA ➢

It sediments as a distinct band in caesium chloride density gradients

Centromeres a sequence that functions during cell division as an attachment point during mitosis A=T Rich sequence



Telomeres sequence at the end of chromosomes that help stabilize chromosome

NOTES

Replication is a very accurate and fidel operation, an example is e-coli, approximately one mistake occurs in a 10^9-10^10 added nucleotide.

Replication in eukaryotes is much more complex because its genome is more bigger and DNA polymerase 1,2 and 3 is different between eukaryote and prokaryote.

The more a cell is divided the less it can continue to do so. In eukaryotes, DNA is complex; it has more than one origin of replication. This increases the speed of replication and the rate of polymerization. In prokaryotes the two chains are opened and then replication begins. Elongation = extension = synthesis of a new strand. The two strands are opposite in direction (3'>5' and 5'>3') because they are anti-parallel.

DNA Metabolism

Chromosome e-coli is circular and listed as the clock having minute (A genetic measurement=40K B.P) it is not a measure unit of time but a part of DNA divided on a particular approach (o/25/50/75/100)

The name of gene is written in small font to distinguish between the gene and protein for example polA and uv gene (responsible for radiation resistance)

As for the capital letters like (A.B.C), these letters according to the discovery of the gene does not symbolize the way the gene works or function.

Meselson –Stahl experiment:

Many studies exist in order to understand the nature of DNA replication, but the problem was that DNA is double stranded therefore this experiment had:

1.The scientists brought bacteria and planted them in a medium containing nitrogen isotope 15, a heavy isotope where the bacteria split and DNA was separated.
2.Then they made a cesium chloride centrifugation (Method of separation of DNA based on density) The heavy one will be in the bottom of the tube while the lighter on the top
3. the result was that the strand was in the bottom of the tube because they used the heavy isotope
4. Then the bacteria was planted again but in lighter isotope (N 14) and allowed to split one time, then the strand was in the middle of the tube.
5. Then transfer into another medium(N14/15) with several divisions and get two band one in the middle and the other in the top of the tube and the origin strands of DNA was faded out.

This indicates that the DNA is semi-conservative. When it split, it did not give two new strand, but each molecule of DNA had an old chain and a new chain based on the type of isotope in which it was placed.

Bacteria grew (Emphasize the use of bacteria for easy handling because they contain one circular chromosome) in a medium with thymidine labelled with tritum H3. The division was tracked by autoradiograph, it was found that replication starts from a point called origin of replication, division of chromosome took the loop shape then separated at Origin of replication in eukaryote = replecator
Celleral to ORC

These two chromosomes grew up in different directions that what we call the bidirectional
This mechanism happened in human cell also but more complicated because DNA linear have different region to replicate from.

If we need to build a certain DNA we need
Enzyme (DNA polymerase)
Template
Primer (DNA/RNA) 3" prime hydroxyl group
Nucleotides (DTTP/DGTP/DATP/DGTP) D= deoxy in DNA

DNA polymerase starts building from the 5" to 3"
DNA is double stranded (anti parallel) so the other strand starts with 3" to 5" so how could it replicate?

1. To synthesis DNA it must have a primer to start the replication
During the replication of the DNA, we need a specific enzyme to separate and stabilize the two strands due to the hydrogen bonds that attempt to reconnect them.

helicases: to remove the helical structure between two strands.
SSB (single strand DNA binding protein) to stabilize the strands. RBA (replication protein a single strand DNA binding protein) resembles the SSB

use as stabilizing to chromosome, protection to chromosome and prevents fusion with a second chromosome

DNA polymerase starts copying the strand (3"to5") with a complementary (5" to 3") this will produce a continues strand (Leading strand)
Other strand with (5" to 3") make a fragment with primer for each and called them Okazaki fragment this strand called (Lagging strand) connect them by enzymes.
It has been discovered that there are types of DNA pol that participate in DNA replication in E.Coli. (we will discuss DNA pol IV and DNA pol V later)

table 25–1

Comparison of DNA Polymerases of *E. coli*

	DNA polymerase		
	I	**II**	**III**
Structural gene*	*pol*A	*pol*B	*pol*C (*dna*E)
Subunits (number of different types)	1	≥4	≥10
M_r	103,000	88,000†	830,000
3′→5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/sec)	16–20	40	250–1,000
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000

*For enzymes with more than one subunit, the gene listed here encodes the subunit with polymerization activity. Note that *dna*E is an earlier designation of the gene now referred to as *pol*C.

†Polymerization subunit only. DNA polymerase II shares several subunits with DNA polymerase III, including the β, γ, δ, δ', χ, and ψ subunits (see Table 25–2).

DNA polymerase 1 = removing the primers and filling the gaps.

The main enzyme that is responsible of the elongation is DNA pol III because it has the highest processivity and the highest polymerization rate.

The enzymes that are responsible of DNA replication in Eukaryotes are:

DNA polymerase alpha resembles the primase use as synthesis of short primer

DNA polymerase delta resembles the polymerase 3 use as extension of primer

DNA polymerase epsilon resembles the polymerase 1

Function1 of primase = add a primer provide hydroxyl group at the free prime in (free prime hydroxyl group)

The primer is single strand RNA (20_60) nucleated added by the enzyme (primase). Primase add primer at the beginning of the strand then DNA polymerase comes and continue

The enzyme responsible for sealing the nick is DNA ligase, DNA ligase use NAD in prokaryote cell and ATP in eukaryotic cell as a cofactor.

Primosomes constituents is only one helicase or primase

*Topoisomerase is an enzyme in E-coli its name DNA gyrase or topoisomerase 2 its function is involved in super coiling.

The most important steps in sealing of the nick:

1-addenlation of DNA ligases

2-activation

3-displacive of AMP

Test ter complex do not happen termination to it

DNA degradation:

Nucleases degrade nucleic acids.

DNasess specific for DNA

RNases specific for RNA.

Exonuclease: degrade nucleic acids from one end of the molecule either from 3’ or the 5’ of dsDNA or ssDNA.

Endonuclease: degrade nucleic acids at internal sites.

Restriction endonucleases: cleaves only at specific internal sites important in Biotechnology

the mechanism

the polymerization reaction occurs by adding a nucleotide to a previous nucleotide and so on... *the reaction start on a template strand, and the primer provide the 3' hydroxyl which is the function group that added on it the new or (incoming deoxynucleuside 5'triphosphate). At alpha phosphate occur a nucleophilic attack from the OH, and make a phosphodiester bond, and the other 2 phosphate comes out of the reaction as inorganic pyrophosphate.
*The incoming deoxy nucleoside 5'-triphosphate selected by base pairing. (A with T) (C with G)

DNA polymerase enzyme has an inorganic cofactor mg which is positive charge, and the aspartate which is coming from negative amino acid, (the two charge +and - play an important position on stabilizing the active site and help the polymerase. Note: DNA polymerase just activate the process, and comes out as it entered the reaction.

The processivity of the enzyme:

How many nucleotides are added by enzyme before dissociating? There is some variation on the enzyme, some of it add a small number, other add a large number of nucleotides before dissociation.

There are 4 ways to prevent the operation from any mistakes:
and those ways gives the operation its fidelity, but unfortunately sometimes, the 4 ways fail and continue to be a mutation.

1)the hydrogen bonds

2) the geometry of the strand: the form of active enzyme plays an important role. اذا تم ربط النيوكليوتيد بشكل خاطئ، الجلسة للنيوكليوتيد ما بتكون زابطة،فيتم إزالتها قبل تكوين رابطة. phosphodiester

3)intrinsic property of polymerase: proofreading system by the help of exonuclease enzyme by checking the nucleotide on the spot** which means that after the addition of nucleotide directly, it removes the mismatched connection. هي عملية يتم فيها الإصلاح بشكل مباشر عند ارتباط نيوكليوتيد بمكان خاطئ عن طريق التوقف عن الاستمرارية للحظة (kinetic pause) ثم العودة خطوة للوراء يعني يصبح يبني من(٣' إلى ٥') ثم يعود إلى وضعه الطبيعي ويبني من ٥'إلى ٣'

4)Repair system: scanner enzyme after building the DNA. وهي الطريقة الأخيرة يكون هناك انزيمات فاحصة تبحث عن حدوث DNA للحماية من حدوث خلل ،وهي بعد بناء ال أي خلل معين في الخلية وتعمل على إزالة المقطع كامل الموجود به الخلل وليس إزالة فقط النيوكليوتيد الذي سبب الخلل

Replicated by unknown mechanism

Double strands is replicated, so it gives catenated chromosome (enter langed circles) which connected to taste ter then become an enzyme named topoisomerase and breaks down the catenated chromosomes and works for its rotation.

After each division the length of the telomeres which is the end of the chromosome is reduced because our cells have enzymes that compensate for the deficiency except for certain cells such as germ cell own enzyme named telomerase (adds and lenthens the telomires)

متى الخلية بتشيخ؟

1) إذا كان عندي damage in DNA

2) اذا كان عندي جينات السرطان

3) اذا كان عندي قصر في ال telomere

Briefly the telomeres are:

1-repetitive sequences

2-untranslated regions

3-determine the number of chromosome that is allowed

4-capping

Areas on the edges of chromosomes 1>(T)x that mean T repeater X number of times

2>(G)y means G repeater Y number of times

Mostly y>x

Teloming always content over hand (single strand possible break any moment) (mean prominent washi)

The party who stayed over hand is looped by protein because it will break and fuse with another chromosome then loop consists then T loop connect to double stranded then capping occurs, so the function of telomine is protection and suability. Telomine will know if there is a break in DNA, because if it did not find, telomine will know it have a damage so it starts fixing or leading the cell to death

Notes:

1-the edges are not genes

2-the age of the cell is determined by telomine

3-the cell has a fixed number of divisions

The greater the division the less able to reproduce except in the cells contain telomerase as cancer cells

The number of times that human cell can divide until it stops called hayflicle limit

The telomenase in embryonic stem cell and in germ that work

How telomerase is tall a telomine? Reversed transcriptase (consists of RNA and protein) that mean it work syntheses of DNA from RNA template like viruses

The region consists of newly synthesized exist of incomplete newly synthesize lagging strand

بيجي ال telomerase المكون من (rna) بيرتبط في ٣ وبصير يعمل copping في template
بعد ما يصير extended بروح يعمل ال primer وبعدين بيرتبط polymerase وبيعمل syntheses

وبعدين بنشيل ال primers وبيصير عندي over hand فبيرتبط فيها البروتين وبعمل loop فبيعمل ويسكر الكروموسوم tolimine

نهايات الكروموسوم ما بصير الها replication لهيك سببها end chromosome replication
لأنو بنشال الprimer وفش اشي يعوض هاد النقص problem

ال telomerase بطول ال telomine

Primase:بحطprimer

layggazaبعدها بيربط ال