



# ***Genetics***

***Subject* : Genetics**

***Lec no* : 11**

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وَقُلْ رَبِّ زِدْنِي عِلْمًا

# ● Synthesis & Processing of ribosomal RNA (rRNA)

- The primary transcripts of the mammalian rRNA include a 45S rRNA (pre-rRNA) & a 5S rRNA.
- The 45S rRNA is synthesized by RNA **polymerase I** then undergoes RNA processing in the nucleus which cleaves the precursor to release the mature 18S, 5.8S, 28S rRNA

– (18S, 5.8S, 28S) – یا تو ا من بین واحد فقط ہونے  
لہ transcription وینتے

→ Pre rRNA (45S)

ای بیجھل اہ RNA processing  
فیصل لہ cleaning ای (18S, 5.8S, 28S)

rRNA تہن بی تی کتب ribosom کا ذکرنا ہابقاً۔

45S rRNA ← ہدک لہ transcription ہن طریق RNAP I

5S rRNA ← ہدک لہ transcription ہن طریق RNAP III

- The 45S genes for 18S, 5.8S and 28S rRNA are typically clustered together and tandemly repeated (one copy each of 18S, 5.8S and 28S occur, followed by untranscribed spacer DNA, then another set occur and so on).

متجمعة معا متكررة  
جنباً إلى جنب  
يفصل بينهم  
Nontranscribed spacer

- 5S RNA gene is transcribed by RNA polymerase III

Hundreds of copies of these genes are present in every cell. This large number of genes is required to synthesize sufficient copies of each type of rRNA to form the  $10^7$  ribosomes required for each cell replication.

مواد الجينات  
التي تعطى 5S أو 45S  
لأنه بدنا نتيج  
الكثير من ribosomes

5S rRNA ← ينتج من Nucleoplasm outside the nucleolus فيه RNAP III ثم ← ينتقل إلى nucleolus من أجل  
انه يتجمع مع 5, 8S, 28S

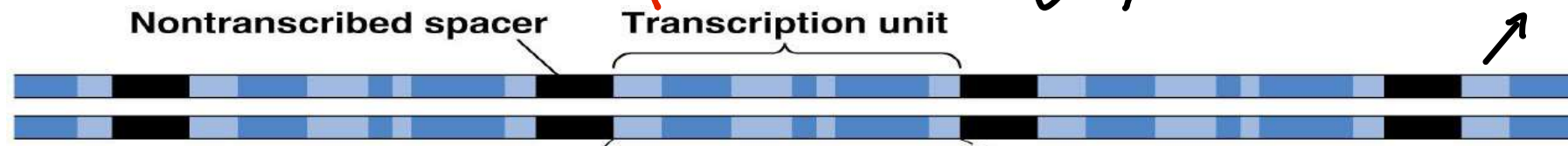
وينتج large ribosomal subunit  
وتتجمع مع small subunit  
وينتج ribosome  
وينتج إلى cytoplasm

← ينتج من nucleolus في 18S, 5.8S, 28S

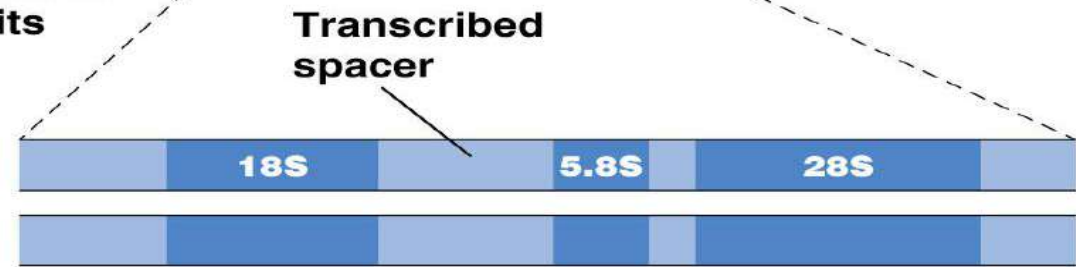
Unlike pre-rRNA genes, 5S-rRNA genes are transcribed by RNA polymerase III in the nucleoplasm outside of the nucleolus. **Without further processing**, 5S RNA diffuses to the nucleolus, where it assembles with the 28S and 5.8S rRNAs and proteins into large ribosomal subunits. When assembly of ribosomal subunits in the nucleolus is complete, they are transported through nuclear pore complexes to the cytoplasm, where they appear first as free subunits.

Nontranscribed spacer  
 المتباعد غير المنقول  
 بتعطينا 45S  
 بيننا وبين الأخرى  
 spacer

clustered together and  
 tandemly repeated  
 الجينات الي بتعطينا 45S rRNA  
 بتعطينا



(a) Tandem array of DNA transcription units



(b) One DNA transcription unit

5S  
 gene

transcription

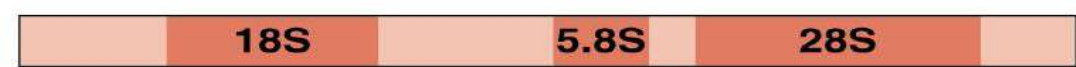
مباشرة بدون  
 انتاج pre rRNA

Transcription by  
 RNA polymerase I

genes

transcription

(c) Pre-rRNA (45S)



45S rRNA (pre rRNA)

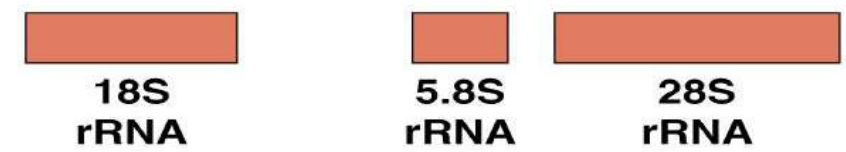
RNA processing  
 (cleavage)

Transcribed spacers  
 degraded

cleavage

mature rRNA  
 molecule (مباشرة)

(d) Mature rRNA molecules



18S, 5.8S, 28S



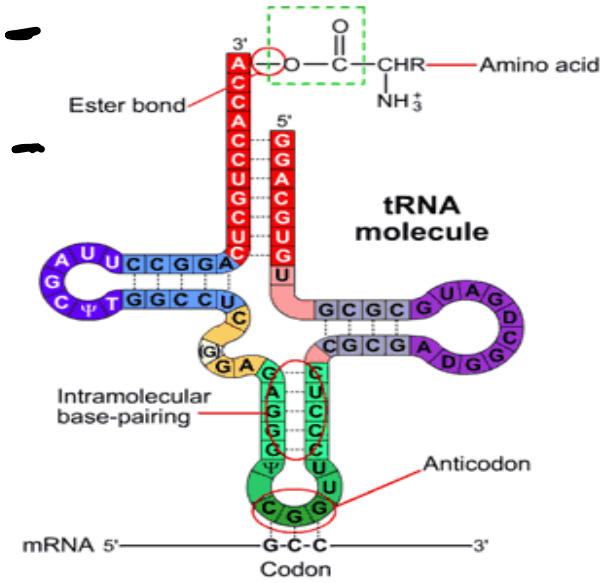
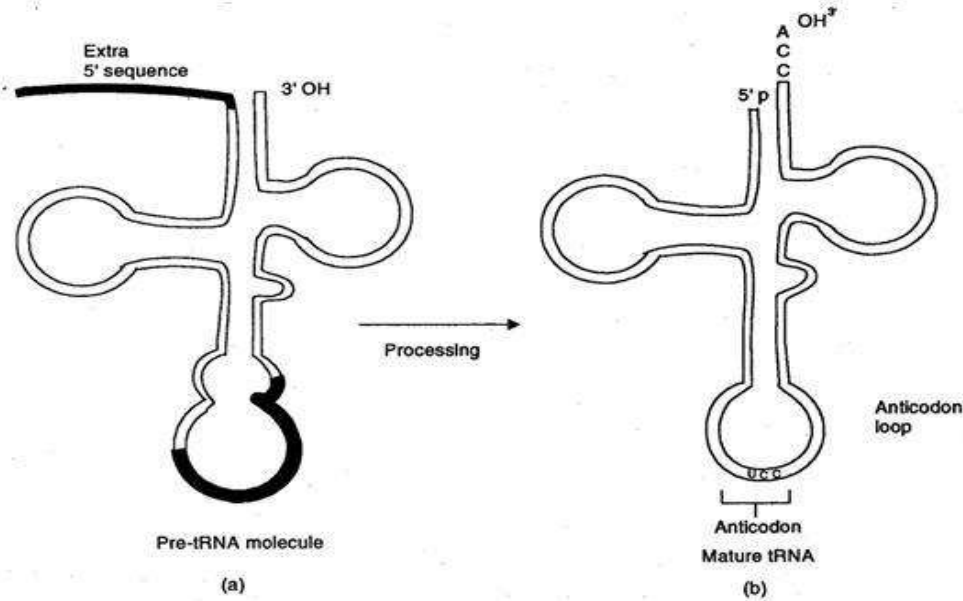
# 🌟 Synthesis & Processing of tRNA

- Eukaryotic tRNA genes are all transcribed by RNA polymerase III.
- The primary transcript (pre-tRNA molecules) requires up to 4 different types of RNA processing steps as follows:
  - 1- Addition of the CCA sequence at the 3' end by the nucleotidyl transferase.  
*إضافة CCA عند 3' عن طريق nucleotidyl transferase*
  - 2- Excision of the nucleotide extension at the 5' end. → *excision عن 5' فيه شئ.*
  - 3- Excision of introns present in the anticodon loop.
  - 4- Modification of some bases by methylation of uracil into thymine or reduction of uracil into dihydrouracil and formation of pseudouracil

RNA polymerase III سے بنتے ہیں ← tRNA

modification  
mature

pre tRNA سے بنتے ہیں → mature  
یعنی پختہ



modification

pre tRNA

- There are several unusual bases in the structure of tRNA  
 C pseudouridine nucleotide → pseudouracil  
 D dihydrouridine  
 S hypoxanthine  
 & methylguanine  
 post-transcriptional modification

# Regulation of eukaryotic gene expression

- The levels of eukaryotic gene regulation include the following:

**1- Alteration of gene content.**

عدد *copies* لانه كثر او قليلو *gene* →  
لو فيه تغيرات في *gene content*  
ايضا دح ناتي على *gene expression*

**2- Transcriptional regulation.**

**3- Post-transcriptional regulation.**



# 1-Alteration of gene content

## (regulation through modification to DNA)

\* بعض الجينات ممكن  
تكرر (gene amplification)  
↓  
• ليس لانه transcription rate  
عالي بل لانه يوجد  
copies كثير لهذه الجينات

The eukaryotic genome may be changed by the following mechanisms:

### ■ Gene amplification:

- It is the increase of a gene product by increasing the number of genes coding for that product e.g. histone & rRNA genes.
- More than 20 genes are known to be amplifiable e.g. dihydrofolate reductase genes.

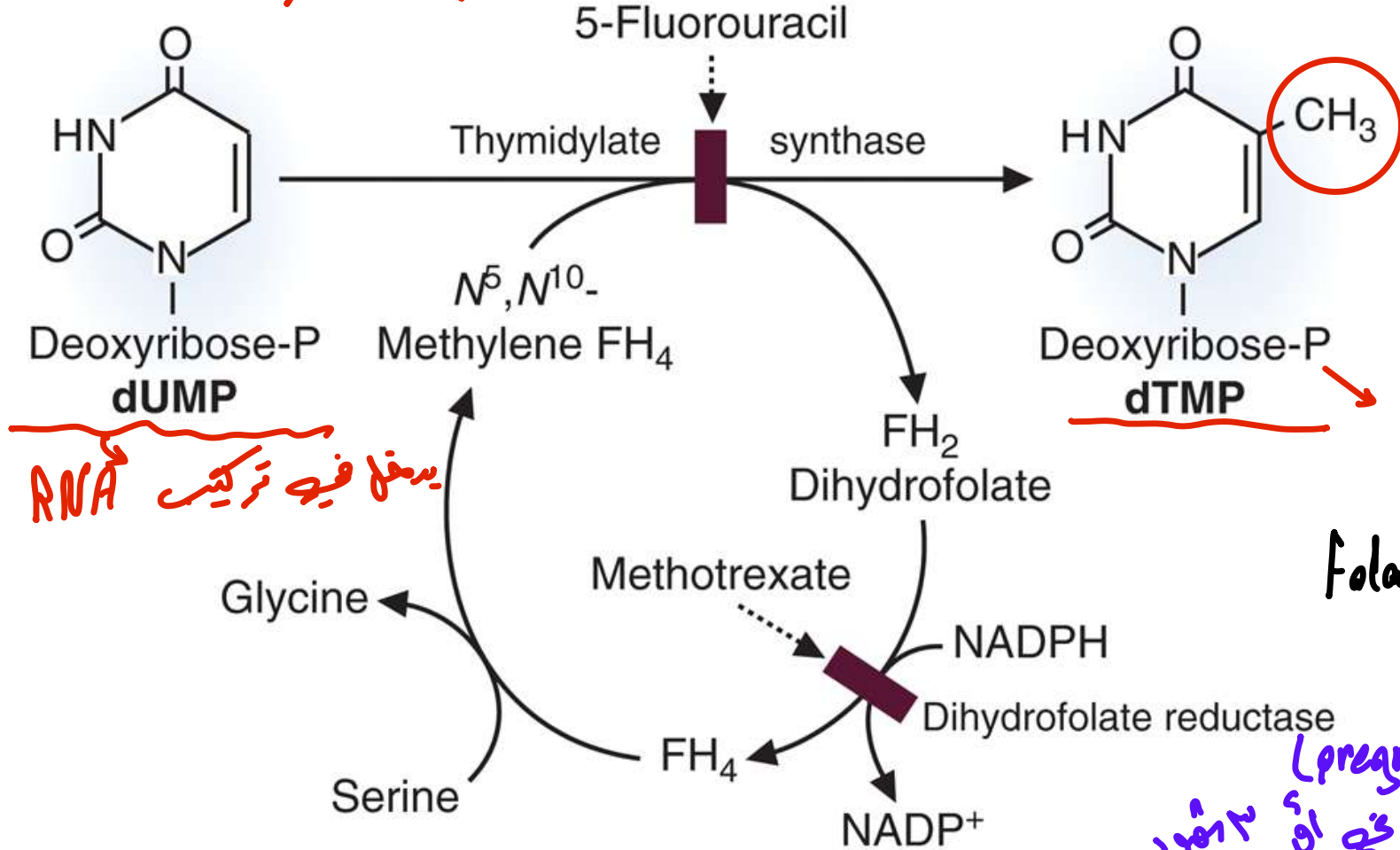
غالبه للتكيف  
- بعض الجينات ممكن تكون  
amplifiable يعني ممكن يكون  
الجين 2 copies في الجسم ولكن  
لسبب ما ينتج اكثر من 2 copies

↓  
مثل الجين المسؤول عن  
انتاج dihydrofolate  
reductase enzyme

- Dihydrofolate (FH<sub>2</sub> is a derivative of folic acid) is reduced to tetrahydrofolate (FH<sub>4</sub>) by dihydrofolate reductase.
- Methylene-FH<sub>4</sub> is required for conversion of dUMP to dTMP which is utilized for DNA synthesis.
- It has been demonstrated in patients receiving methotrexate ( an inhibitor of FH<sub>2</sub>-reductase) as a treatment for cancer that malignant cells can develop drug resistance by increasing the number of genes for dihydrofolate reductase.
- *For cancer, methotrexate competitively inhibits dihydrofolate reductase (DHFR) (methotrexate is structurally similar to folate). The affinity of methotrexate for DHFR is about 1000-fold that of folate.*

- لتكوين DNA نحتاج 4 types of nucleotides وThymine الذي يتم انتاجه من Uracil

الفارق بين Thymine وUracil هو وجود methyl group على Thymine من Methylene FH<sub>4</sub>

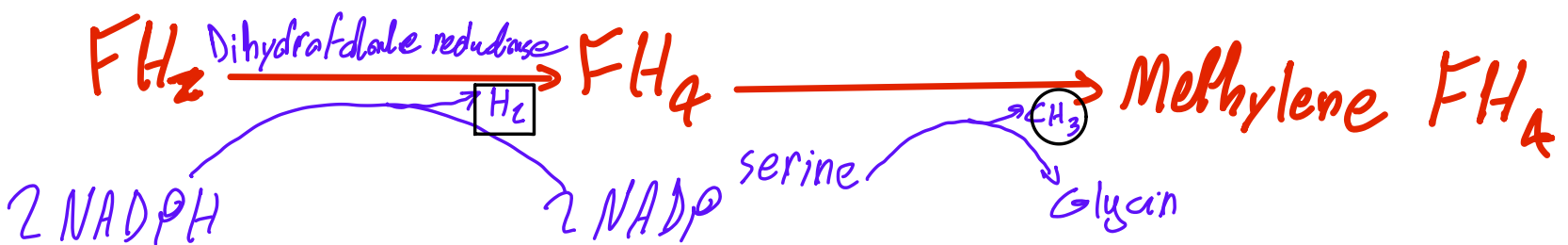


يدخل فيه تركيب DNA

يدخل فيه تركيب RNA

Folate → folic acid (vitamin B<sub>9</sub>)

- النساء الحوامل (pregnant women) ينصحن بأخذ folic acid قبل أو أثناء الحمل من أجل حتى يساعدوا الجنين في بناء DNA.



methotrexate ← دواء يوقف عن حالة cancer  
يعمل inhibition د Dihydrofolate  
reductase

← تركه يثبت Dihydrofolate وعند affinity أي بأن  
هذه من Dihydrofolate reductase

بالتالي فإن هذا يؤثر على إنتاج Thymine الذي يدخل  
في تركيب DNA، لأن هذا جيب فانا بهذا الشكل  
يمنع تكاثر cancer cells

ولكن جعل resistance لهذا الدواء بسبب أنه  
اجبت الذي ينتج Dihydrofolate reductase جعل له

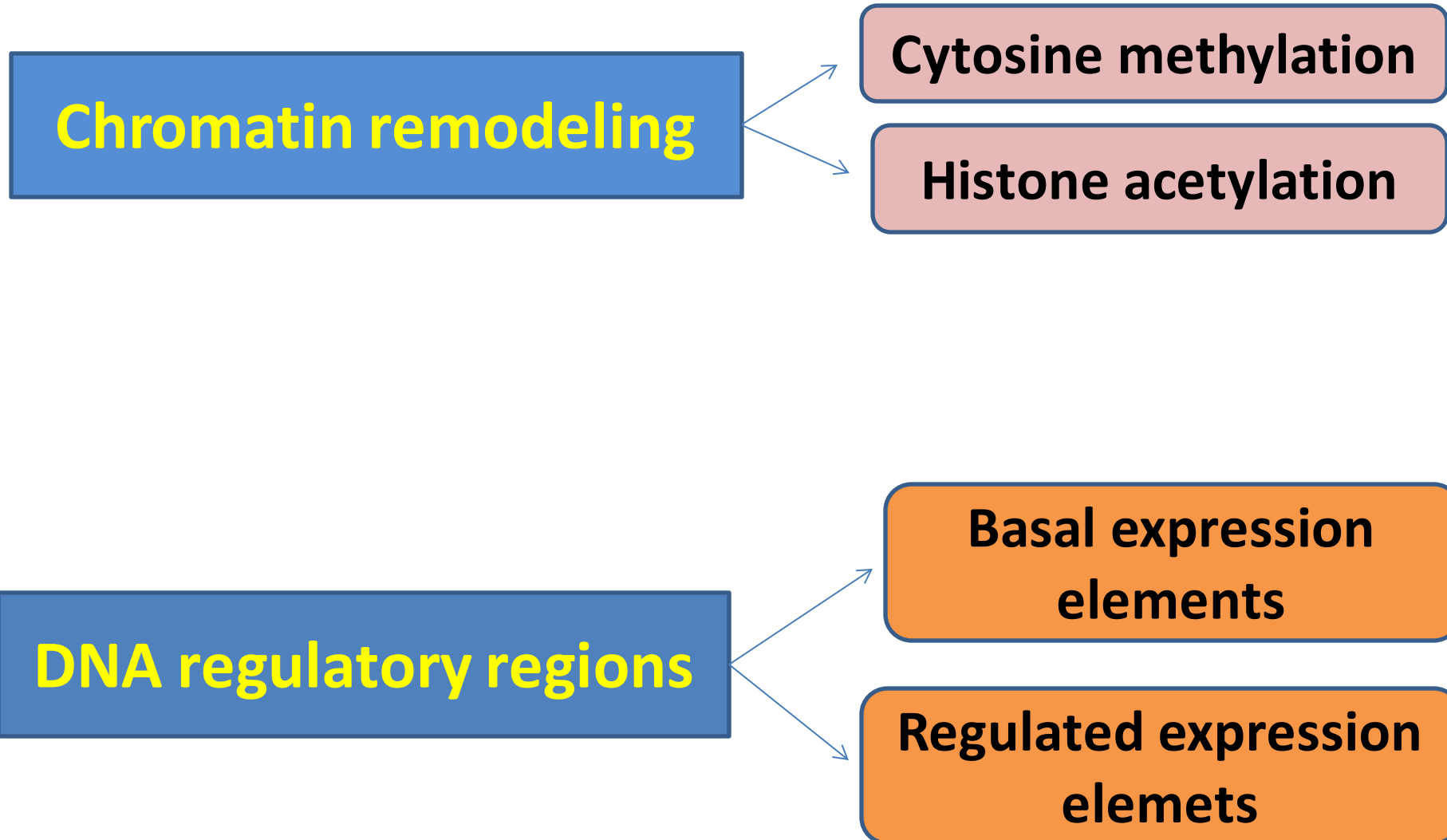
amplification وبالتالي زاد إنتاج Dihydrofolate reductase  
ويعتبر إنتاج DNA وتكاثر cancer cells

ABCs ما فينا nucleus (ما فينا جينات) و لكن reticulocytes cells الي بتتج ABCs تحتوي على nucleus -

ولكن اثناء مرحلة development عدت Gene diminution  
(جيت كنت محتاجه في مرحلة development وبمديت بطلت احتاجه)  
فعدت diminution (9) ■ Gene diminution:

- It is a rare form of regulation by removing a gene or genes from the genome e.g. complete loss of all genes in red blood cells during development.
- *A gene whose expression is only needed at a particular developmental point or in a particular tissue may be shut off by gene diminution. As reticulocytes mature into red blood cells all of their genes are lost as the nucleus is degraded.*

## 2- Transcriptional regulation.



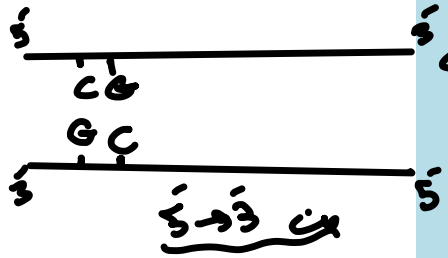


# Cytosine methylation

- أغلب الجينات تحتوي على CG-rich region في coding region (مناطق تبعية) upstream of coding region  
Cytosine في هذه المناطق يمكن يسهل له methylation عن طريق DNA methylase

- Many mammalian genes have CG- rich regions upstream of the coding region, that provide multiple sites for methylation.

- The methyl group is added by DNA methylase on both strands of DNA in 5'-CG-3' dinucleotides.



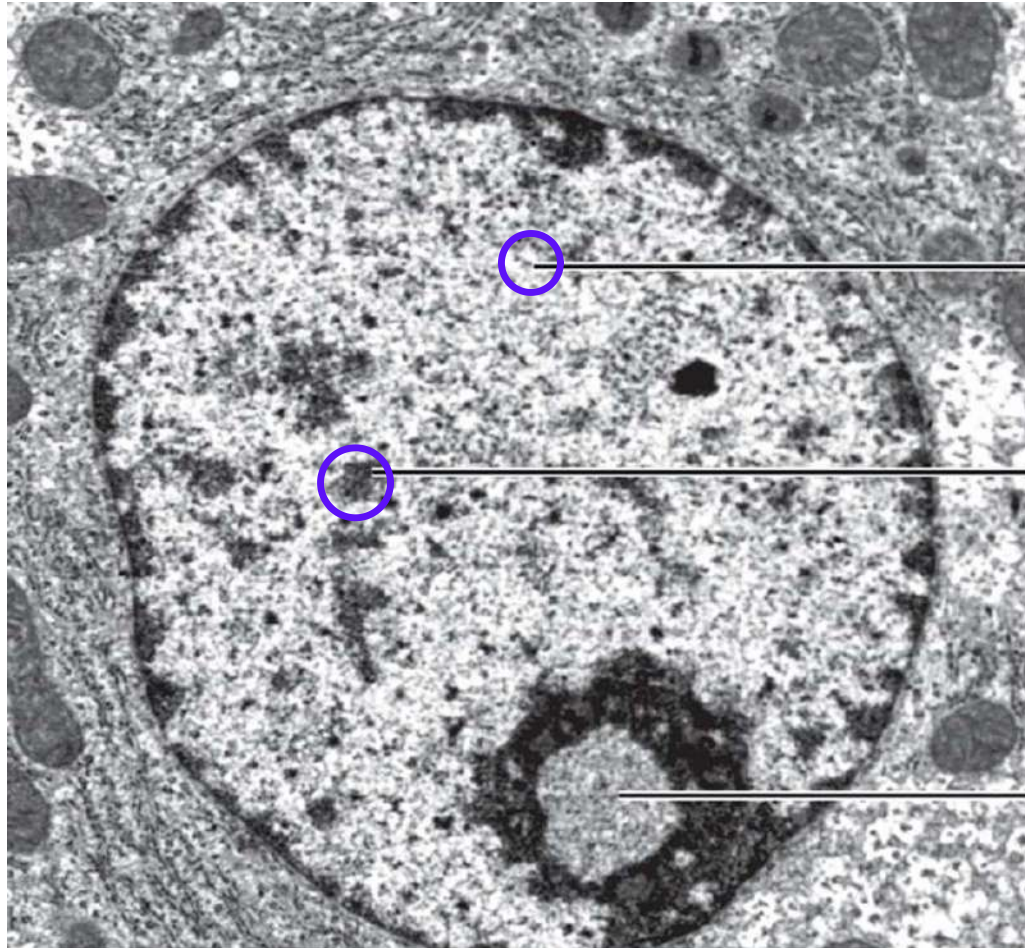
DNA sequence of gene لم يحدث أي تأثير على

→ reversible process

\*methylation associated with gene silencing  
(C residues مرتبطة مع واثق  
فإن transcription لهذا الجين قليل جداً أو مشى موجود

- Heavy methylation is associated with genes for which the rate of transcription is low.
- Transcriptionally inactive chromatin is densely packed (a highly coiled and compact structure) during interphase as observed by electron microscopic studies and is referred to as heterochromatin; transcriptionally active chromatin stains less densely and is referred to as euchromatin.
- methylation converts the active euchromatin into inactive heterochromatin and it may result in transcriptional silencing. Reactivation occurs by demethylation.

→ transcriptionally inactive الجينات التي تكون heavily methylated (يعني واثق على C بنزلة)



euchromatin

↳ nonmethylated  
↳ transcriptionally active.

heterochromatin

↳ intensely pigmented

↳ methylated  
↳ transcriptionally inactive.

nucleolus

- Heavy methylation is one of epigenetic mechanisms that marks a gene for silencing.

التأثير على transcription من دون  
التأثير على genetic material  
(dna sequence)

- The Greek prefix *epi-* (ἐπι- "over, outside of, around") in *epigenetics* implies features that are "on top of" or "in addition to" the traditional genetic basis for inheritance.

sequence of  
DNA  
سلسلة  
الجينوم

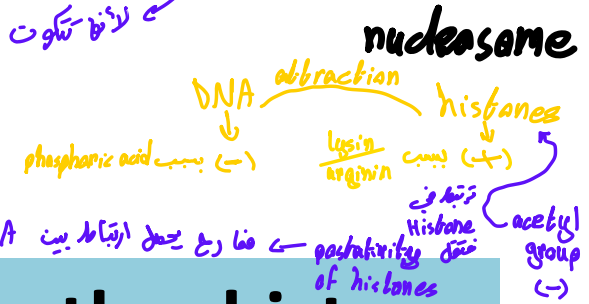
*Therefore epigenetic refers to heritable changes in gene expression that are not due to changes in the DNA sequence itself.*

reversible process

# Histone acetylation

positive charged proteins DNA و بيجلوا  
positive charged a.a لانا تكون من

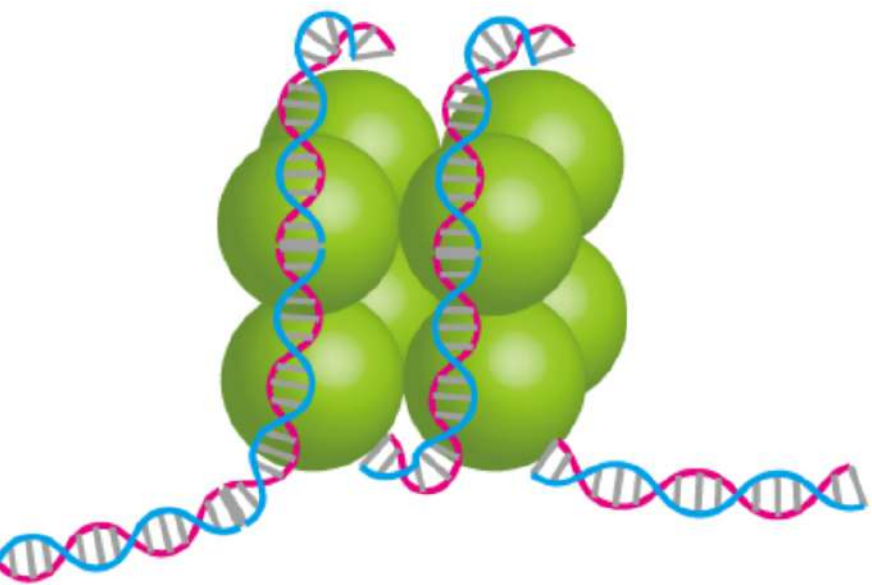
lysine/arginin



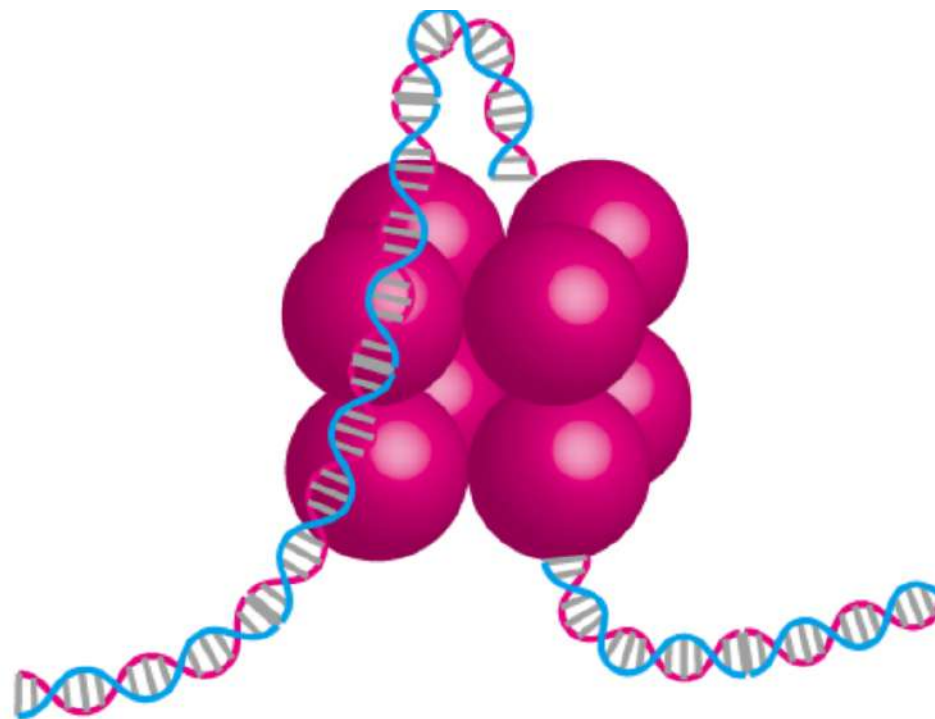
gene expressions بالزيادة Histones و DNA ما ربح يحصل ارتباط بين  
gene expression مع cytosin methylation

- Acetylation at the N-terminal (lysine) reduces the histone positive charges & decreases the binding affinity of histones for the negative charged DNA, which allows the access of the different transcription factors to act.
- Deacetylation reverses the process.

acetyl group (-)



Acetylation





# DNA regulatory regions

transcription to RNA لا يفسر لها

- Each gene can be divided into coding & regulatory regions, as defined by the transcription start site.
- In case of class II gene (transcribed by polymerase II), the coding region contains the DNA sequence that is transcribed into mRNA, which is translated into protein.

**The regulatory region consists of two classes of elements as follows:**

**A- Basal expression  
elements**

**B- Regulated expression  
elements  
(cis-acting elements)**

- Basal expression elements: it contains

قريبة من (+1) start point  
TATA: على

Basic expression element

proximal element or TATA box that directs the RNA polymerase II to the correct start site (+1)

The upstream element  
e.g. CAAT box or GC box that specify the frequency of initiation

Cis-regulatory elements (CREs) or Cis-regulatory modules (CRMs) are regions of non-coding DNA which regulate the transcription of neighboring genes. *sequences that are present on the same gene*  
 in promoter  
 من سبب من الجين  
 (من نفس منطقة الجين)  
**Regulated expression elements (cis-acting elements):** they are specific DNA sequences that are present on the same gene, so termed cis-elements, and are responsible for regulation of expression & include the following elements:

elements لا يعمل فيها بعض البروتينات تعمل enhancing of gene expression  
 elements لا يعمل فيها بعض البروتينات تعمل enhancing of gene expression

### Enhancers

they interact with gene regulatory proteins or **trans-factors** (so termed because they are produced by other genes) and **increase the rate of expression** (they facilitate initiation of transcription)

يتم انتاجها من جينات بغيره عن هذا الجين الى تسريع من زيادة expression  
 يتم انتاجها من جينات بغيره عن هذا الجين الى تسريع من زيادة expression

### Silencers

elements لا يرتبط فيها بروتين مما يمنع عمل للجين القريب منها inhibition of expression  
 elements لا يرتبط فيها بروتين مما يمنع عمل للجين القريب منها inhibition of expression  
 they interact with gene regulatory proteins or **trans-factors** and decrease the rate of expression (they inhibit initiation of transcription)

### Other regulatory elements

elements  
 elements لا يرتبط فيها بعض البروتينات بتزيد expression و بروتينات اخرى لها ترتبط. تنقل expression  
 They mediate response to various signals including chemicals, metals and hormones. In the latter case, it is termed the **hormone response elements (HRE)**

هرمونات ترتبط فيها receptors elements  
 hormones elements

## 3-Post-transcriptional regulation

❖ Alternative splicing: for example, in the thyroid gland, the calcitonin gene produces a transcript that codes for the hormone calcitonin , the same gene is expressed in neurons and produces a transcript that codes for calcitonin-related peptide which is involved in taste.

## ❖ Regulation of RNA stability:

- RNAs have different half-life time e.g. the longer the poly A tail, the longer the half-life time of mRNA.
- Certain proteins interact with mRNA, forming ribonucleoproteins. Some of these proteins protect mRNA from digestion by Rnase enzyme, enhancing translation.



### ❖ mRNA editing:

- The only example known in humans involves the editing of apolipoprotein B mRNA.
- Apo B-48 is synthesized by the intestine, and Apo B-100 is synthesized by the liver.
- The apolipoprotein B mRNA synthesized by the intestine is primarily the same as that synthesized by the liver. However, intestinal cells convert a site-specific cytosine of mRNA to uracil. This results in the formation of a stop codon near the middle of the mRNA that terminates the synthesis of the growing polypeptide at 48% that of apo B-100.
- The differences in the translated proteins is not due to alternative splicing but is due to the tissue specific RNA editing event.