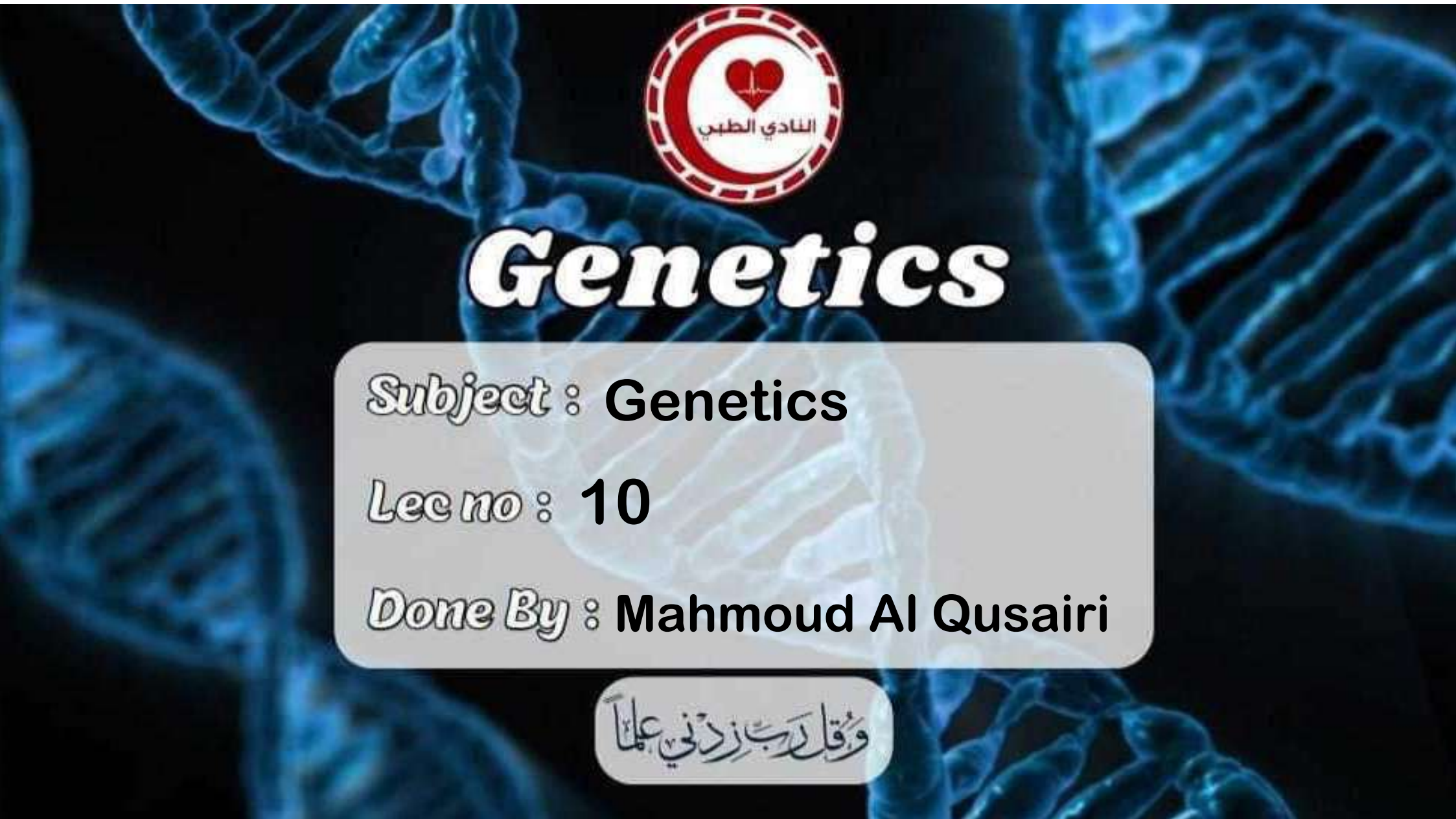

النادي الطبي
Genetics
Subject : Genetics
Lec no : 10
Done By : Mahmoud Al Qusairi
وقل رب زدني علماً

Termination in eukaryotes:

- Leads to the dissociation of the complete transcript and the release of RNA polymerase from the template DNA. The process differs for each of the three RNA polymerases.
- As Pol II reaches the end of a gene, two protein complexes carried by the CTD (carboxy terminal domain), CPSF (cleavage and polyadenylation specificity factor) and CSTF (cleavage stimulation factor), recognize the poly-A signal (polyadenylation signal sequence AAUAAA) in the transcribed RNA.
- The sequences that, once transcribed into RNA, trigger transfer of these factors to the RNA are called poly-A signals

- RNAP II دع يعرف recognise of specific sequence (Termination sequence) ← poly-A signal

- يكون فيه بروتينات ما بكين بـ (carboxy terminal domain)

CPSF ← هو اللي دع يساعد في عملية فصل RNA بعد تكوينه وإضافة poly-A tail

CSTF ← هو أيضاً دع يساعد في عملية فصل RNA عن DNA

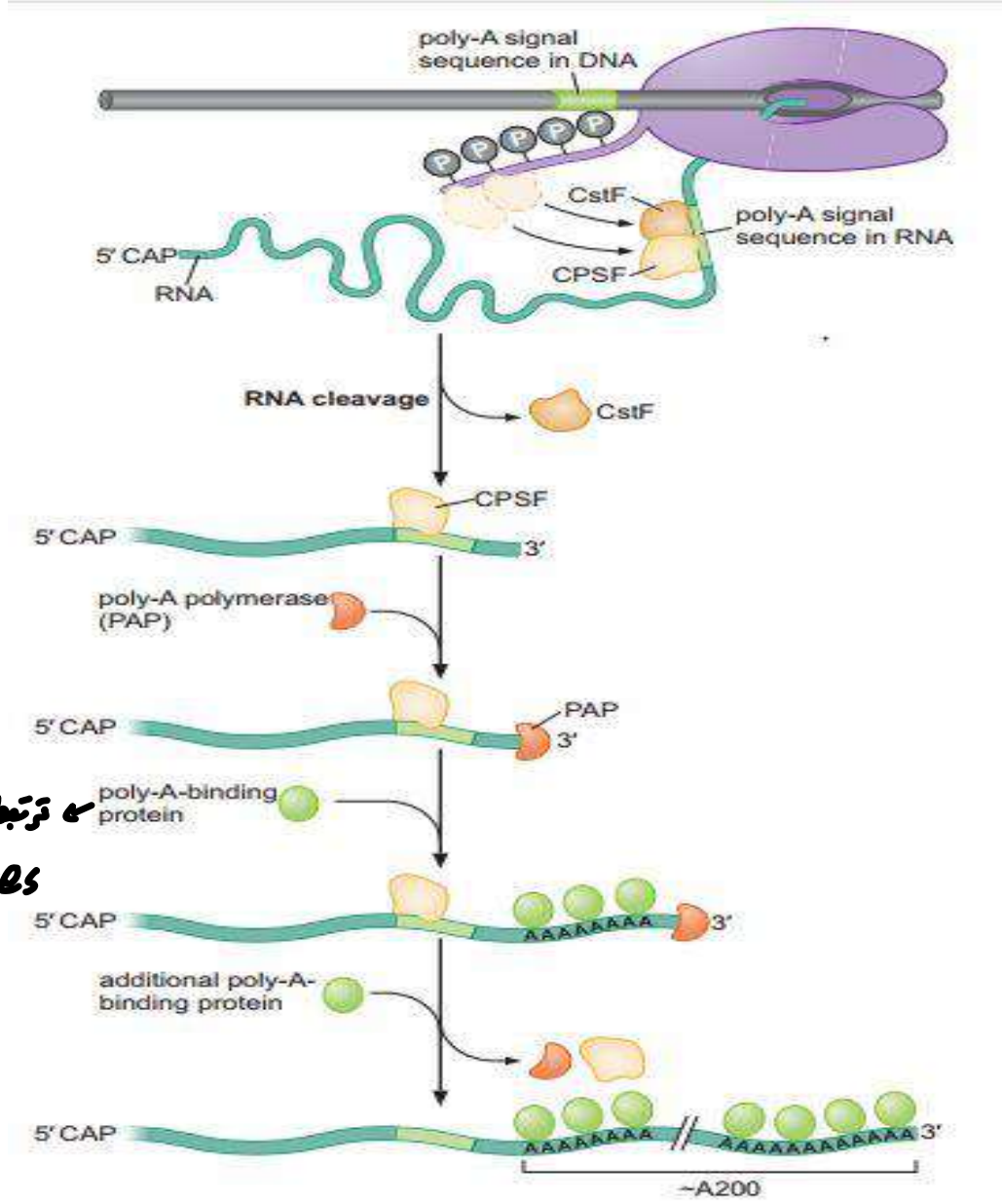
- عندما يوصل RNAP II إلى (Termination signal) ← CSTF, CPSF دع يساعدوا في عملية الفصل وإضافة poly-A tail

- عندما يتعرف RNAP II على (Termination signal AAUAAA) ← CSTF, CPSF دع يتقلوا على RNA

- Poly-A-bound CPSF and CSTF recruit other proteins to carry out RNA cleavage and then polyadenylation. **Poly-A polymerase** adds approximately 200 adenines to the cleaved 3’ end of the RNA without a template. The long poly-A tail is unique to transcripts made by Pol II.
- The RNA molecule made by RNA pol II is called a **primary transcript**, which needs extensive RNA processing in order to produce a mature mRNA for translation & protein synthesis.

- عند ارتباط CPSF, CSTF بـ (poly-A signal in RNA) ← دي بيفرزوا بروتينات اخرى تساعد في عملية RNA cleavage واضافة Tail
- RNA cleavage تكون بعد 15-20 نيوكليوتايد بعد poly-A signal
- بعد ما نتجاوز poly-A signal بـ 15-20 نيوكليوتايد دي تنتقل البروتينات من RNAP tail إلى poly-A signal في RNA هذا دي بيفرز بروتيناتَ بأنها تعمل cleavage وبروتينات poly-A polymerase يعمل tail عند 3' of RNA (من 200 A nucleotides)

لـ RNA النابج هو primary transcript مجتاح processing (post-transcriptional modification)



RNA يخرج من
RNA exit channel
الموجودة في RNAPII

ترتبط poly-A tail من قبل ال تحميها من
Action of 3′ exonucleases

- Processing of mRNA
- Synthesis & Processing of ribosomal RNA (rRNA)
- Synthesis & Processing of tRNA

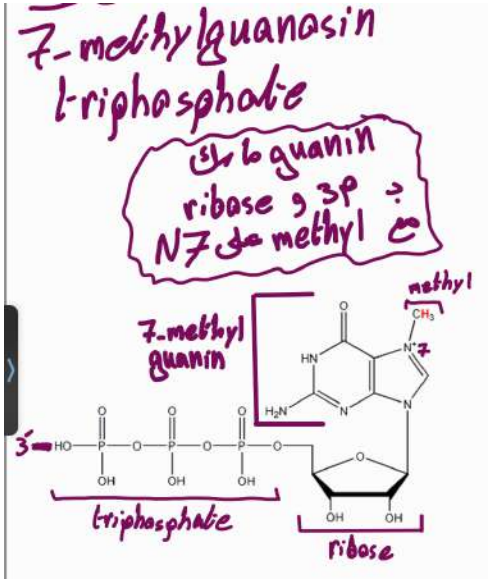
By
Dr. Walaa El Gazzar

Processing of mRNA
(Post transcription modifications)

A. 5\-Capping :

- The RNA is capped as soon as it emerges from the RNA-exit channel of polymerase. This happens when the transcription cycle has progressed only as far as the transition from the initiation to elongation phases.
- The cap is a **7- methylguanosine triphosphate** attached to the 5\– terminal end of the mRNA **(which terminates at a triphosphate group).**

- One of the terminal phosphate groups is removed by **RNA triphosphatase**, leaving a bisphosphate group



GTP

- عملية إضافة cap-5 تكون بعد ظهور RNA exit channel من 5' end of RNA
  7-methylguanosine triphosphate = cap
- أول nucleotide عند 5' بتكون triphosphate لأنها متل ما بتكون قبل nucleotide بـ
  وعند إضافة cap إلى هذا nucleotide راح يتم إزالة اثنين من 3P عن طريق RNA triphosphatase
- منه إضافة GTP إلى 5' end عن طريق guanylyltransferase بتفقد pyrophosphate
  وتصير GMP .

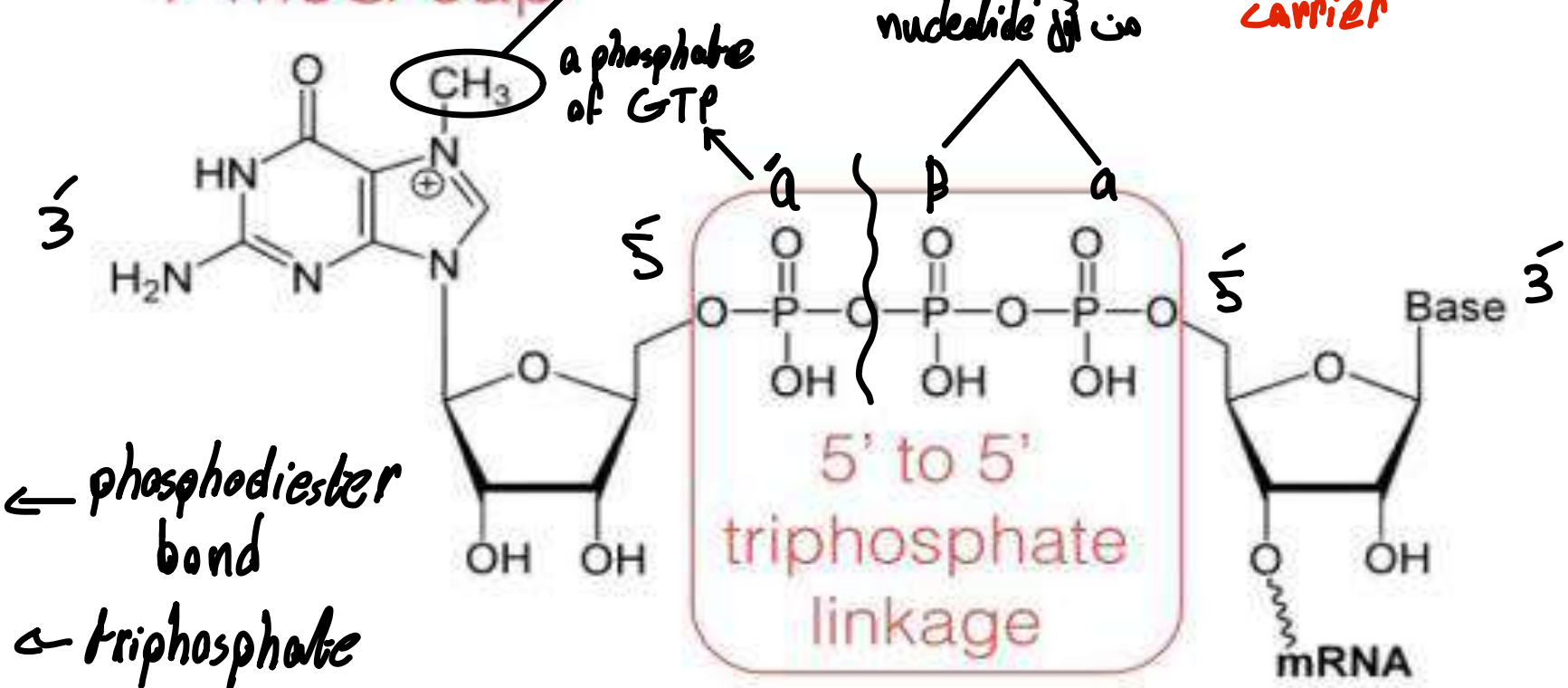
- GTP is added to the terminal bisphosphate by mRNA **guanylyltransferase**, losing a pyrophosphate from the GTP substrate in the process. This results in the **unusual 5\to 5\ triphosphate linkage.**

guanine-7-methyltransferase تسمى Methylation تتم وإضافتها عن طريق عملية

عند CH_3 ل transfere يعمل ذي enzyme
guanine من 7 رقم N

primary methylation

S adenosylmethionine من CH_3 تأتي methyl group
(SAM)
(active methionin)
methyl group عبارة عن
carrier



5' to 3' ← phosphodiester bond

5' to 5' ← triphosphate linkage

5' end of mRNA

خروج عن مجاورة nucleus في تكون ← 5- capping

RNA exit channel ← 5'-end

cytoplasm يكون في secondary methylation/ nucleus تكون في primary methylation

- **Methylation** of this terminal guanine is catalyzed by **guanine-7-methyltransferase**.
- S adenosylmethionine, SAM, (active methionine) is the source of methyl group. **Methylation of N-7 of guanine of the GTP cap occurs in the nucleus.**
- In the cytoplasm, methylation may occur at 2\ OH of ribose of some nucleotides, and at N-6 of adenine of some nucleotides **(secondary methylations)**

RNA-transcript خروج عند
إلى cytoplasm بعض
RNA transcript في nucleotides
يتم إضافة methyl group عند
ribose في C2 إلى OH
وأيضاً عند N-6 في
Adenin في
nucleotides بعض.

تضيف cap إلى أول nucleotide عند 5' من أجل حماية exonuclease من 5'-end

بعد خروج RNA إلى cytoplasm يتم توجيهه إلى ribosome

❖ Importance of capping:

- It protects the 5\ end of the mRNA from **5\ exonuclease** enzyme.
- It helps its recognition by the ribosome.
- It helps the initiation of protein synthesis.
- Eukaryotic mRNA lacking the cap are not efficiently translated.

↳ بدون cap يصبح RNA لا يحدث له translation أو يحدث ولكن ليس بالكفاءة المطلوبة

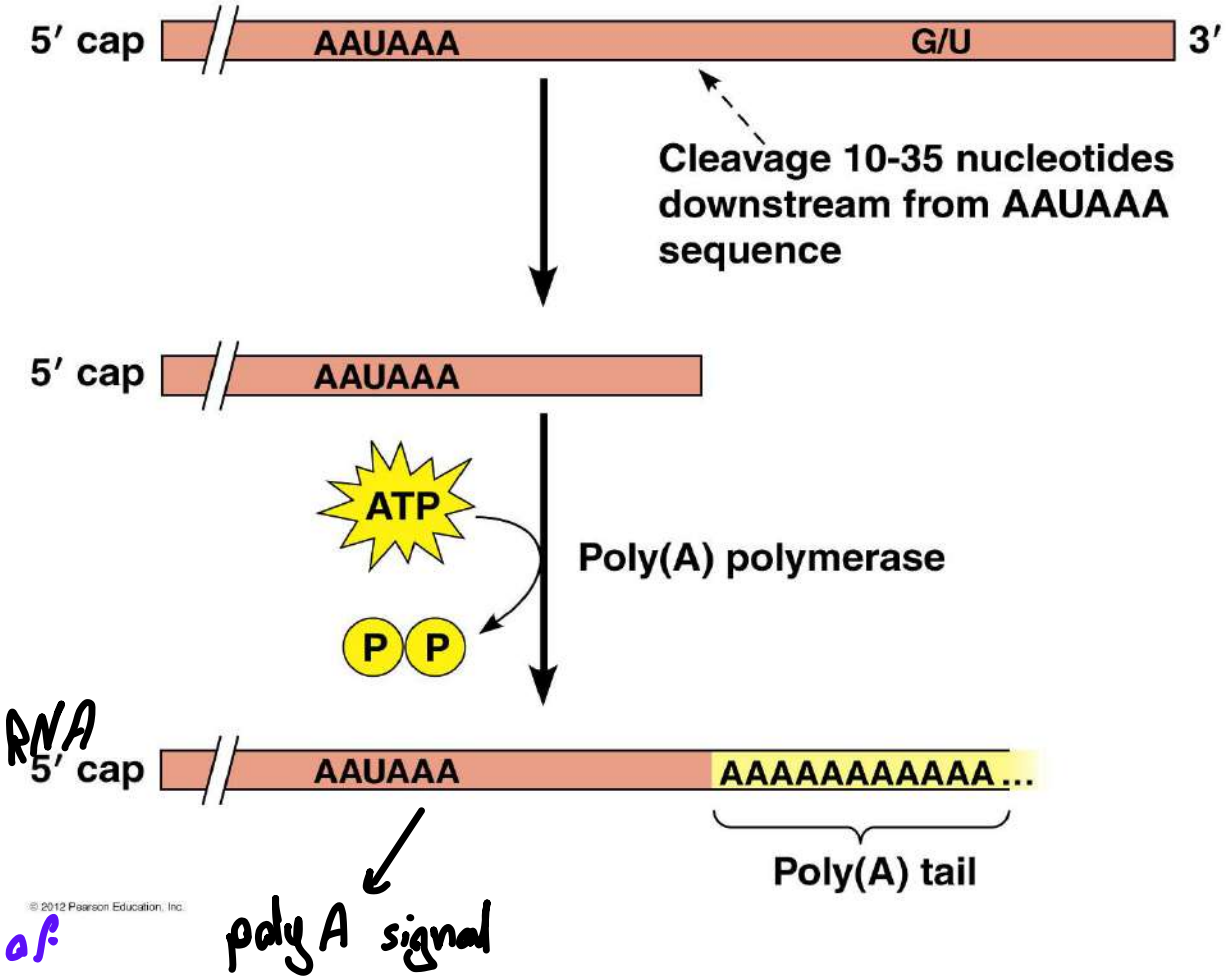
- Helps transport of mRNA to the cytoplasm.

B. Addition of poly(A) tail:

- ✓ The final RNA processing event, polyadenylation of the 3\ end of the mRNA, is intimately linked with the termination of transcription
- ✓ It is the addition of poly- A tail at the 3\ end of mRNA (100-200 A bases).
- ✓ This poly–A tail is not transcribed from DNA but added after transcription by the enzyme polyadenylate polymerase using ATP as a substrate.
- ✓ This occurs after the mRNA is cleaved 15-20 nucleotides downstream from the AAUAAA recognition sequence.
- ✓ The poly–A tail immediately binds several copies of a poly (A) binding proteins that protect mRNA against 3\ exonuclease.

source of energy هنا لست ATP
بل وحدة بناء الـ tail

cleavage يبدأ بـ RNAPII على DNA يحوي recognition signal أولاً بتوقفها
بعد 15-20 نيوكليوتايد من recognition signal في RNA (البروتينات الموجودة
على poly-A signal بتساعد على poly-A tail وإضافة polyadenylate polymerase عن طريق إضافة ATP.



انتبه: انتبه للفرق بين

poly A tail / poly A signal in RNA

↓ بتم إضافتها بعد transcription صح

↓ ناتجة من transcription of poly A signal in DNA

half life of RNA يحدد length of poly A tail

إذا ما كان tail طويل أي ما فقد RNA فترة أطول في السيتوبلازم من دون ما يتكسر

وأي ما فقد أطول كل ما كان translation أفضل وأكثر كفاءة.

بتساعده الخروج من nucleus بسهولة

❖ Importance of poly-A tail:

- It stabilizes the mRNA & protects it from exonucleases enzymes. The length of poly (A) tail determines the half life time of mRNA.
- Increases the efficiency of translation.
- It facilitate their exit from the nucleus .After the mRNA enters the cytosol, the poly-A tail is gradually shortened.

* وبعد ما يفقد RNA في cytoplasm يبدأ يفقد tail أي يبدأ يتكسر RNA وهذا يكون بعد عملية translation

C. Removal of introns and splicing of exons :

- It means excision of introns and joining the ends of exons to **leave only the functional mRNA molecule.**

snRNA بيعملوا ← RNAP II, RNAP III

RNA ماركي فيها بروتينات ← ribonucleoproteins

- This process occurs in the nucleus by the help of **the small nuclear ribonucleoproteins** (snRNP, or snurps) which are composed of small nuclear RNA (snRNA) and proteins.

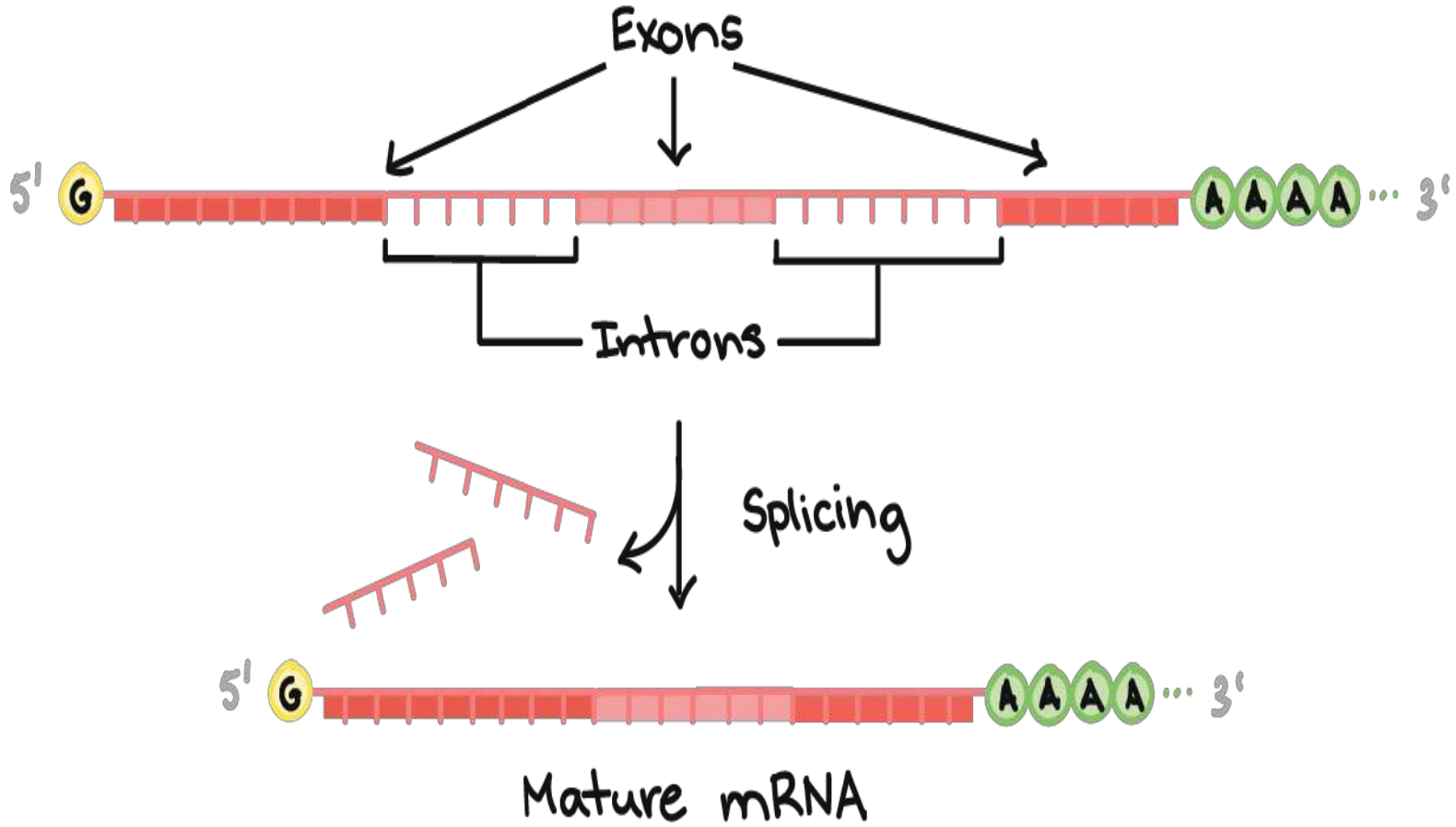
لما تترتبط مع proteins معينة وتكمل ← snurps

تساعد في عملية إزالة introns وربط exons

- Snurps acting on mRNA are called **spliceosomes** . This is an example of catalytic RNAs or RNA enzymes, which are termed **ribozymes**.

- The sequence of bases at the exon-intron junction determines the site of splicing.

nucleus في ← removal of introns and splicing of exons



- One type of β thalassemia appears to result from nucleotide change at the exon-intron junction leading to failure to remove introns, reducing the synthesis of the β globin chain.

- Patients with systemic lupus erythematosis (SLE) produce antibodies against snRNP.

- Histone mRNAs (replication-dependent histones that are expressed during the S-phase of the cell cycle) do not contain introns.

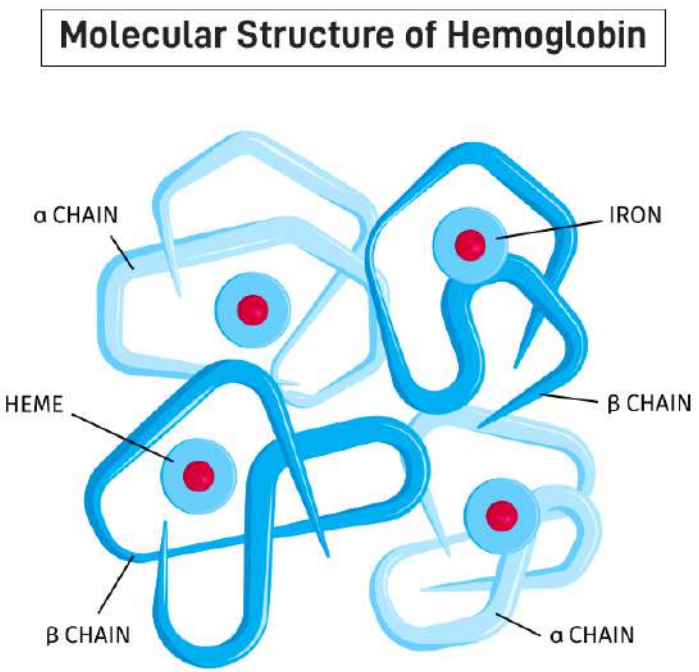
expression of histones during S phase because DNA replication take place during S phase

مشكوده بوقت محدد

2 B chains , 2 α chains تتكون من hemoglobin ←

في أحد أنواع B Thalassemia ← mRNA إلى بيطلع من B gene بيكون فيه مشكلة في exon-intron junction (فيه مشكلة بـ sequence) وبالتالي فإن snurps ما رح تقدر تقص عند هذه junctions وتفرق introns عن exons

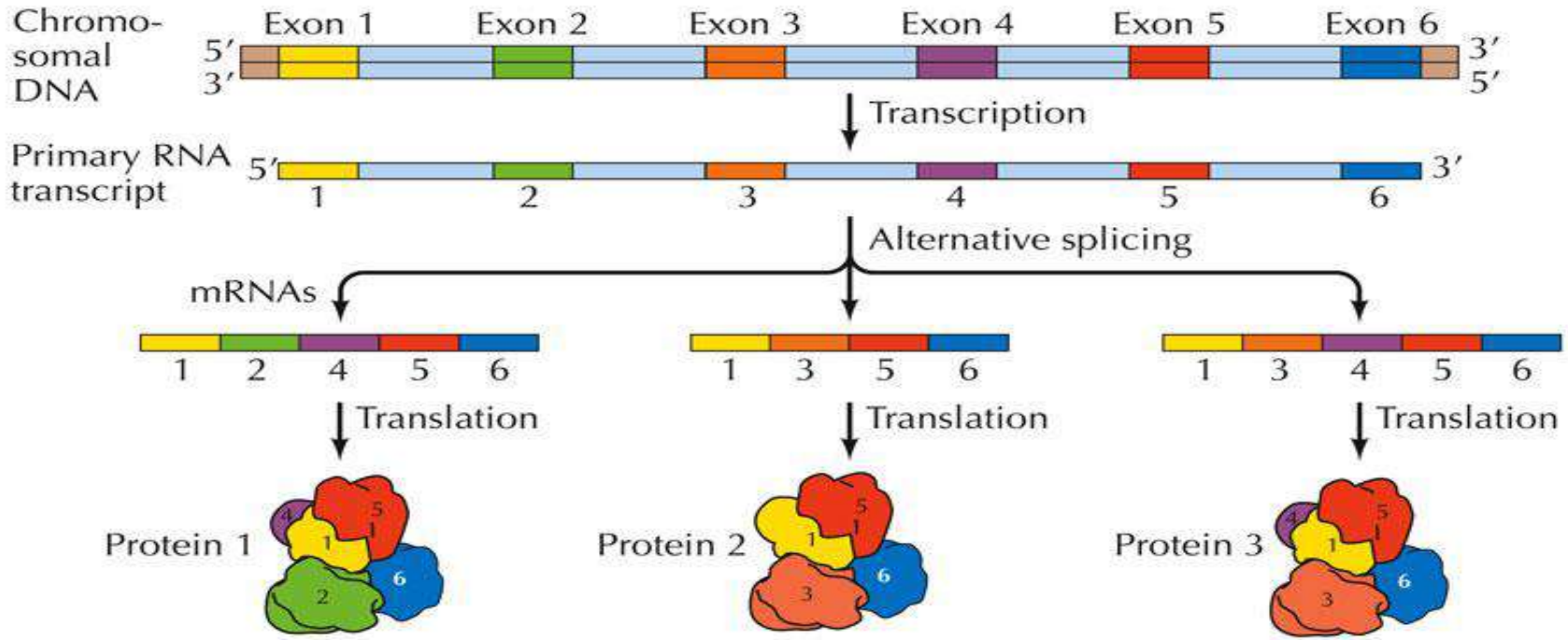
وبالتالي لن يتكون functional mRNA ←
وبالتالي لن تستطيع عملية translation
وإنتاج B polypeptide chains
وبالتالي يوجد مشكلة كبيرة في hemoglobin



Two advantages are suggested for having protein- coding genes organized as exons & introns:

1- **Alternative splicing** may lead to the formation of different types or new types of mRNA molecules or proteins.

2- Also this will **decreases the possibility of effective mutations** ,(that result in protein abnormalities or disease), if it occurs at the regions of introns.



THE CELL, Fourth Edition, **Figure 5.5** © 2006 ASM Press and Sinauer Associates, Inc.

- **Alternative splicing**, or **differential splicing**, is a regulated process during gene expression that **results in a single gene coding for multiple proteins.**
- In this process, particular exons of a gene may be included within or excluded from the final, processed messenger RNA (mRNA) produced from that gene.
- Notably, alternative splicing allows the human genome to direct the synthesis of many more proteins than would be expected from its 20,000 protein-coding genes.

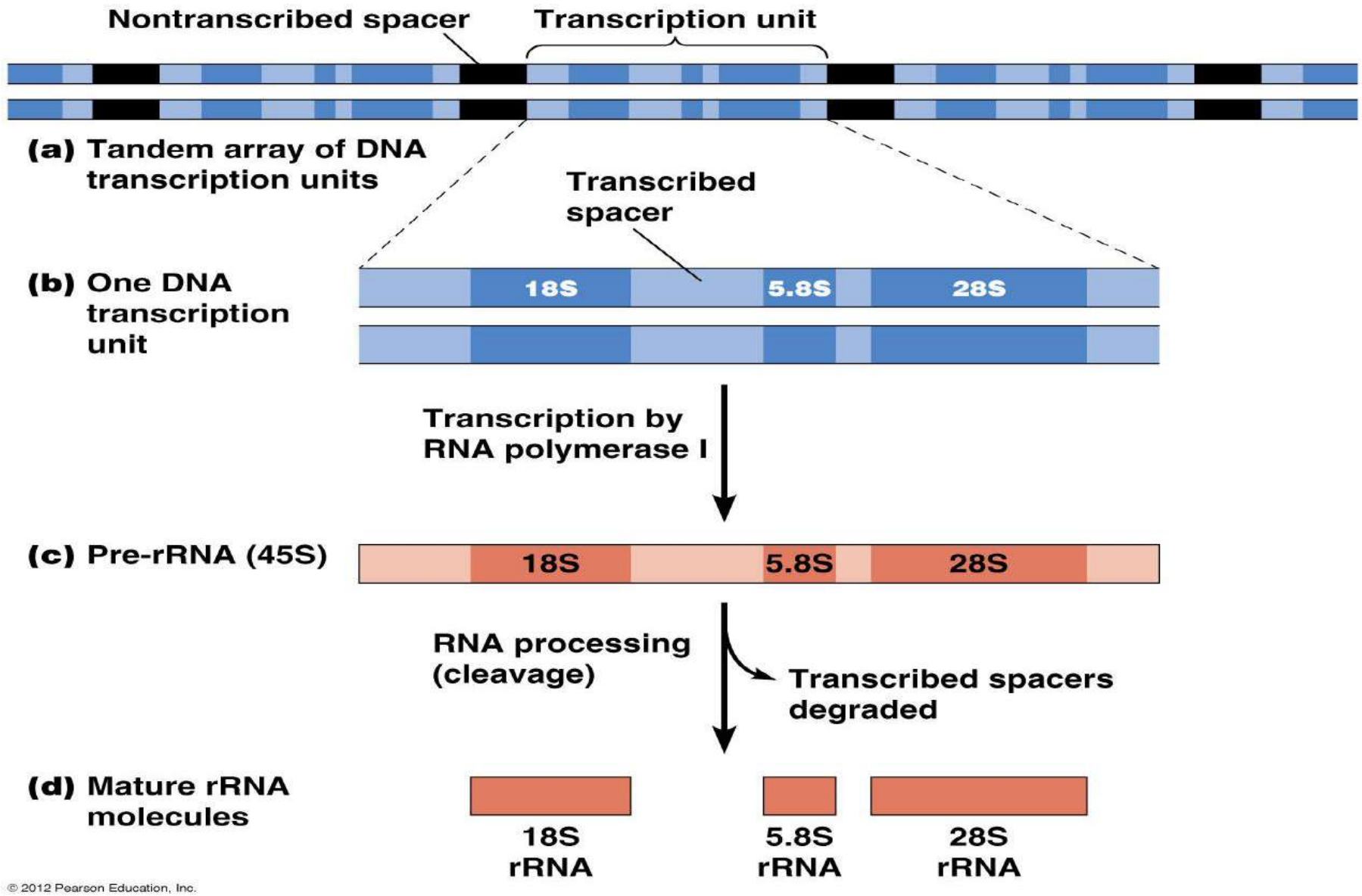
يوجد 20,000 جين ولكنه يوجد عدد أكبر بكثير من البروتينات وذلك بسبب alternative splicing

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

- 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).
- **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.

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.



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Synthesis & Processing of tRNA

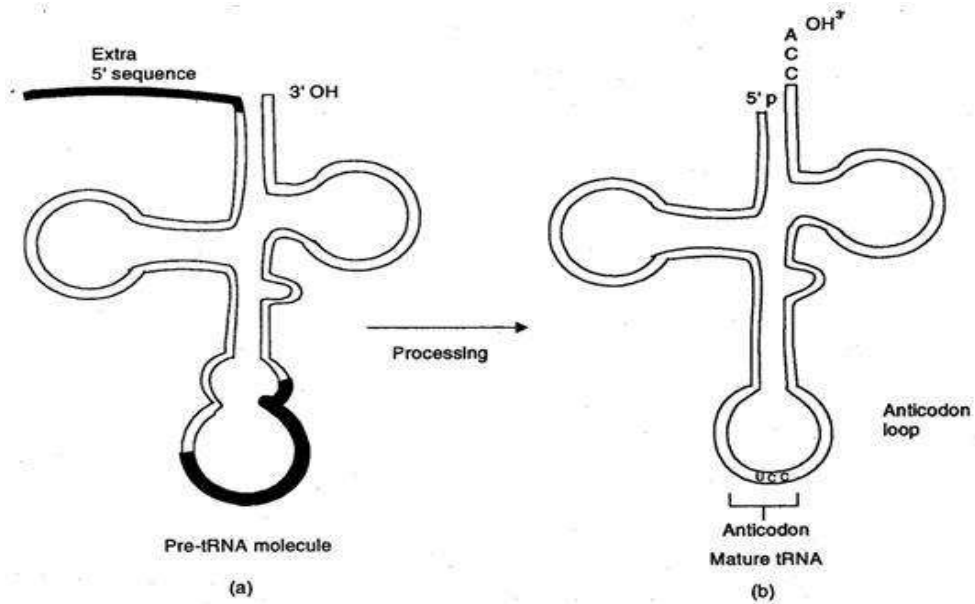
- Eukaryotic tRNA genes are all transcribed by **RNA polymerase III.**
- The primary transcript (pre-tRNA molecules) requires up to 4 different types of RNA processing steps as follows:

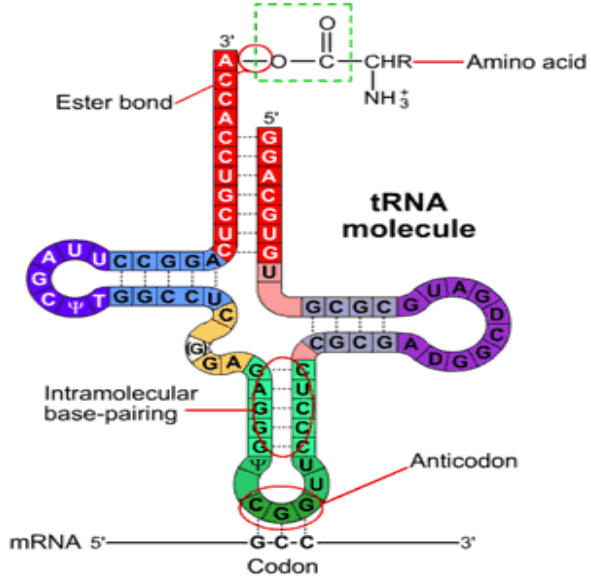
1- Addition of the CCA sequence at the 3\ end by the nucleotidyl transferase.

2- Excision of the nucleotide extension at the 5\end.

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


Extra
5' sequence
3' OH
Processing
Pre-tRNA molecule
(a)
OH
A
C
C
5' p
Anticodon
loop
Anticodon
Mature tRNA
(b)


3'
Ester bond
Amino acid
CHR
NH3+
5'
tRNA
molecule
Intramolecular
base-pairing
Anticodon
mRNA 5'
3'
Codon