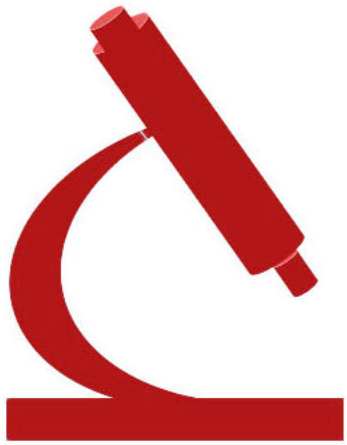

CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

14

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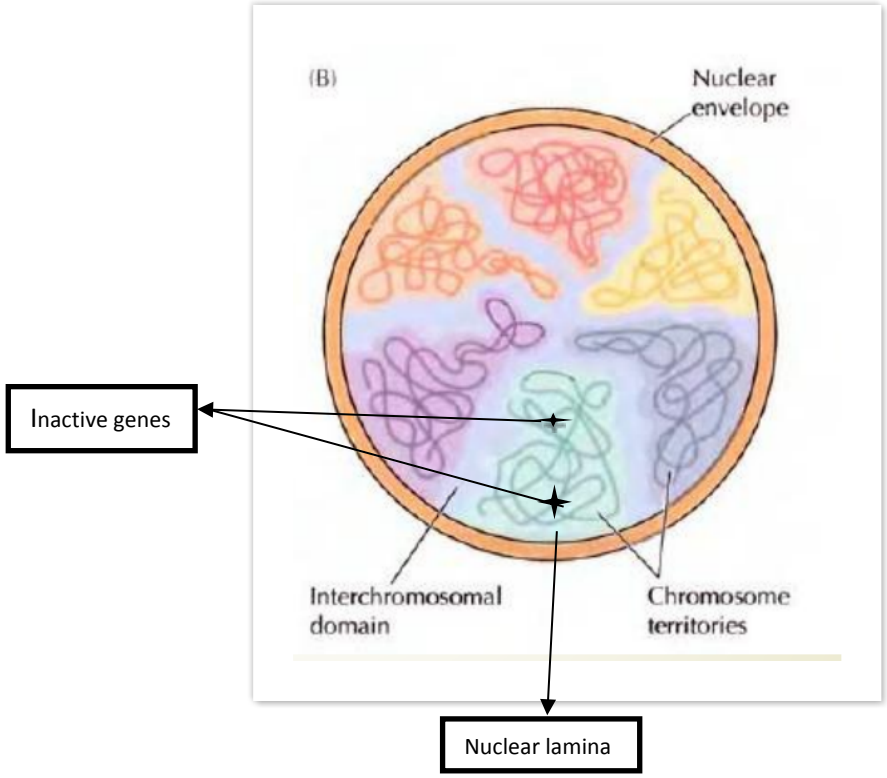
- In the last lecture we discussed the **nuclear chromosomal territories**. We said that these territories are separated by inter-chromosomal domains.

- Chromosomes within these territories are **active** so they are going to move depending on the activation state of certain genes.

- If the gene is going to be activated, it's going to be moved next to the inter-chromosomal domains. Explain?

To allow the access of transcription factors and their binding, and that's why this region is going to be decondensed.

- However, if the gene is inactive that gene is going to be found in the inactive regions, that means in the middle or next to the lamina as shown in the figure below:



- If I want to active a gene, then it had to be moved to the activation region next to the inter-chromosomal domain.

- Examples on non-coding sequences that are found in the inactive region (in the middle or next to the nuclear lamina) : telomers and centromeres.

Chromatin types depending on it's activation state :

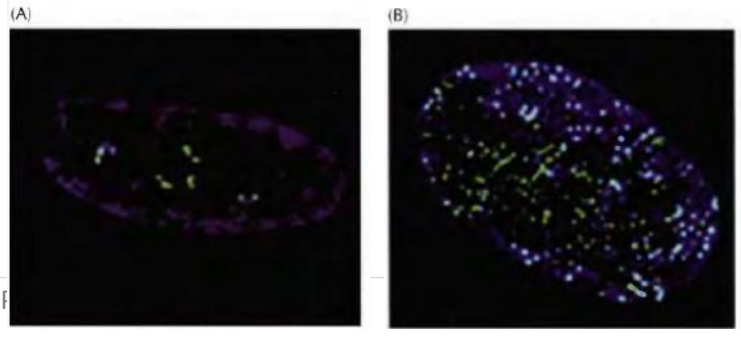
Heterochromatin	Euchromatin
Highly condensed	Decondensed
Transcriptionally **inactive**	Transcriptionally **active**
Includes **non-transcriptional** DNA sequences such as telomeres and centromeres	Contains **transcriptional** DNA regions
Located close to the nuclear envelope and around the nucleolus and binds to lamins and proteins of the inner nuclear membrane	Localized to the periphery of chromosome teritorries adjacent to channels between the chromosomes

- ✓ So, Heterochromatin is expected to be found in the middle or next to the nuclear lamina.

- ✓ While Euchromatin is expected to be found next to the inter-chromosomal domains.

Nuclear bodies:

- ✓ Nuclear bodies: assembles of certain functional units within the nucleus.
- ✓ **For example**, all the machineries that I need for splicing will be together and next to each other in the nucleus forming certain nuclear body. Also, all the machineries that I need for repairing DNA errors will form another nuclear body and so on.
- ✓ So, remember that proteins and RNAs that function in specific nuclear processes will be in the same site inside the nucleus forming a **nuclear body**.



In the figures shown, each dot represent replication factors in different phases of cell cycle.

✓ We have a lot of types of nuclear bodies, but you only need to know the following :

Nuclear body	Number per nucleus	Function
1. Cajal body	0-10	snRNP assembly
2. Histone locus body	2-4	Transcription and processing of histone pre-mRNAs
3. Nuclear stress body	2-10	Response to stress
4. Polycomb body	10-20	Gene silencing
5. Nucleolus		rRNA synthesis

✓ **nuclear stress bodies** :

Examples on stresses:

1-Oxidative stresses, whether it's low concentration of O_2 , or over production of reactive oxygen species (Chemical species containing O_2).

2-Mechanical stress, excess liquid in the extracellular matrix surrounding the cell.

The cell then must deal with these stresses, by using specific proteins, that requires activating certain genes.

✓ **Polycomb bodies :**

*Responsible of gene silencing by methylation (Adding methyl groups).

*Adding methyl groups to nucleotides or nitrogen bases or histones, result in inactivation of a gene.

-Example: **X**-chromosome inactivation (Barr body). Polycomb body plays a role in this process.

✓ **Nucleolus:**

1- It has no surrounding membrane.
2- Is associated with chromosomal regions that contain about 200 copies of the genes for: 5.8S, 18S and 28S rRNAs. **Why?**

To synthesize large amounts of ribosomes.

3- Nucleolus does Ribosomal RNAs synthesis.
4- Ribosomes are composed of rRNAs and proteins .

5- There are different types of rRNA molecules inside the ribosome.

6- All those rRNAs need to be **synthesized** , **prepared** and **binding** them to proteins **then finally assemble them into ribosomes**.

7- 3 of 4 different types of rRNAs are coded by the same gene: **18 S**, **5.8 S** , 28 S. And then they will be transcribed by **polymerase I.**

8- The last type is **5 S** is transcribed outside the nucleolus by **polymerase III.**

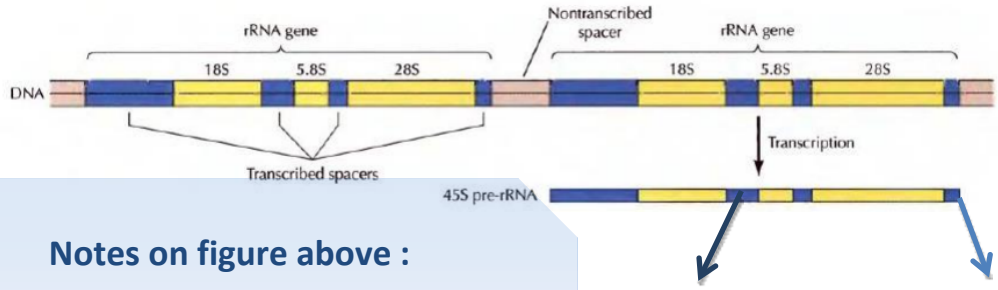


FIGURE 9.25 Ribosomal RNA genes Each rRNA gene is a single transcription unit containing the 18S, 5.8S, and 28S rRNAs as well as transcribed spacer sequences. The rRNA genes are organized in tandem arrays, separated by nontranscribed spacer DNA.

Notes on figure above :

*The required sequence is in yellow, separated by spaces.

*Spaces can be : in-between or on the edge.

IN BETWEEN SPACE

ON THE EDGE SPACE

Nucleolus functions

- rRNA synthesis
- Ribosome production
- RNA modification and assembly of ribonucleoproteins particles
- Small RNA production such as tRNA, snRNA, RNase P RNA (the catalytic part of the tRNA processing enzyme), SRP(it targets proteins to ER)
- Cell division & response to stress

snoRNAs :

- snoRNAs = Small nucleolar RNAs.
- Localized RNAs to the nucleolus.
- They complex with proteins to form snoRNPs

snoRNAs+ Proteins= snoRNPs

snoRNAs Functions :

- Pre-rRNA processing(like spliceosomes of pre-mRNA) by cleavage of pre-rRNA into 5.8S , 18S and 28S products
- Base pairing with pre-rRNA (they contain 15 nucleotides complementary to pre-rRNA) to target it by enzymes that catalyze base modification

- So snoRNA is going to identify the spaces **(regions to be spliced).**
- Then bind that identified region complementary **(G-C and A-U).**
- After binding, that region is going to be marked as a cut site by methylation **(Adding methyl groups).**

Notes:

1-There are different types of snoRNA, each type can recognize certain spacer.

2-Each spacer has a beginning and an end (3' and 5'), so there are several types sequences that require several types of snoRNAs to recognize .

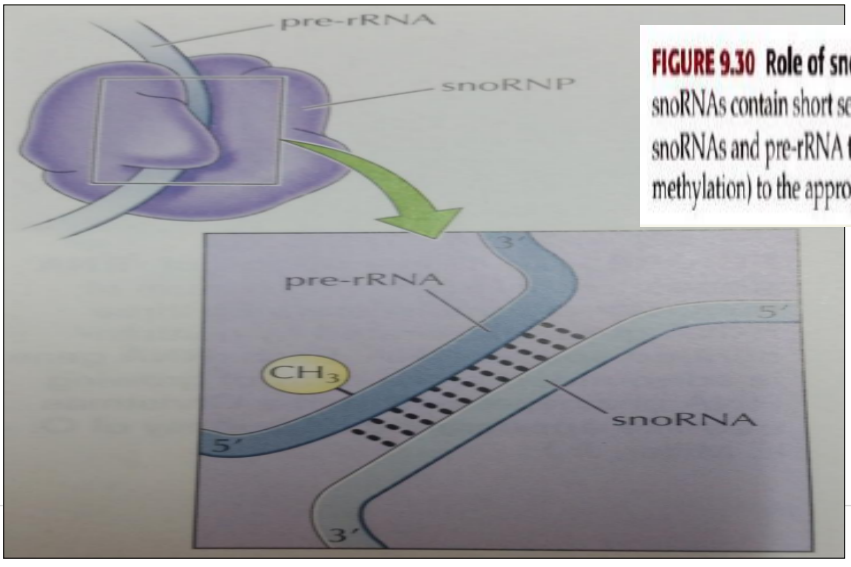


FIGURE 9.30 Role of snoRNAs in base modification of pre-rRNA The snoRNAs contain short sequences complementary to rRNA. Base pairing between snoRNAs and pre-rRNA targets the enzymes that catalyze base modification (e.g., methylation) to the appropriate sites on pre-rRNA.

Transcription and processing of rRNA:

Processing of pre-rRNA involves:

I. Cleavage within the external transcribed spacer (ETS) near the 5' end.
II. Removal of the (ETS) at the 3' end.
III. Base modification (Methylation of ribose and some bases)

You need to know that there are Internal transcribed spacer (ITS) and (ETS) that need to be cleaved ending up with 3 types of rRNAs (18S, 5.8S and 28S)

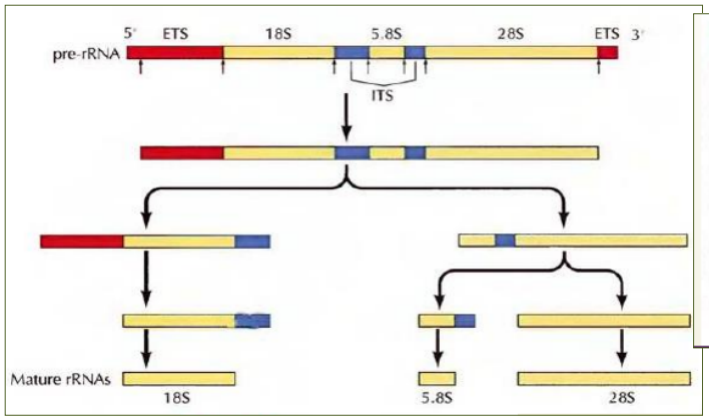


FIGURE 9.29 Processing of pre-rRNA The higher eukaryote 45S pre-rRNA transcript contains external transcribed spacers (ETS) at both ends and internal transcribed spacers (ITS) between the sequences of 18S, 5.8S, and 28S rRNAs. The pre-rRNA is processed via a series of cleavages to yield the mature rRNA species.

****Notice that 18S is like a separate piece, while 5.8S and 28S were together at first then were separated.**

Ribosome assembly:

- We mentioned before the process of forming the 3 types of rRNAs 18S, 5.8S, 28S.
- Also, we said that there is one type 5S that is going to be transcribed outside the nucleolus by polymerase III.
- Ribosomal proteins are transcribed outside the nucleolus by polymerase II. Then they will be translated in the cytoplasm.
- The ribosomal proteins bind to the pre-rRNA while cutting and processing is going on.
  1) 18S + proteins = will form the small ribosomal subunit (40s subunit)
  2) 28S + 5.8S +5S + proteins = large ribosomal subunit (60s subunit)

*Small subunit processing is **simpler** by **4 endonuclease cleavages** in the ***nucleus***.

*Large subunit processing is **more complex** with **extensive nuclease cleavage** in the ***nucleolus***.

-Ribosomal subunit maturation: pre-ribosomal particles are exported to the cytoplasm to form active 40S and 60S subunits of ribosomes.

- The formation of these different types of ribosomal rRNA subunits and their contents of ribosomal rRNA molecules is achieved by different types of enzymes.
- **Finally,** once we have the large and small ribosomal subunits are ready they will be exported to the cytoplasm.
- **Then they will combine once mature-mRNA binds to the small subunit and then the large subunit will bind forming the ribosome structure and after that translation will occur.**

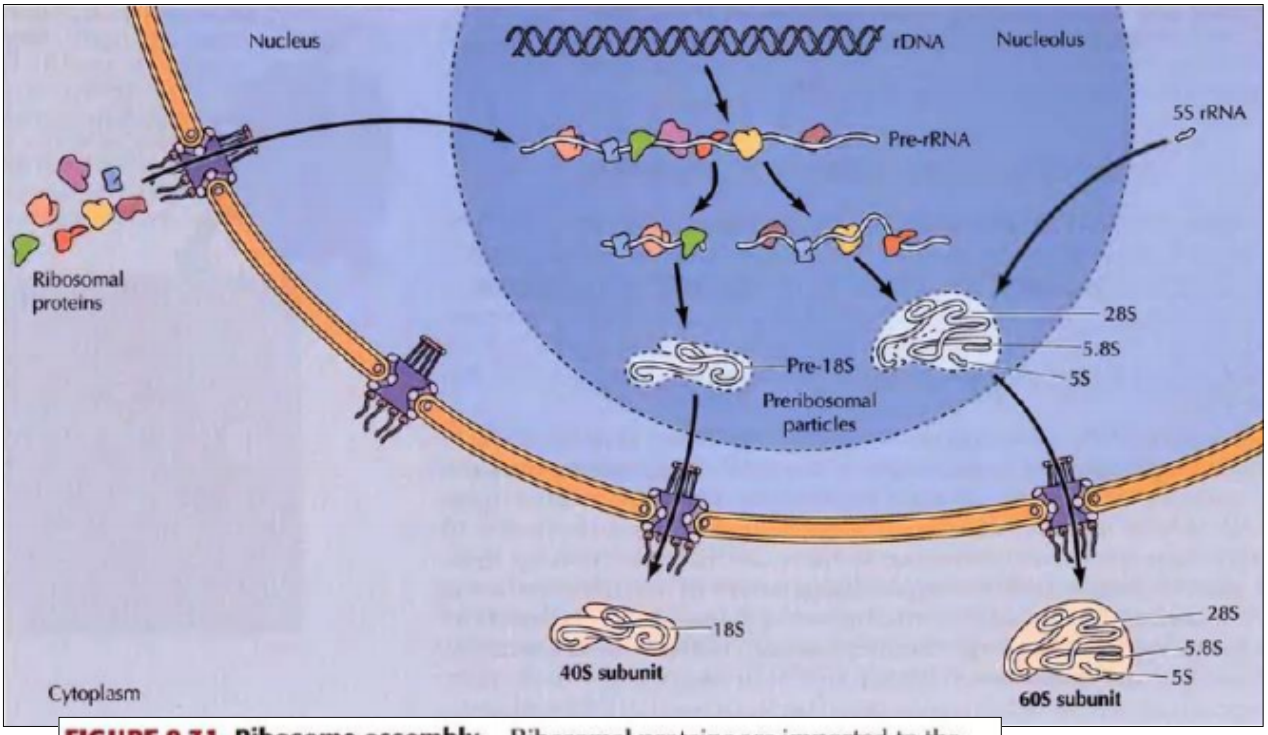


FIGURE 9.31 Ribosome assembly Ribosomal proteins are imported to the nucleolus from the cytoplasm and begin to assemble on pre-rRNA prior to its cleavage. As the pre-rRNA is processed, additional ribosomal proteins and the 5S rRNA (which is synthesized elsewhere in the nucleus) assemble to form preribosomal particles. The final steps of maturation follow the export of preribosomal particles to the cytoplasm, yielding the 40S and 60S ribosomal subunits.

Whenever the art of medicine is loved, there is also a love of humanity.

-Hippocrates