**Semester IV**

**Elective Paper: Cell and Molecular Biology**

## Unit 3.2: Ultrastructure of Nucleolus: Organisation of rDNA

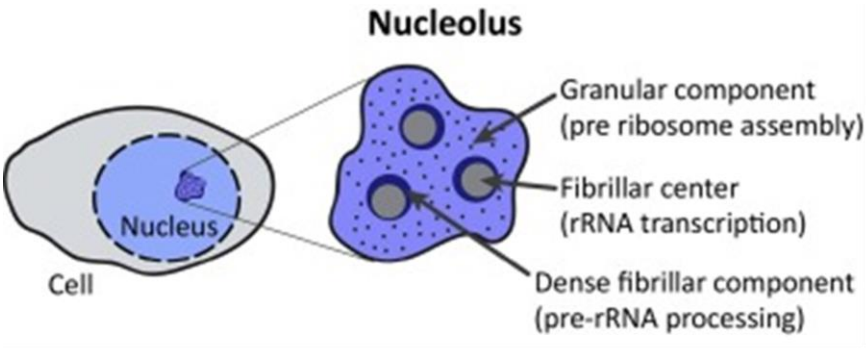
The nuclear space is mostly occupied by chromosome territories and nuclear bodies. The most prominent nuclear body is the nucleolus, which is the site of ribosomal RNA (rRNA) transcription and processing as well as ribosome assembly. Like other nuclear bodies, the nucleolus is also not surrounded by any membrane; however, is associated with chromatin and specific proteins that provides functional identity to nucleolus. Nucleoli are organised around the core rRNA gene regions, referred as nucleolus organiser regions (NORs). Actively growing mammalian cells contain 5-10 million ribosomes that must be synthesized each time the cell divides. The nucleolus is a ribosome factory, designed to fulfill the need for regulated and efficient production of rRNAs and assembly of ribosomal subunits. The rRNA genes are the most highly transcribed in the genome, with rRNA accounting for approximately 80% of total RNA in a cell.

**Early milestones of nucleolus research**

- First observed by F. Fontana nearly 200 years ago.
- Nucleolus terminology was coined in 1839.
- The dynamic disappearance and reappearance of nucleoli in mitotic cells were described in 1893-1894.
- Montogomery described nucleolus morphology in 1898.
- The association between nucleoli and chromosomes were reported in 1917.
- McClintock described nucleolar organising body in 1934.
- Casperson and Brachet detected the presence of RNA in nucleolus in 1950s.
- Perry, Ritossa and Spiegelman identified rDNA in NOR in 1960s.
- In 1960s the isolation of nucleoli and biochemical characterisation was done. The data revealed that nucleolus is hub for ribosome biogenesis.
- 1980-2000: the functional organisation of nucleolus was deciphered by EM labelling experiments.

**Ultrastructure of Nucleolus**

Nucleolus typically displays a tri-partite organisation as observed from ultrastructure studies.




**Schematic of nucleolus: The nucleolus is comprised of three subcompartments: the fibrillar center (FC), dense fibrillar component (DFC), and granular component (GC).**

The high resolution imaging of nucleolus by electron microscopy revealed that it consists of three morphological distinguishable regions.

- The fibrillar center (FC)
- The dense fibrillar center (DFC)
- Granular component (GC)

1) The fibrillar cener (FC):

- The FC is the pale staining region in the center of the nucleoli consisting of fine fibrils (4-8nm thick) network which is relatively opaque in the EM.
- The rRNA, RNA polymerase I and the other component of rDNA transcription machinery such as UBF, SL-1 and topoisomerase I localizes to the periphery to FC.
- rRNA transcripts are synthesized in FC.

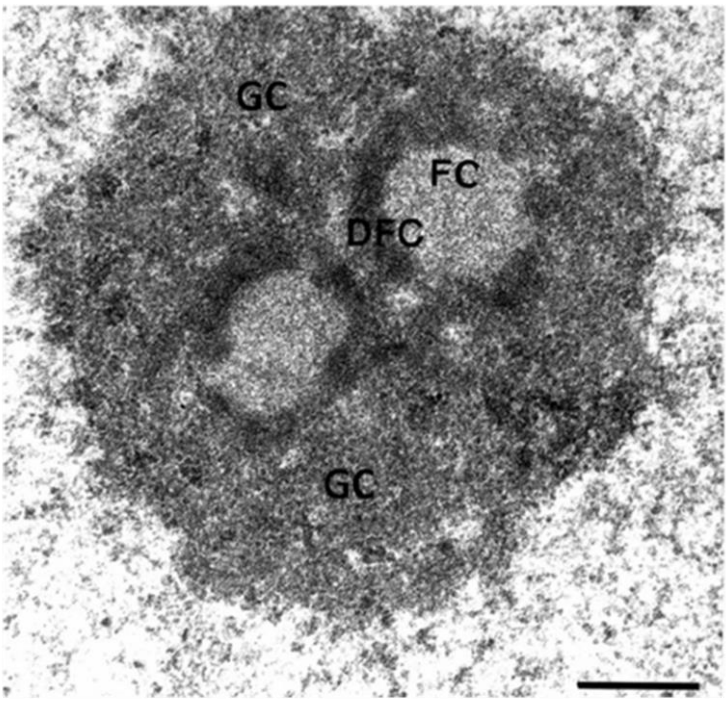
2) The dense fibrillar center (DFC):

- The DFC surrounds the FC and is characterized by densely packed fine fibrils (3-5nm thick) and contains a higher concentration of 34kDa protein fibrillarin.
- The fibrillarin associates with protein required in the early stages of rRNA processing such as U3, U8 and U13 snoRNP (small nucleolar ribonucleoprotein).

- rRNA transcripts are spliced, modified and folded in DFC.

3) Granular component (GC):

- The GC is localized to the periphery of the nucleoli and consists of granular structures ranging in the diameter of 10-15nm.
- The GC are the assembly site for maturing ribosome precursor particles.




**Nucleolus ultrastructure: An electron micrograph of a HeLa cell demonstrates that the nucleolus is comprised of three subcompartments: the fibrillar center (FC), dense fibrillar component (DFC), and granular component (GC). Bar 0.5 µm.**
***Chromosoma (2015) 124:323–331***

The nucleolar compartments (FC, DFC and GC) coexist in liquid phases and this phase separation induces the formation of multi-layered liquids that facilitates sequential RNA processing inside nucleolus.

The sequential movement of rRNA through the FC, DFC and GC sub-compartments can be demonstrated when cells are labelled with a short pulse of halogenated nucleotide, which reveals a wave of nascent rRNA spreading from the FC-DFC complexes to the GC regions.

The size of nucleolus positively correlates with rRNA synthesis. The dividing cells produces elevated amount of rRNA and often possesses large nucleoli whereas the down regulation of rRNA gene transcription leads to reduction in nucleolar size.

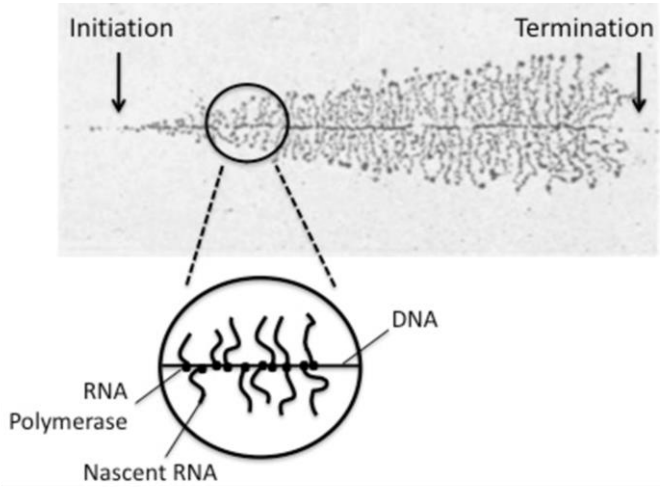
## Organisation of rDNA

The genes encoding the rRNA genes are known as rDNA and are present in multiple copies in tandem arrays. rDNA is the basis for nucleolus biogenesis, organization, and function. The rDNA are located on different chromosomes in mammalian cells and those chromosomal regions are known as Nucleolar Organising Regions (NORs).

### *Nucleolar Organising Regions (NORs)*

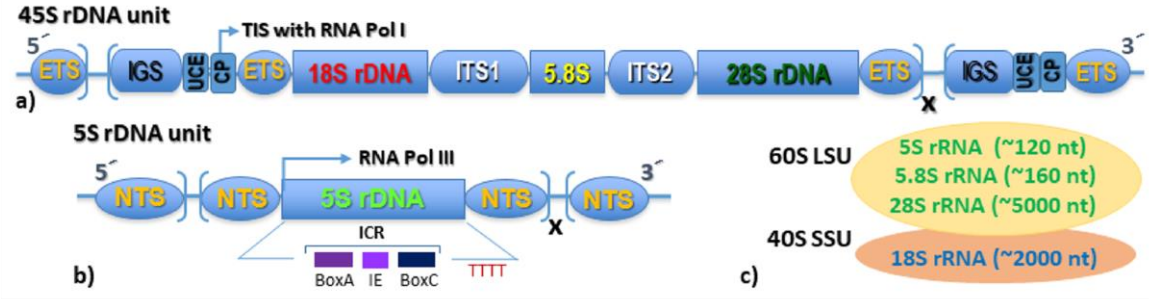
NORs are the chromosomal regions containing ribosomal RNA genes (rRNA). The rDNA are arranged in arrays of head to tail tandem repeats and constitute NORs.

- Genes coding for rRNA (5.8S, 18S and 28S rRNA) are present on different chromosomes. The 5.8S, 18S and 28S rRNA are transcribed as a single unit within the nucleolus by RNA polymerase-I yielding a 45S pre-rRNA.
- The 45S pre-rRNA is processed to generate 18S rRNA (a component of 40S ribosome subunit) and 5.8S, 28S rRNA (a component of 60S ribosome subunit).
- Under Miller spreads condition (eukaryotic nucleus are swollen and spread in an alkaline, hypotonic solution in the presence of detergent), the rDNA repeats are fully extended and, along this central DNA axis, nascent growing rRNA transcripts can be seen emerging from each rDNA unit. As transcription proceeds from the initiation point, the rRNA transcripts become increasingly longer, resulting in a `Christmas tree' model.




**Transcription from tandemly arranged rRNA genes.**
**Transcription of rRNA genes visualized by electron microscopy as seen in Miller spreads of nucleoli from salamander oocytes.**
***Human Genetics volume 63, pages197–215(1983)***

- The genes of 5.8S, 18S and 28S rRNA are clustered in tandem array on the five different chromosomes in humans. They are present on chromosome 13, 14, 15, 21 and 22.
- The 5S rDNA encoding 5S rRNA are present in a single tandem array on human chromosome 1.
- To meet the high demand of rRNA all of these genes are present in multiple copies in each cell. Such as in human genome there are 200 copies of 5.8S, 28S and 18S rRNA and 2000 copies of 5S rRNA.
- The coding rDNA sequence is highly conserved among eukaryotes, while the intergenic spacers (IGSs) that separate the proper units of the 45S rDNA cluster can differ in length and sequence. In mammals, IGSs contain regulatory regions called UCE (upstream control element), CP (core promoter) and T (termination of transcription site).
- In rats, mice, and mammalian cells, the IGS contains one or more RNA polymerase-I (Pol I) promoters with high homology to the core region of the main rDNA promoter.




The genomic organization of the rDNA loci. (**a**) Structural organization of the 45S rDNA gene cluster (or rRNA transcription unit); the repeating or single clusters of rDNA are present of various chromosomes in mammalian cells, they form the precursor pre-rRNA (**b**) Structural organization of the 5S rDNA unit: the 5S rDNA are also present on human chromosome 1, outside the nucleolus (**c**) 80S eukaryotic ribosome composed of the large subunit (LSU) and the small subunit (SSU) with outlined rRNAs.
CP-core promoter, ETS-external transcribed spacer, ICR-internal control region, IE-internal element, IGS-intergenic spacer, ITS1, ITS2-internal transcribed spacer 1 and 2, RNA Pol I and III-RNA polymerase I and III, LSU-large (ribosomal) subunit, nt-nucleotides, NTS-non-transcribed spacer, SSU-small subunit, TIS-transcription initiation site, TTTT-polyT transcription termination site, UCE-upstream control element.
***Genes 2019, 10(5), 345***

- Following each cell division, the nucleoli associates with the chromosomal regions that contains 5.8S, 28S and 18S rDNA. The formation of nucleolus requires the synthesis of 45S rRNA (pre-rRNA) that helps in the assembly and fusion of small pre-nucleolar bodies that contain processing factors and other components of nucleolus.

The genes in NORs are organised in such a way that despite the high levels of rRNA gene transcription not all rRNA genes participates in transcription. Only a fraction of the

numerous rDNA copies is transcribed into rRNA. The non-transcribed rDNA copies are extremely important for integrity of the entire genome. In mammalian cells, rRNA genes are subdivided into three major classes according to their chromatin organisation and transcription status.

1. Active rRNA genes
2. Inactive (Pseudo-genes) rRNA genes
3. Silent rRNA genes

1. Active rRNA genes:

- Active rRNA genes do not have DNA methylation at its promoter and these genes are transcribed. The coding- region of active rRNA genes are nucleosome free.
- The key factor required for establishing active rRNA genes is the presence of upstream binding factor (UBF).
- The UBF associates with rRNA gene body, spacer promoter and enhancer repeats allowing the formation of PIC with RNA-polymerase I.
- In metaphase, where rRNA genes are not transcribed, active rRNA genes remains associated with UBF, SL-1 and TIF1.

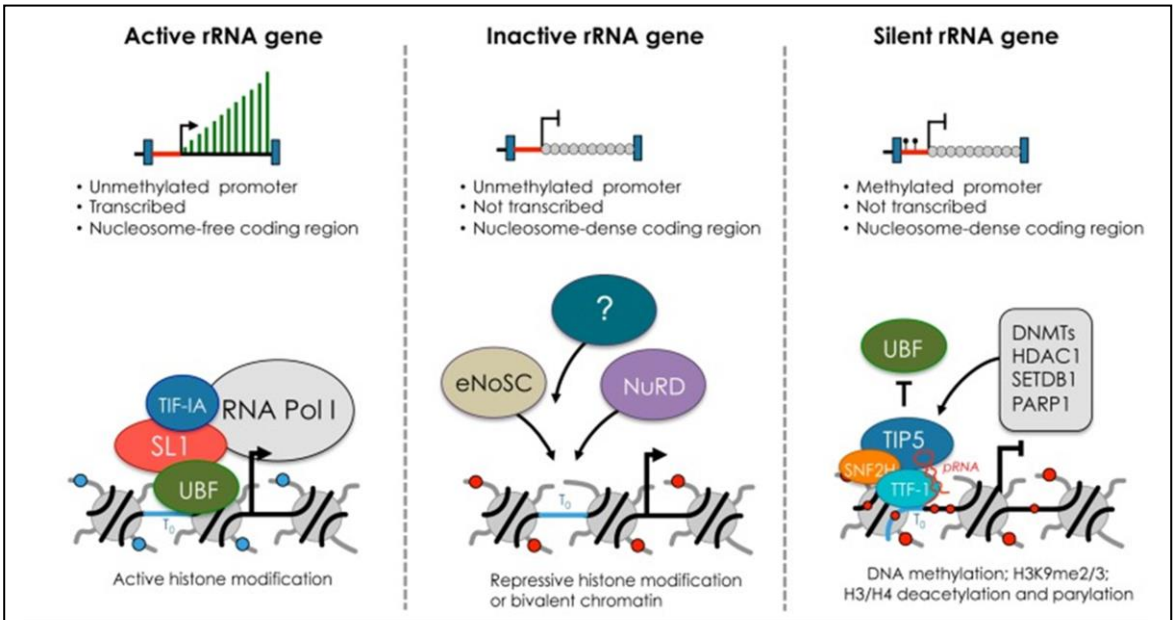
2. Inactive rRNA genes:

- These repeats do not have DNA methylation at promoters and hence their transcription state can be reversed.
- UBF does not bind to inactive genes therefore these genes remains non-transcribed.
- eNoSC (energy dependent nucleolar silencing complex) and the NuRD (nucleosome remodelling and deacetylase) complexes establish a repressive or poised chromatin state at inactive rRNA genes.

*3. Silent rRNA genes*:

- The promoter of silent rRNA genes have DNA methylation which is absent in rest of the repeats (inactive and active rRNA genes).
- Silent genes display heterochromatic structures and associates with repressive histone modifications such as H3 K9me2, H3K9me3 and deacetylated histones.

- Silent genes are non-transcribing and have nucleosome-packed rDNA chromatin.
- In mammalian cells, silent rRNA genes replicates in mid-late S-phase that coincides with heterochromatin DNA replication.
- The presence of CPG methylation on silent rRNA genes promoters inhibits the formation of RNA-polymerase I pre-initiation complex.
- NoRC (nucleosome remodelling complex) helps in the formation and maintenance of silent rRNA genes. NoRC contains TIP5 and SNF2H and they interact with repressive chromatin modifiers such as DNA methyltransferases, HDACs and SETDB1, etc.



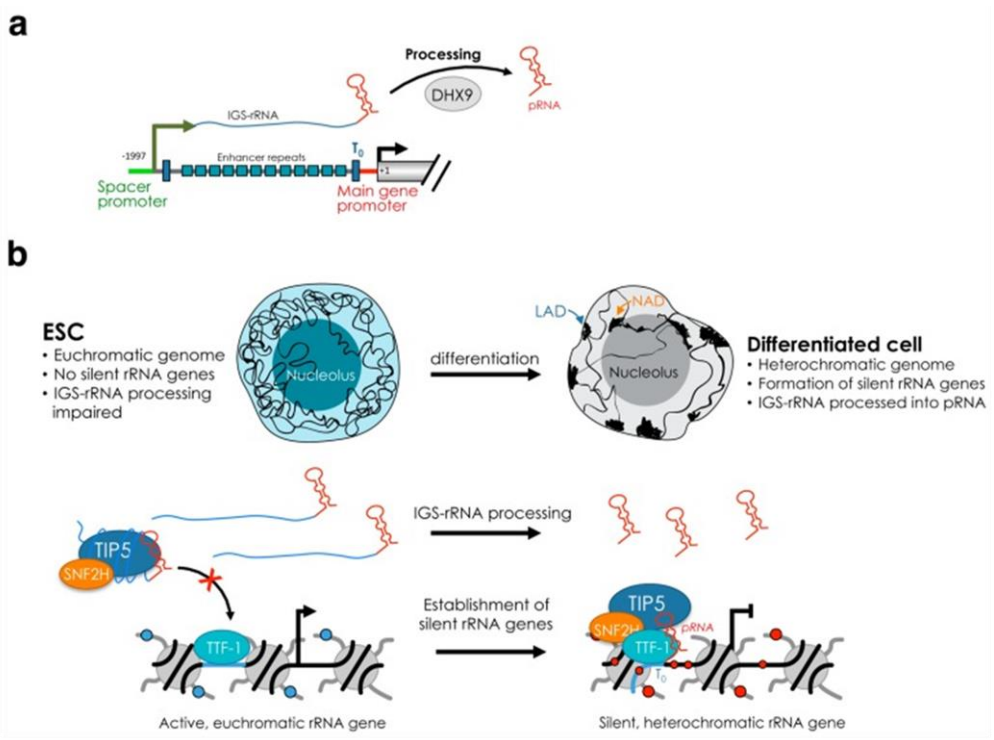

Description of active, inactive and silent rRNA genes based on transcription, chromatin and epigenetic state and factors regulating their state. ***Cells. 2019 Jun; 8(6): 579.***

*Establishing chromatin states of rRNA genes.*

- During development, in early blastocyst stage, the replication of all NORs are highly synchronised and occurs in early S-phase.
- Upon differentiation, the de novo methylation at rRNA gene promoter takes place, one copy of each NOR becomes late replicating and this multi-chromosomal allelic pattern is then maintained clonally in somatic cells.
- Upon exit from pluripotency state, rRNA genes start to acquire DNA methylation and associate with repressive histone marks such as H3K9me2/3. In rats, mice, and mammalian cells, the transcripts originating from spacer promoters are co-

directional with pre-rRNA synthesis and enhance transcription from the main rDNA promoter.

- Intergenic spacers rRNA transcripts originating from a promoter upstream from the pre-rRNA start site are processed into a heterogeneous population of 150–250 nucleotide RNAs, dubbed promoter RNA (pRNA) as their sequence matches the rDNA promoter.
- pRNA helps in the silencing of rRNA in the differentiated state.




Regulation of rRNA gene chromatin state embryonic stem cells. (**a**) Scheme showing the synthesis of IGS-rRNA from the mouse spacer promoter and its processing into pRNA, a reaction mediated by DHX9. (**b**) Model showing the chromatin organization of the nucleus and nucleolus of ESCs (open, euchromatic) and differentiated cells (closed, heterochromatic). In ESCs, IGS-rRNA is not processed with consequent lack of mature pRNA. IGS-rRNA impairs the association of TIP5 with TTF1 and TIP5 recruitment to rRNA genes. Consequently, all rRNA genes are kept euchromatic and active in ESCs. Upon differentiation, mature pRNA is produced and promotes TIP5-TTF1 interaction that is productive for TIP5 guiding to rRNA genes and formation of heterochromatin at nucleoli.
***Cells. 2019 Jun; 8(6): 579.***

## *References*


[1]*Cooper and Hausman, The Cell: A molecular approach*

[2]*Trends in Genetics, Volume 35, Issue 10, October 2019, Pages 710-723*

[3]*Genes 2019, 10(5), 345*

[4]*Cells. 2019 Jun; 8(6): 579.*

[5]*Chromosoma (2015) 124:323–331*