Nucleolus: Nucleolus is a non-membranous organelle found inside the nucleus of a eukaryotic cell. It is a macromolecular aggregate and contains rRNA genes, precursor rRNAs, mature rRNAs, rRNA-processing enzymes, ribosomal proteins, non-ribosomal proteins and partly assembled ribosomes.

Functions of the nucleolus are summarized below and later discussed in required detail.

- Synthesis and processing of non-coding RNAs such as rRNA, tRNA etc.
- Synthesis of ribonucleoprotein complexes such as signal recognition particle and U6 snRNP.
- Synthesis and assembly of ribosomes i.e. ribosome biogenesis.
- Assembly of telomerase enzymes which is a RNA-protein complex.
- Protein sequestration with the help of long noncoding RNAs.
- Sensing cellular stress.

Structure of the nucleolus: In higher eukaryotes, the nucleolus is composed of three distinct regions. This division into distinct regions is closely linked to the sequential steps in the ribosome biogenesis and have been identified using electron microscopy and light microscopy.

We can imagine it like two concentric regions that are embedded in a matrix.

- The inner most part is called as the fibrillar centres (FC) and it is surrounded by the dense fibrillary component (DFC).
- The FC and DFC are embedded in the granular component (GC).
- The FC contains unengaged RNA pol I transcription factors and DFC contains pre-RNA processing factors.
- The transcription of the rRNA genes or rDNA takes place at the boundary of the FC and DFC, so we consider the FC as the site of transcription.
- After transcription, the precursor of rRNA must be processed into different subunits i.e. 18S, 5.8S and 28S.
- This processing takes place in the DFC.
- After processing, we need assembly of the rRNA and proteins into smaller and larger subunits of the ribosomes and this takes place mainly in the GC.

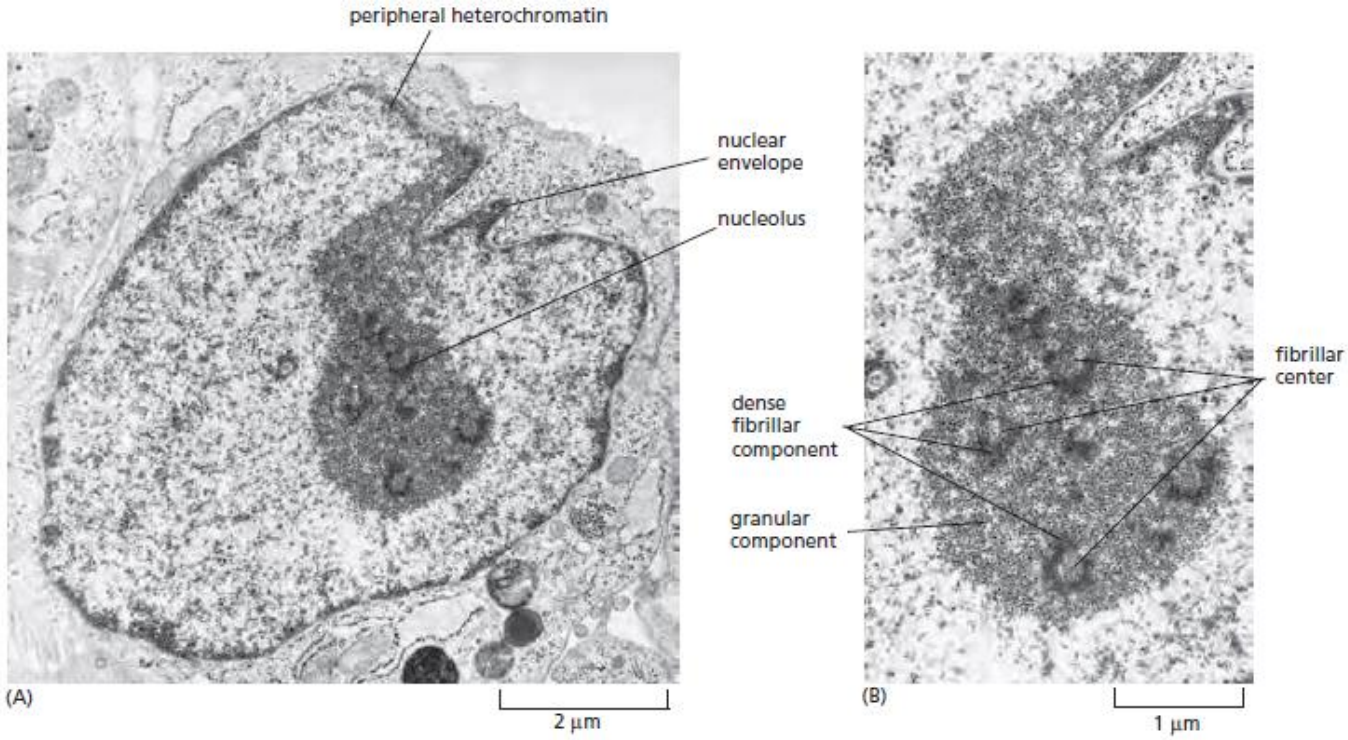


Figure: Nucleolus as seen under electron microscope.
A. Shows entire nucleolus within nucleus.
B. Shows high power view of the nucleolus to clearly demarcate all the regions.

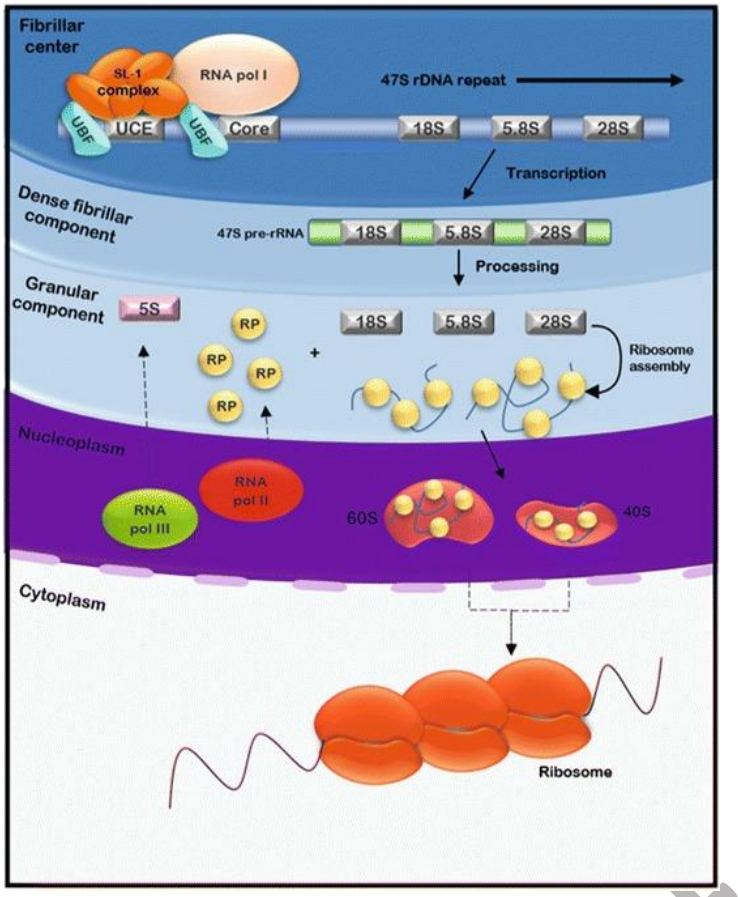


Figure: The sequential role of the FC, DFC and GC in the ribosome biogenesis.

Note:

1. **The precursor rRNA/rDNA shown here is of 47S while the one discussed in the text is 45S.**
2. **This is because in lower eukaryotes such as yeast it is 45S while in higher eukaryotes such as humans and other mammals it is 47S.**
3. **The source of the figure is a discussion on the nucleolus by Marie-Line Dubois and Franc'ois-Michel Boisvert.**
4. **Their focus during the discussion is on higher eukaryotes so it is 47S in the figure.**
5. **Since basic fundamental details about the nucleolus remain the same there is no cause for concern.**

Synthesis and processing of the ribosomal (rRNA) in the nucleolus:

rRNA is the most abundant RNA in the cells and in rapidly dividing cells it makes up to 80% of the total RNA in the cell. The rRNA genes are found in clusters and human cells have approx. 200 rRNA gene copies per haploid genome found in small clusters on 5 different chromosomes. Thus, there are total of 400 copies in 10 clusters of the rRNA genes in a diploid cell in humans.

The repeat units are 43kb long and are arranged in arrays of head-to-tail tandem repeats along the acrocentric chromosomes i.e. chromosomes 13,14,15,21 and 22. This arrangement of the rRNA genes is called as nucleolar organizing region (NOR).

Each repeat unit of 43kb has 13-14kb coding region for rRNA while the remaining 30kb separates it from the next transcription unit and is called as intergenic spacer (IGS). The IGS contains various non-coding elements important for the transcription such as gene promoter, enhancer elements and terminator sequences.

During transcription, the rRNA containing regions are spread out in the FC region like loops on which transcription takes place.

The ribosomes in eukaryotes have one copy each of 4 different rRNAs viz. 28S, 18S, 5.8S and 5S.

The first three are synthesized from a single 45S precursor rRNA in the FC of nucleolus while the 5S rRNA is synthesized in the nucleoplasm of the nucleus and from a different cluster of genes. The 5S rRNA later diffuses into the GC region of the nucleolus to participate in the ribosome assembly process.

For the synthesis of 45S rRNA we need RNA pol I for transcription and for the synthesis of the 5S rRNA we need RNA pol III for the transcription.

The RNA pol I does not need TATA box sequence in its promoter but it needs other DNA based regulatory elements. These elements are divided into two parts:

1. CORE: They are located next to the transcription start site and located between -45 to +20 region.
2. UCE: It stands for upstream control elements and they are located between -200 to -107 region.

The transcription by RNA pol I needs formation of a pre-initiation complex (PIC) and this is similar to the RNA pol II.

The PIC is composed of RNA pol I, upstream binding factor (UBF) and promoter selectivity factor (SL1) and is formed at the promoter of the RNA pol I. The formation of PIC for RNA pol I is discussed below:

- UBF undergoes dimerization and binds to the UCE and CORE.
- This dimer form of UBF introduces loops in the upstream region so that UCE and CORE elements come into contact.
- The structure so formed is called as enhanceosome and it allows binding of SL1.
- Now, it recruits the SL1. SL1 is made of TATA-binding protein (TBP) and TBP-associated factors (TAFs).
- Recruitment of SL1 stabilizes the binding of the UBF and helps create a stable PIC.
- The TAFs interact with the UBF and RNA pol I. The interaction with RNA pol I is via hRRN3 protein. This helps recruit the RNA pol I to the promoter.
- Now, PIC is complete.

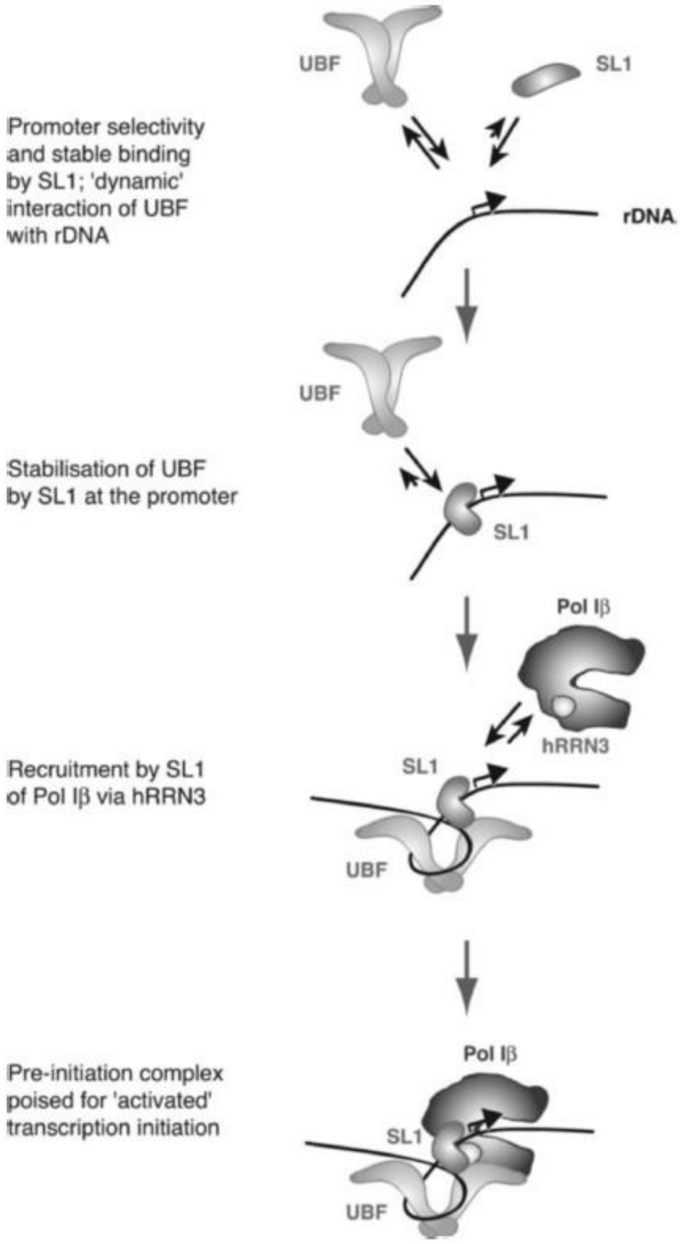


Figure: Formation of RNA pol I PIC in humans.

All the steps described above are shown here.

RNA pol Iβ means transcriptionally active RNA pol I while those which are transcriptionally inactive are called as RNA pol Iα.

hRRN3, the human homologue of a yeast Pol I transcription factor.

rDNA means the genes for rRNA.

If UBF or hRRN3 (also similar proteins in the lower eukaryotes) are deleted or silenced, then genes for rRNA also become silent and no rRNA synthesis takes place.

When transcription is initiated and elongation is on, UBF and SL1 remain associated with the promoter region while RNA pol I has left. This allows them to recruit another RNA pol I at the promoter so that each rRNA gene can be synthesized multiple times simultaneously. This gives rise to the Christmas tree like structures seen under electron microscope. **This is also a major difference between transcriptional behavior of RNA pol I and pol II.**

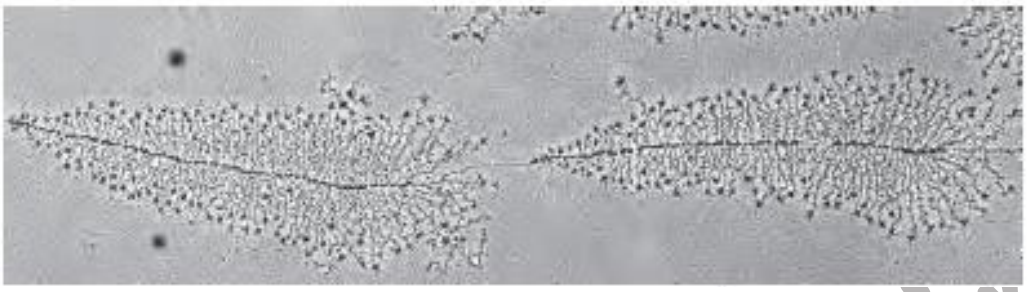


Figure: Transcription of rRNA genes by RNA pol I being visualized as Christmas tree like structure in EM.

The RNA pol I lacks the C-terminal tail found in RNA pol II. Due to this reason the rRNA transcripts produced by RNA pol I are not capped or polyadenylated. **This is another major difference between transcriptional behavior of RNA pol I and RNA pol II.**

When RNA pol I has completed the transcription process we get the precursor rRNA. This precursor undergoes extensive modifications and then 28S, 18S and 5.8S rRNAs are cleaved out of the precursor.

The modifications carried out include approx. 100 methylations of the 2'-OH positions on ribose sugar and similar number of isomerizations of uridine base in the nucleotides to pseudouridine.

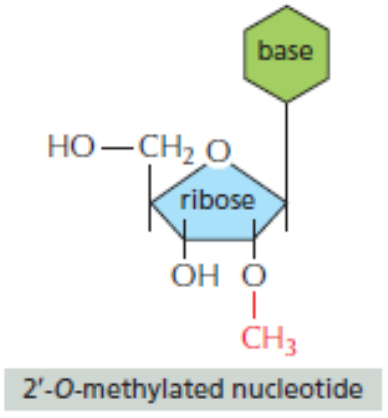


Figure: Modifications in the ribose sugar of the nucleotides in rRNA.

The modification is shown here at 2'-OH and is coloured red.

As mentioned in the text, modification shown here is methylation.

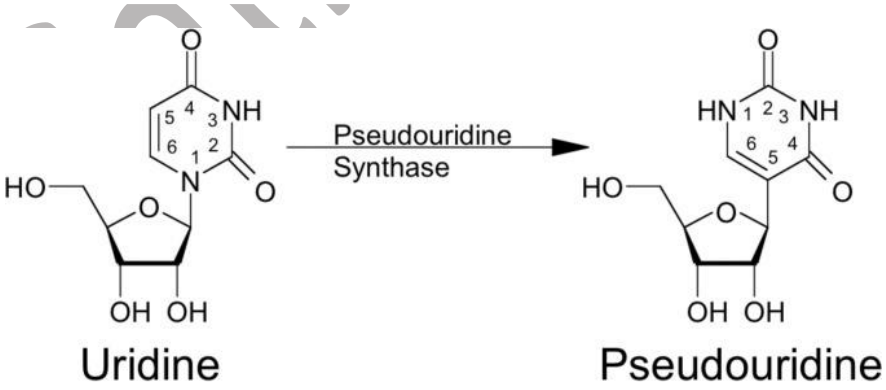


Figure: Pseudouridine is an isomer of uridine.

In uridine, the base is attached to the ribose at N_1 while in pseudouridine base is attached to the sugar via C_5.

These modifications help in the folding and assembly of the rRNAs.

The modifications are made at specific positions in the precursor with the help of guide RNAs who position themselves on the precursor via base pairing and then help recruit an RNA modifying enzyme at exact position. Pseudouridine synthase shown in the figure above is one such enzyme.

Another set of guide RNAs cause conformational changes in the precursor which exposes the cleavage sites to the nucleases. Thus, they help in the formation of the final products from the precursor.

These guide RNAs are examples of small nucleolar RNAs or snoRNAs. They are called so because they perform their function in the nucleolus. Many snoRNAs are coded by the introns of the other genes especially those coding for ribosomal proteins. Thus, they are synthesized by RNA pol II and then processed from the intron sequence after its removal during splicing.

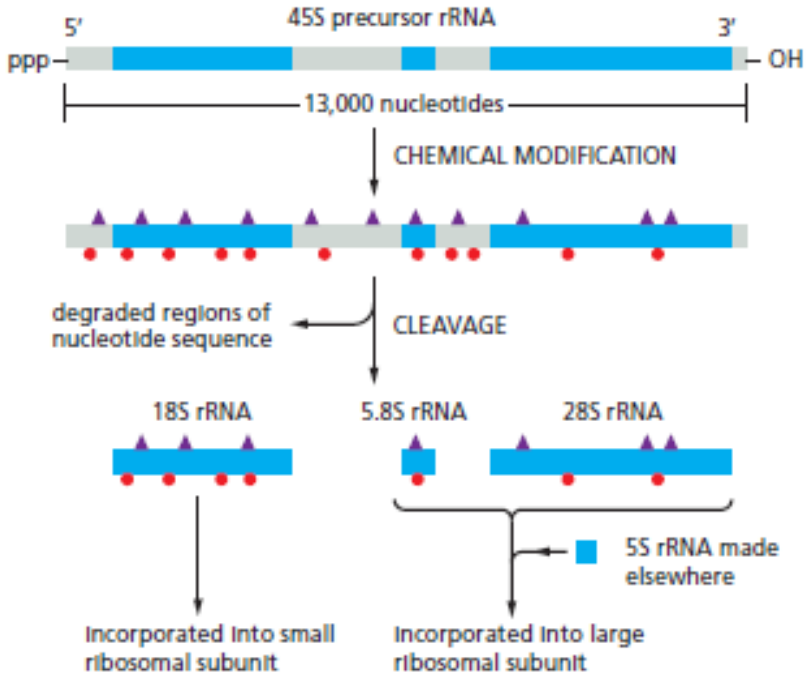


Figure: The chemical modifications and nucleolytic cleavage of the precursor rRNA into 3 different rRNAs.

RNA pol III is specialized for transcription of short, abundant, non-coding RNAs. It is responsible for transcription of 5S rRNAs, tRNAs and many other essential RNAs such as U6 snRNA and RNA components of signal recognition particle and RNase P.

RNA pol III is multi-subunit enzyme like other RNA pols and for its function it uses 3 different transcription factors i.e. TFIIIA, TFIIIB and TFIIIC.

- TFIIIA is specific for transcription of 5S rRNA gene RDN5.
- TFIIIB is composed of Brf1, Bdp1 and TBP.
- TFIIIC is responsible for recognition of promoter elements of all the genes transcribed by RNA pol III.

The promoters for RNA pol III in most of its target genes are present in the transcribed region itself, e.g. tRNA genes have conserved internal promoter elements called as A box and B box and they are represented in the tRNA structure as D loop and T loop respectively.

In case of 5S rRNA gene, internal promoter elements consist of A box and gene specific C box which recognizes TFIIIA for the transcription of the 5S rRNA gene.

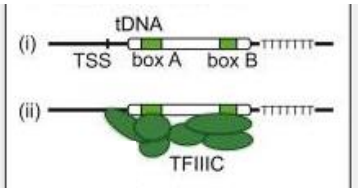


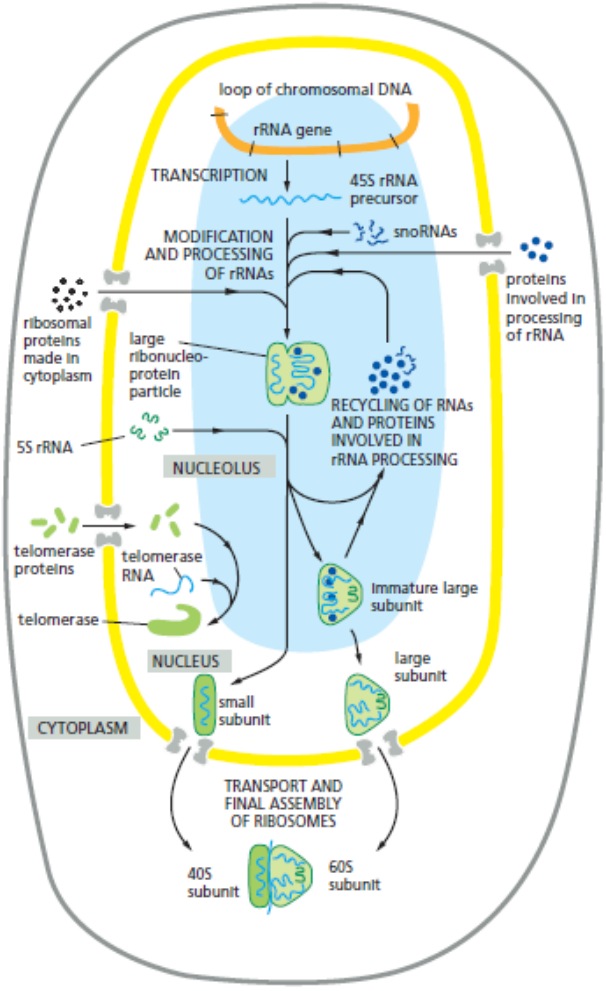
Figure: Internal promoter elements in tRNA and 5S rRNA genes respectively.

Ribosome assembly: The assembly of ribosomes can be described in the following steps:

1. Formation of 90S pre-ribosome complex. It consists of precursor rRNA, 5S rRNA, ribosomal proteins and about 150 non ribosomal proteins involved in the processing and maturation processes. Examples of non-ribosomal proteins in the complex are endo- and exonucleases, pseudouridine synthases, methyltransferases, RNA chaperons, GTPases, AAA-ATPase helicases etc.
2. This 90S complex is mainly located in the DFC region.
3. This complex is then resolved into pre-40S and pre-60S subunits in the GC region.
4. This is the form in which ribosome subunits are exported to the cytosol.
5. In cytosol, additional processing causes them to mature in small 40S and large 60S ribosome subunits.

The 40S subunit contains 18S rRNA and 33 ribosomal proteins and 60S subunit contains 28S, 5.8S and 5S rRNA and 49 ribosomal proteins.

The complete discussion is summarized in the figure below:



Nucleolus and cell cycle: As the cells enter prophase along with the nucleus we find dissociation of the nucleolus as well. This happens because chromatin is condensed and no transcription is possible. When nuclear division is almost over and cells are in telophase we see nucleolar association as several small clusters. As the cells reach mid G_1 and progress to S-phase we find that clusters have merged to form one large nucleolus which remains till the end of G_2 phase.