Eukaryotic Gene Expression: Basics & Benefits

P N RANGARAJAN

Lecture 13

Eukaryotic gene regulation:
Synthesis of ribosomal RNA by RNA polymerase I

Almost 50% of nascent RNA synthesis in a cell is accounted for by the transcription of ribosomal RNA (rRNA) genes which produce ribosomal RNAs (rRNAs) that become key components of ribosomes.

Transcription of ribosomal genes occurs in the nucleolus, which is the site of ribosome biogenesis.

There are several hundred copies of rRNA genes in a eukaryotic cell nucleus and they are arranged in clusters as tandem head-to-tail repeats and constitute the nucleolar organizing regions (NORs)

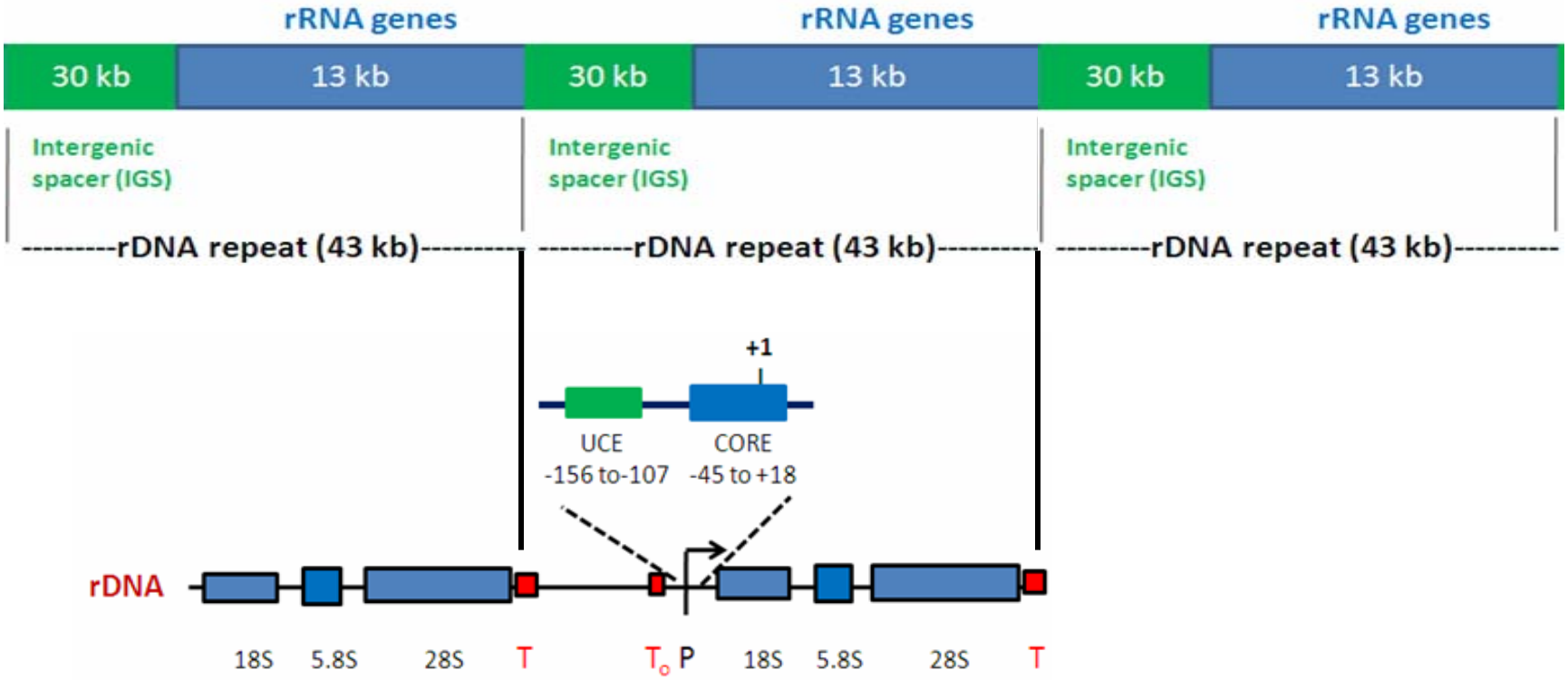
It estimated that in a cultured human HeLa cells, ~14,000 ribosomal subunits leave the nucleoli per minute.

The nucleolar RNA polymerase I activity in eukaryotic cells is harmonized with that of nucleoplasmic (extranucleolar) activities of RNA polymerase III and RNA polymerase II synthesizing 5S rRNA and mRNA for more than 70 ribosomal proteins, respectively.

Thus, to make a functional ribosome, all three RNA polymerases have to work together.

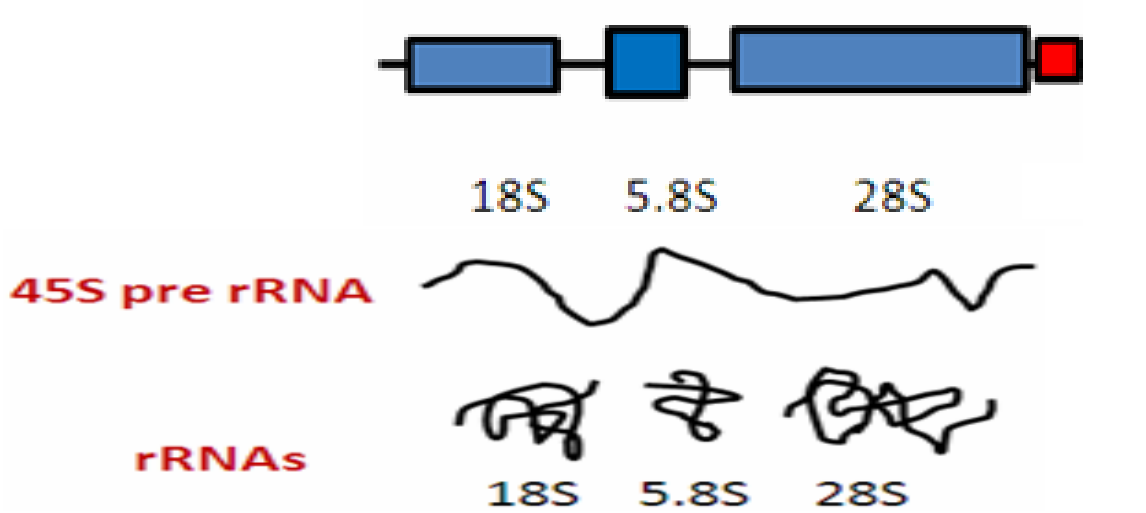
Organization of rRNA genes

Several hundred copies of rRNA genes are arranged in clusters as tandem head-to-tail repeats



RNA Pol I initiates transcription at the human rDNA promoter (P), which contains an core promoter element (−45 to +18) and an upstream control element (UCE, −156 to −107).

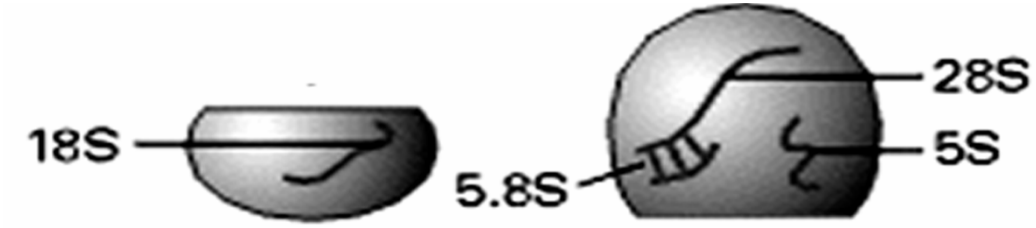
Several transcription-termination elements are present at the 3′ end of the transcribed region of the rRNA genes (T) and immediately upstream of the rRNA gene transcription start site (T_0), between the spacer and gene promoters.



The primary rRNA transcript (45S prerRNA) synthesized by mammalian RNA polymerase I is processed into the mature 18S, 5.8S and 28S rRNAs.

These three rRNAs, together with the 5S rRNA transcribed by RNA polymerase III, constitute the RNA components of the ribosome.

The 28S and 5.8S rRNAs associate with the large (60S) ribosomal subunit while the 18S rRNA associates with the small (40S) ribosomal subunit.



40S 60S

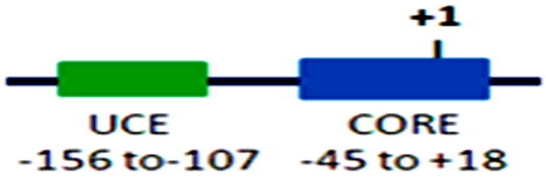
ribosomal subunits

Just as protein coding genes are transcribed by RNA Pol II, rDNA is transcribed by RNA Pol I.

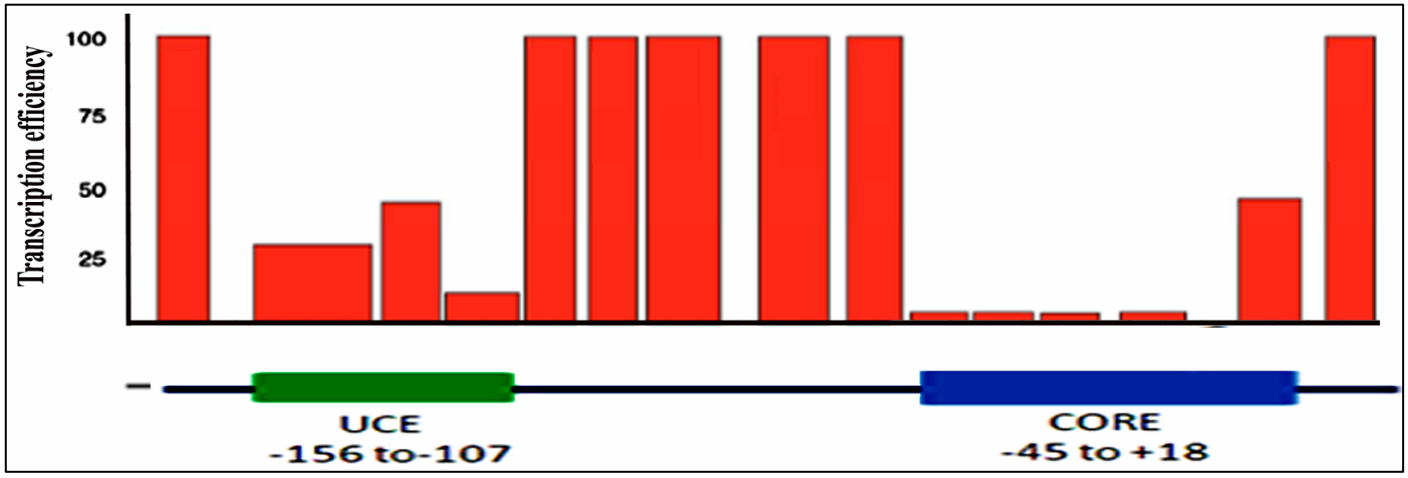
Yeast RNA Pol I comprises 14 subunits, and there are mammalian homologues for all of these except one of the yeast RNA Pol I subunits, the RPA14

As observed in case of RNA pol II, PolI transcription also requires polymerase I-specific transcription factors for promoter recognition

THE CIS-ACTING ELEMENTS OF POL I PROMOTER



The core promoter is GC-rich except for a short stretch of AT-rich region around The transcription start site (Initiator). The core promoter is sufficient for basal levels of transcription but the efficiency is increased by the UCE which Is also rich in GC residues.



THE TRANS-ACTING FACTORS OF POL I PROMOTER

Activation of transcription from Pol I promoter requires two transcription factors:

1. Core binding factor

In humans, a tetrameric protein known as SELECTIVITY FACTOR OR SL1 (*a.k.a.* TIF-IB (transcription initiation factor-IB in mouse, rib1 etc.) binds to the core promoter.

SL1 contains TBP and at least three TBP-associated factors includingTAFI110, TAFI63 and TAFI48.

Thus, TBP is require for not only Pol II but also for Pol I transcription. However, the TAFs present in SL1 are different from those present in TFIID. SL1 cannot support Pol II transcription and TFIID cannot support Pol I transcription.

2. Upstream binding factor (UBF)

UBF is a single polypeptide that binds to the GC-rich region of the UCE.

Interestingly, the spacing between the UCE and core promoter has a key role in pol I transcription.

This spacing can be changed by distances involving integral numbers of turns of DNA but not by distances that introduce half turns.

This ensures that the UBF and core binding factor bind on the same side of DNA helix and interact with each other.

Recombinant mammalian SL1 that comprises only TBP and the three TAFs (TAF_I110, TAF_I63 and TAF_I48) does not support efficient promoter-specific RNA pol I transcription in an in vitro transcription system.

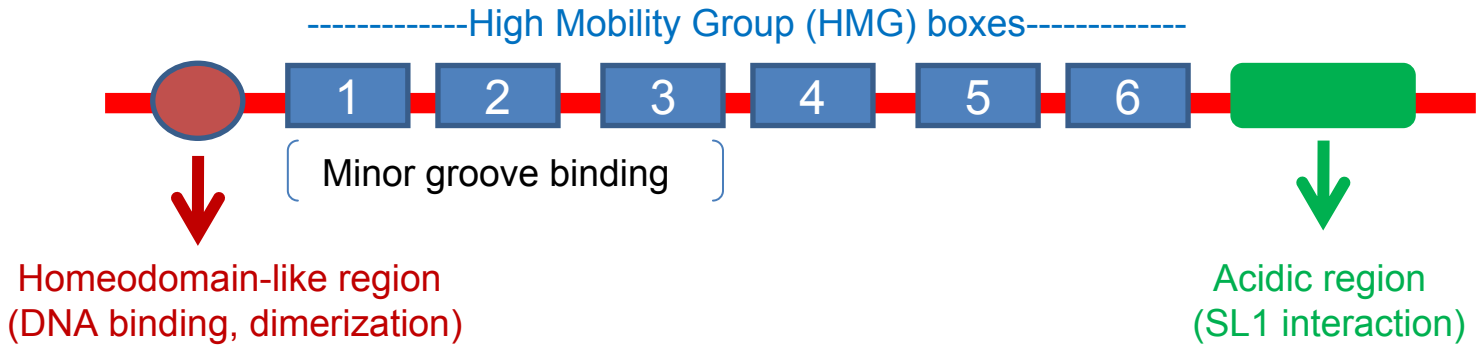
Interaction of RNA Pol I with a protein called RRN3 (TIF-IA in mouse) is required for the assembly of preinitiation complex.

TIF-IA (RRN3) knockout mice survive until day 9.5, but the embryos are small because they contain fewer cells.

High levels of transcription requires the interaction of SL1 with the highly acidic C terminal region of UBF. TAF_I48 and TBP are also involved in these interactions.

UBF1 (97 kDa, 764 amino acids)

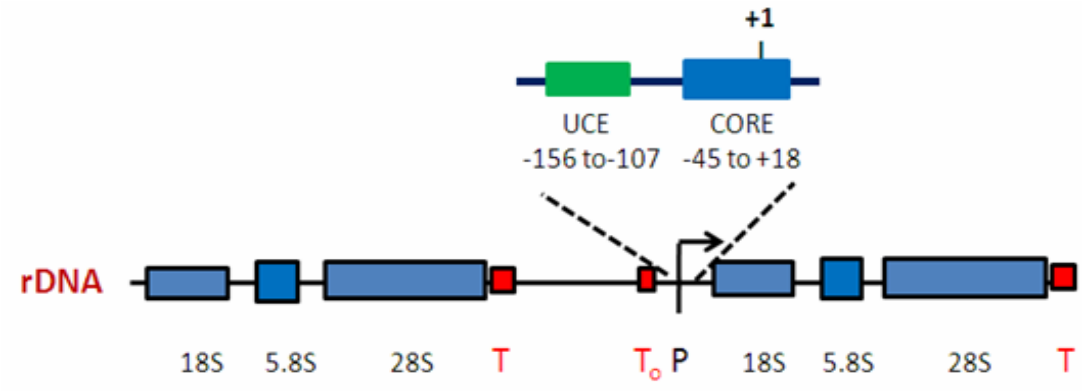
HMG boxes share significant homology to w80 amino acid domains in the nuclear non-histone HMG proteins HMG1 and HMG2, which are key structural elements of chromatin and chromosomes



UBF is involved in the formation of an ‘enhancesome’, in which a dimer of UBF organizes ~140 base pairs of enhancer DNA into a single 360^{o} turn as a result of six in-phase bends generated by three of the six HMG boxes in each UBF monomer.

Formation of such structures at the UCE and at the core would juxtapose these precisely spaced promoter elements, thereby, presumably, supporting interaction between SL1 and UBF.

Interaction of UBF with individual protein components of the pol I machinery at sites other than the promoter throughout the rDNA repeat might alter the chromatin structure and enable access of such factors to the promoter and/or increase the local concentration of such factors, and thereby indirectly enhance the efficiency of PIC formation in vivo.



A protein known as transcription termination factor I (TTF-I) binds to the terminator sequence upstream of the rRNA gene promoter

Interestingly, TTF-I plays an important role in the assembly of preinitiation complex.

TTF-I interacts with p300/CBP, a HAT which acetylates the TAFI68 subunit of TIF-IB (SL1) and thereby enhance the binding of this SL1 subunit to the rDNA promoter

TTF-1 is not only involved in the termination of transcription, but also in the remodelling of ribosomal chromatin leading eventually to the silencing of the rRNA gene

The RNA polymerase I (pol I) transcription cycle:

pre-initiation complex formation (PIC)
transcription initiation
promoter escape and clearance
elongation
termination
reinitiation

Key steps in the RNA polymerase I (pol I) transcription

Preinitiation complex formation involves binding of selectivity factor 1 (SL1) to the rDNA promoter, incorporation of upstream binding protein (UBF) and recruitment of Pol Ib by SL1.

Pol I then initiates transcription and following promoter escape, pol I is converted into a processive enzyme (pol Iε), which elongates the nascent rRNA

As pol I escapes and clears the promoter, UBF and SL1 remain bound to promoter *in vitro*, enabling recruitment of another pol I complex leading to reinitiation of transcription from the same promoter, facilitating multiple rounds of transcription.

Transcription by pol I terminates at the 3' end of the gene at specific sequences bound by termination factor TTF-I and PTRF (Pol I & transcript-release factor) with the concomitant release of pol I and the nascent rRNA.

Regulation of rDNA transcription by covalent modification of transcription factors

The rate of cell growth and proliferation is directly proportional to the rate of protein synthesis, which is intricately linked to ribosome biogenesis and controlled at the level of rDNA transcription by RNA pol I.

In mammalian cells, rDNA transcription is regulated by cell cycle. Transcription is absent during mitosis and gradually increases during G1, peaking in the S and G2 phases of cell cycle.

During mitosis, the nucleoli disassemble and thus rDNA transcription is inhibited. At the end of mitosis, nucleoli reform and rRNA synthesis resumes.

The phosphorylation status of SL1 fluctuates during the cell cycle. Phosphorylation of the SL1 subunit TAFI110 by certain cell-cycle specific protein kinases such (cdc2-cyclin B) during metaphase correlates with the inactivation of SL1. As a result, SL1 can no longer interact with UBF leading to mitotic repression of rDNA transcription.

The activity of many other factors involved in Pol I transcription such as TTF1, RRN3, UBF is also regulated by phosphorylation during different phases of cell cycle as well as during different phases of growth such as log phase, stationary phase etc.

Epigenetic regulation of rDNA transcription (rDNA silencing)

The transcription termination factor, TTF-I recruits the ATP-dependent nucleolar remodelling complex (NoRC),

DNA methyltransferases (Dnm1, Dnm3b) and histone deacetylases (HDAC1) interact with NoRC, resulting in de novo DNA methylation and histone deacetylation.

Methylation of rDNA promoter prevents UBF binding leading to repression of transcription.

Methylation of histone H3 on lysine 9 and subsequent binding of heterochromatin protein HP1 ultimately help to condense the nucleosomal DNA into silenced heterochromatin

Treatment of cells with inhibitors of cytosine methylation and histone deacetylation can lead to derepression of rDNA transcription.

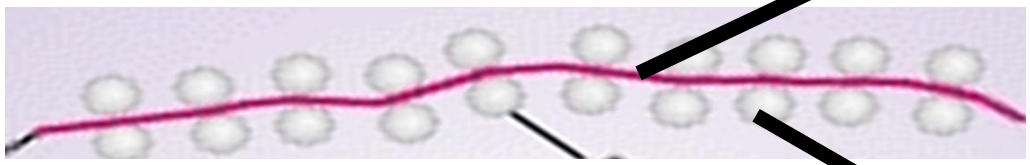
Processing of pre-rRNAs and assembly of ribosomes

Following their synthesis in the nucleolus, nascent pre-rRNAs are immediately bound by proteins to form pre-ribonucleoprotein particles, or pre-rRNPs.

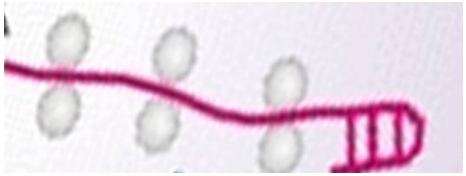
During processing, pre-rRNA undergoes modifications such as methylation of the 2′-hydroxyl group of specific riboses and conversion of specific uridine residues to pseudouridine.

The positions of cleavage sites in pre-rRNA and the 2′-*O*-methylation pattern and pseudouridine formation are determined by ~150 different small nucleolus-restricted RNAs called **small nucleolar RNAs (snoRNAs),** which hybridize transiently to pre-rRNAs.

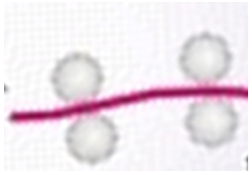
Processing of pre-rRNPs



80S pre-rRNP

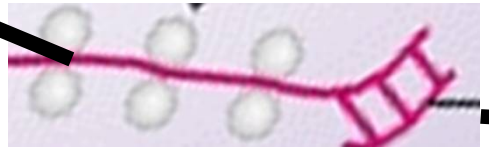


32S rRNA

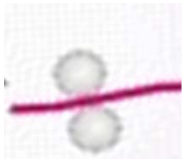


20S rRNA

28S rRNA

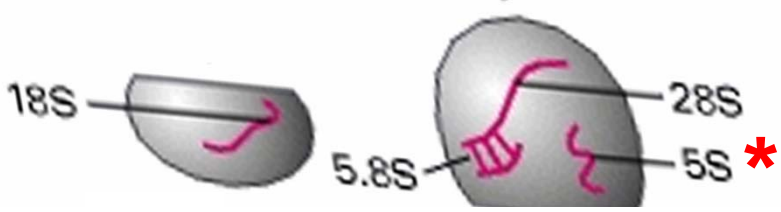


5.8S rRNA



18S rRNA

40S & 60S ribosomal subumits



* **synthesized by RNA pol III**

The number of active rRNA genes varies between different cell types with the same genotype.

During cellular differentiation (spermatogenesis for example), there is a decrease in rRNA synthesis.

By contrast, stimulation of proliferation of lymphocytes causes an increase in rRNA synthesis with an increase in the number of active rRNA genes.

Approximately 50% of the rRNA genes in each yeast cell are transcribed during log phase, whereas, during the stationary phase, the percentage of actively transcribed rRNA genes is reduced owing to changes in chromatin.

Histone deacetylation mediated by Rpd3–Sin3 histone deacetylase (HDAC) is involved in the inactivation of rDNA transcription.

Yeast cells engineered to contain 40 copies of the rRNA gene produce the same levels of rRNAs and grow at a rate similar to cells containing with the copies, of which ~75 are active.

This is achieved by increasing the frequency of transcription initiation events on each gene.

Cell growth (increase in size and mass) and cell proliferation (increase in cell number) are intricately linked in most cells.

Cell growth without cell division leads to the production of large cells with a single nucleus. In such cells there is an increase in rRNA, ribosomes and protein synthesis.

rDNA transcription is also increased several fold in cancer cells. rRNA is produced at abnormally high levels in almost every tumour type investigated indicating that overexpression of rRNA may be a general feature of all types of cancer cells.

When cells are treated with chemicals that block rDNA transcription, it results in cell cycle arrest

Regulation of Pol I transcription by oncogenes and tumour suppressors.

	Pol I transcription
ONCOPROTEINS	
c-Myc	activation
Ras	activation
Raf	activation
TUMOUR SUPPRESSORS	
RB	inhibition
P53	inhibition

Visualization of ribosomal genes by Miller spreads

The molecular organization of active ribosomal genes in the form of Christmas trees was described by Miller and collaborators on nucleolar spreads from amphibian oocytes more than 40 years ago

Miller Jr., O.L., Beatty, B.R., 1969. Visualization of nucleolar genes. Science 164, 955–957.