Eukaryotic Gene Expression: Basics & Benefits

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Lecture 14

Eukaryotic gene regulation: Regulation of tRNA and 5S rRNA synthesis by RNA polymerase III

Eukaryotic Gene Expression: Basics & Benefits

So far……..

1. Eukaryotic RNA polymerases and basal transcription factors
2. Diversity in core promoter elements
3. Diversity in general transcription factors
4. Proximal & Distal Promoter Elements, Enhancers and Silencers, Gene-specific Regulators
5. Transcription factors – DNA binding domains
6. Transcription factors – transcription activation domains
7. Role of chromatin in eukaryotic gene regulation
8. Role of histones in eukaryotic gene regulation
9. Role of DNA methylation in eukaryotic gene regulation
10. Chromatin remodelling & gene regulation
11. mRNA processing – Role of RNA Pol II in mRNA capping and mRNA splicing
12. mRNA processing – Role of RNA Pol II in polyadenylation mRNA editing

Regulation of mRNA synthesis

13. Regulation of RNA Pol I transcription

Regulation of rRNA synthesis

14. Regulation of RNA Pol III transcription

Regulation of tRNA & 5S rRNA synthesis

RNA Pol III

In *S. cerevisiae*, 17 genes encode the various subnunits of RNA Pol III

Five of these constitute the core subunits of RNA Pol III:

1. C160 (homologue of Pol I 190; Pol II 220; bacerial β')
2. C128 (Pol I 110; Pol II 150; bacterial β)
3. C40 is referred to as AC40 as it is common to Pol I and Pol III
   (Pol II B45 (Rpb3), Bacterial α)
4. AC19 (Pol II B12.5 (Rpb11)
5. ABC23 (Rpb6, bacterial ω)

RNA polymerase III transcribes a set of genes whose main common features are that they encode structural or catalytic RNAs and in general, they are shorter than 400 base pairs.

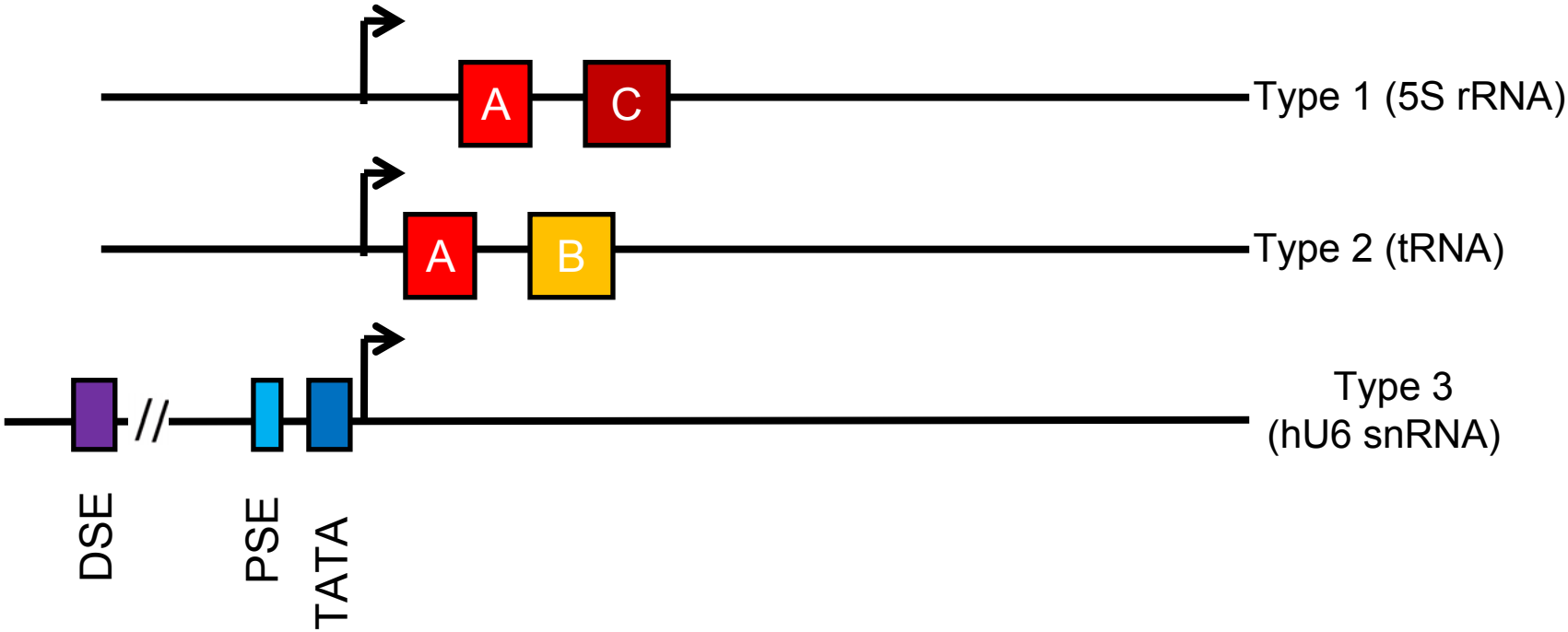
These include:

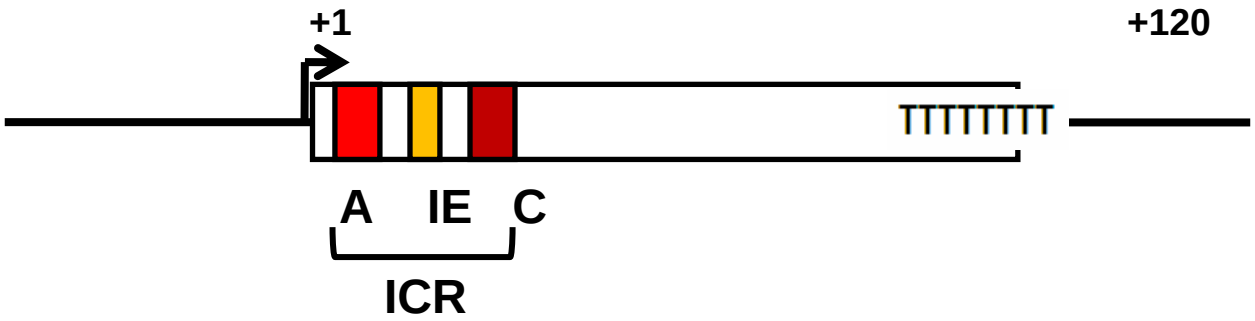
Transfer RNAs
5S ribosomal RNA
U6 spliceosomal RNA
RNase P and RNase MRP RNA
7SL RNA (the RNA component of the signal recognition particle)
Vault RNAs
Y RNA
SINEs (short interspersed repetitive elements)
7SK RNA
Several microRNAs
Several small nucleolar RNAs
Several gene regulatory antisense RNAs

A unique feature of RNA Pol III promoters is that they can be either intragenic or extragenic.

The intragenic promoters are generally TATA-less while the extragenic promoters generally contain a TATA box.

THREE TYPES OF POL III PROMOTERS



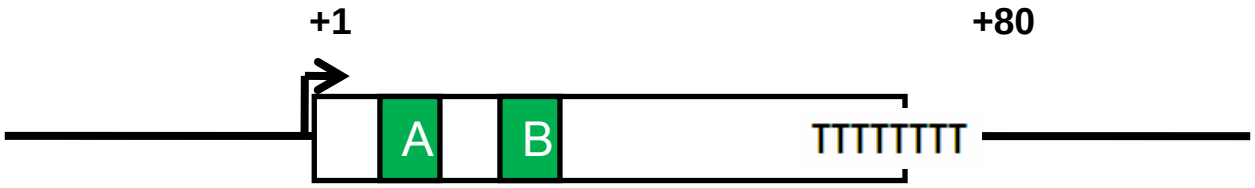


The Pol III promoter of the *Xenopus laevis* 5S RNA gene encoding 5S ribosomal RNA (Type 1)

The main cis-acting elements are:

An internal control region (ICR)
comprising of an
A box (+50 to +64),
intermediate element (IE, +67 to +72),
C box (+80 to +90).

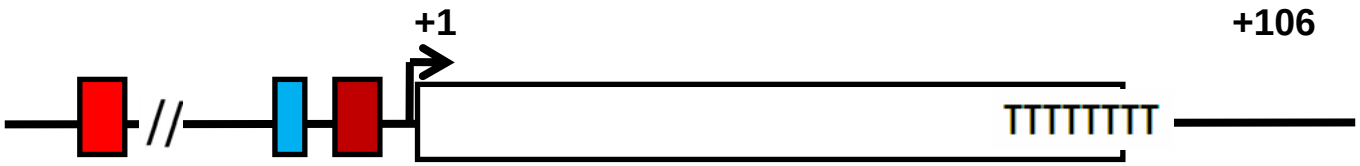
A transcription termination signal consisting
of a simple run of T residues.



The Pol III promoter of the *X. laevis* tRNALeu gene (type 2)

The major cis-acting elements are:
A box (+8 to +19)
B box (+52 to +62)

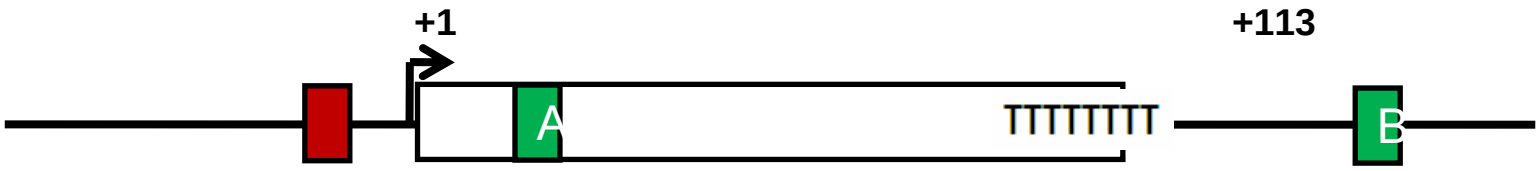
A transcription termination signal consisting
of a simple run of T residues.



The Pol III promoter of human U*6 snRNA gene (Type 3)*

The cis-acting elements are:
Distal sequence element (DSE, −215 to −240),
a core promoter consisting of a
proximal sequence element (PSE, −65 to −48)
and a
TATA box (−32 to −25).

A transcription termination signal consisting
of a simple run of T residues.



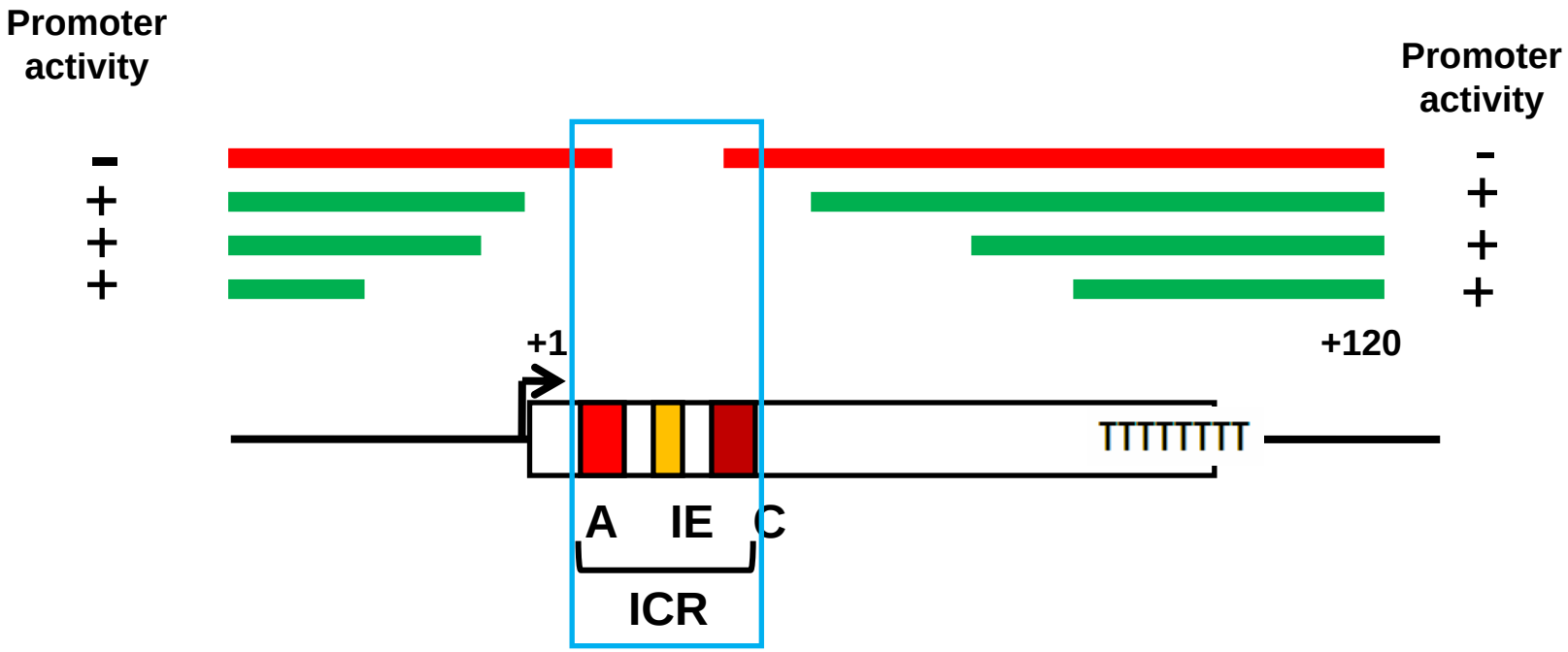
The Pol III promoter of *Saccharomyces cerevisiae* U6 snRNA gene (hybrid)

The cis-acting elements are intragenic as well as extragenic:

a TATA box (−30 to −23),
an A box (+21 to +31), and a B box located downstream of the
U6 coding region (from +234 to +244)

A transcription termination signal consisting
of a simple run of T residues.

Identification of ICRs by deletion studies



The Pol III promoter of the *Xenopus laevis* 5S RNA gene encoding 5S ribosomal RNA (Type 1)

Transcription factors involved in the regulation of Pol III transcription

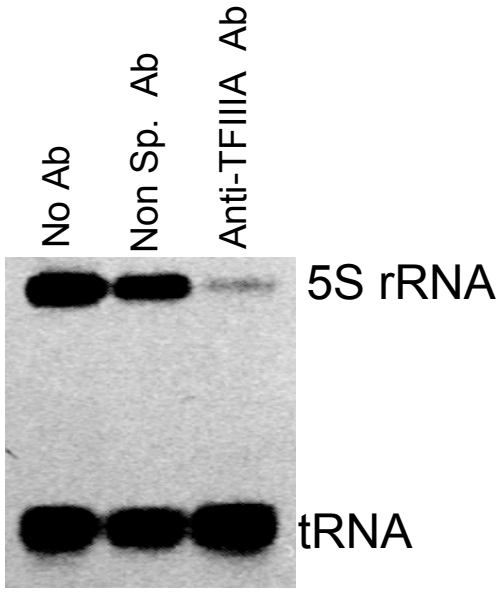
TFIIIA, TFIIIB, TFIIIC

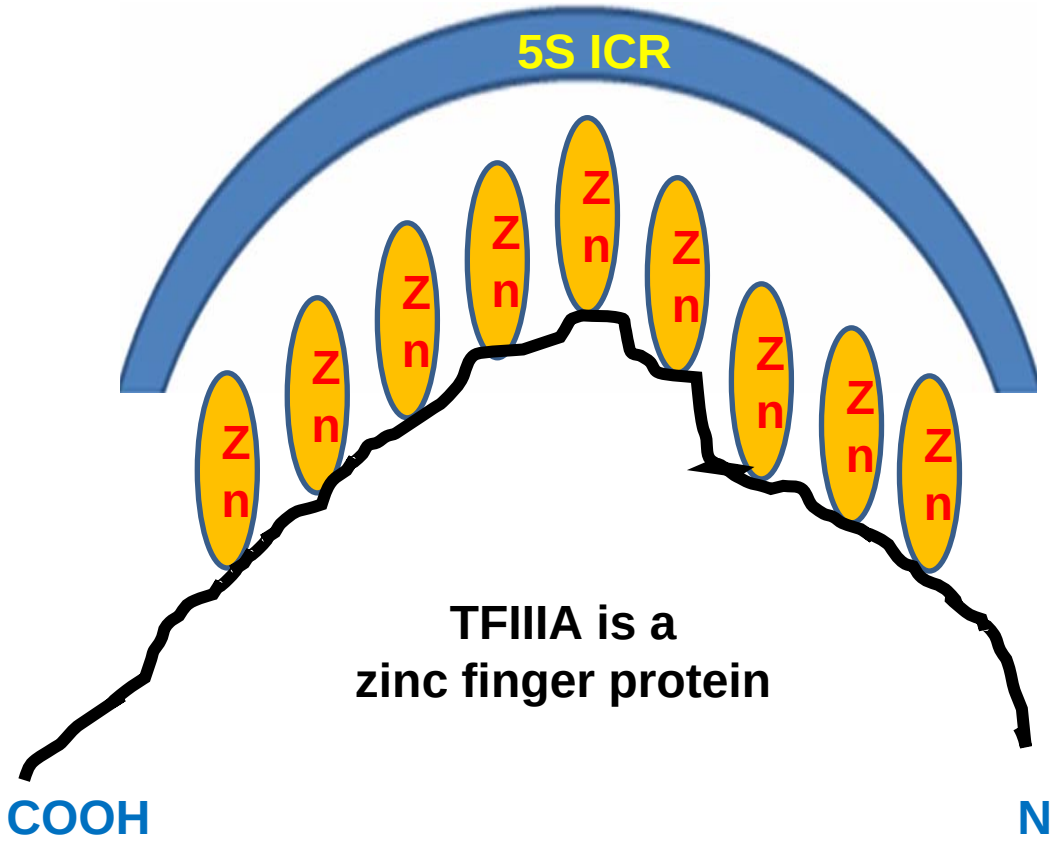
Type 1 promoters (5s rRNA) use TFIIIIA, TFIIIIB and TFIIIIC

Type 2 promoters use TFIIIC and TFIIIB

Thus, TFIIIA is required for the synthesis of 5sRNA, but not tRNA

TFIIIB and C are required by both 5srRNA gene and tRNA gene





The TFIIIA consists of nine tandem sequences that are closely spaced and together constitute NINE C_2H_2 zinc finger motifs

(Tyr, Phe)-X-Cys-X2,4-Cys-X3-Phe-X5-Leu-X2-His-X3,4-His-X2-6,

(where X is a variable residue).

Zinc fingers are discussed in detail in LECTURE # 5

TFIIIA, is the first eukaryotic transcription factor purified to homogeneity

Xenopus transcription factor IIIA (XTFIIIA): after a decade of research.
Shastry BS.
Prog Biophys Mol Biol. 1991;56(2):135-44. Review.

Structure, function, evolution of transcription factor IIIA.
Hanas JS, Gaskins CJ, Smith JF, Ogilvie MK.
Prog Nucleic Acid Res Mol Biol. 1992;43:205-39. Review.

Transcription factor IIIA (TFIIIA): an update.
Shastry BS.
Experientia. 1993 Oct 15;49(10):831-5. Review.

TFIIIA: nine fingers--three hands?
Pieler T, Theunissen O.
Trends Biochem Sci. 1993 Jun;18(6):226-30. Review.

Transcription initiation at Pol III promoter involves the assembly of

TFIIIA

and

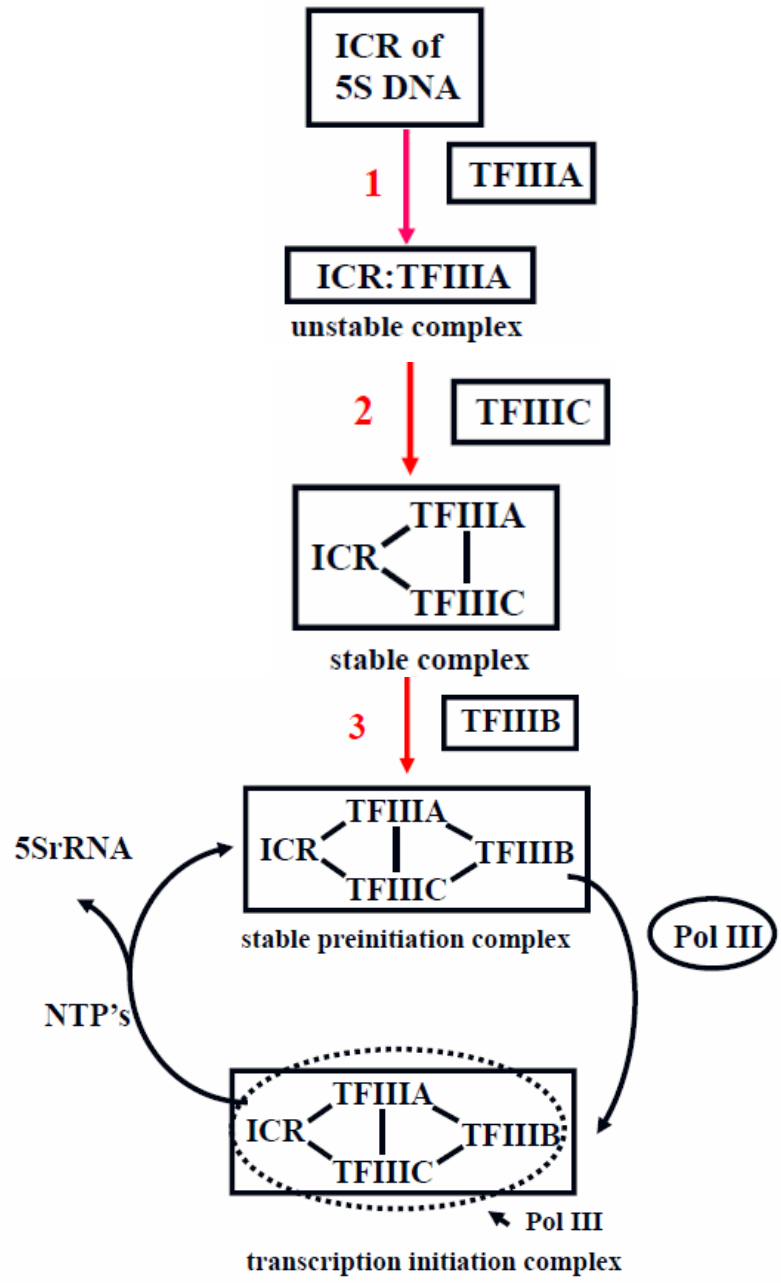
TFIIIC,

the initiation factor TFIIIB
(TBP, Bdp1(B”), Brf1),

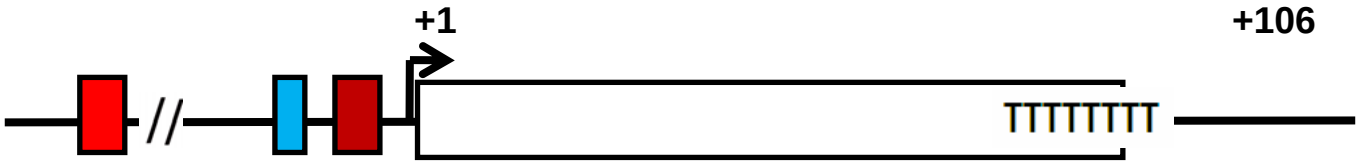
and

RNA Polymerase III

Ordered Binding of Transcription Factors to the promoter of 5S rRNA Gene



The Pol III promoter of human U6 *snRNA gene (Type 3)*



The cis-acting elements are:

Distal sequence element (DSE, −215 to −240), a core promoter consisting of a proximal sequence element (PSE, −65 to −48) and a TATA box (−32 to −25).

A transcription termination signal consisting of a simple run of T residues.

Trans-acting factors binding to U6 snRNA promoter

- Oct-1 and Staf/ZNF/SBF bind to DSE
- SNAPc (the snRNA activator protein complex) also known as PTF (the PSE-associated transcription factor) binds to PSE
- TBP binds at the TATA box

Schramm L and Hernandez N (2002)

Recruitment of RNA polymerase III to its target promoters.

Genes & Development 16:2593-620.

Termination of Pol III transcription

Pol III is termination-prone, consistent with its role in producing short transcripts.

Short stretches of U residues can lead to termination:
For ex., U4 in case of Xenopus and human pol III,
U4 to U5 for S. pombe pol III
U5-6 for S cerevisiae pol III

The C11 subunit of RNA Pol III is essential for termination since if the C11 subunit is missing, termination effciency decreases strongly.

Thus, C11 subunit can be regarded as the Pol III transcription termination factor

In addition to transcribing genes encoding tRNAs, 5S rRNA and U6 RNAs, Pol III also transcribes a number of other genes. Some of these are:

RNase P RNA. The RNA component of RNase P (an endoribonuclease) that processes the 5' termini of pre-tRNAs).

RNase MRP RNA. The RNase mitochondrial RNA processing (MRP) is an RNA-protein endoribonuclease that processes RNA primers of DNA replication in the mitochondrion. MRP is also present in the nucleolus, where it participates in pre-rRNA processing.

7SL RNA. The 7SL RNA, forms the scaffold of the signal recognition particle.

7SK RNA. The 7SK RNA binds to the transcription elongation factor P-TEFb and represses Pol II transcription.

Vault RNAs. Vaults are very large cytoplasmic RNPs implicated in multidrug resistance of human tumors and are thought to be involved in macromolecular assembly and/or transport.

Y RNAs. These are small RNA components of the Ro ribonucleoprotein particle (RoRNP) which is a target of autoimmune antibodies in patients with systemic lupus erythematosus (SLE)

BC1 and BC200 RNAs. These are rodent- and primate-specific RNAs specifically expressed in neural cells and are part of cytoplasmic RNPs with a possible role in translation of dendritic mRNAs.

Virus-encoded RNAs. The VA-I and VA-II RNAs of adenovirus act by inhibiting the RNA-activated protein kinase (PKR) in response to viral infection, thus allowing the translation of adenoviral mRNAs.

Short interspersed repeated DNA elements (SINEs)-encoded RNAs. SINEs are nonautonomous retroposons originating from Pol III–transcribed genes.

SUMMARY

Synthesis of tRNA by RNA Pol III machinery involves the use of promoter elements located within the transcribed region of tRNA genes.

In most tRNA genes transcribed by RNA Pol III, these internal promoter elements are known as: box A and box B .

Two transcription factors, TFIIIB and TFIIIC are required for the initiation of transcription by RNA Pol III from promoters of tRNA genes.

In case of 5S rRNA genes, instead of box B, box C and an intermediate element are present and the transcription factor TFIIIA is required, in addition to TFIIIB and TFIIIC.

TFIIIB recruits Pol III and promotes transcription initiation and reinitiation.

One of the components of TFIIIB is the TATA-box binding protein (TBP) or a TBP-related factor (Ex. *D. melanogaster* TRF1) that interacts with a TATA-like sequence element present around position -30 of several Pol III promoters.

In addition to TATA box, the key upstream promoter elements of class III genes are:

i) Proximal sequence element (PSE) located 20 bp upstream of TATA box that interacts with a multisubunit factor called SNAPc / PBP / PTF.

ii)Distal sequence element (DSE), located ~ 200 bp upstream of TATA box binds to transcription factors such as Staf and Oct1 and further enhances the rate of pol III transcription

TFIIIA ,TFIIIC, TFIIIB and RNA Polymerase III

Pol III is recruited to the promoter by TFIIIB

TFIIIC is composed of six subunits and it recruits TFIIIB to its DNA sites upstream of the transcriptional start site.

TFIIIA, the 5 S rRNA gene-specific DNA-binding protein, forms the platform for binding TFIIIC to these genes.

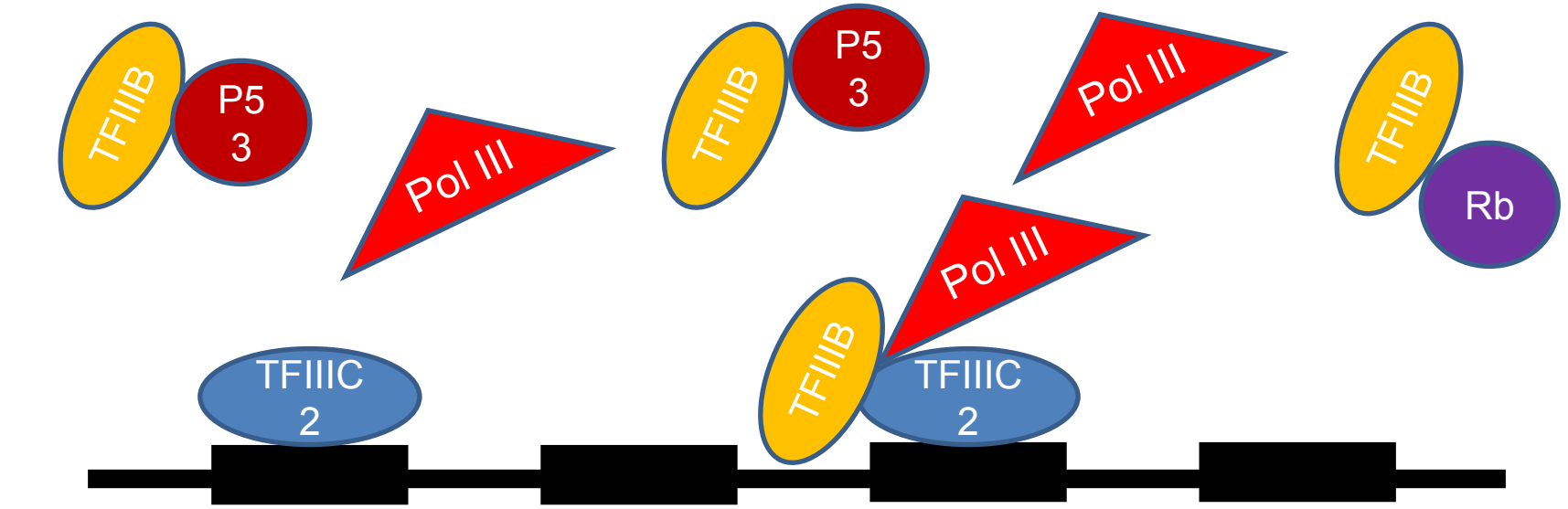
Pol III transcription and cancer

Protein synthesis is a prerequisite of rapid cell growth. Growth rate is directly proportional to the rate of protein accumulation and a 50% reduction in protein synthesis causes cells to withdraw from cell cycle and become quiescent.

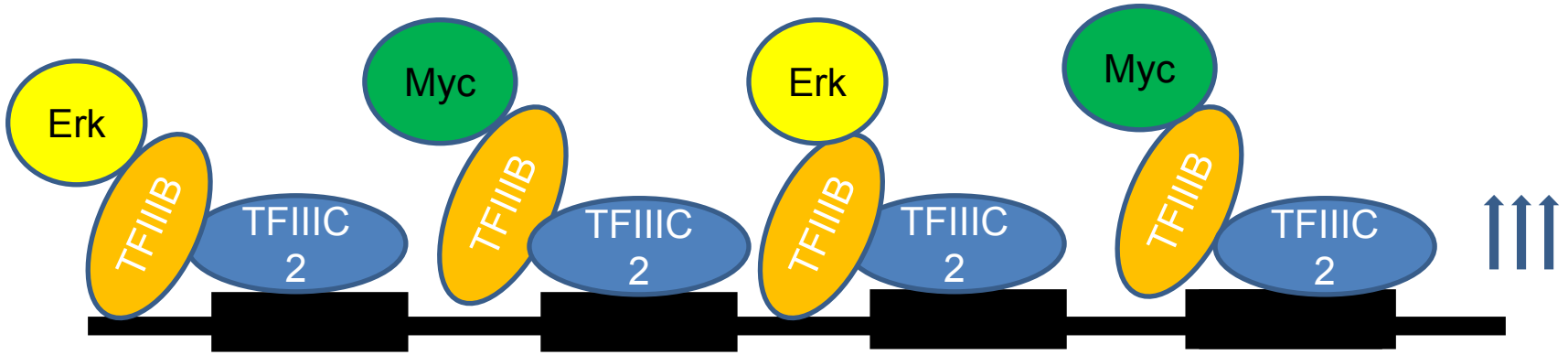
The availability of tRNA and rRNA is an important determinant of the rate of translation. High levels of pol III transcription are necessary to sustain rapid growth. Thus, in many cancer cells, there is frequent deregulation of pol III in transformed cells.

Pol III transcription of tRNA and 5S rRNA genes (as well as Pol I-dependent synthesis of large rRNAs) is tightly linked to growth conditions, being decreased when cells are deprived of serum or nutrients and increased on mitogenic stimulation.

Pol III transcription of tRNA and 5S rRNA is known to be stimulated by oncoproteins such as c-Myc and repressed by tumor suppressor proteins such as p53 and Rb by by directly targeting the essential Pol III initiation factor TFIIIB.

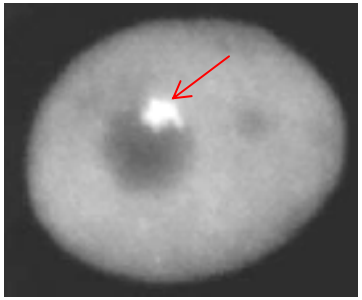


NORMAL CELLS



CANCER CELLS

The perinucleolar compartment (PNC)



PNC, a distinct nuclear body discovered in the year 1992, localizes to the nucleolar periphery.

The PNC is predominantly found in transformed cells and rarely observed in normal primary cells. Thus, the abundance of PMCs can be a pan-cancer marker for solid tumors.

PMC is enriched in **several pol III RNAs** and heterogeneous nuclear ribonucleoprotein I/polypyrimidine tract binding protein (hnRNP I/PTB).

Callie Pollock and Sui Huang (2009)
The Perinucleolar Compartment.
Journal of Cellular Biochemistry 107:189–193

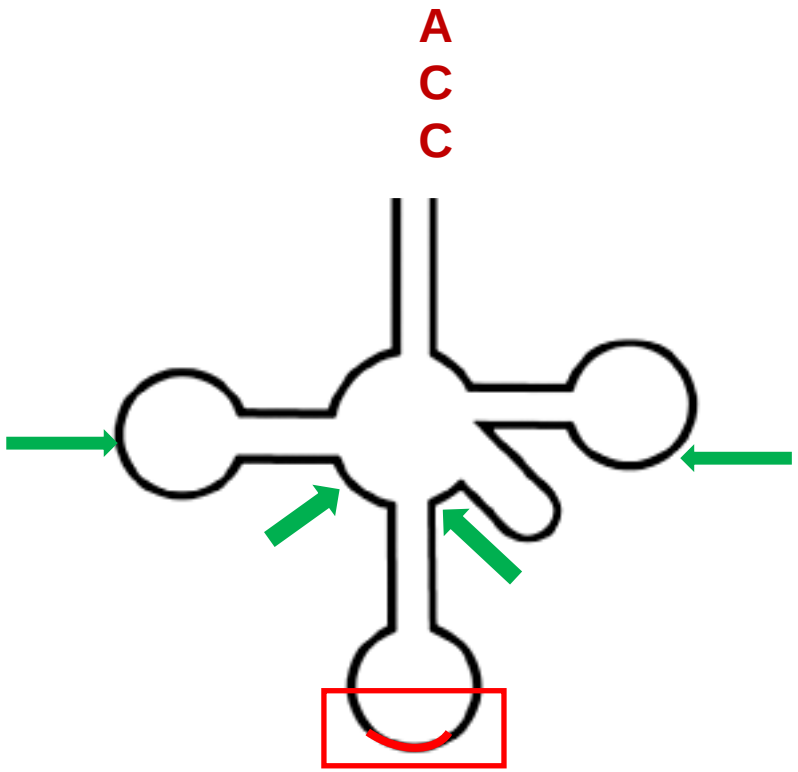
Pol III transcription and disease

In addition to their regulation during cancer, many Pol III transcripts are implicated in several diseases.

For ex., the BC1 and B200 RNA which are invovled in the regulation of protein translation in neurons are implicated in **Alzheimer disease and Fragile X syndrome.**

The RNase MRP (RNase mitochondrial RNA processing) is implicated in a genetic disorder known as **cartilage hair hypoplasia**

tRNA processing



RNase P
RNase Z

Modification of bases

Splicing

Dihydrouridine in the D arm
Pseudouridine in the T arm
Methylation of bases
The first anticodon base is sometimes modified to inosine

Original research articles on purification & characterization of Pol III transcription factors

Segall, J., Matsui. T., and Roeder, R. G. (1980) *J. Biol. Chem.* 255, 11986-11991

Matsui T., Srgall, J., Weil, P.A., and Roeder, R.G. (1980) J. Biol. Chem. 255, 11992-11996

Engelke, D. R.,N g, 54.-Y., Shastry, B.S ., and Roeder, R. G. (1980) *Cell 19, 717-728*

Review articles

Murphy S et al., (1989) Common mechanisms of promoter recognition by RNA polymerases II and III. Trends Genet. 1989 5:122-126.

Sollner-Webb, B. (1988) Surprises in polymerase III transcription. Cell. 1988 52:153-154.