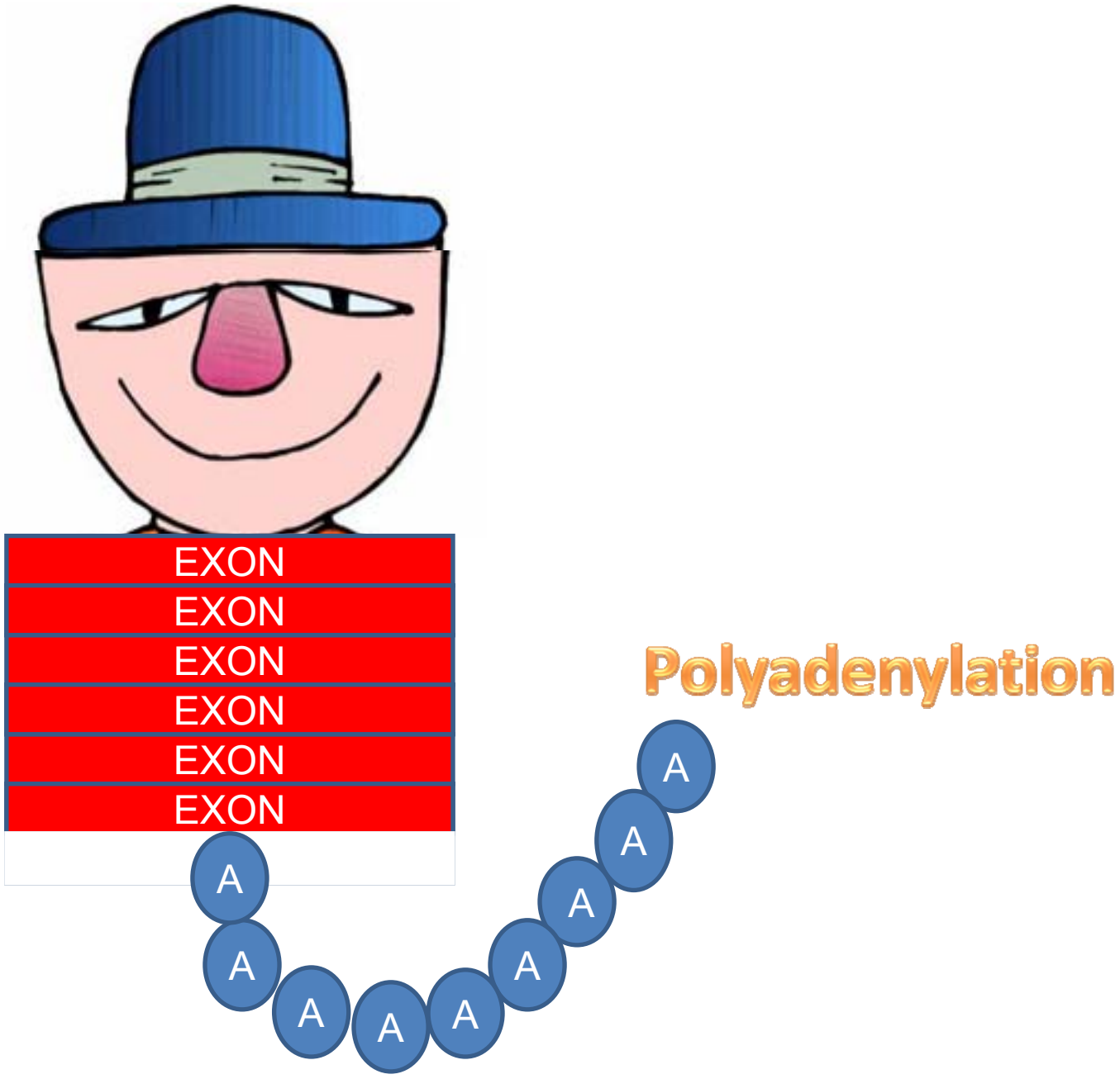
Eukaryotic Gene Expression: Basics & Benefits

P N RANGARAJAN

Lecture 12

Eukaryotic gene regulation:
Co-transcriptional and post-transcriptional modifications of pre messenger RNA - II


EXON
EXON
EXON
EXON
EXON
EXON
Polyadenylation
A
A
A
A
A
A
A
A
A

Polyadenylation of mRNA takes place in two steps:

Recognition of polyadenylation signal and endonucleolytic cleavage of the mRNA precursor

Poly(A) addition to the 3' end of the upstream cleavage product.

Polyadenylation of mRNA requires multiple protein factors:

cleavage/polyadenylation specificity factor (CPSF),
cleavage stimulation factor (CstF),
two cleavage factors, CFI and CFII,
poly(A) polymerase
poly(A) binding protein II (PABII)

POLYADENYLATION FACTORS

CPSF
cleavage and polyadenylation specificity factor binds to polyadenylation signal, AAUAAA and is required for both cleavage and polyadenylation,

CF-I cleavage factor I
RNA binding protein, required for cleavage only.

CF-II cleavage factor II
required for cleavage only

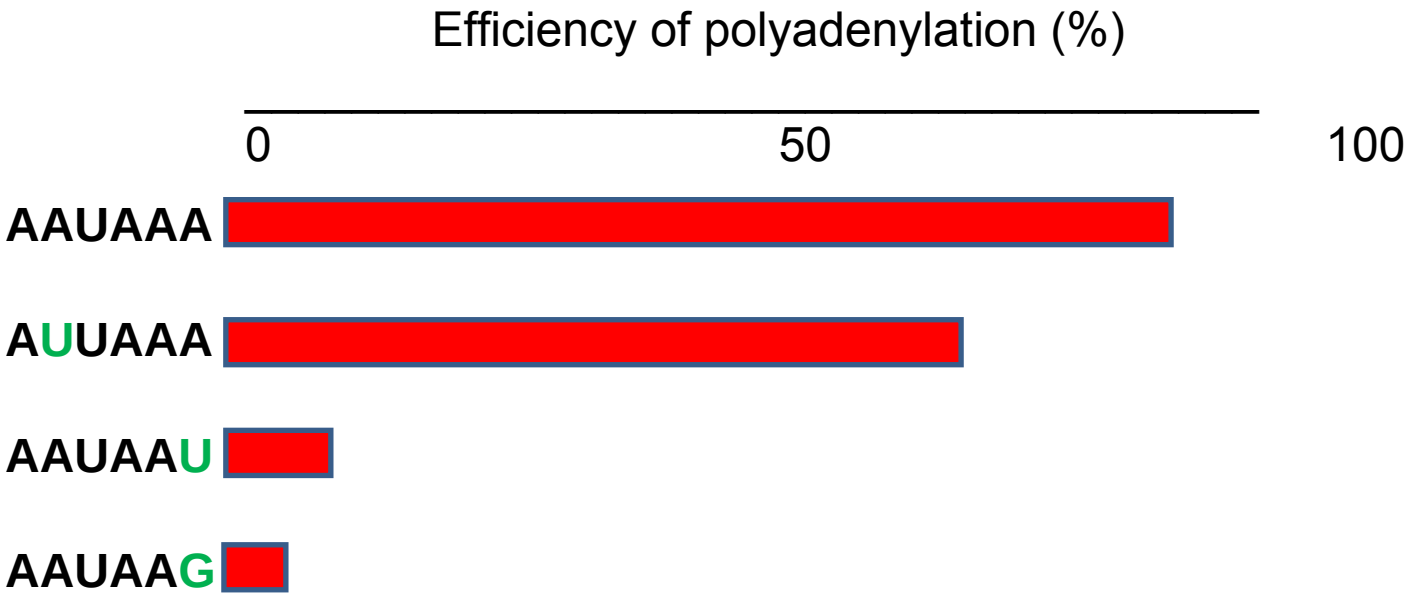
CstF cleavage stimulation factor
required for cleavage only, binds to GU/U region

PAP poly [A] polymerase
catalyzes poly [A] synthesis, also required for cleavage

PAB II pol [A] binding protein II
stimulates polyadenylation, required for lengthening of poly [A] tail

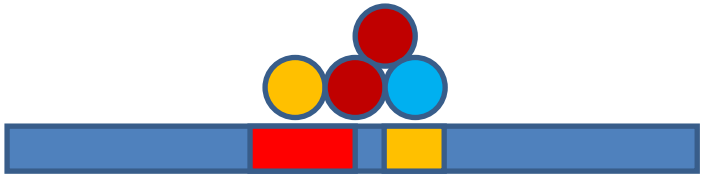
mRNA

Consensus polyadenlation signal: $A_{98}A_{86}U_{98}A_{98}A_{95}A_{96}$



Mechanism of polyadenylation

Recruitment of CPSF, CFI, CstF



Cleavage occurs, CPSF remains, PAP joins

PAP adds 10 As

PAB II joins and extends the tail upto 200 As

cleavage/polyadenylation specificity factor (CPSF),
cleavage stimulation factor (CstF),
two cleavage factors, CFI and CFII,
poly(A) polymerase (PAP)
poly(A) binding protein II (PABII)

Role of RNA polymerase II in polyadenylation and transcription termination

RNAs transcribed by CTD-truncated RNAP II were not efficiently polyadenylated in transiently transfected cells.

It was also shown that CPSF and CstF present in unfractionated nuclear extracts could bind GST-CTD, and both were present in an RNAP II holoenzyme preparation.

Unlike capping and splicing, the phosphorylation status of the CTD was found not to affect binding.

These results suggested that the CTD functions to help recruit polyadenylation factors to sites of RNAP II transcription, increasing their local concentration and thereby facilitating efficient processing.

Proudfoot NJ (1989) TIBS 14: 105-110

McCracken et al. (1997) *Nature* 385: 357-361

How and when do poly(A) factors associate with the polymerase?

The initially unexpected answer is that at least some of the action occurs at the promoter.

While studying the general transcription factor TFIID, it was found that an extensively purified preparation contained in good yield of at least three of the four subunits of CPSF.

Thus, atleast some poly(A) factors appear to associate early with RNAP II and remain
associated with it during elongation

Dantonel et al. (1997) *Nature* **389:** 399-402

Biochemical evidence demonstrating a more direct role of RNAP II in polyadenylation.

Purified RNAP IIA and II0 were both found to activate the first step of polyadenylation, 3' cleavage, in a reconstituted system containing all the other polyadenylation factors.

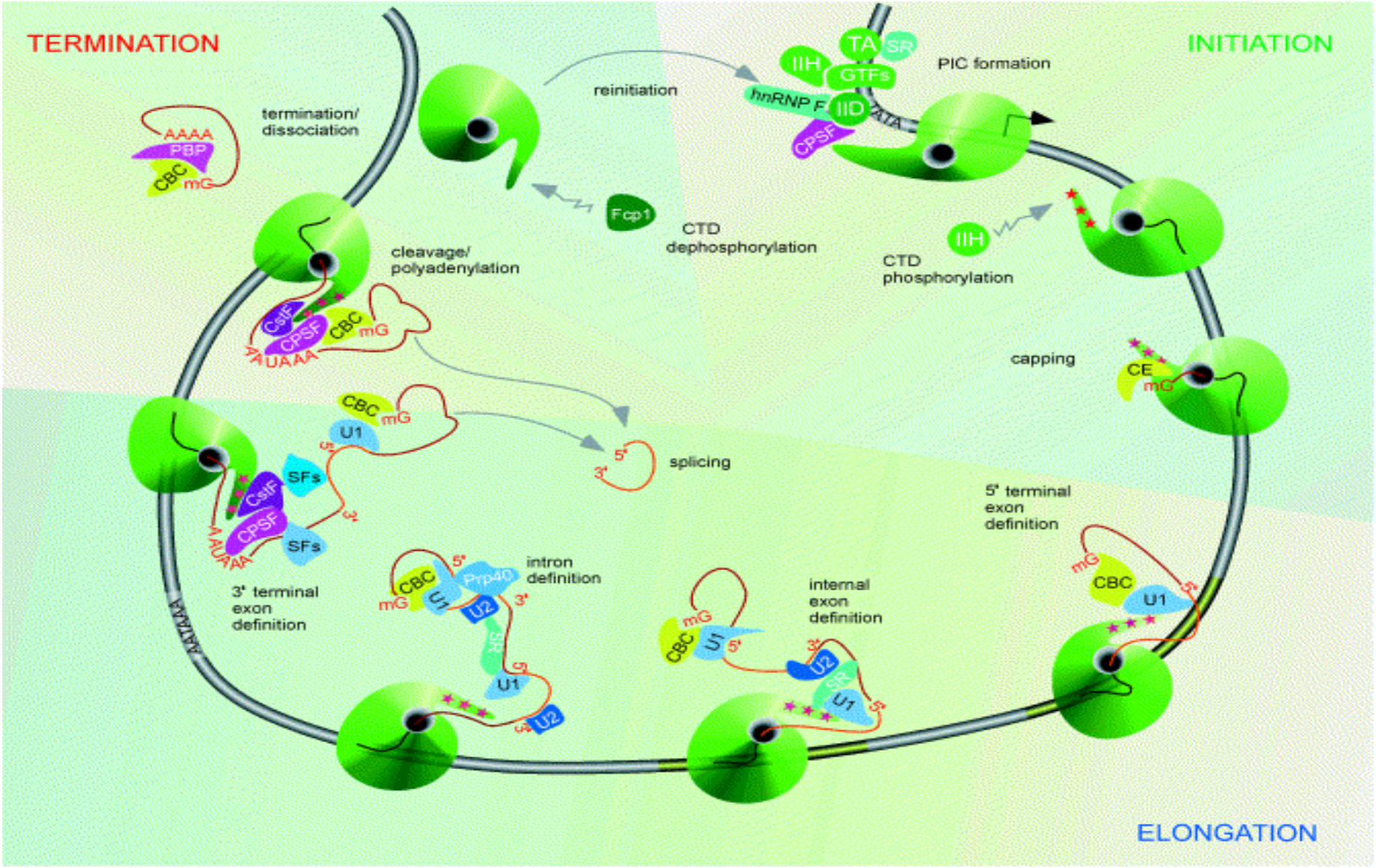
In addition, both unphosphorylated and hyperphosphorylated GST-CTD proteins activated cleavage just as efficiently as did RNAP II, although in this case the hyperphosphorylated CTD was more active than the nonphosphorylated form.

Further, 3' cleavage in nuclear extracts could be inhibited by immunodepletion of RNAP II and rescued by add-back of the purified enzyme.

These results suggested that the CTD of RNAP II participates directly in the formation and/or function of a stable, catalytically active processing complex through direct interaction with polyadenylation factor(s).

Hirose & Manley (1998) *Nature* 395: 93-96

Transcriptional coupling of pre-mRNA processing.



See next slide for figure legend

Figure reproduced from the article authored by Howe KJ BBA (2002)1577:308-324
on obtaining license No.2434111235771 from publishers (Elsevier) for posting at http://nptel.iitm.ac.in

Transcriptional coupling of pre-mRNA processing.
Figure legend

The transcription cycle is depicted starting at the top right and proceeding in a clock-wise fashion.

Processing factors may be recruited to the PIC at the promoter (TATA) by GTFs (TFIID, IID; TFIIH, IIH) and transcriptional activators (TAs).

Hypophosphorylated RNAP II (green “tadpole” depicted with a CTD “tail”).

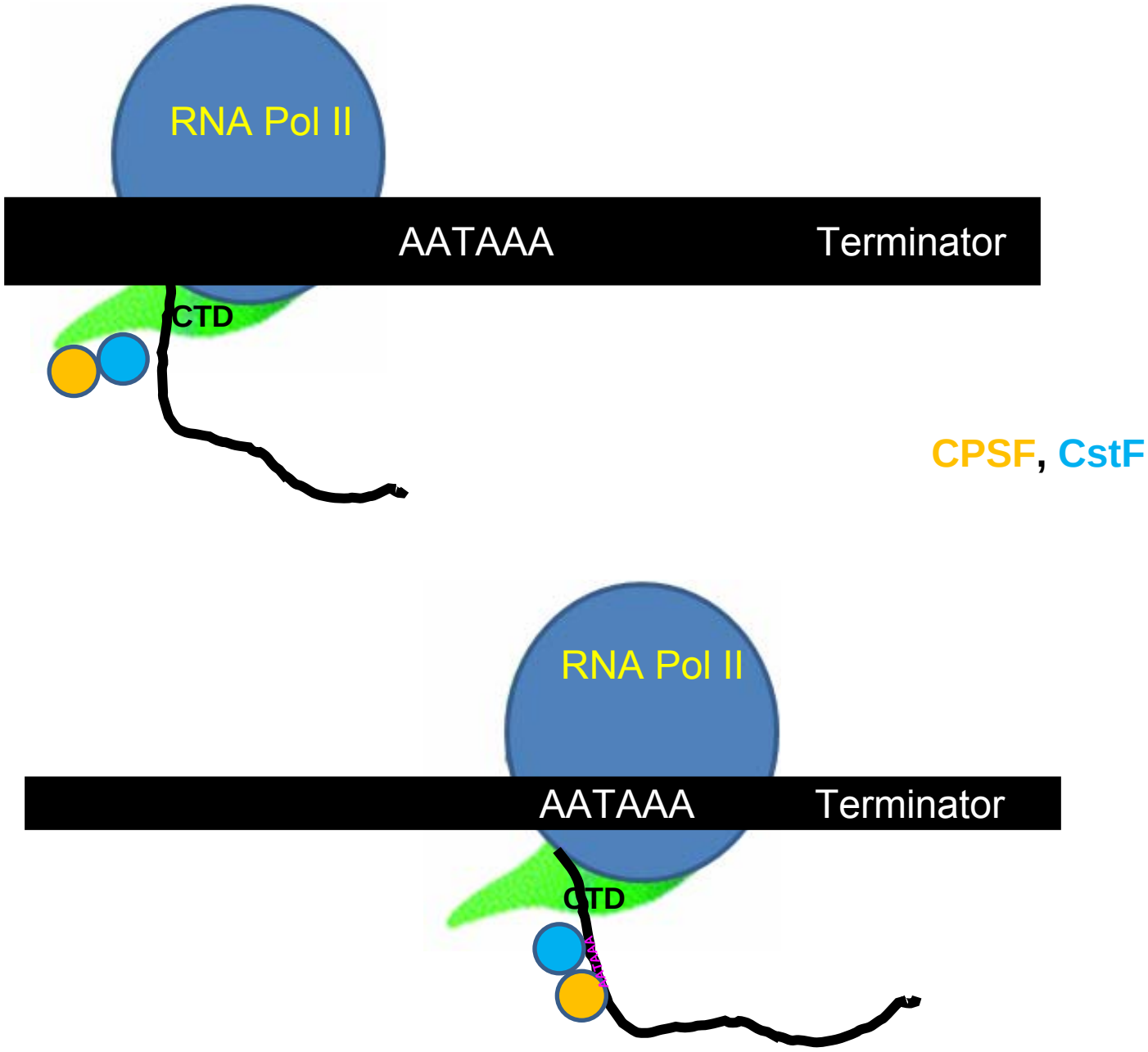
TFIIH phosphorylates the CTD (red stars) soon after initiation, which recruits CE.

CBC mediates 5′ terminal exon definition. IVSs are denoted by the gold DNA. SR proteins and the CTD facilitate internal exon definition. Intron definition occurs when splice sites are paired across introns (Prp40 is shown assisting this process).

3′-End processing is mediated by cleavage/polyadenylation factors assembled at the poly(A) site (AATAAA) and is assisted by the CBC and splicing factors (SFs). These interactions mediate 3′ terminal exon definition.

Transcription termination is accompanied by CTD dephosphorylation (proposed to occur by Fcp1).

Figure legend reproduced from the article authored by Howe KJ BBA (2002)1577:308-324
on obtaining license No.2434111235771 from publishers (Elsevier) for posting at http://nptel.iitm.ac.in


RNA Pol II
AATAAA
Terminator
CTD
CPSF, CstF
RNA Pol II
AATAAA
Terminator
CTD

Polyadenylation and the CTD

Hirose, Y., and Manley, J.L. (1998). Nature 395, 93–96.

McCracken, S., Fong, N., Yankulov, K., Ballantyne, S., Pan, G.H., Greenblatt,
J., Patterson, S.D., Wickens, M., and Bentley, D.L. (1997). Nature 385, 357–361.

Olga Calvo and James L. Manley (2003) Genes Dev. 17: 1321-1327

Yutaka Hirose, and James L. Manley Genes Dev. (2000) 14: 1415-1429

Colgan, D.F. and J.L. Manley. 1997 *Genes & Dev.* **11:** 2755-2766

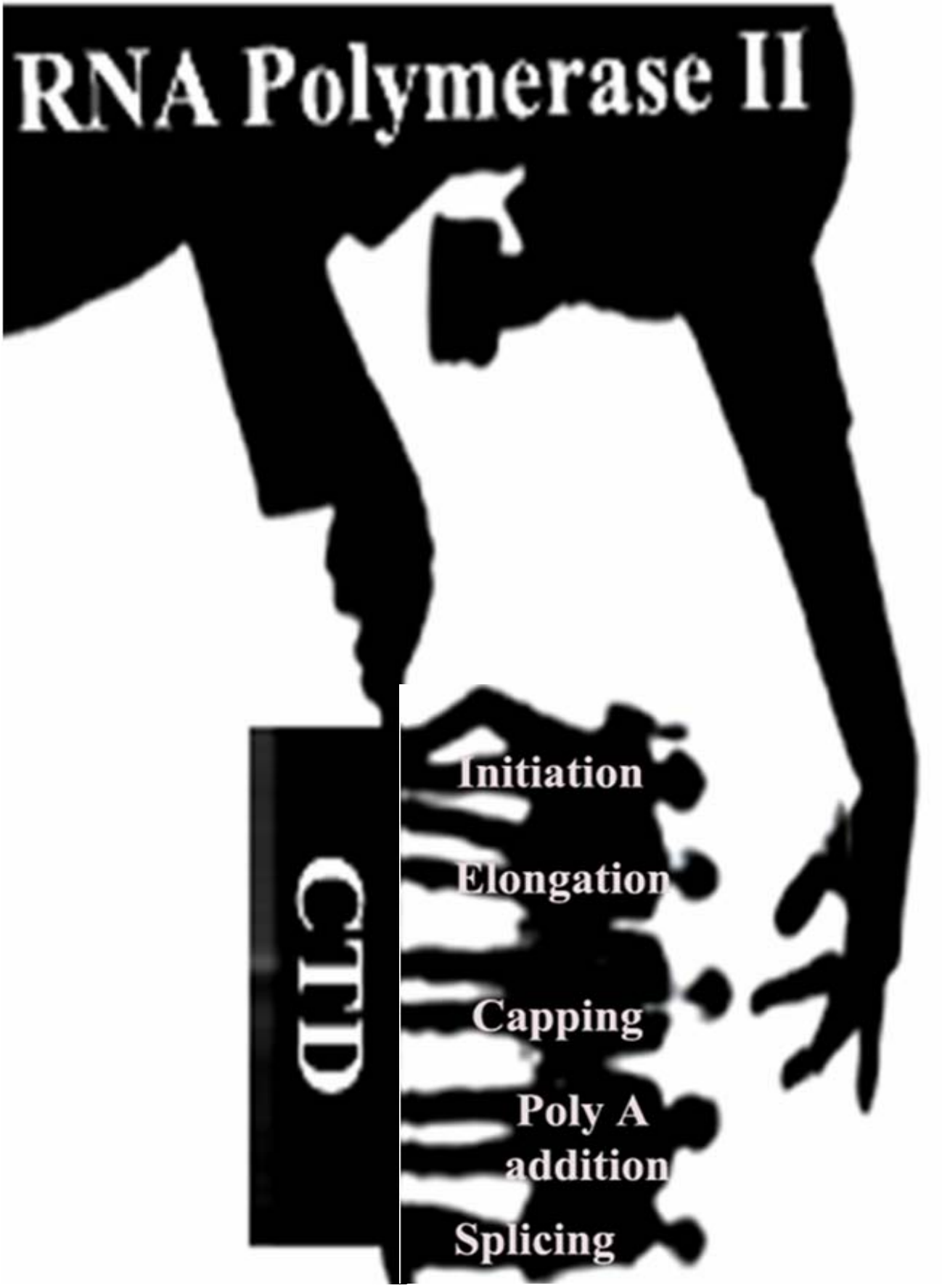
Zhao, J., L. Hyman, and C. Moore. 1999 *Microbiol. Mol. Biol.* **63:** 405-445

The idea that RNAP II, and specifically the CTD of its largest subunit, participates in mRNA processing was unexpected and controversial just a few years ago, but now seems quite solidly established.

A mature eukaryotic mRNA

Steps in mRNA processing

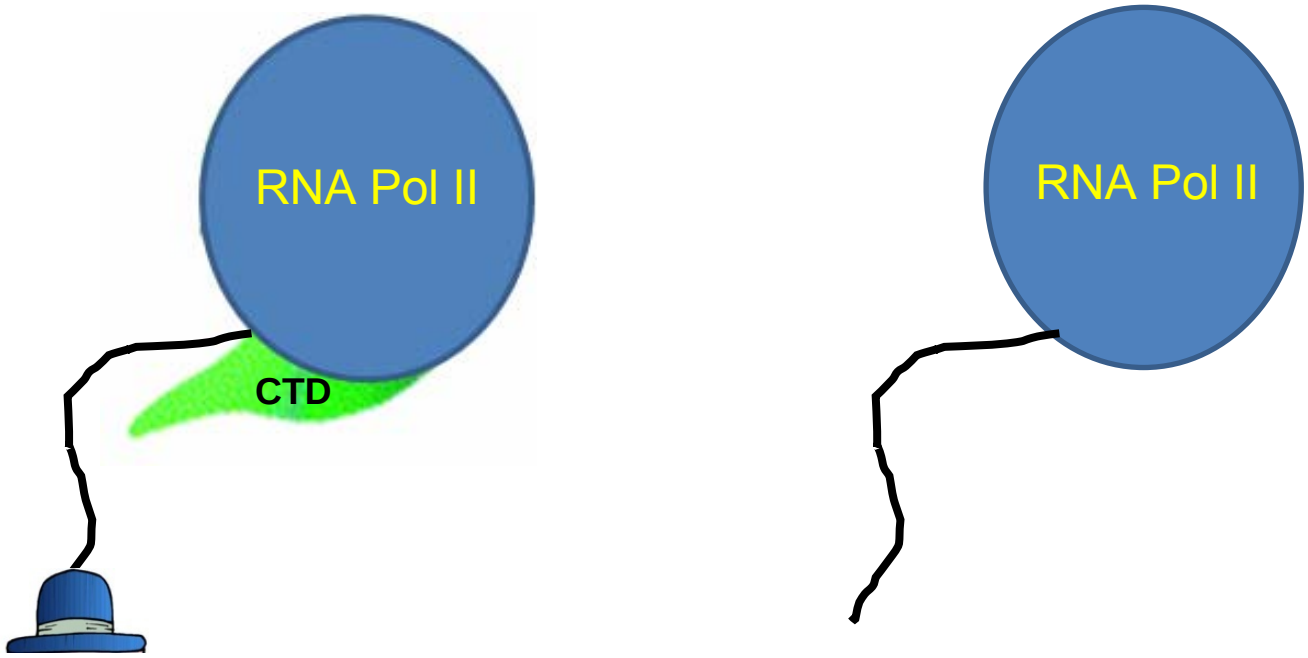
1) CAP is added to 5' end of nascent transcript when mRNA is ~20 nt long
2) Spliceosomes are assembed onto introns as transcription continues; splicing is usually not completed until after the nascent transcipt is released
3) Shortly after Pol II passes a "polyadenylation signal", the 3' end of the transcript is generated by cleavage and polyadenylation


RNA Polymerase II
CTD
Initiation
Elongation
Capping
Poly A addition
Splicing

Throughout the transcription cycle the RNA Polymerase II CTD undergoes a variety of covalent and structural modifications which can, in turn, modulate the interactions and functions of other factors involved not only in transcription initiation but also elongation, termination, capping and splicing.

Thus, inside the nucleus there is a harmonic integration of transcriptional and post-transcriptional activities, many of which once were considered to be functionally isolated within the cell.

Describe an experiment that demonstrated a role for RNA Pol II in mRNA capping



RNA Pol II CTD plays a key role in mRNA capping

Phosphorylation of serine 5 of CTD is essential for the recruitment of capping enzyme to the Pol II complex as well as for the allosteric modulation of guanylyltransferase activity of the capping enzyme.

mRNA capping is co-transcriptional

mRNA splicing and polyadenylation are also co-transcriptional

Positive patches and negative noodles: linking RNA processing to transcription?

A.L. Greenleaf *Trends Biochem. Sci.* **18** (1993), pp. 117–119.

Greenleaf suggested that the negatively charged hyperphosphorylated CTD could act as an ionic scaffold for the recruitment of arginine-rich (i.e., positively charged) SR proteins involved in splicing and phosphorylation states of the CTD and/or the serine residues of the RS domains of SR proteins might regulate these associations.

The experimental evidence for physical linkage between transcription and splicing was first demonstrated by Chabot et al., (1995), who showed that RNAP II, spliceosomal snRNPs and splicing intermediates could be co-immunoprecipitated using a monoclonal antibody directed against the phosphorylated CTD.

B. Chabot, S. Bisotto and M. Vincent *Nucleic Acids Res.* **23** (1995), pp. 3206–3213.

M. Vincent, P. Lauriault, M.F. Dubois, S. Lavoie, O. Bensaude and B. Chabot
Nucleic Acids Res. **24** (1996), pp. 4649–4652.

Splicing, polyadenylation begin co-transcriptionally and are completed post-transcriptionally.

McCracken, N. Fong, K. Yankulov, S. Ballantyne, G. Pan, J. Greenblatt, S.D. Patterson, M. Wickens and D.L. Bentley

The C-terminal domain of RNA polymerase II couples mRNA processing to transcription *Nature* 385 (1997), pp. 357–361.

These authors demonstrated for the first time that the carboxy-terminal domain (CTD) of RNA pol II large subunit is required for efficient RNA processing.

Splicing, processing of the 3' end and termination of transcription downstream of the poly(A) site, are all inhibited by truncation of the CTD.

Cleavage-polyadenylation factors CPSF and CstF specifically bound to CTD affinity columns and copurified with pol II in a high-molecular-mass complex.

There is an association between the CTD and 3'-processing factors suggesting that **an mRNA 'factory' exists inside the nucleus which carries out coupled transcription, splicing and cleavage-polyadenylation of mRNA precursors.**

REGULATION OF TRANSCRIPTION ELONGATION

Eukaryotic transcription factors involved in regulation of transcription elongation:

I: Those that suppress transient pausing and stimulate the rate of transcript elongation by the polymerase: TFIIF, ELL, and Elongin

II: Factors that reactivate a RNA polymerase molecule that has arrested during transcription: the eukaryotic TFIIS proteins which stimulate a cleavage activity that is intrinsic to the RNA polymerase in order to rescue it from arrest.

III: Factors that stimulate elongation on chromatin templates: HMG14, HMG17, FACT and nucleosome remodeling complexes like SWI/SNF.

Other factors: P-TEFb, DSIF, NELF, Rtf1, Spt4/5/6, and Elongator

Rachel N. Fish and Caroline M. Kane (2002)
Promoting elongation with transcript cleavage stimulatory factors
BBA 577:287-307

Co- or post-transcriptional modifications

mRNA
capping,
splicing,
polyadenylation
and
what else?

RNA editing

- RNA editing is the co- or post-transcriptional modification of RNA primary sequence from that encoded in the genome through nucleotide deletion, insertion, or base modification mechanisms.

RNA editing was first discovered in kinetoplastids which are single celled protozoa (T*rypanosoma brucei, T. cruzi, Leishmania)*

RNA editing

Two types

Base modification (substitution editing) (Adenosine deaminases)

A to I	seen in viruses, human genes
C to U, U to C	seen in chloroplasts, plant mitochondria, human genes

Insertion/deletion editing

U insertion/deletion,	seen in kinetoplastid protozoa
mono/di nucleotide insertion,	seen in *Physarum*
nucleotide replacement,	seen in *Acanthamoeba* tRNAs

Edited mRNA

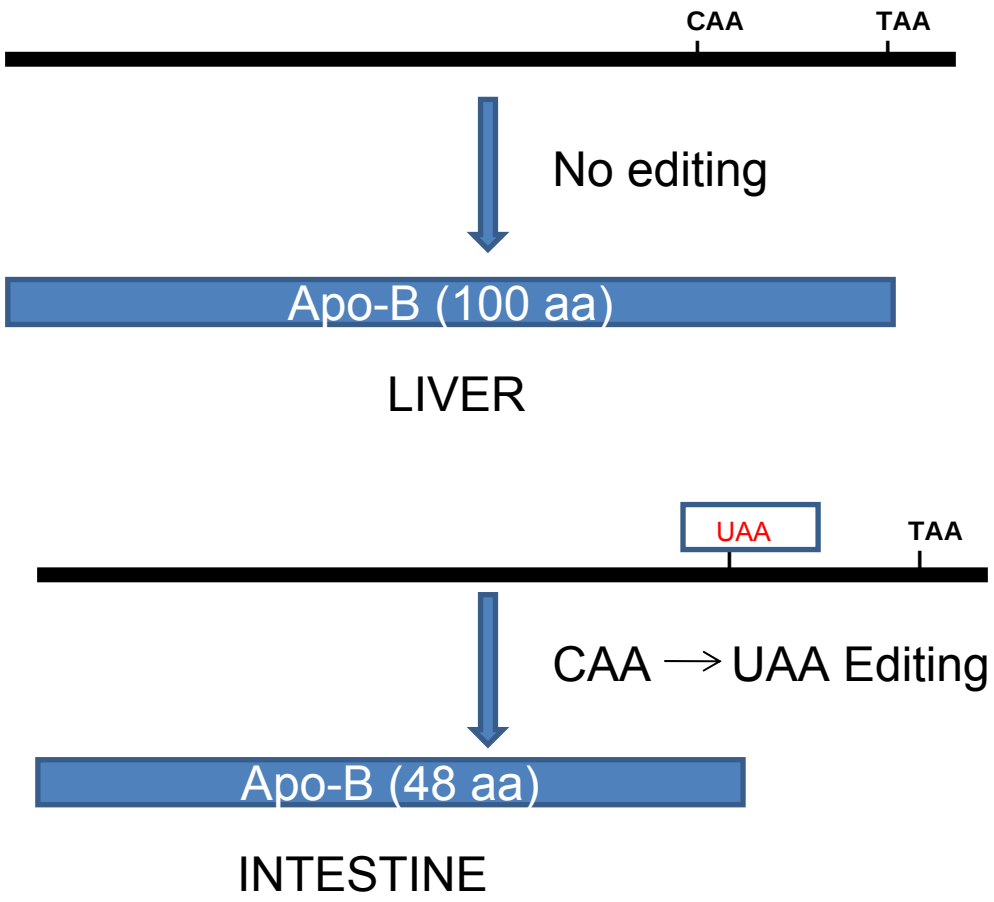
5′ -GGGGGAGGAGAGAAGAAGGGAAAGuuUGuuAUUUUGGAGUUAUAGA -3′

Substitution Editing (A-to-I): RNA editing by members of the ADAR (adenosine deaminases acting on RNA) family leads to site-specific conversion of adenosine to inosine (A-to-I) in precursor messenger RNAs.

Levanon, E.Y., et al. Nat Biotechnol, 2004

Prasanth KV et al., Regulating gene expression through RNA nuclear retention. Cell. 2005 Oct 21;123(2):249-63.

Substitution Editing (C-to-U): Editing of *apoB* mRNA



An 11 nucleotide sequence known as the **'mooring sequence'** and the flanking nucleotides within the apolipoprotein B (*apoB*) mRNA are recognized by proteins known as Apobec-1 and ACF1 (Apobec-1 complementing factor).

MULTIPLE mRNAs CAN BE GENERATED FROM A SINGLE GENE BY PROCESSES SUCH AS RNA EDITING, RNA SPLICING ETC.

Systematic identification of abundant A-to-I editing sites in the human transcriptome

Levanon, E.Y., et al. Nat Biotechnol, 2004.)

They predict 12723 editing sites in 1637 genes and experimentally validate 26 genes.

They offer many new editing sites and experimental evidence.

Based on EST and other expression data, up to 80% of human genes are believed to produce alternatively spliced mRNAs

Sub nuclear structures having a role in transcriptional regulation

Cajal Bodies: involved in small nuclear and nucleolar RNA metabolism, snRNP biogenesis

Gems: assembly of snRNP and snRNP maturation

Nucleolus: ribosome synthesis and assembly

Heterochromatin: inactive chromatin

PcG bodies: contain polycomb group proteins (silencing proteins) such as RING1, BMI1 and hPc2

Nuclear Speckles: contains groups of pre-mRNA splicing factors

Paraspeckles: storage of processed mRNA (Ex. CTN RNA), contains proteins such as P54nrb and noncoding RNAs such as VINC/NEAT1/Men epsilon RNA

IGC: interchromatin granule clusters assembly, modification of pre-mRNA splicing factors

OPT Domains: (Oct1/PTF/transcription) appear in G1 but disappear in S phase; contain transcription factors but not RNA processing factors

Cleavage Bodies: cleavage and polyadenylation of pre-mRNA processing

Transcription and processing are indeed linked physically and functionally in the nuclei of mammalian cells.

Misteli and Spector 1999 *Nature* **387:** 523-527

Spector 1993 Annu. Rev. Cell. Biol. **9:** 265-315

Nuclear speckles represent storage sites, or perhaps sites of assembly or recycling of splicing complexes, rather than sites of active splicing.

Both hyperphosphorylated RNAP II and polyadenylation factors are associated with periphery of speckles. Transcriptional activation seems to result in a redistribution of factors from the speckles, and indeed RNAP II and the SR protein ASF/SF2 appear to migrate from speckles to sites of transcription.

In cells expressing an RNAP II with a truncated CTD, relocalization of splicing factors to transcription sites does not occur, further confirming a role for CTD in RNA splicing.

Thus the localization and dynamics of transcription and mRNA processing factors within the nucleus is consistent with the functional interactions observed in vitro.

Eukaryotic Gene Expression: Basics & Benefits

Lectures 1- 12

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

Eukaryotic Gene Expression: Basics & Benefits

EXERCISES (Lectures 1-12)

Test Your Knowledge (based on lectures 1-12)

Distinguish between prokaryotic and eukaryotic RNA polymerases

Name the different types of eukaryotic RNA polymerases and mention their functions

What are core promoter elements and how do they contribute to differential gene regulation?

Eukaryotic RNA polymerases are blind. They initiate transcription randomly in vitro.
How can you make them initiate transcription accurately in vitro?

What is the difference between TBP and TFIID?

What are upstream activation sequences? Give an example

What are enhancers? How do they function?

Test Your Knowledge (based on the lectures 1-12)

Give atleast three examples of transcriptional activators that bind to specific enhancer elements and activate transcription

Discuss:
Thyroid hormone receptor – co-repressor interactions
Activation of transcription by cyclicAMP

What are the major functional domains of eukaryotic transcriptional activators?

Define the function of the following domains:

a) zinc finger
b) helix-turn –helix
c) leucine zipper
d) acidic activation domain

Test Your Knowledge (based on lectures 1-12)

Functional significance of RNA Polymerase CTD

Name the general transcription factor which phosphorylates RNA polymerase II CTD

What are RNA polymerase II A and RNA Polymerase II O

What are the components of a nucleosome

Histones have only a structural role in cells – state true or false, give reasons

What are core histones and linker histone

Mention various post translational modifications (PTMs) of histones

What is the significance of histone acetylation?

Which PTMs of histones are involved in negative regulation of gene expression?

Test Your Knowledge (based on lectures 1-12)

What are HATs and HDACs? How are they recruited near promoters?

What is histone code?

How are cytosines methylated in a chromatin template?

How are methylated cystosines recognized in a chromatin template?

What is the interrelationship between histone deacetylation and DNA methylation?

What is epigenetic code?

Test Your Knowledge (based on lectures 1-12)

Distinguish between Transcription initiation complex and transcription elongation complex

What is mRNA capping and discuss its functional significance

What is the role of RNA polymerase II in mRNA capping?

How do you prove that RNA Pol II CTD is involved in mRNA splicing?

Transcription initiation and polyadenylation are coupled – Discuss

What is DNA foot printing?

What is an electrophoretic mobility shift assay?

How do you assay the activity of a promoter in vivo and in vitro?

What is a G-free cassette?

A reconstituted cell-free trasnscription system containing TBP instead of TFIID does not

support activator-dependent transcription - Discuss

Test Your Knowledge (based on lectures 1-12)

What is alternate splicing? Give an example

Comment on sub nuclear structures and their role in RNA synthesis and RNA processing

Define RNA editing and discuss different types of RNA editing with examples

Discuss the mechanisms by which multiple mRNAs can be generated from a single gene