Eukaryotic Gene Expression: Basics & Benefits

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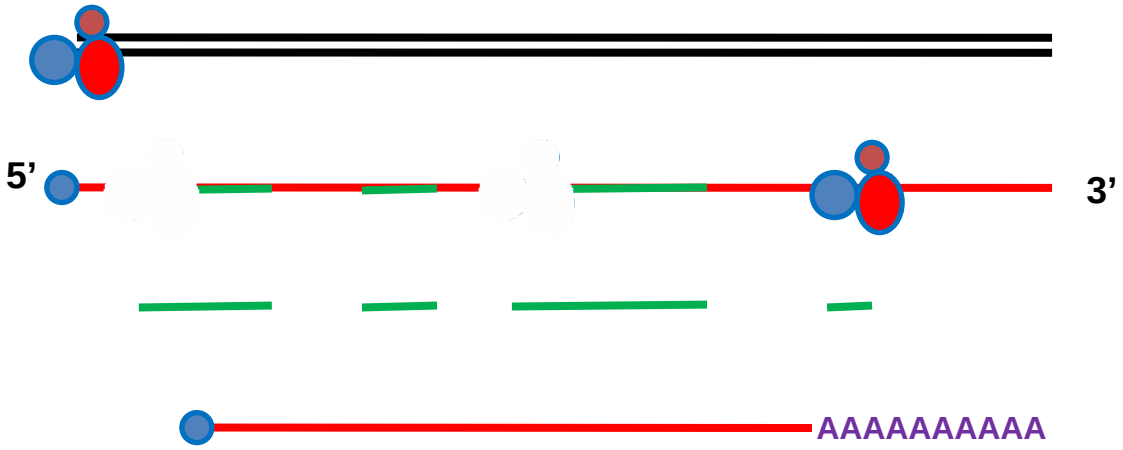
Lecture 11

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

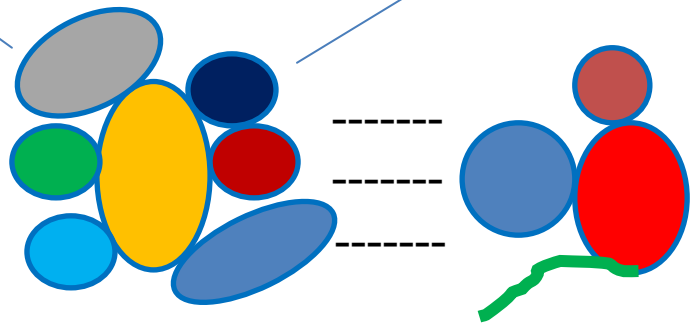
mRNA synthesis and processing

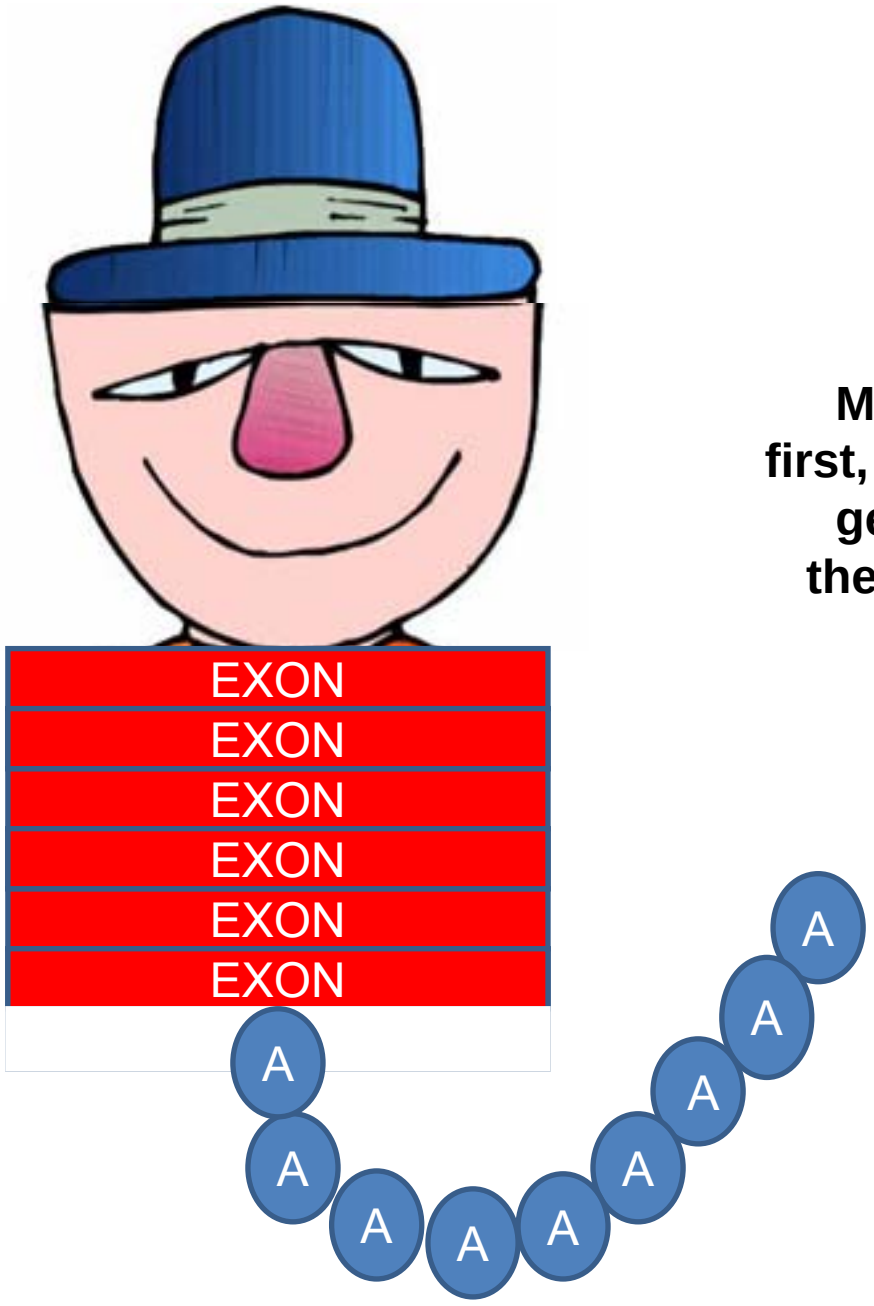


mRNA capping

mRNA splicing

Polyadenylation





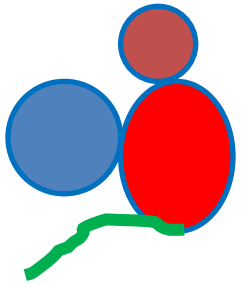
Mr. pre-mRNA,
first, put your cap on,
get spliced up,
then wag your tail

SEVERAL POST TRANSCRIPTIONAL MODIFICATIONS OF PRE mRNA ARE **CO-TRANSCRIPTIONAL , RATHER THAN POST-TRANSCRIPTIONAL**

In the nucleus, mRNA capping, splicing and polyadenylation occur at the same time and place, in close proximity

The target for factors involved in RNA processing is not a solitary RNA molecule

Instead, the transcription elongation complex (TEC) comprising the growing nascent RNA, RNA polymerase and proteins associated with it are targeted by a plethora of factors involved in RNA processing.



RNA Polymerase II C-terminal domain (CTD) plays a key role in pre-mRNA processing.

Hypophosphorylated CTD (pol IIA)

Hyperphosphorylated CTD (pol IIO)

Mutations in CTD affect not only transcription initiation but also certain steps of RNA processing

RNA Polymerase II CTD acts as a ‘‘landing pad,’’ for the binding of factors involved in pre-mRNA capping, 3’ end processing, transcription elongation, termination, and chromatin modification

TRANSCRIPTION ELONGATION COMPLEX (TEC)

HOW DOES TRANSCRIPTION ELONGATION OCCUR?

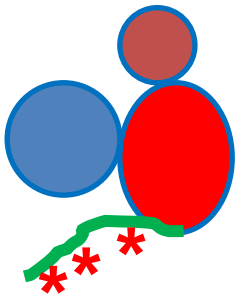
HOW IS IT REGULATED?

HOW ARE TRANSCRIPTION ELONGATION, CAPPING,

SPLICING AND POLYADENYLATION ARE INTEGRATED?

RNA Pol II CTD contains heptad repeats (26 in yeast, 52 in mammals) with the consensus sequence :

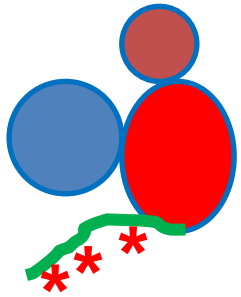
$Y_1S_2P_3T_4S_5P_6S_7$



The RNA polymerase II CTD interacts directly with a number of proteins involved in mRNA processing :

i)Cgt1, an enzyme involved in mRNA capping
ii)Pcf11, a protein involved in polyadenylation
iii)Set2, a histone methyltransferase and
iv)Nrd1, an RNA binding factor involved in transcription termination

The heptad repeats are phosphorylated and dephosphorylated by kinases and phosphatases at the S2, S5, and S7 positions during the various phases of initiation, elongation, and termination cycles.



Phosphorylated RNA Pol II CTD was shown to allosterically activate the guanylyl transferase activity of mRNA capping enzyme.

At the time of transcription initiation, S5 is phosphorylated by the TFIIH-associated kinase Cdk7 (Kin 28 in yeast).

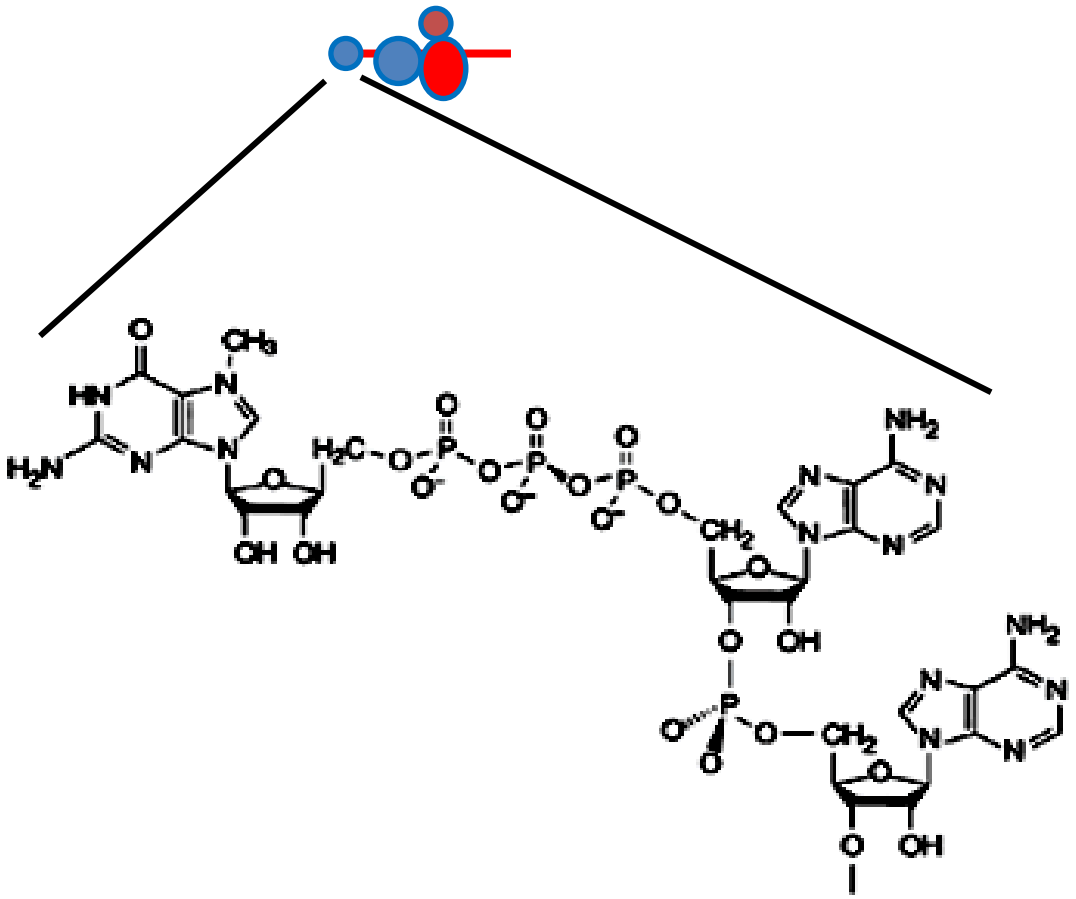
During transcription elongation, S2 is phosphorylated by Cdk9/PTEFb, a factor involved in transcription elongation

The S5 and S2 residues are dephosphorylated by the Rtr1 and Fcp1 phosphatases during different stages of mRNA synthesis and processing.

The S7 residues of the CTD heptads are also phosphorylated by Kin28/Cdk7 in yeast and mammalian cells and this results in the recruitment of factors Involved in transcription elongation such as Nrd1.

mRNA CAPPING

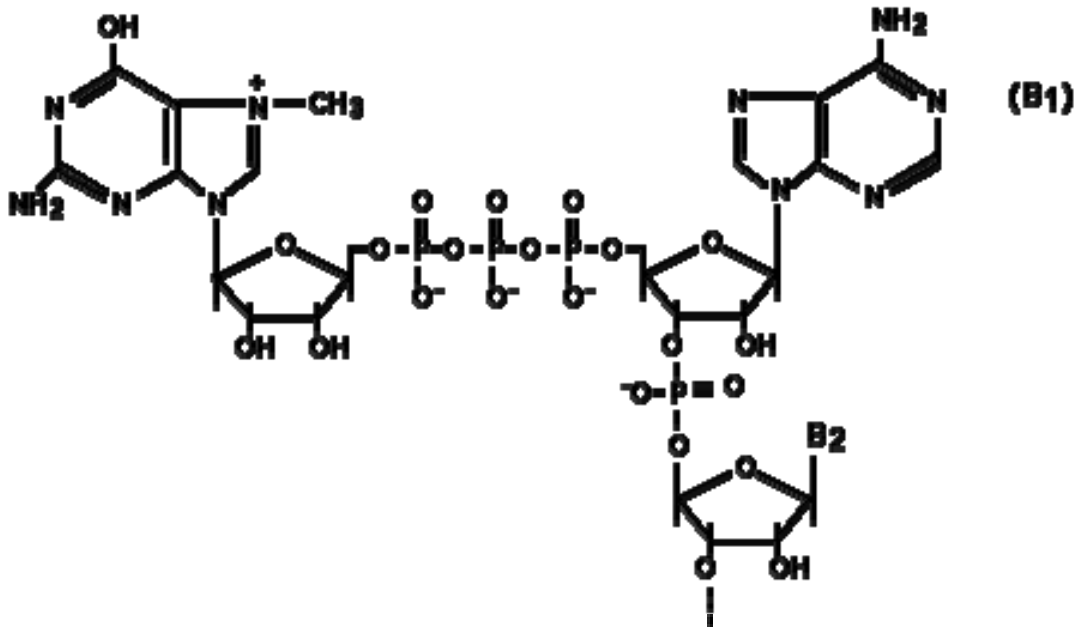
Pre-mRNAs are modified at their 5’ ends by addition of a 7-methyl G5’ppp5’N cap when the pre-mRNA is only 25–50 bases long.



The 5' cap is a unique feature of eukaryotic cellular and viral messenger RNAs that is absent in prokaryotes

The cap structure

- discovery: A. Shatkin in 1976
- found on all cellular cytoplasmic mRNAs.
- Mitochondrial mRNAs and a few viral RNAs do not have it



Cap structure: the first base (B1) is usually a purine.

Capping is a three-step process

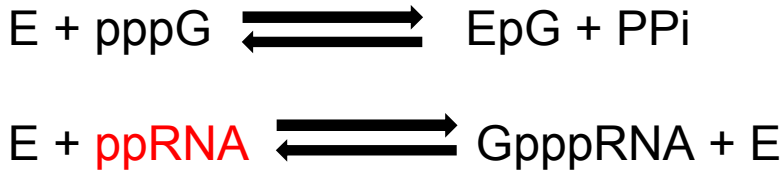
- **Removal of the g-phosphate of the first nucleotide by RNA triphosphatase.**

- **Addition of GMP by RNA guanylyltransferase in two steps:**

i) a lysine side chain on the enzyme (E) reacts with the phosphorus of GTP to form a covalent enzyme–guanylate intermediate (EpG) . Pyrophosphate (PP_i) is released.

ii) enzymatic transfer of GMP to the 5' diphosphate RNA end to form a G cap and regeneration of the apoenzyme.

- **Methylation of guanine at N7 position by guanine N7 methyltransferase.**



The guanylyltransferase acts specifically on 5' diphosphate RNA ends, and it does not catalyse the transfer of GMP to a 5' monophosphate RNA.

Thus, caps are added only to the 5' end of the pre-mRNAs and not to processed 5' ends that arise from endonucleolytic cleavage of mRNAs.

Prokaryotic 'mRNAs contain an eight-nucleotide Shine–Dalgarno sequence, which is located proximal to the site of translation initiation.

This sequence can base-pair with the sequence near the 3' end of 16S rRNA of the bacterial ribosome, and position the AUG start codon at the correct site on the ribosome.

Eukaryotes use the cap as an alternative signal to direct the translation apparatus to the 5' end of protein-encoding RNAs.

A protein known as the m^7G cap-binding protein (eIF4E) binds to the 5’ cap and recruits the 40S ribosome subunit to the 5' end of the mRNA.

Role of RNA polymerase II CTD in mRNA capping

Guanylyltransferase and methyltransferase do not associate with one another

How are these two enzymes brought to the vicinity of the RNA substrate?

Both these enzymes bind directly and specifically to the phosphorylated Pol II CTD. When transcription initiates, phosphorylation of the CTD on S5 residue permits loading of capping enzyme onto the TEC and allosteric activation of the guanylyltransferase.

Thus, the capping reaction is facilitated by both colocalization of the capping enzymes on the phosphorylated CTD and allosteric activation.

Is mRNA capping a regulatory step in transcription?

Capping enzymes stimulate or inhibit transcription initiation and early elongation

There is a phenomenon called 5' pausing wherein, transcription pauses after initiation.

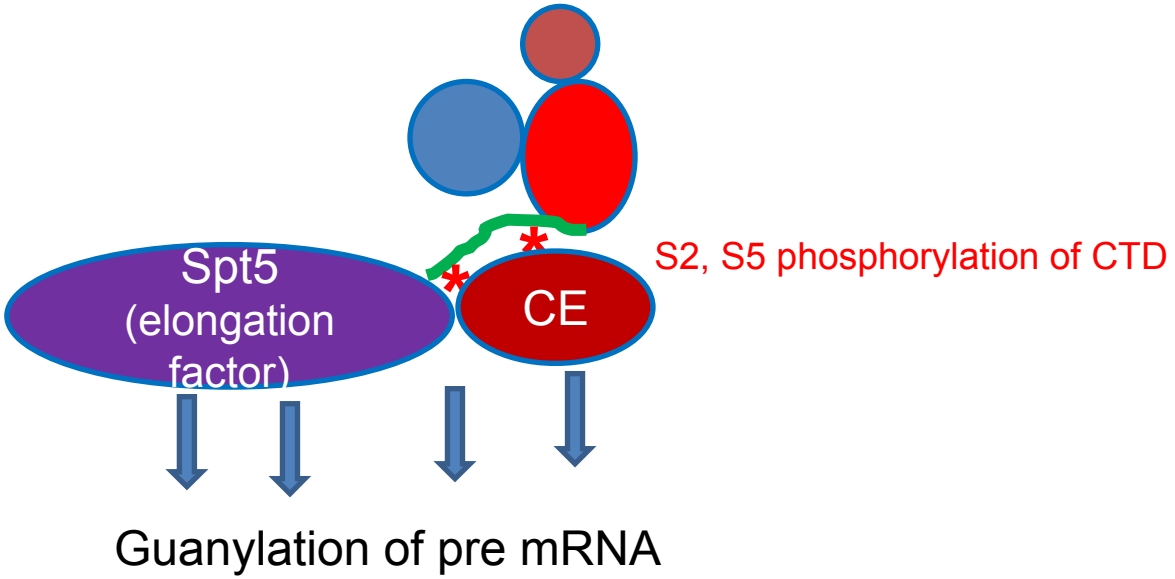
5' pausing is regulated by proteins such as transcription elongation factor Spt5 and HIV1 Tat protein which activate the guanylytransferase and enhance capping and promote transcription elongation.

Thus, CTD phosphorylation and recruitment of factors such as Spt5 act as key regulatory steps in transcription elongation and mRNA capping.

The capping enzymes continue to be associated with the RNA polymerase II even after capping and thus may have a regulatory role in other RNA processing activities as well.

THUS, FACTORS INVOLVED IN TRANSCRIPTION ELONGATION AND mRNA PROCESSING ALSO PLAY A ROLE IN REGULATION OF mRNA SYNTHESIS

Interrelationship between mRNA capping enzyme, RNA polymerase CTD and transcription elongation

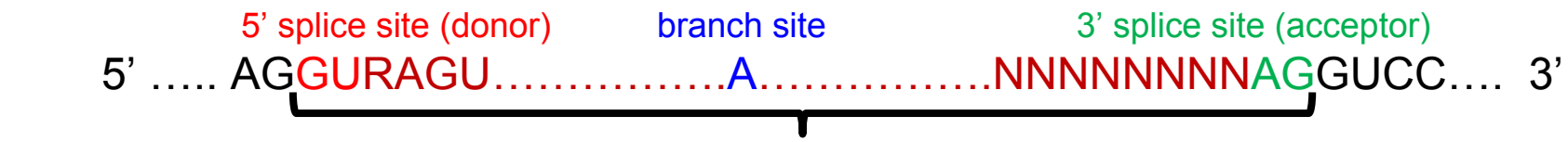


Shuman, S. (2001). **Structure, mechanism, and evolution of the mRNA capping apparatus.** Prog. Nucleic Acid Res. Mol. Biol. 66, 1–40.

Shuman, S. (2002) **What messenger RNA capping tells us about eukaryotic evolution.** Nat Rev Mol Cell Biol. 3, 619-25.

Interactions between RNA Polymerase and mRNA splicing machinery

The multi ribonucleoprotein complex involved in mRNA splicing is called spliceosome. There are five snRNAs in a spliceosome (**U1 to U6)** and as many as 100 proteins.



5’ …C/A AG GU A/G AGU……A…… UUUUUUUUUUU / CCCCCCCCCCC N C/U AG A/G…3’

CONSENSUS SEQUENCE FOR 5’ SPLICE SITE

BRANCH POINT

CONSENSUS SEQUENCE FOR 3’ SPLICE SITE

GURAGU……A……NNNNNNNNAG INTRON

5’ …..AGGUCC 3’….. SPLICED EXONS

mRNA splicing
Cotranscriptional or post transcriptional ?

Beyer AL, Osheim YN.
Splice site selection, rate of splicing, and alternative splicing on nascent transcripts.
Genes Dev. 1988 Jun;2(6):754-65.

These authors demonstrated for the first time by electron microscopy that many, but not all, introns are removed cotranscriptionally rather than posttranscriptionally.

Interactions between mRNA splicing machinery and RNA Pol II

Yeast U1 snRNP protein Prp40, which bridges the 5' splice site and branch point, was shown to bind directly to the phosphorylated CTD.

Human U1snRNP, but not other snRNPs, also coimmunoprecipitates with RNA Pol II.

U1snRNP at a 5' splice site can promote recruitment of RNA Pol II and general transcription factors to the promoter indicating that there is a special relationship between transcription initiation/elongation machinery and components of mRNA splicing.

Proteins known as SR (serine-arginine rich) proteins involved in mRNA splicing were shown to interact with RNA Pol II and promote transcription elongation.

Several elongation factors such as PTEFb, CA150, and TAT-SF1 associate directly or indirectly with spliceosomal U snRNPs

A functional role for RNA Pol II CTD in splicing

Transcriptional activation of pol II genes induces the association of splicing factors to sites of transcription only when RNA pol II has a full CTD

Deletion of the RNA Pol II CTD inhibits splicing of the β-globin gene

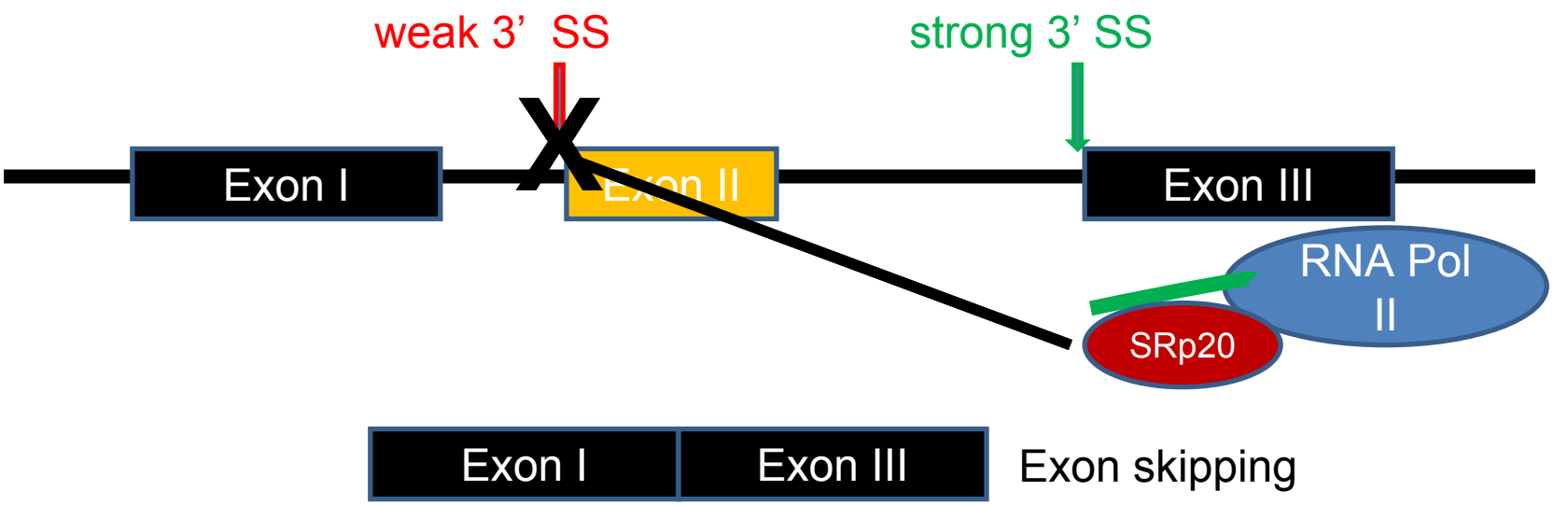
Using *in vitro* splicing reactions it was demonstrated that phosphorylation of RNA polymerase II CTD enhances mRNA splicing.

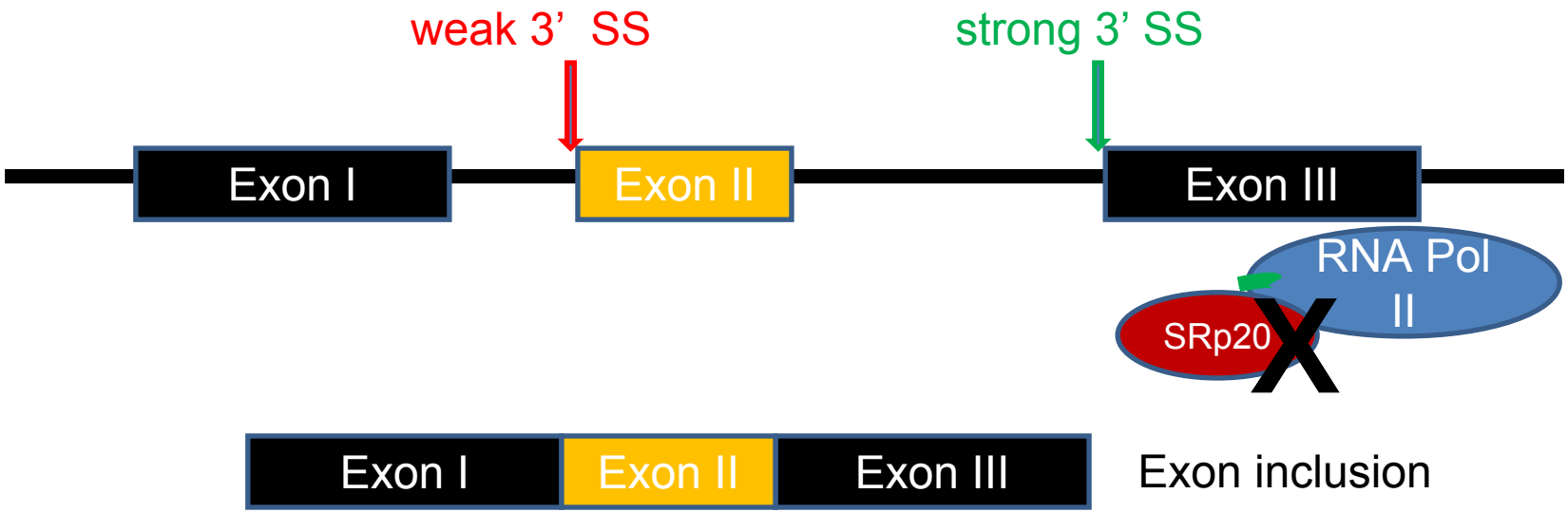
Two examples:

1. Role of RNA pol II CTD in splicing

2. Role of transcription elongation in splicing

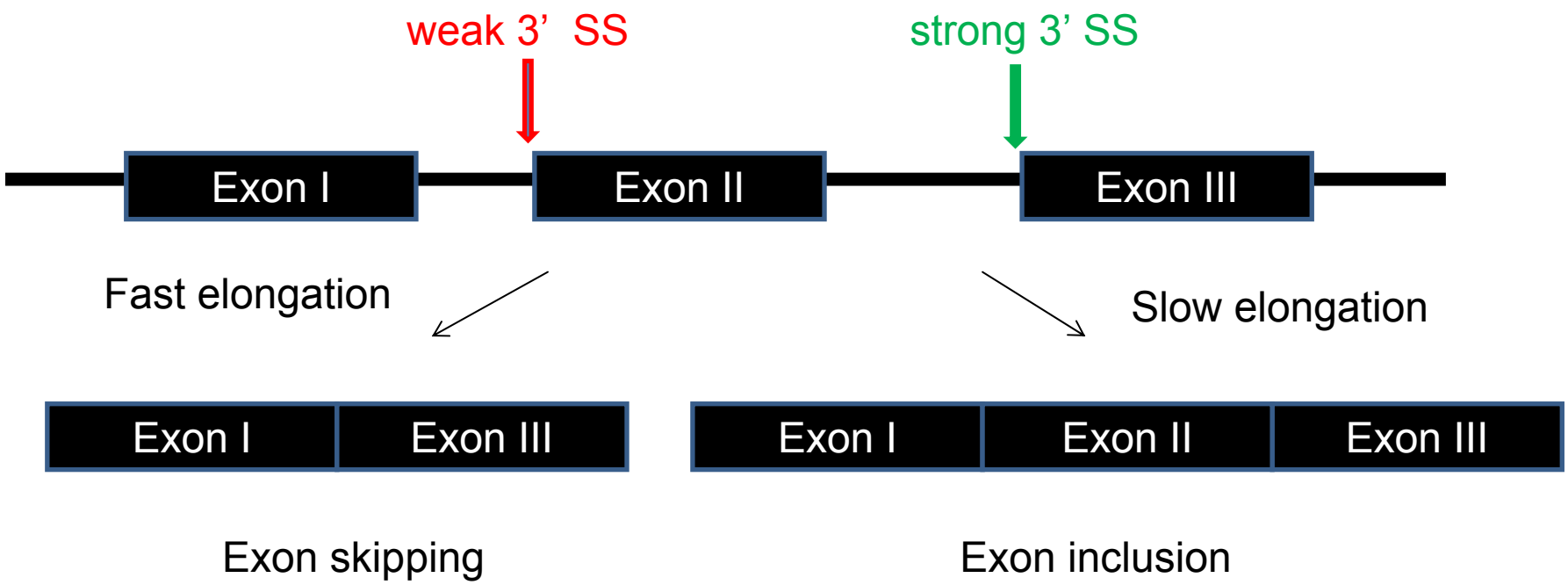
Role of RNA pol II CTD in generating two different forms of mRNA by alternate splicing in case of fibronectin gene





The rate of transcription elongation in eukaryotes varies from 1.9 kb/min to 4.3 kb/min

The rate of elongation may often influence splicing and can lead to alternate splicing.



Misteli, T., J.F. Caceres, and D.L. Spector. 1997.
The dynamics of a pre-mRNA splicing factor in living cells.
Nature **387:** 523-527

Following activation of a reporter gene in cells expressing either full-length or CTD-truncated RNAP II as the only source of active enzyme, sites of accumulation of both the newly synthesized reporter transcripts and splicing factors were simultaneously visualized by immunohistochemistry techniques.

Although both sites colocalized well in cells expressing wild-type RNAP II, the transcription sites did not colocalize with either SR proteins or snRNP particles in cells expressing the CTD-truncated RNAP II.

Truncation of the CTD prevented accumulation of spliced products despite the presence of significant amounts of unspliced pre-mRNAs.

These results supported the idea that the CTD is required for targeting splicing factors to transcription sites and that this can be important for efficient splicing.

Original research articles

In response to UV-induced DNA damage, the CTD becomes hyperphosphorylated, transcription elongation slows down, and alternative splice choices are switched in favor of the proapoptotic isoforms of Bcl-x and caspase.

Munoz, M.J., et al., (2009).
DNA damage regulates alternative splicing through inhibition of RNA polymerase II elongation. Cell 137, 708–720.

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

McCracken *et al.*, 5′-Capping enzymes are targeted to pre-mRNA by binding to the phosphorylated carboxy-terminal domain of RNA polymerase II, *Genes Dev.* **11** (1997), pp. 3306–3318

de la Mata, M. et al., (2003).
A slow RNA polymerase II affects alternative splicing in vivo.
Mol. Cell 12, 525–532.

M. de la Mata and A.R. Kornblihtt
RNA polymerase II C-terminal domain mediates regulation of
alternative splicing by SRp20 *Nat. Struct. Mol. Biol.* **13** (2006), 973–980.