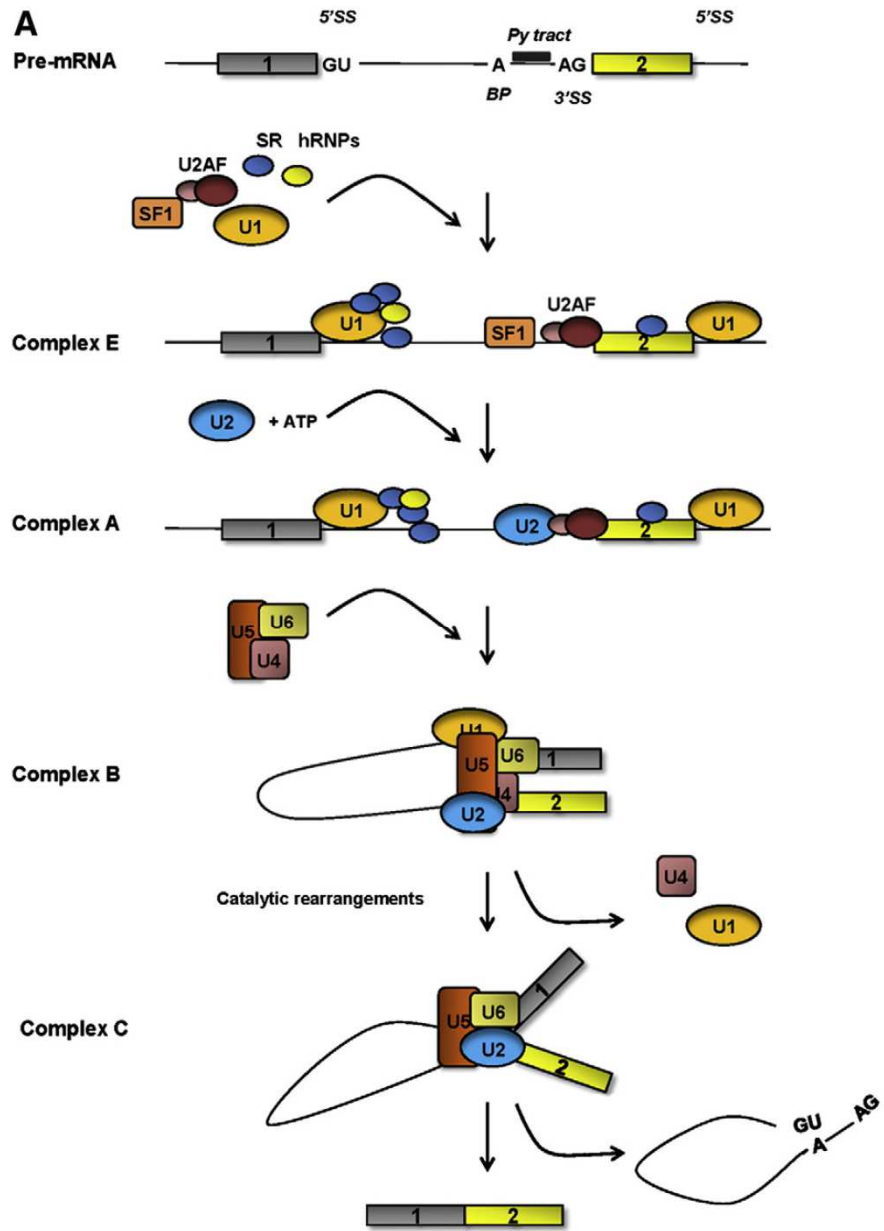


Where Splicing Joins Chromatin And Transcription

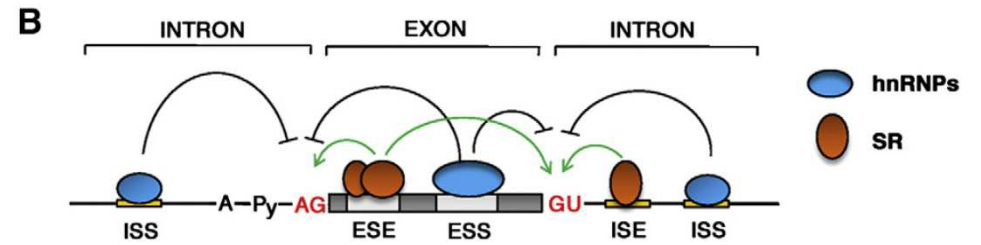
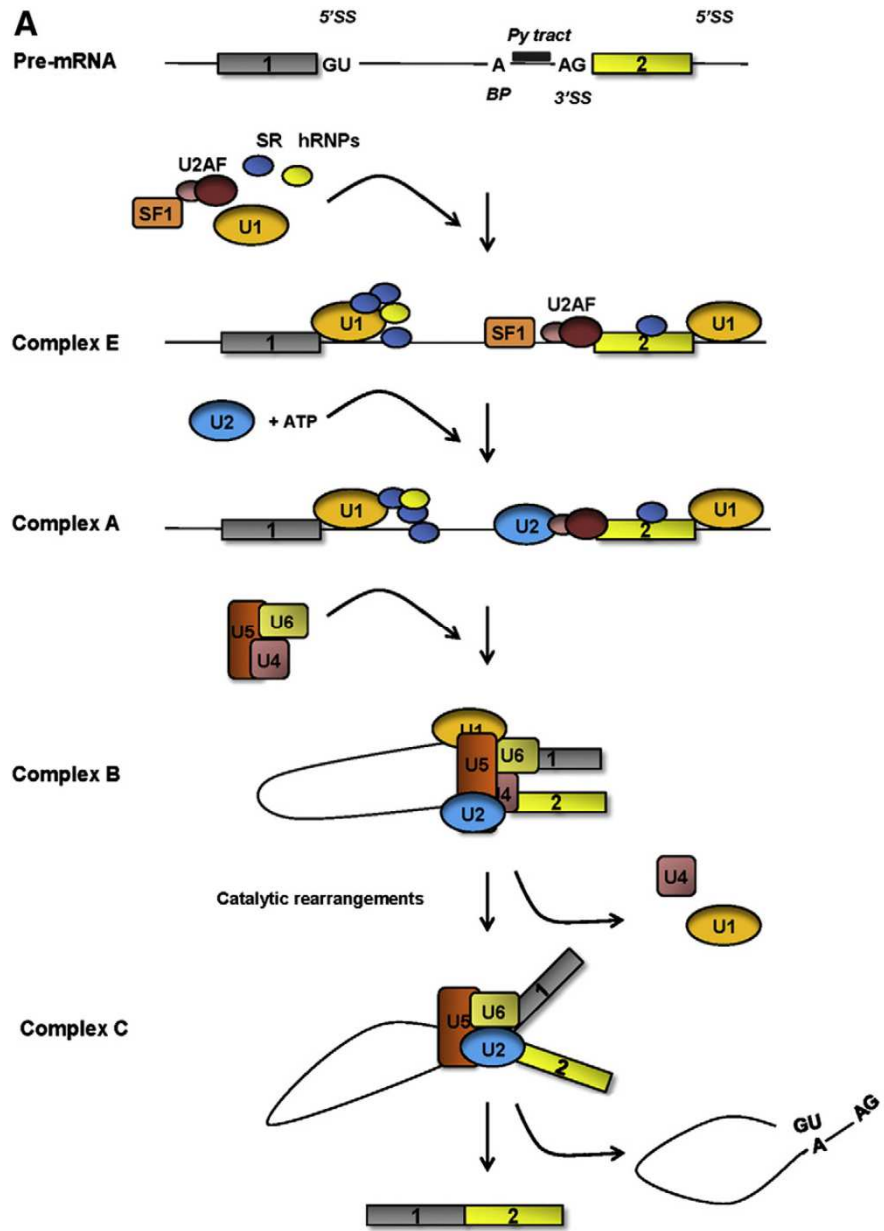
18/10/2013

Dario Balestra

Splicing process overview



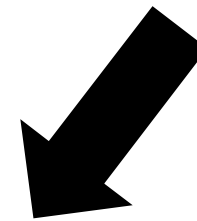
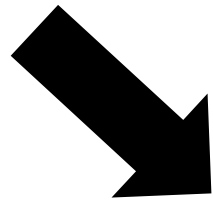
Splicing process overview



Tissue-specific Proteins

Sequence context

Development stage



Splicing

Tissue-specific Proteins

Sequence context

Development stage

Splicing

ncRNA

Chromatin

Transcription

Elongation rate

Promoter effect

Pausing effect

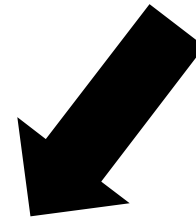
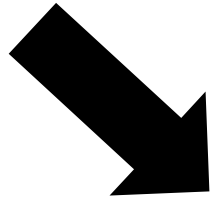
Histone modification

Nucleosome position

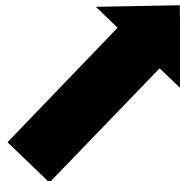
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Development stage



Splicing



Transcription

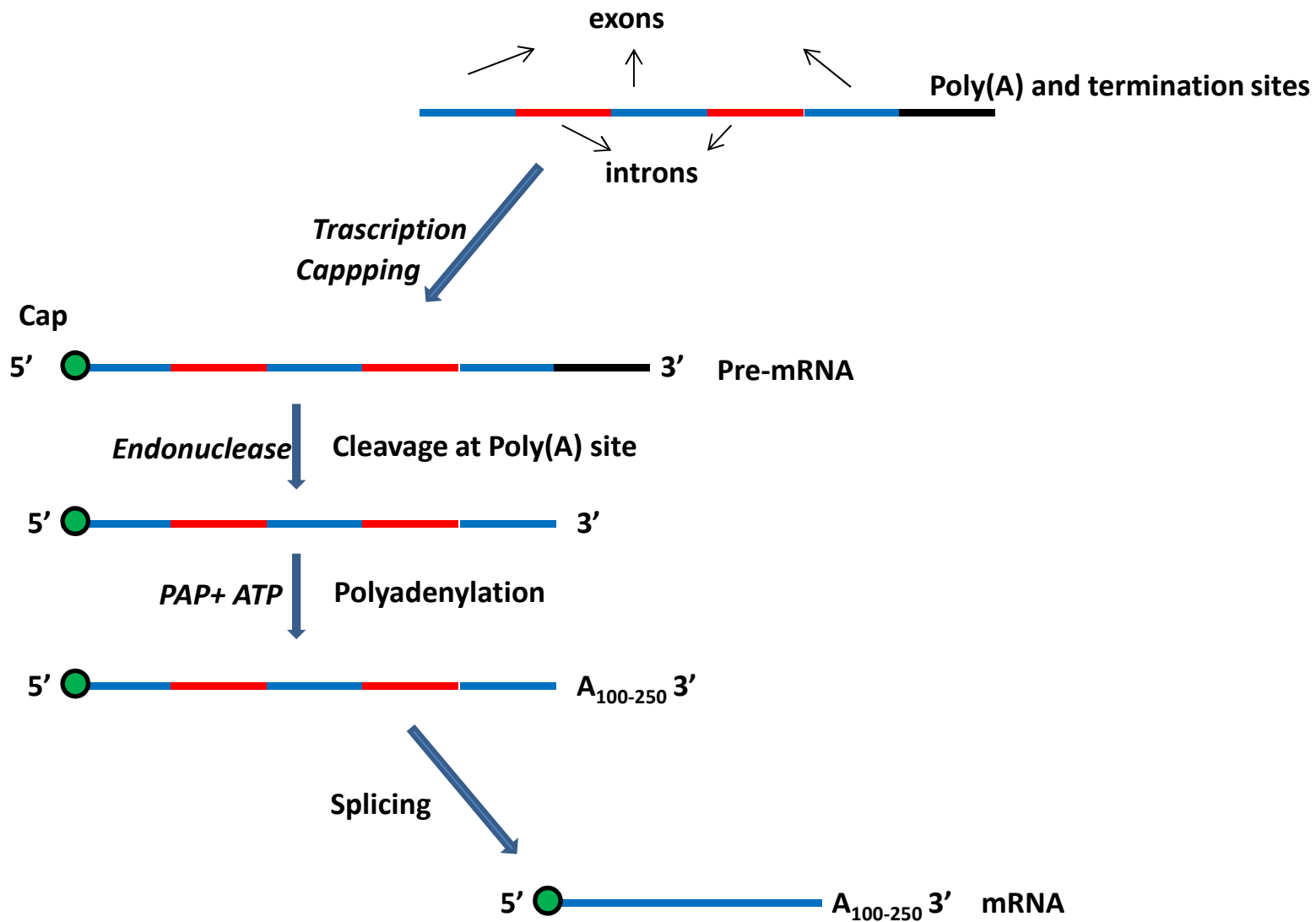
Elongation rate

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Splicing and transcription

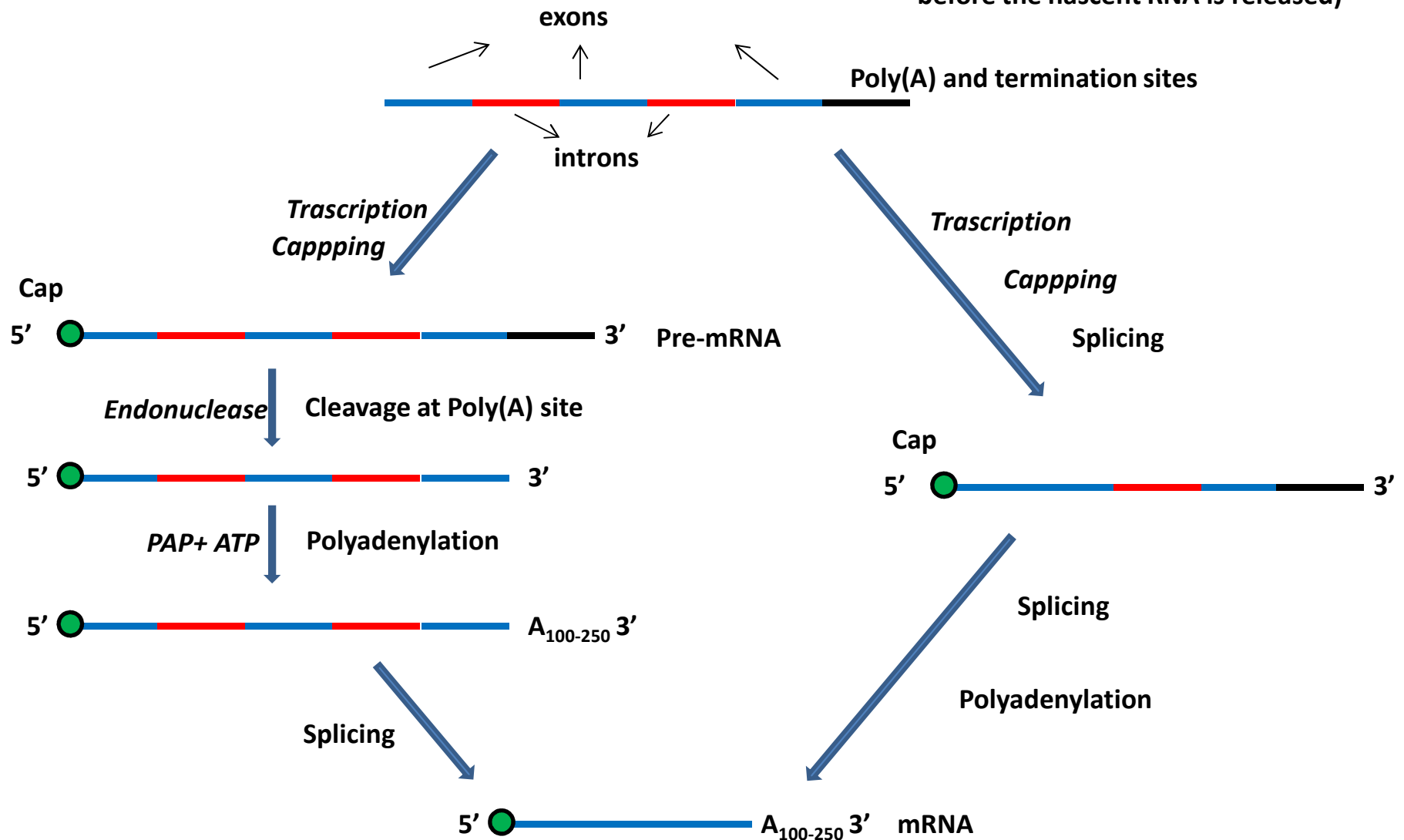
Classical view of pre-mRNA processing



Splicing and transcription

Classical view of pre-mRNA processing

Cotranscriptional pre-mRNA processing
(splicing takes place, or is committed to occur, before the nascent RNA is released)



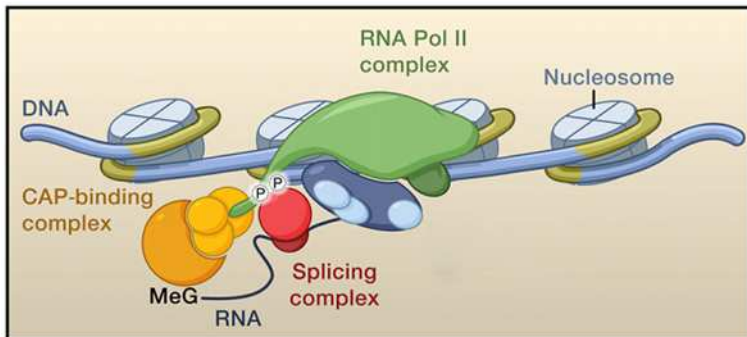
Transcription and splicing:

How can RNA Pol II affect splicing outcome?

Different recruitment
of splicing factors
(recruitment coupling)



Phosphorilated CTD domain
of RNA pol II can recruit
CAP-binding complex and
several splicing factors

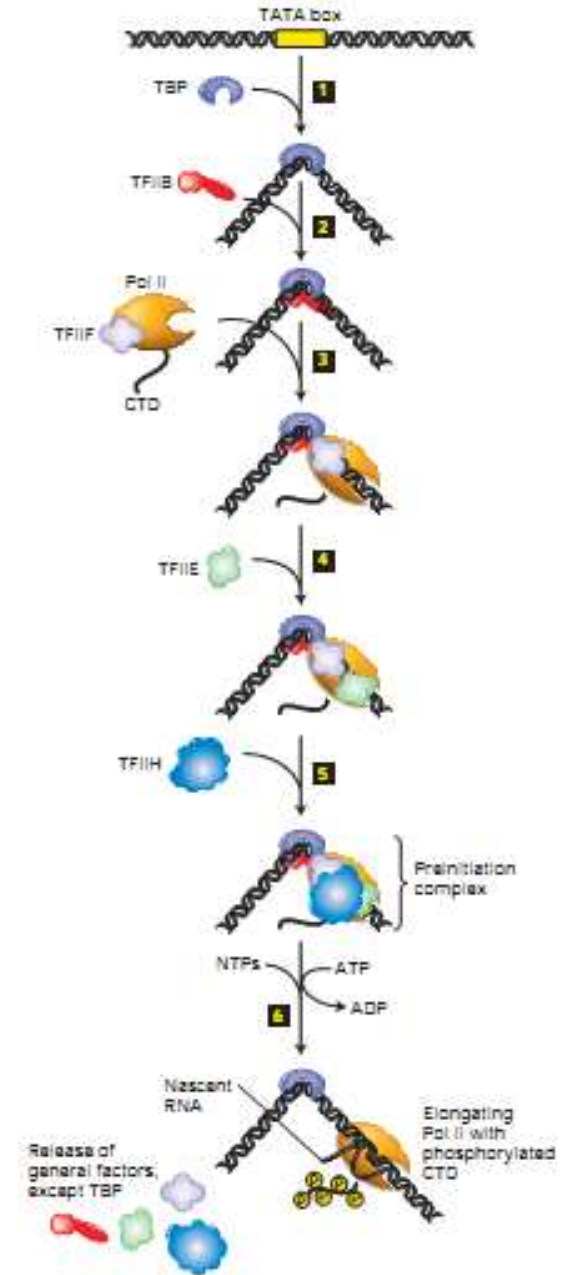


Different rates of
RNA Pol II elongation
(kinetic coupling)



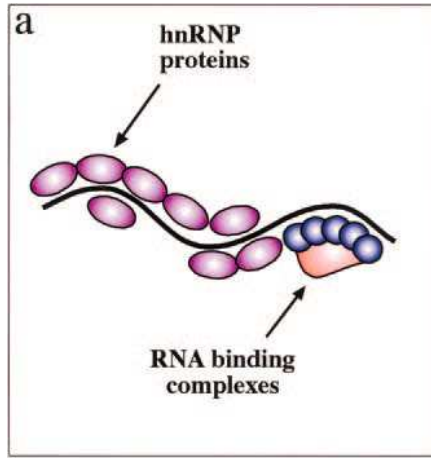
Elongation rate

RNA structure



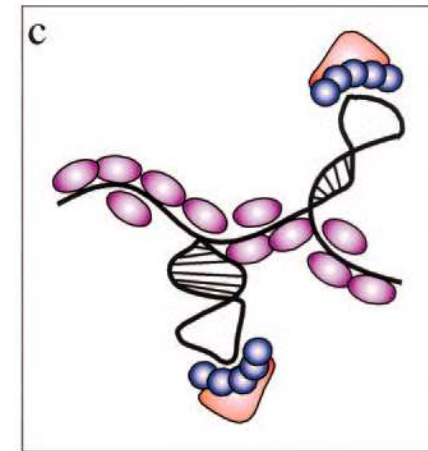
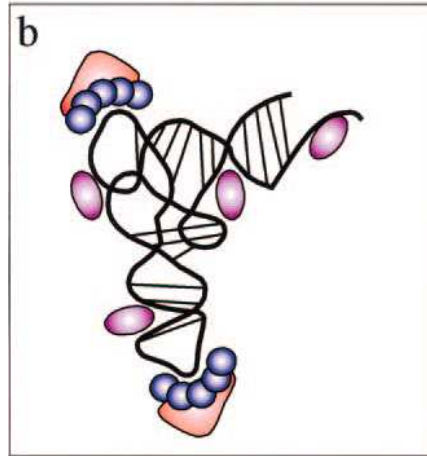
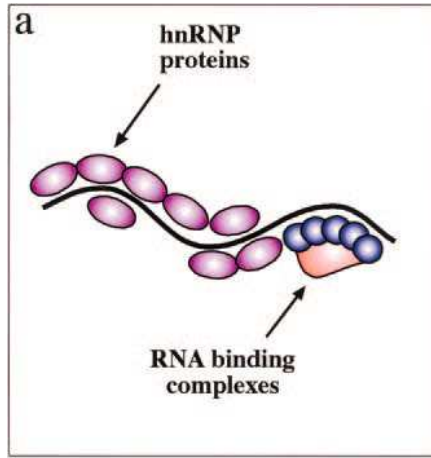
RNA Structure:

Sites not accessible:



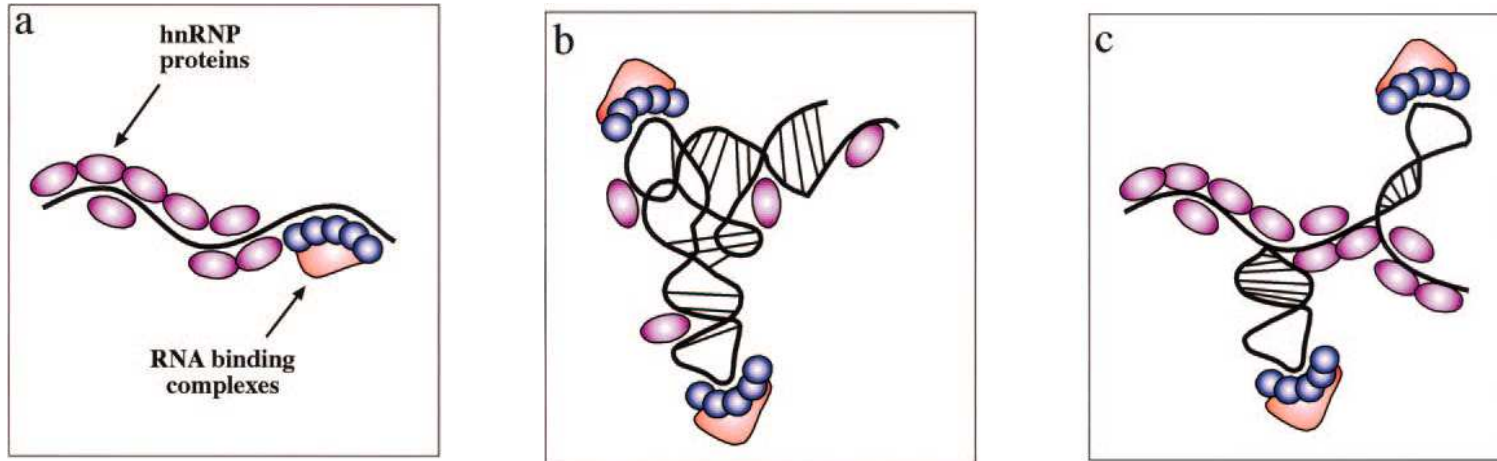
RNA Structure:

Sites not accessible:

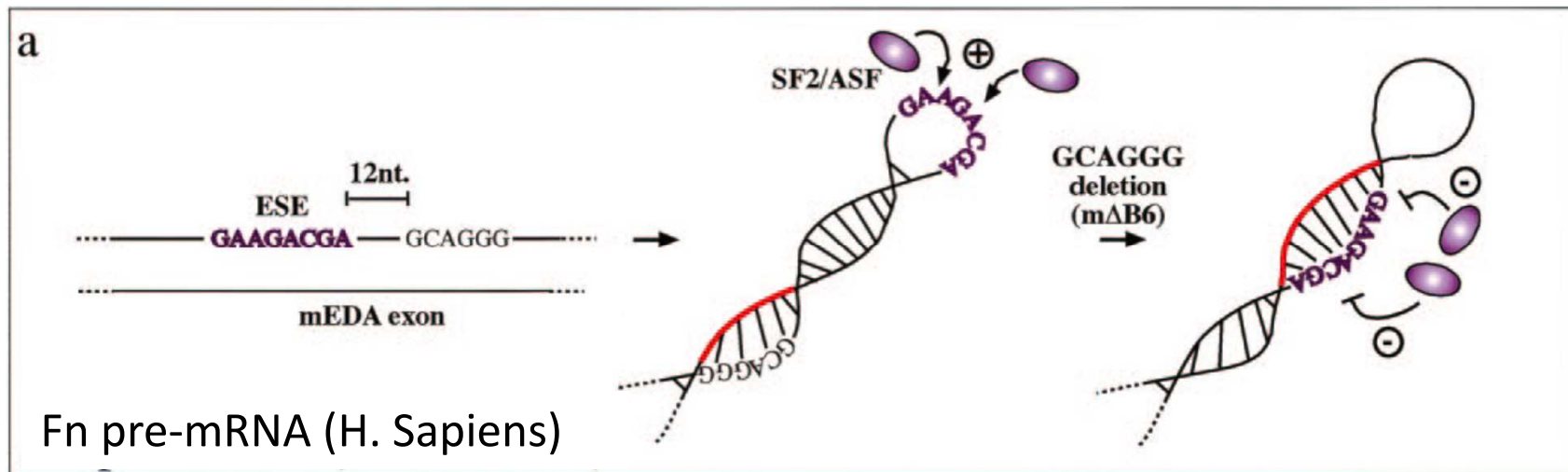


RNA Structure:

Sites not accessible:



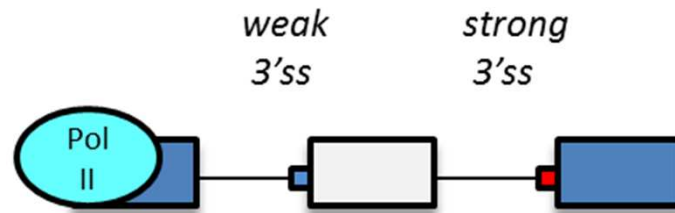
Steric hindrance:



Elongation rate:

Affected by:

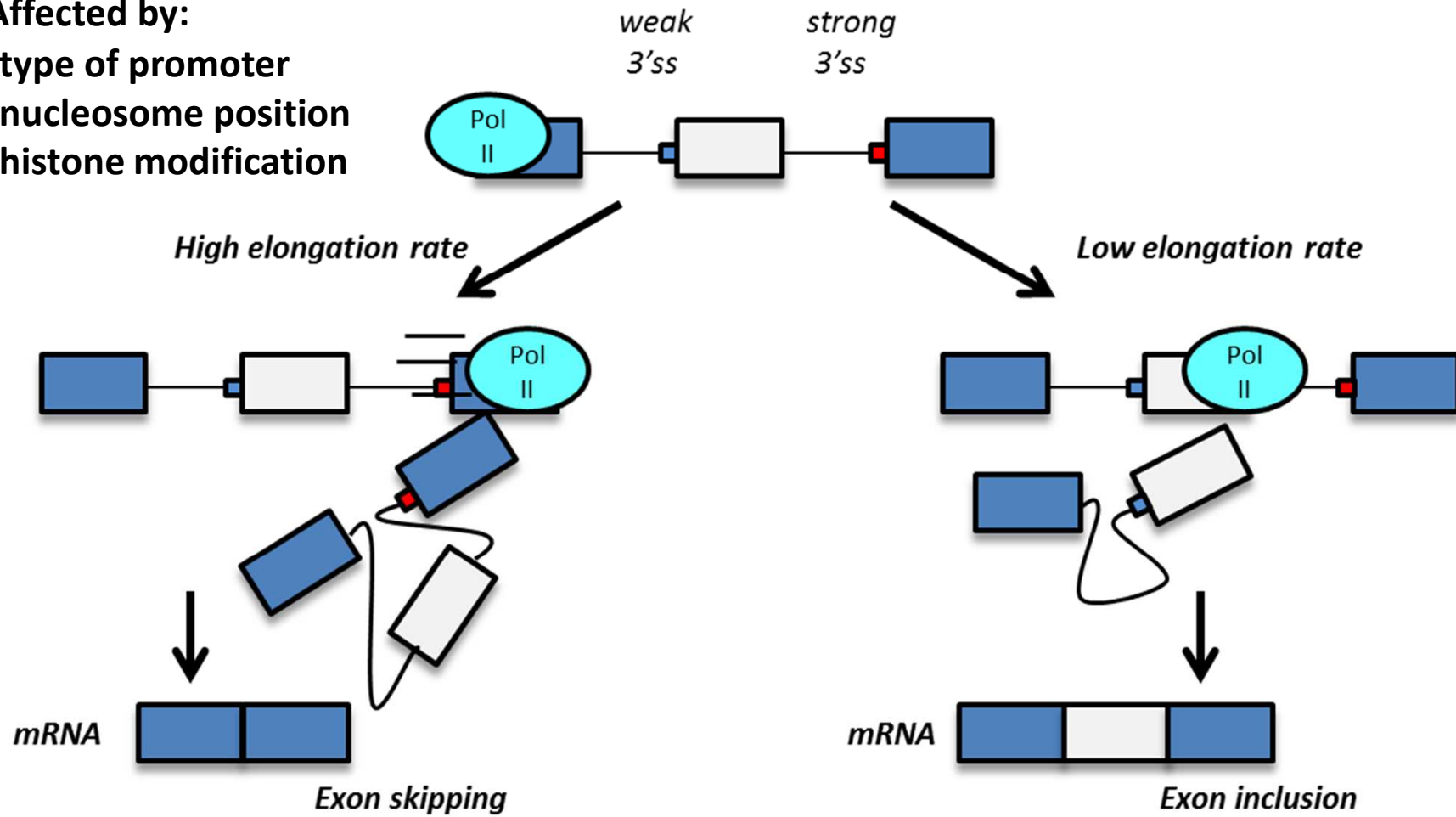
- type of promoter
- nucleosome position
- histone modification



Elongation rate:

Affected by:

- type of promoter
- nucleosome position
- histone modification

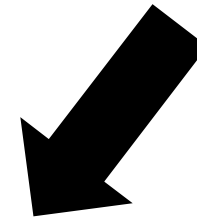
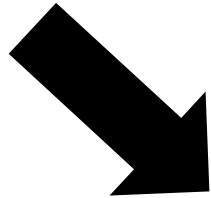


Slowing of RNA Pol II **increases the window of time** an upstream weak exon can recruit the splicing machinery before the splicing sites of a stronger downstream exon emerge from the polymerase complex, favouring exon inclusion

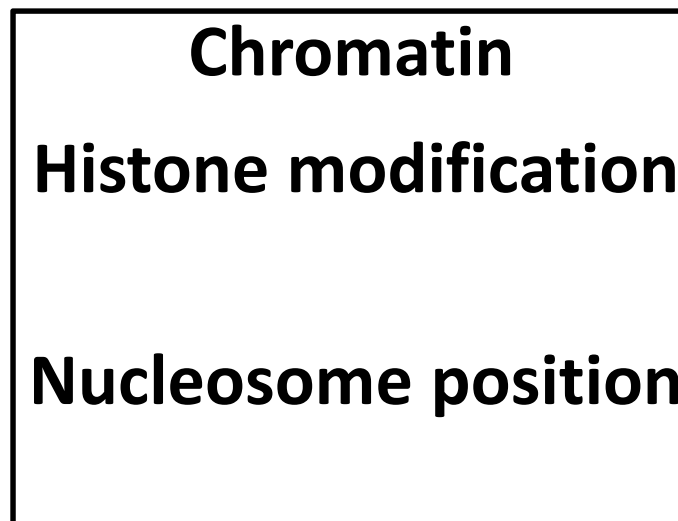
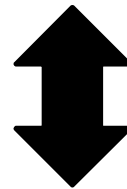
Tissue-specific Proteins

Sequence context

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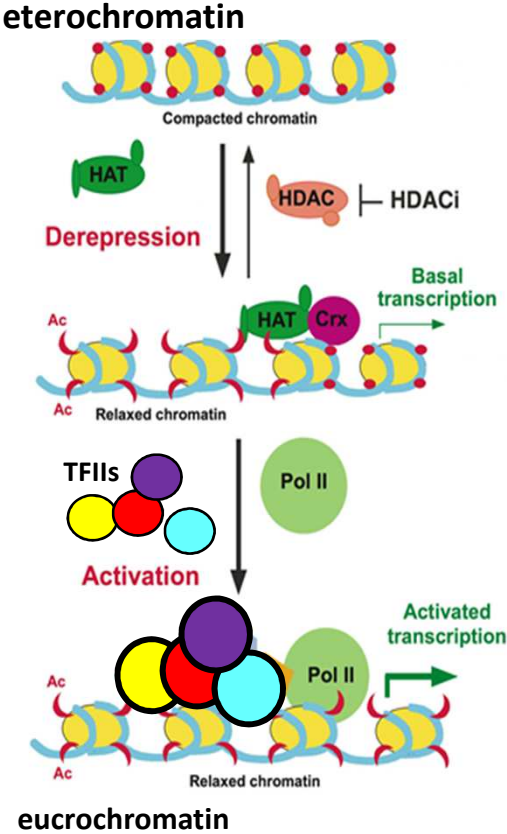


Splicing



Histone modifications: major regulators of alternative splicing

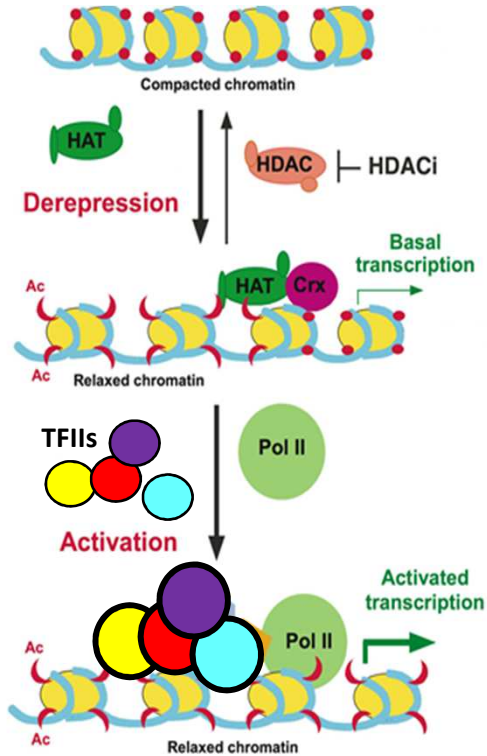
Chromatin structure and transcription



Histone modifications: major regulators of alternative splicing

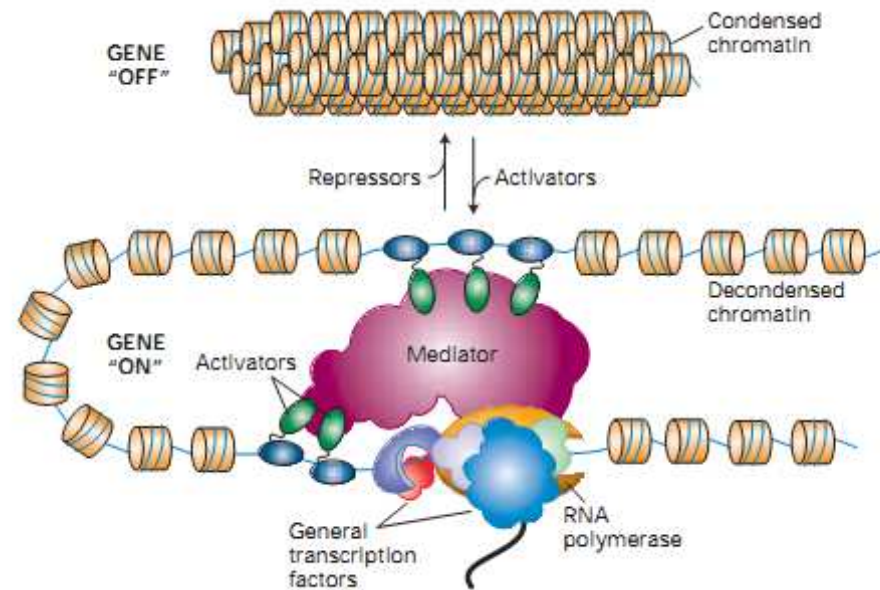
Chromatin structure and transcription

heterochromatin



eukromatin

General overview

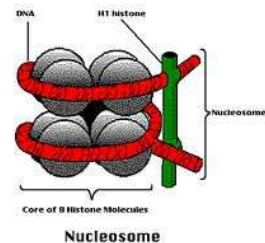


Histone modifications: major regulators of alternative splicing

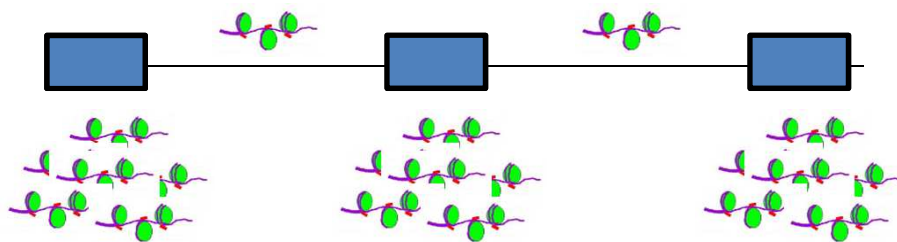
Exons are marked by increased nucleosome occupancy, distinct histone modifications and elevated DNA methylation relative to introns.

Nucleosome position:

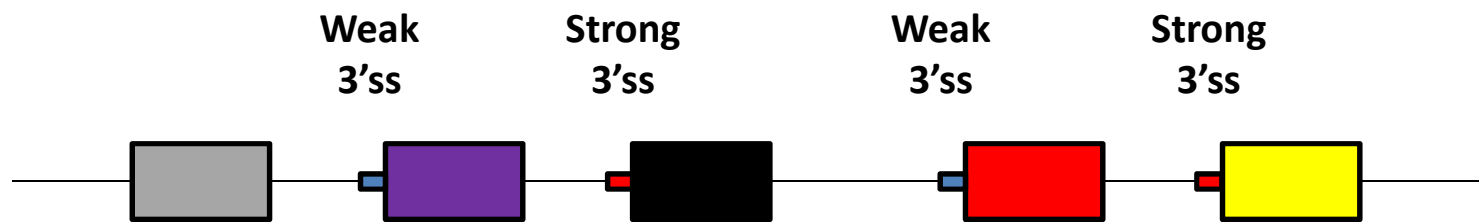
Nucleosome: stretch of ~147bp of DNA wrapped around an octamer of histone proteins

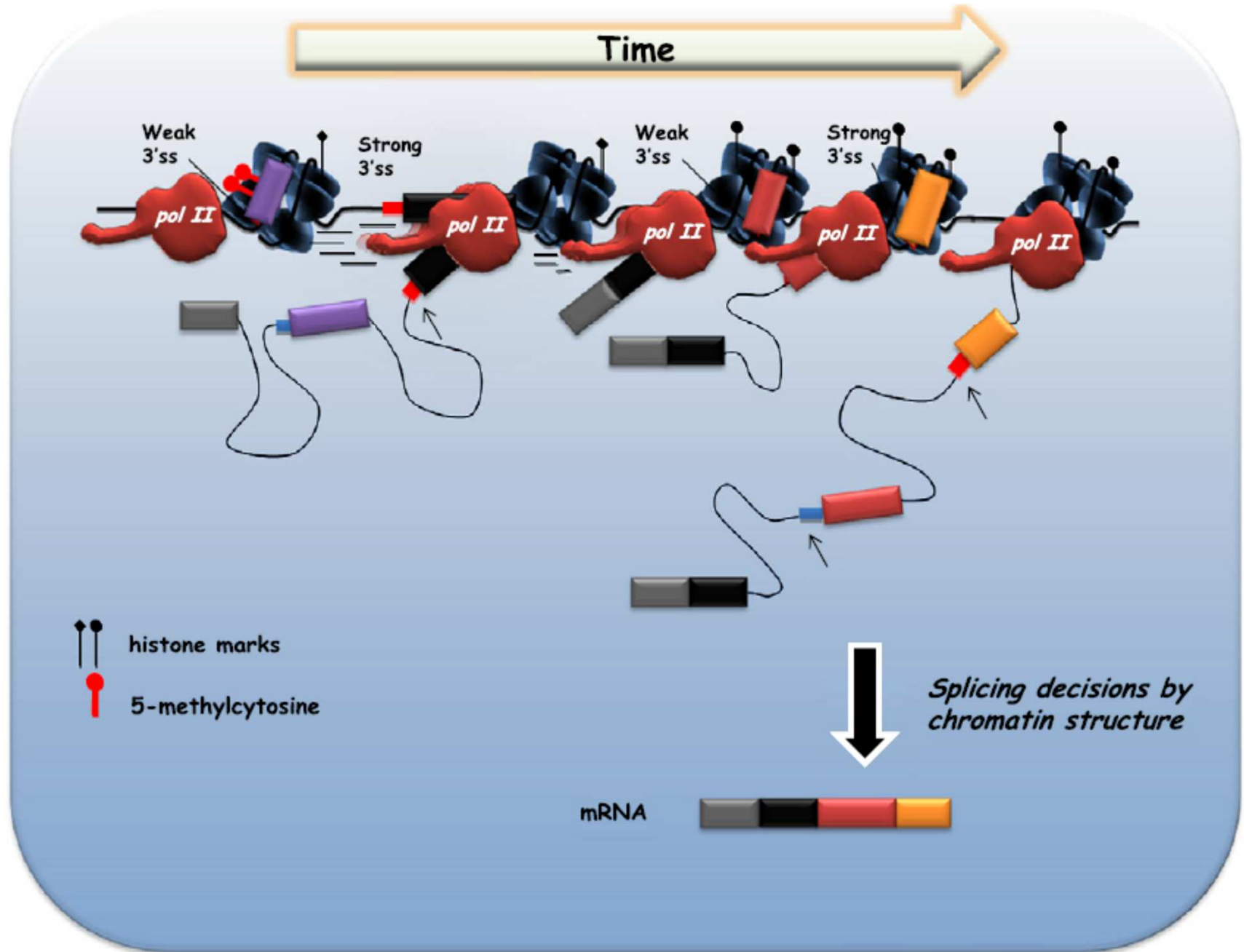


Average size of mammalian exons: 145bp



Nucleosomes behave as barrier that slowing down the elongation rate of Pol II



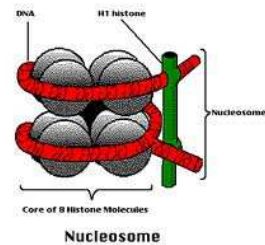


Histone modifications: major regulators of alternative splicing

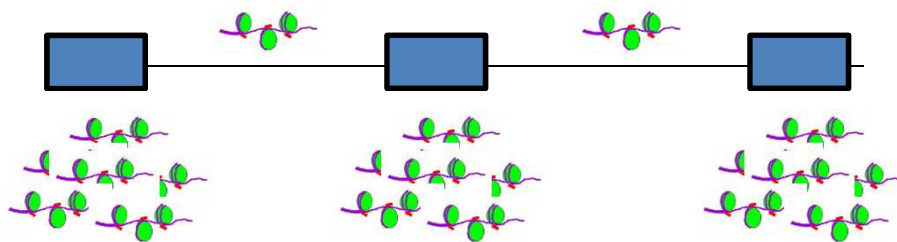
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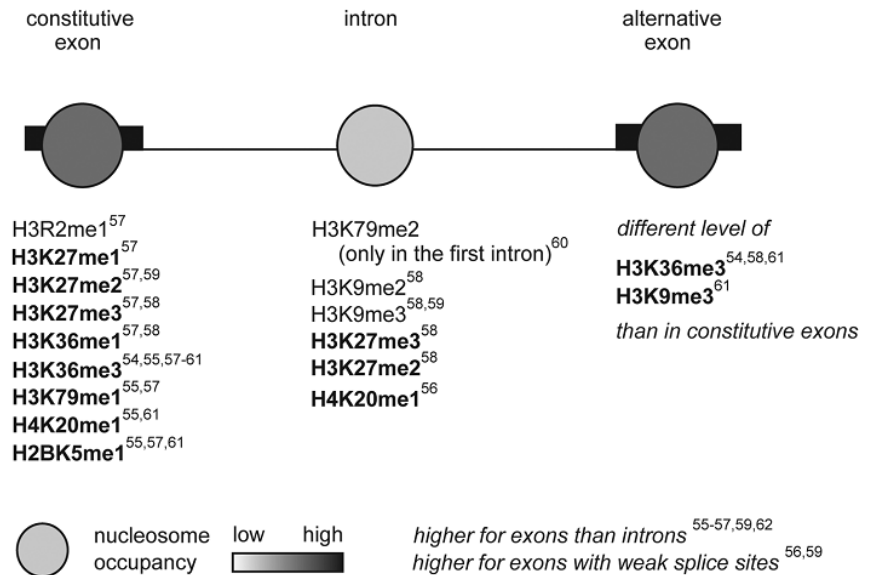
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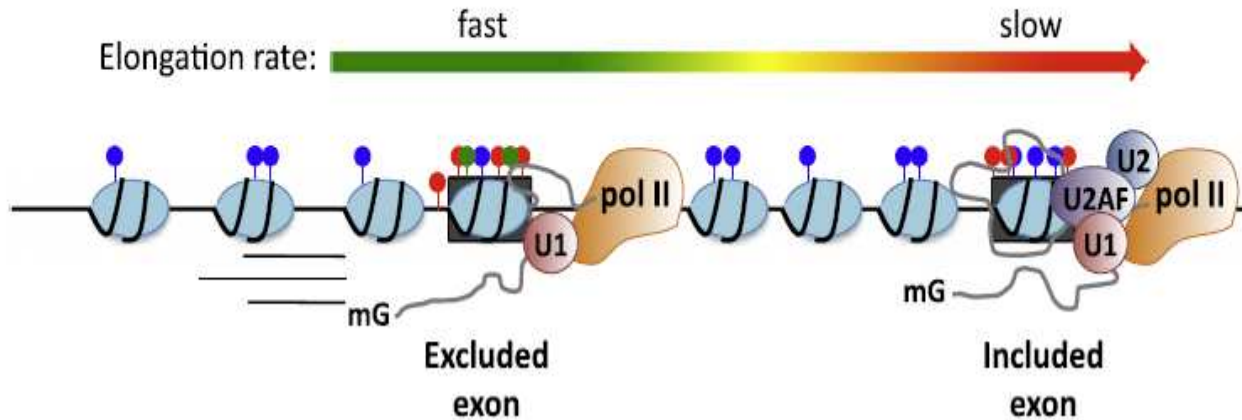
Nucleosomes behave as barrier that slowing down the elongation rate of Pol II

Histone modifications: major regulators of alternative splicing

Histone modifications are not randomly distributed among genome:

a

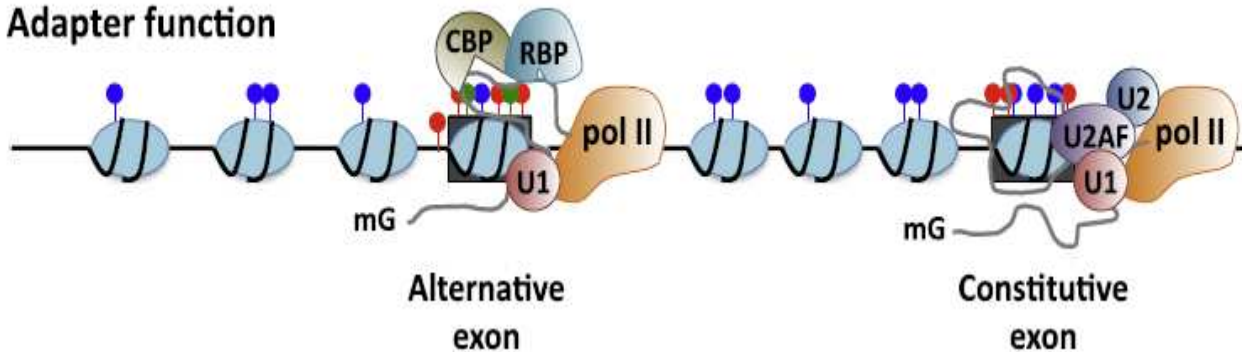
Kinetic regulation



Facilitate the recruitment of splicing regulators at weak exons

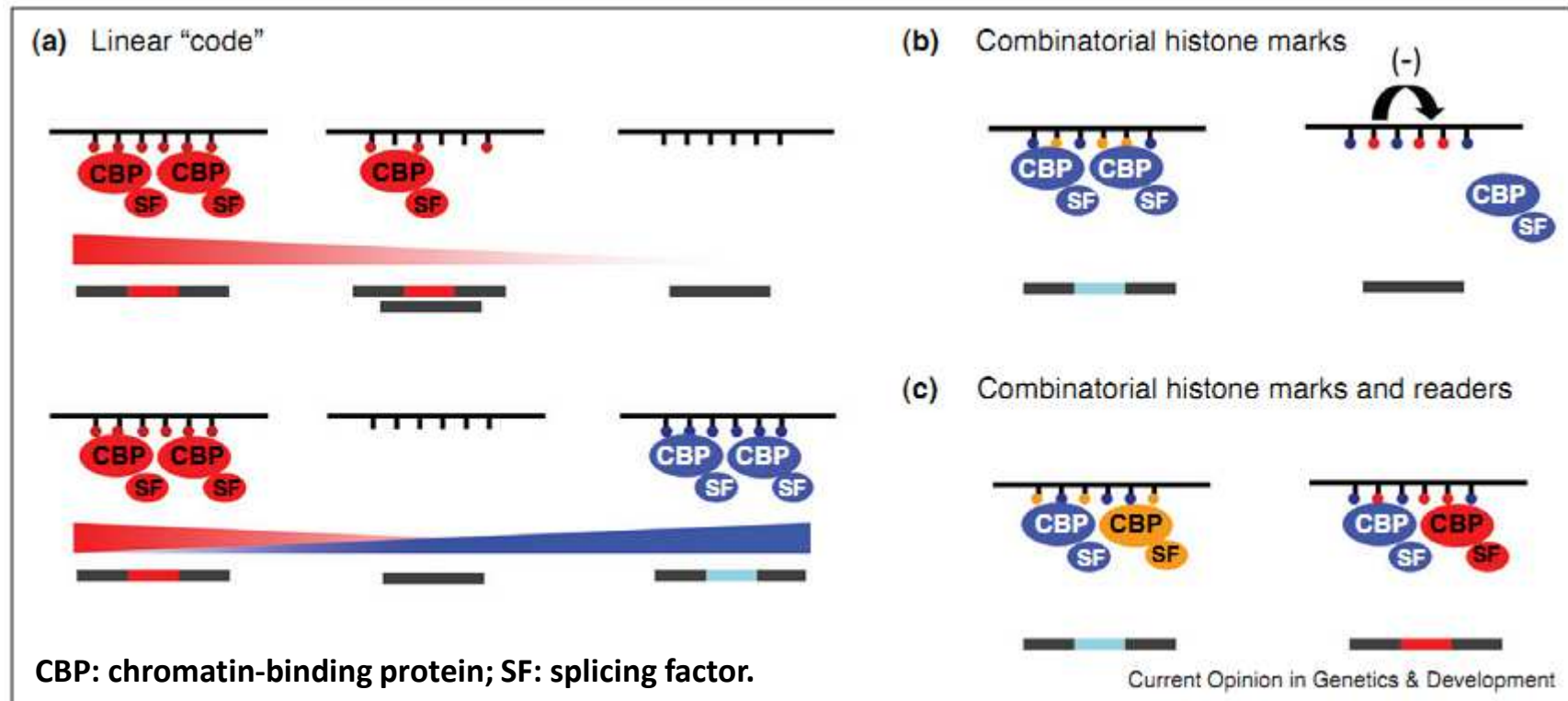
b

Adapter function



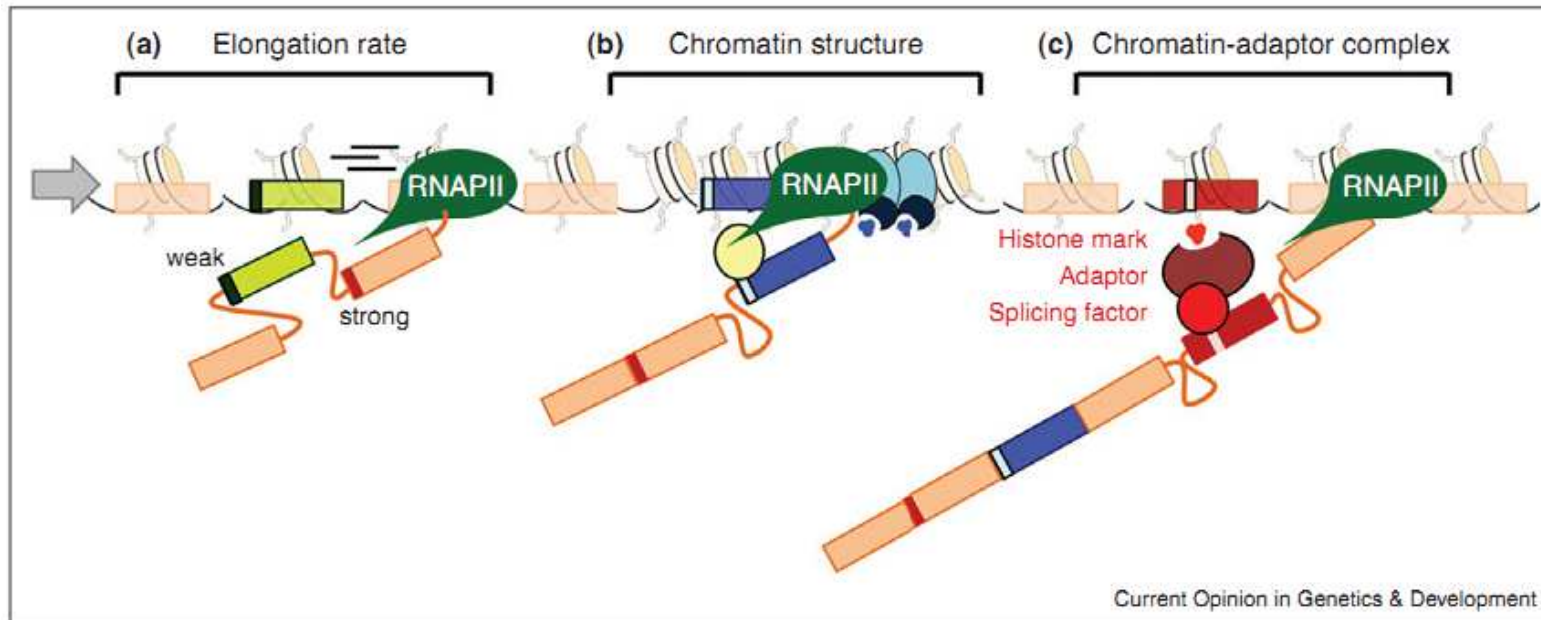
Recruitment of Chromatin-binding Proteins (CBP) that act as adaptor molecules for RNA binding protein (RBP) that promote or inhibit spliceosome assembly

Adapter function: the “Histone code”



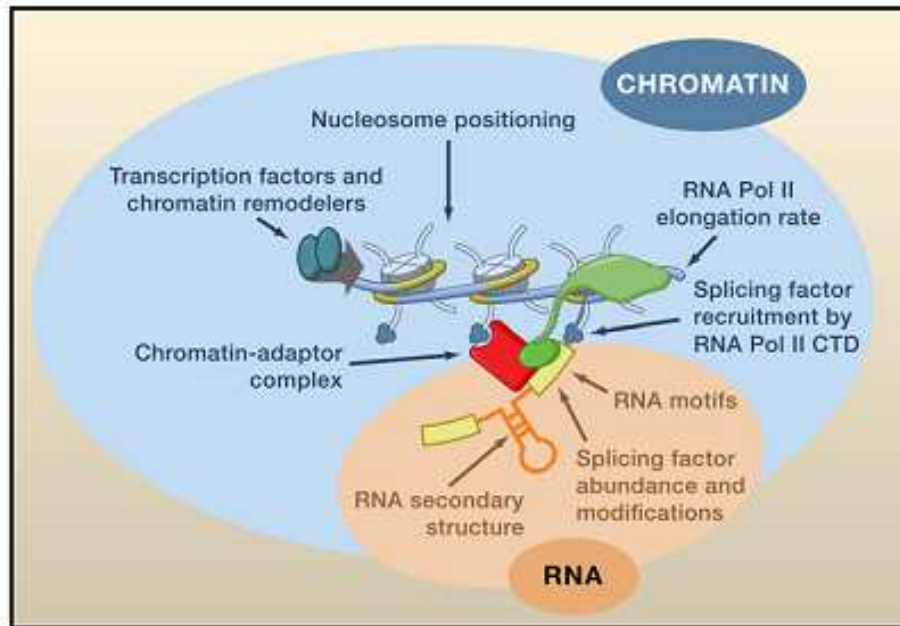
- (a) Histone marks may act linearly with increasing levels of a single histone mark recruiting increasing levels of a chromatin-adaptor protein complex leading to increased usage of a given site. Competing levels of different histone marks modulate the recruitment of competing chromatin-adaptor complexes determining the final splicing outcome
- (b) Histone modifications may act in combination by favoring (left) or inhibiting (right) the recruitment of a single chromatin-splicing complex
- (c) Multiple histone marks may recruit in combination multiple chromatin-adaptor complexes that will favor or inhibit exon inclusion.

The role of chromatin in alternative splicing:



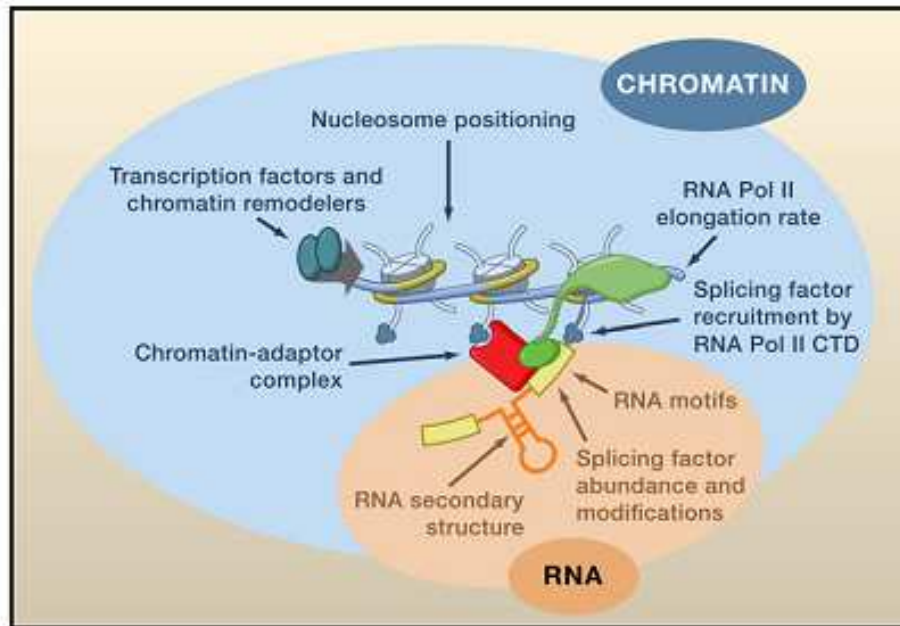
- (a) RNAP II elongation rate affects recruitment of the splicing machinery. Fast elongation favors inclusion of a downstream exon with strong splice sites.
- (b) A change in chromatin conformation, such as localized heterochromatinization (blue ovals and higher density of nucleosomes), slows down RNAP II which favors recruitment of splicing factors (yellow oval) to the weaker exon (blue rectangle), inducing exon inclusion.
- (c) Histone modifications (small red circles) can directly recruit splicing factors via a chromatin-adaptor system (red ovals) which consists of a chromatin-binding protein that reads the histone marks and modulates recruitment of the splicing factor to the pre-mRNA (red rectangle).

Integrated model:

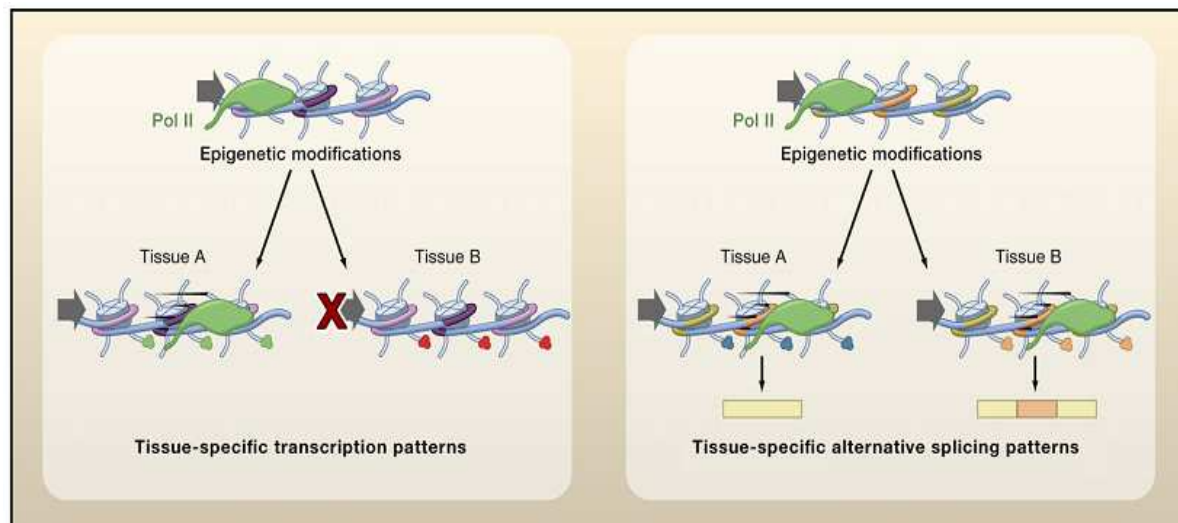


Alternative splicing patterns are determined by a combination of parameters including cis-acting RNA regulatory elements and RNA secondary structures (highlighted in orange) together with transcriptional and chromatin properties (highlighted in blue) that modulate the recruitment of splicing factors to the pre-mRNA.

Integrated model:



Alternative splicing patterns are determined by a combination of parameters including cis-acting RNA regulatory elements and RNA secondary structures (highlighted in orange) together with transcriptional and chromatin properties (highlighted in blue) that modulate the recruitment of splicing factors to the pre-mRNA.



The combination of histone modifications along a gene establishes and maintains tissue-specific transcription patterns (left panel), as well as heritable tissue-specific alternative splicing patterns (right panel)

Histone Deacetylase Activity Modulates Alternative Splicing

Jarmila Hnilicová, Samira Hozeifi, Eva Dušková, Jaroslav Icha, Tereza Tománková, David Staněk*

Department of RNA Biology, Institute of Molecular Genetics AS CR, Prague, Czech Republic

11 February 2011 PlosOne

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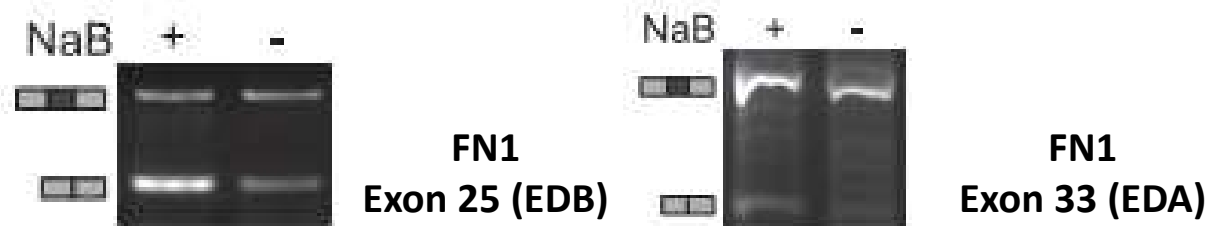
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11 February 2011 PlosOne

Cells treated with potent HDAC inhibitor – sodium butyrate (NaB)

The splicing of 681 genes (out 17771) was altered.

Focus on fibronectin FN1 gene.



It is known that SRp40 and PTB are important for exon 25 (EDB) inclusion

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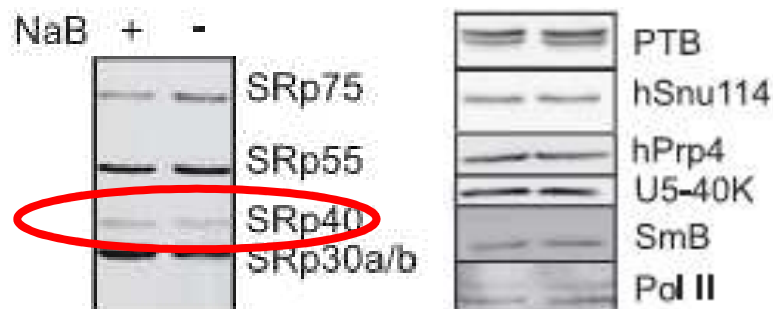
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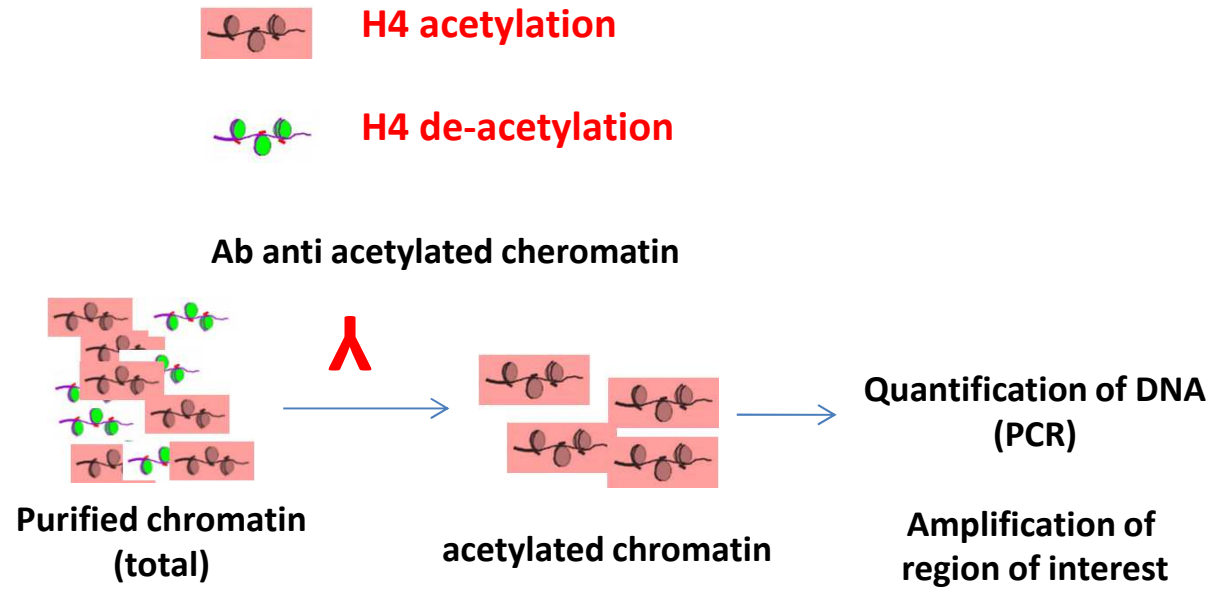
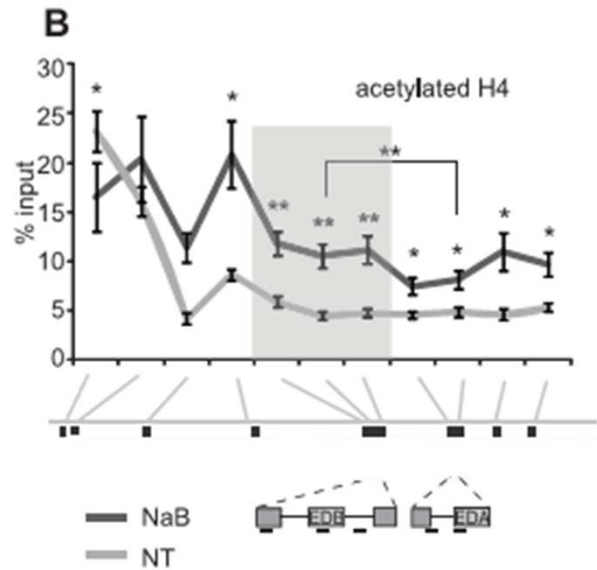
Wich is the relationship between HDAC inhibition and EDB splicing??

Does the HDAC inhibition affect expression of splicing regulators (SR protein)?

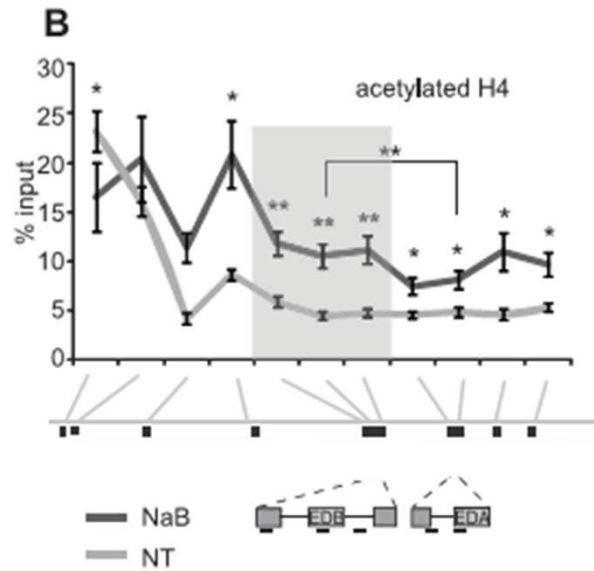


No differences in expression of SR proteins, PTB, Pol II or core spliceosomal components

Analysis of chromatin marks along FN1 gene

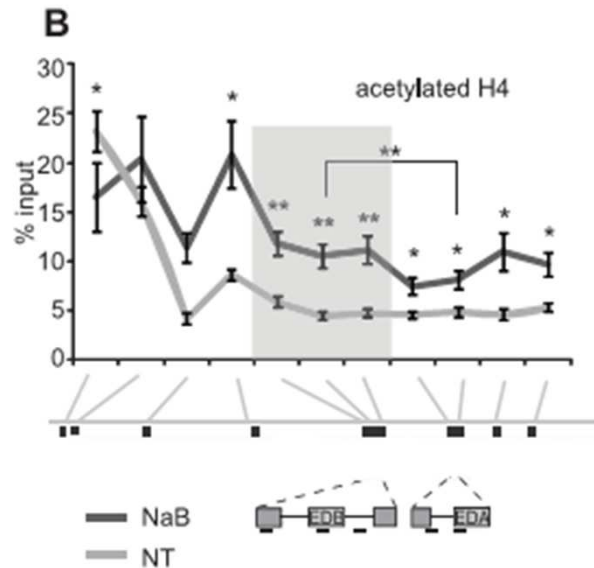


Analysis of chromatin marks along FN1 gene



Higher H4 acetylation levels
H4 acetylation correlates with EDB exon skipping

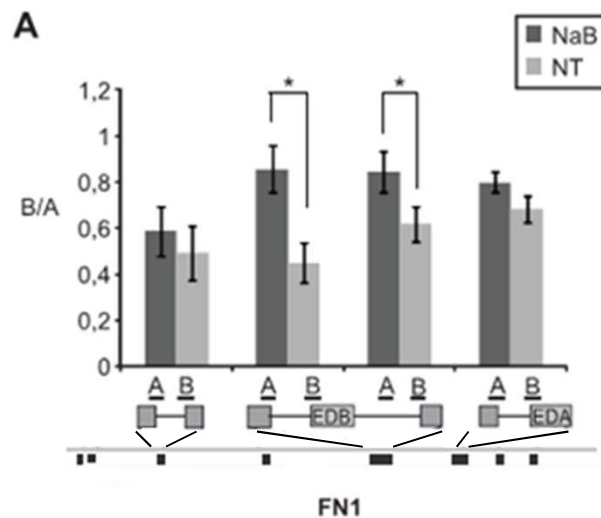
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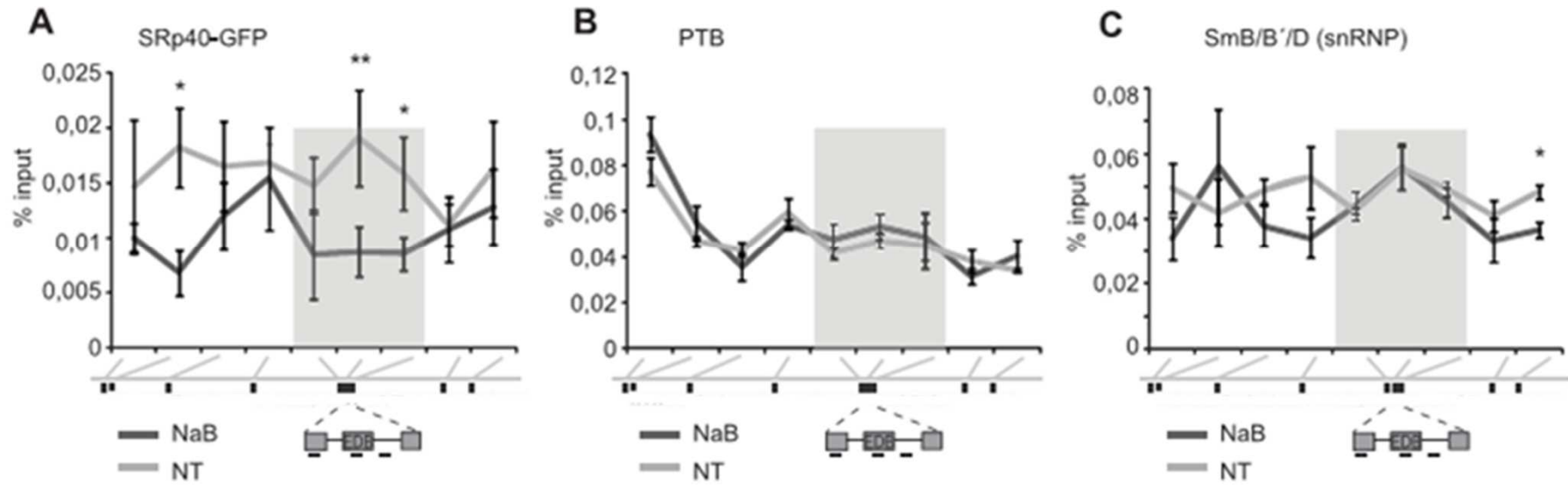
Pol II processivity and HDAC inhibition:

Exist a correlation between H4 acetylation and RNA Pol II processivity



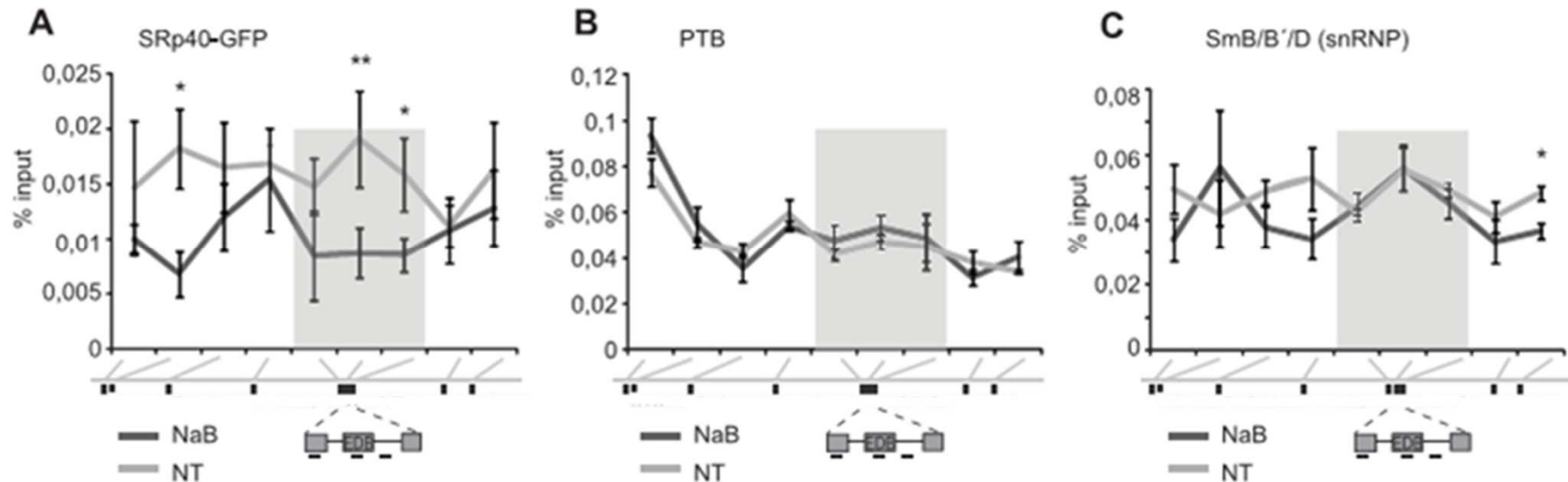
increased Pol II processivity at upstream and downstream introns

HDAC inhibition and SRp40,PTB and snRNP association with the EDB exon:



Reduced SRp40 association with the EDB exon

HDAC inhibition and SRp40,PTB and snRNP association with the EDB exon:



Reduced SRp40 association with the EDB exon

Conclusion:

increase of Pol II dynamics in the vicinity of the alternative EDB exon correlates with reduced co-transcriptional recruitment of SRp40 supporting the model of kinetic coupling between transcription and splicing

co-transcriptional recruitment of splicing factor is modulated by histone modifications and Pol II processivity, which provides a link between chromatin modifications, transcription and splicing

Link between global changes in chromatin structure and local changes within specific genes.

