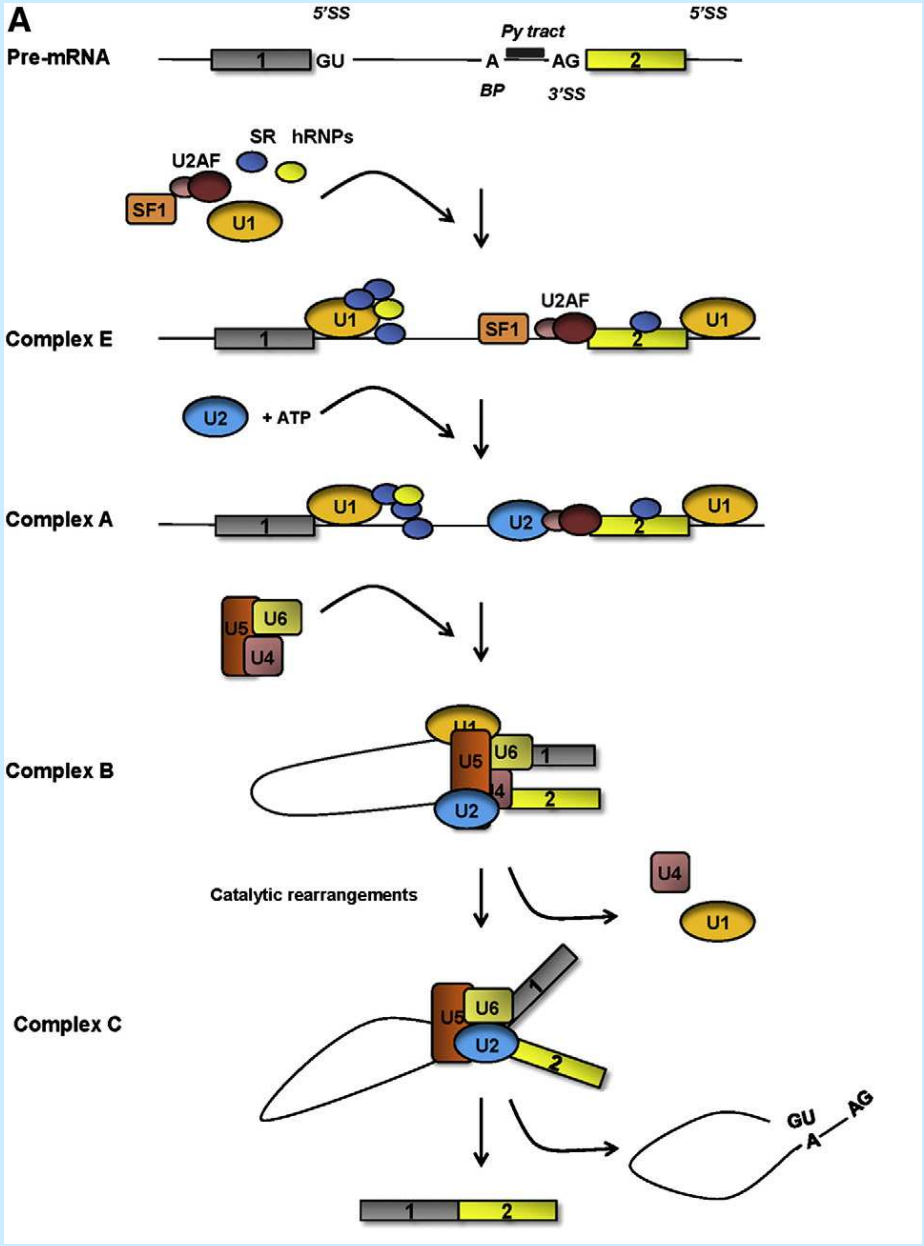
Where Splicing Joins Chromatin And Transcription

9/11/2012

Dario Balestra

Splicing process overview



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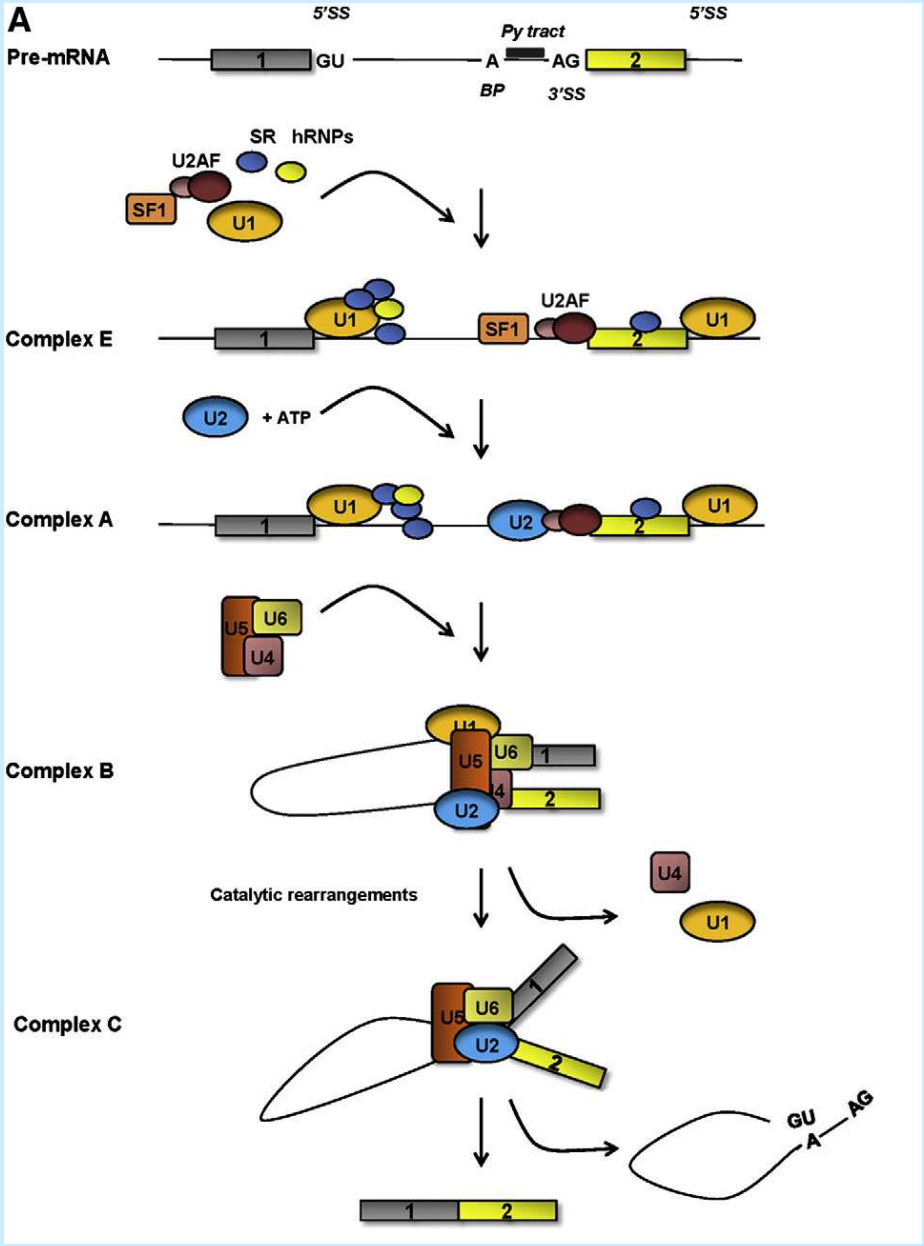
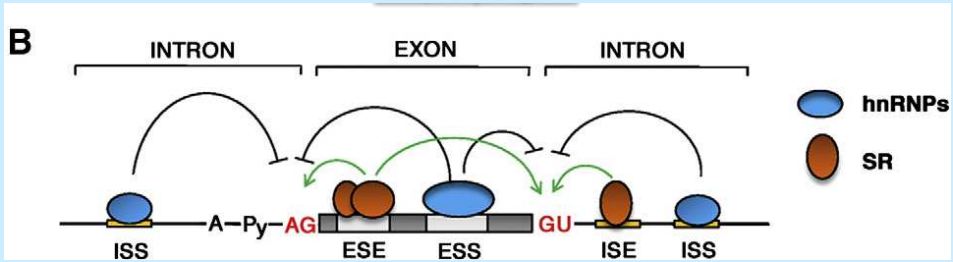
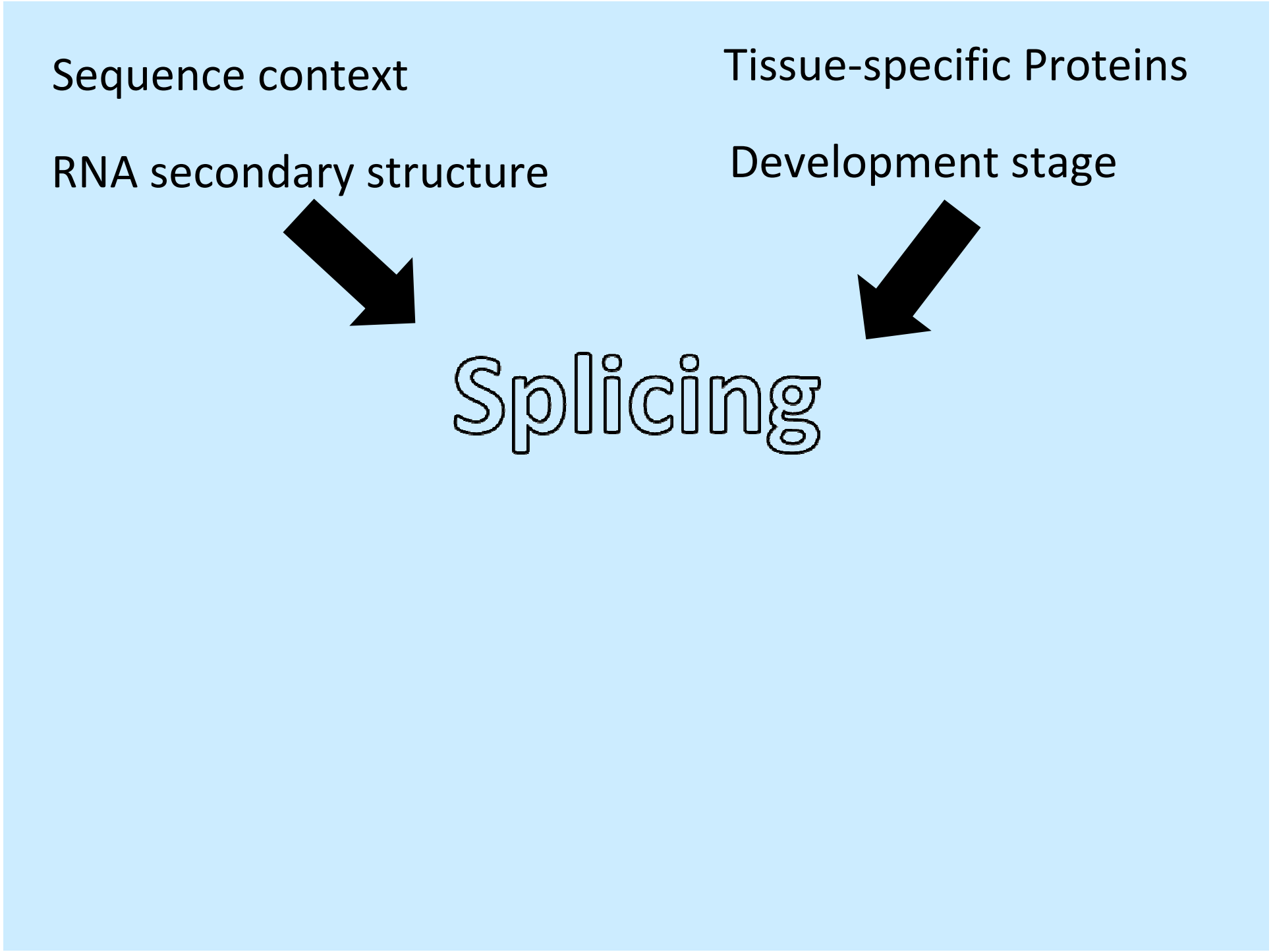
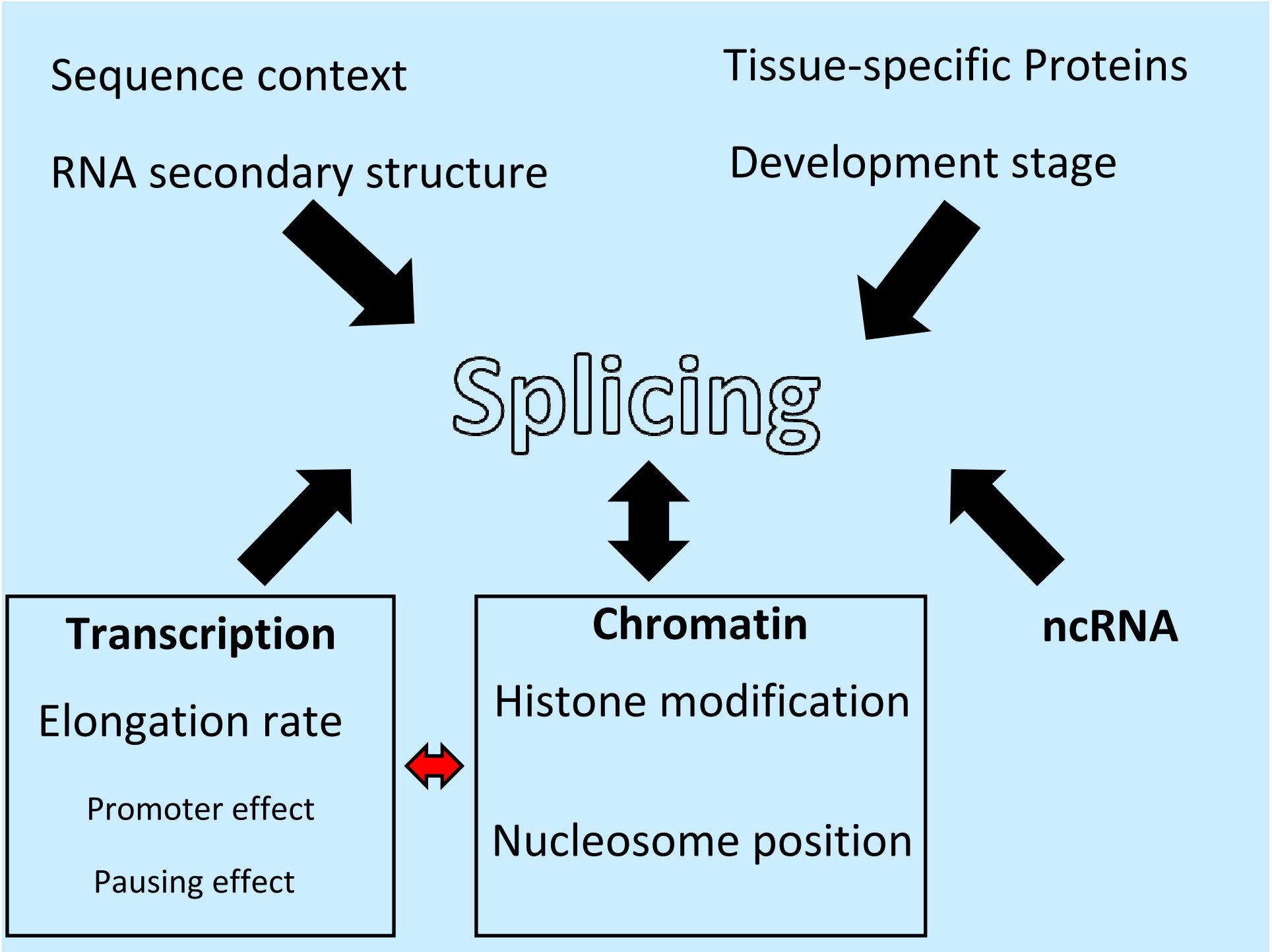





Sequence context
RNA secondary structure
Tissue-specific Proteins
Development stage
Splicing


Sequence context
RNA secondary structure
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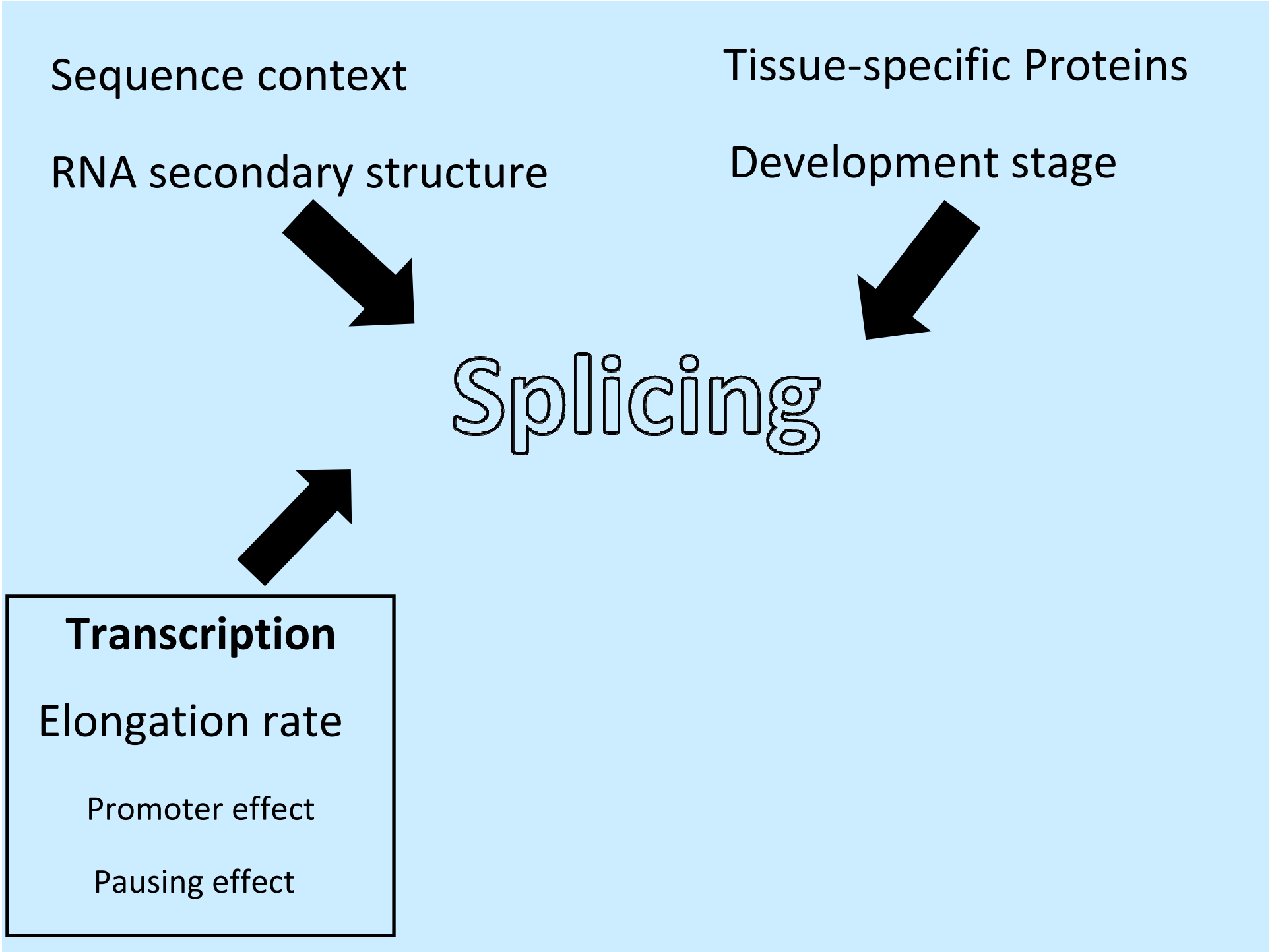
Development stage
Splicing
Transcription
Elongation rate
Promoter effect
Pausing effect
Chromatin
Histone modification
Nucleosome position
ncRNA


Sequence context
RNA secondary structure
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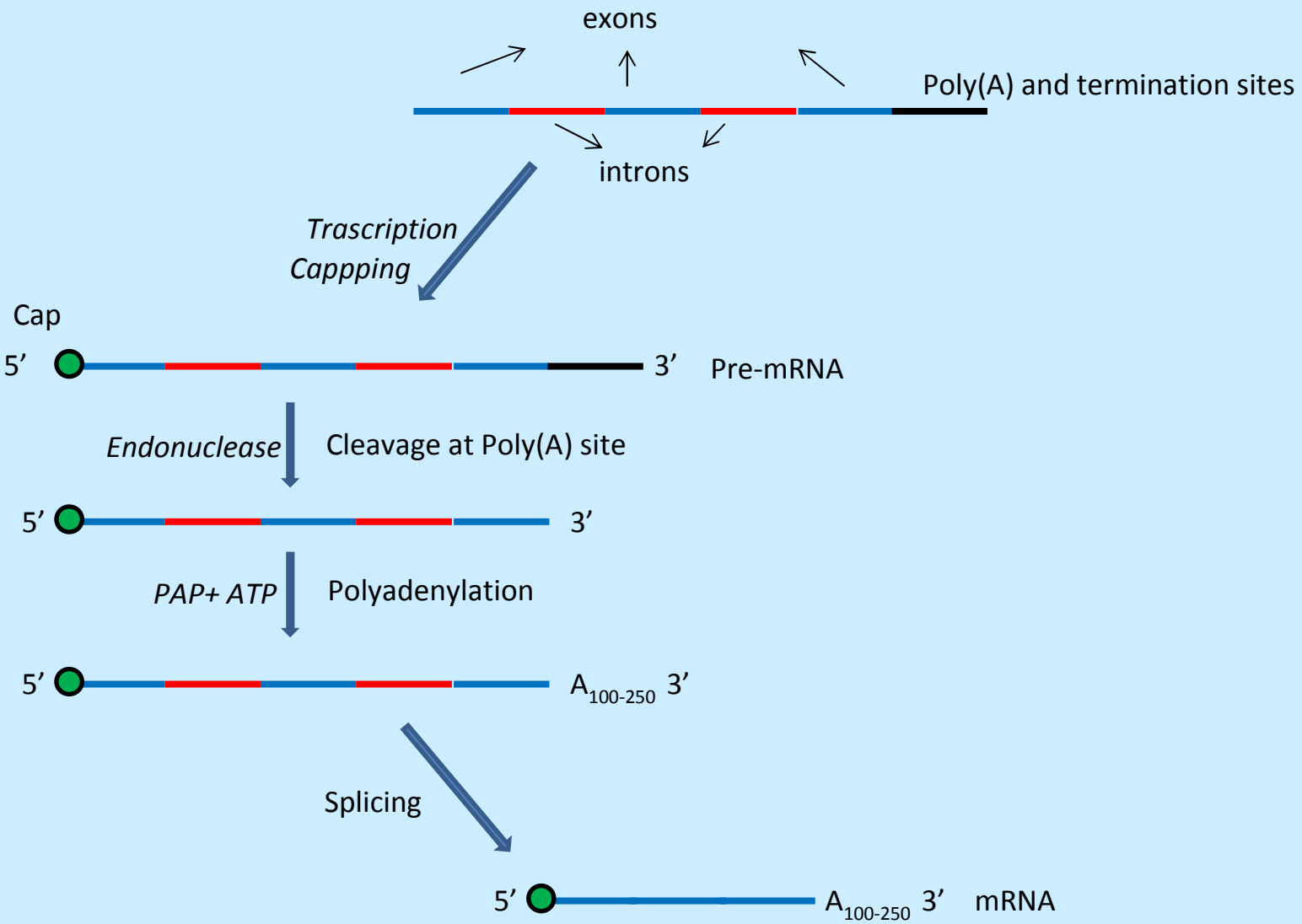
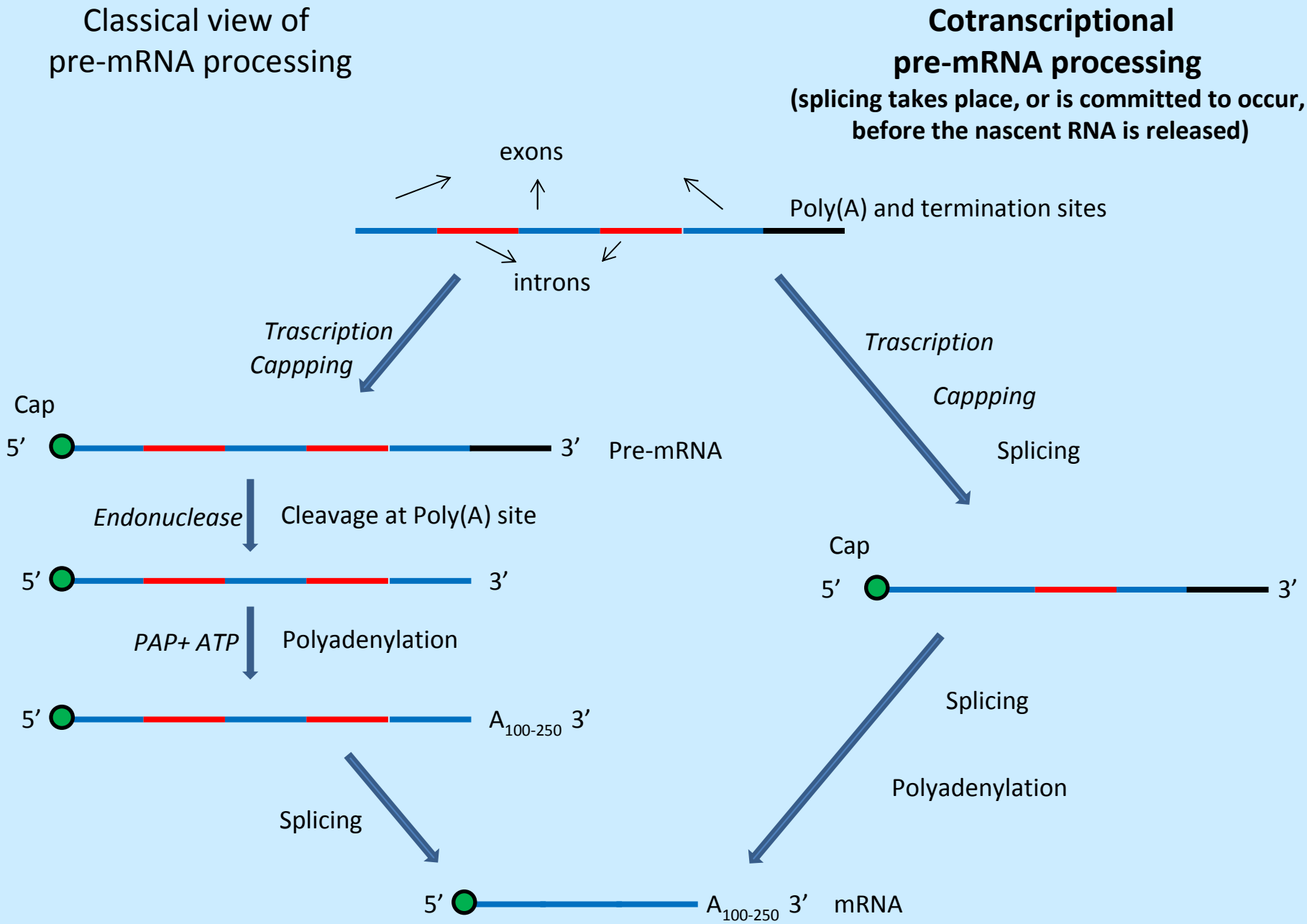
Splicing and transcription

Classical view of
pre-mRNA processing



Splicing and transcription

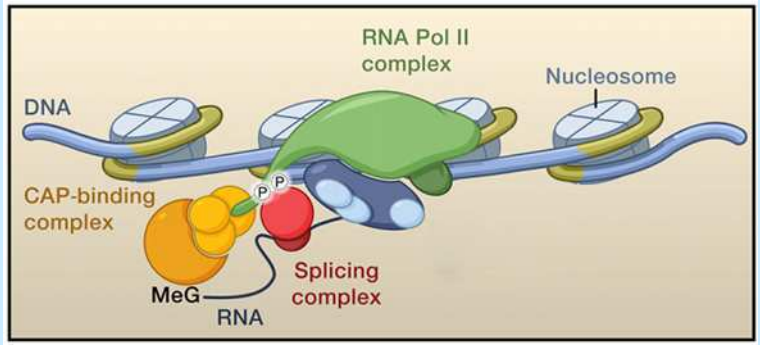


Transcription and splicing:

How can RNA Pol II affect splicing outcome?

Different recruitment of splicing factors (recruitment coupling)

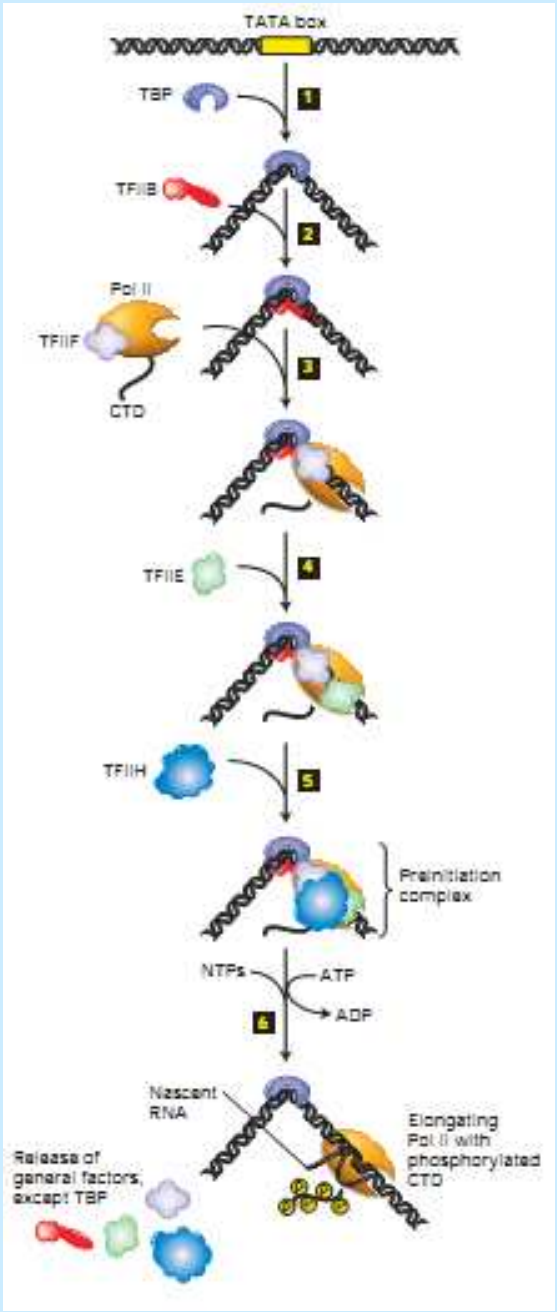
Phosphorilated CTD domain of RNA pol II can recruit CAP-binding complex and several splicing factors



Different rates of RNA Pol II elongation (kinetic coupling)

→ **Elongation rate**

→ RNA structure



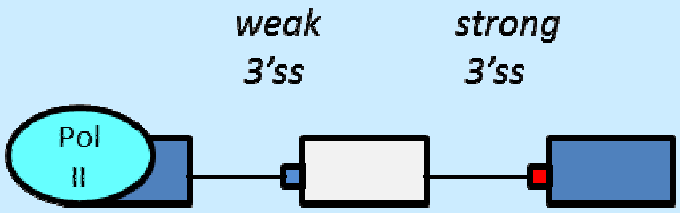
Elongation rate:

Affected by:

- type of promoter

- nucleosome position

- histone modification



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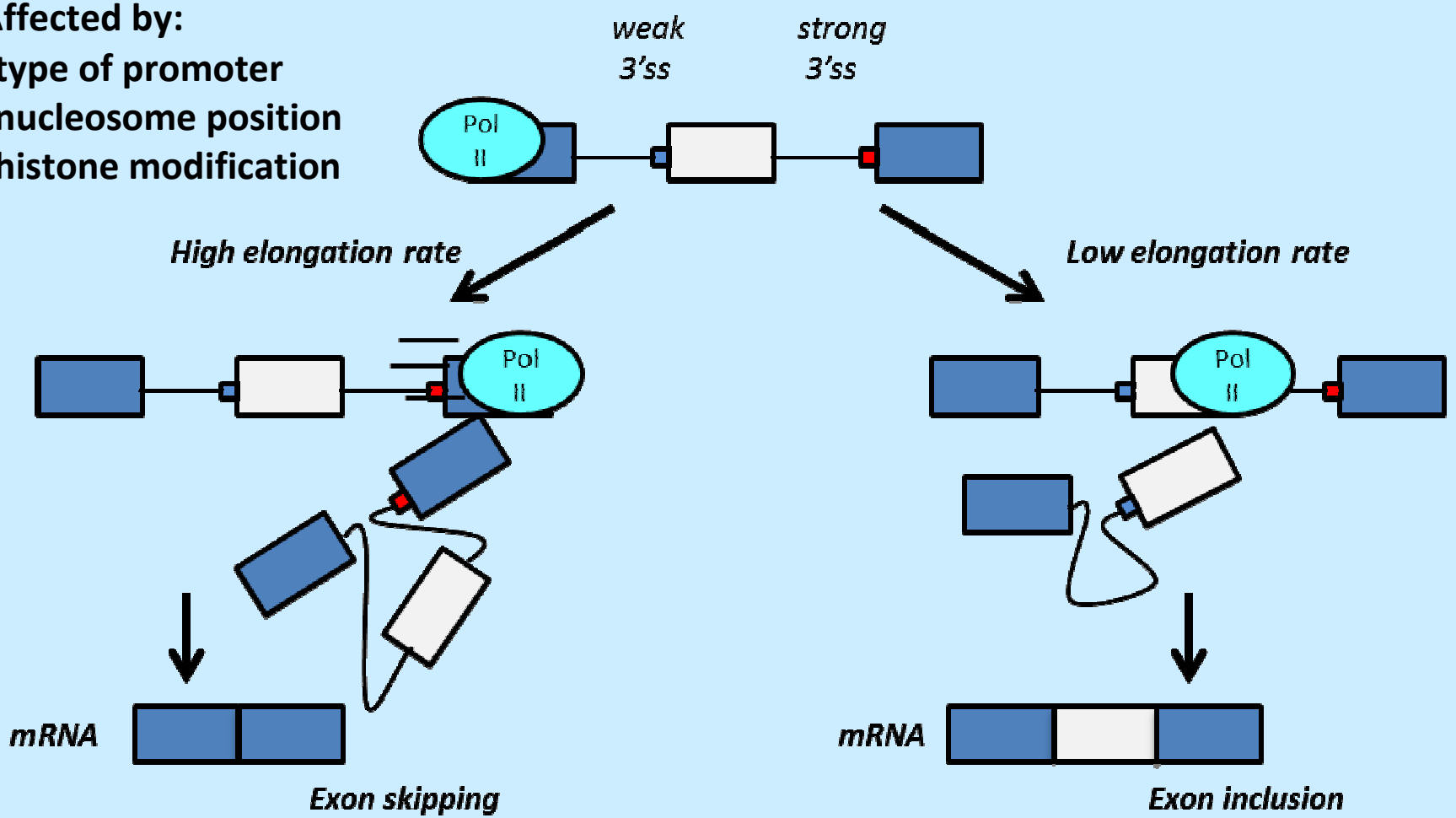
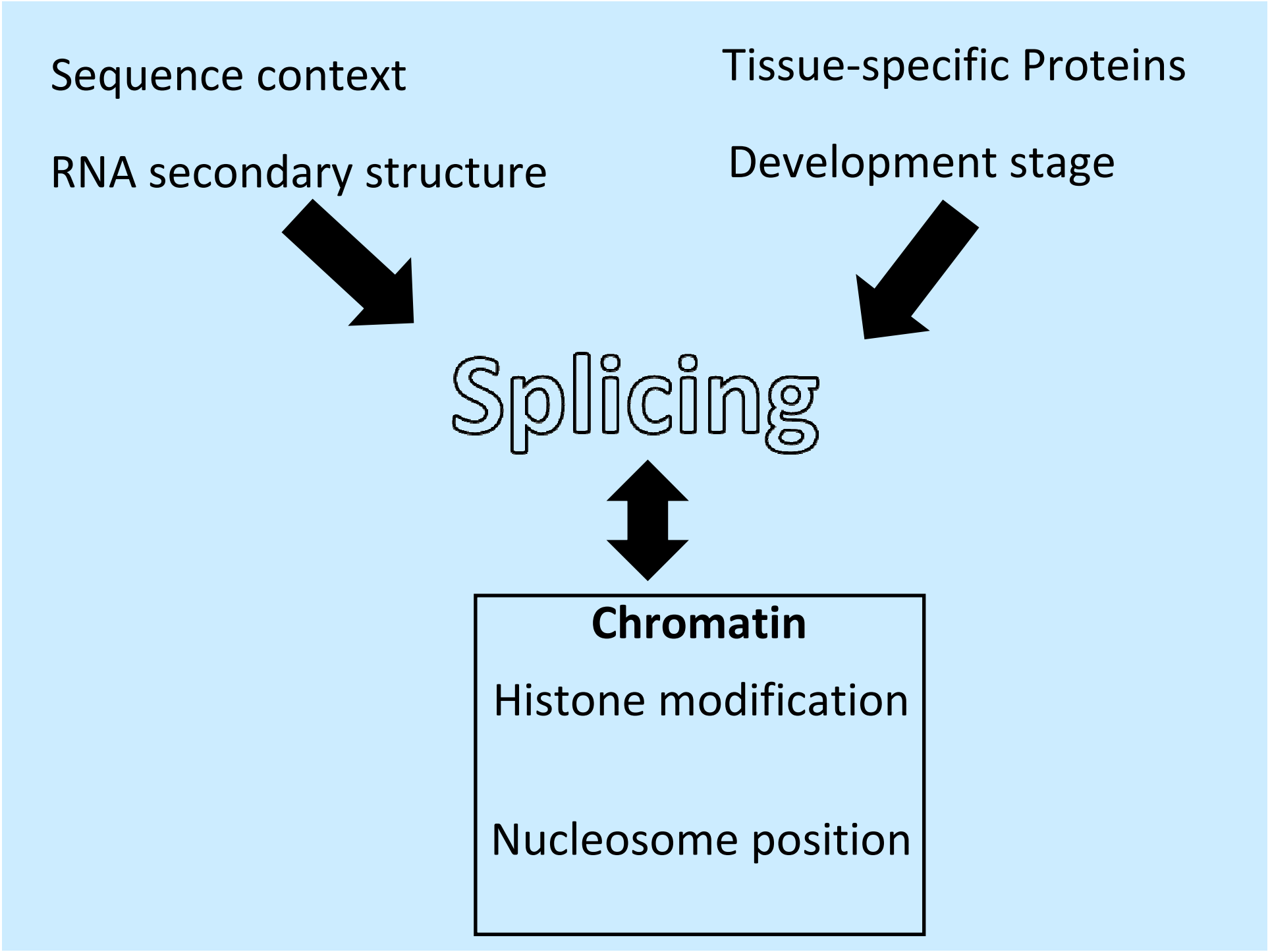


Slowing of RNA Pol II increases the window of time an upstream weak exon can recruit the splicing machinery before the splicing sites of a stronger downstream exon emerge from the polymerase complex, favouring exon inclusion


Sequence context
RNA secondary structure
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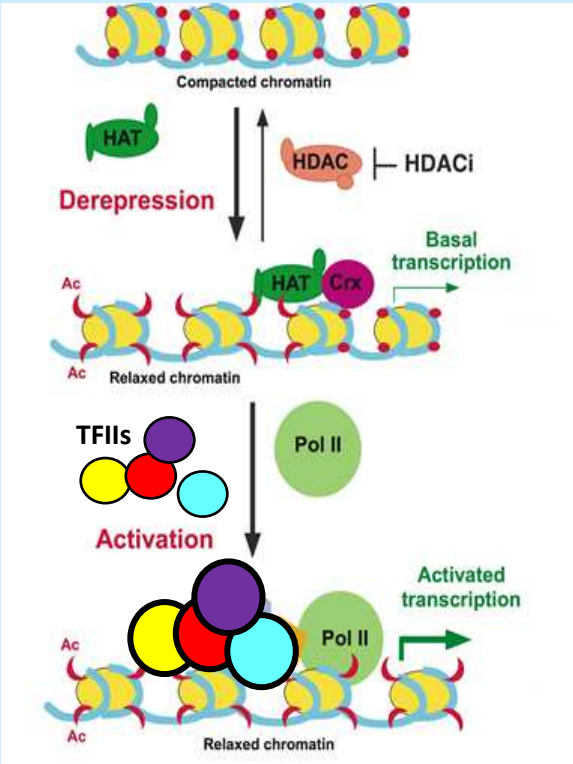
Histone modification
Nucleosome position

Histone modifications: major regulators of alternative splicing

Chromatin structure and transcription

eterochromatin

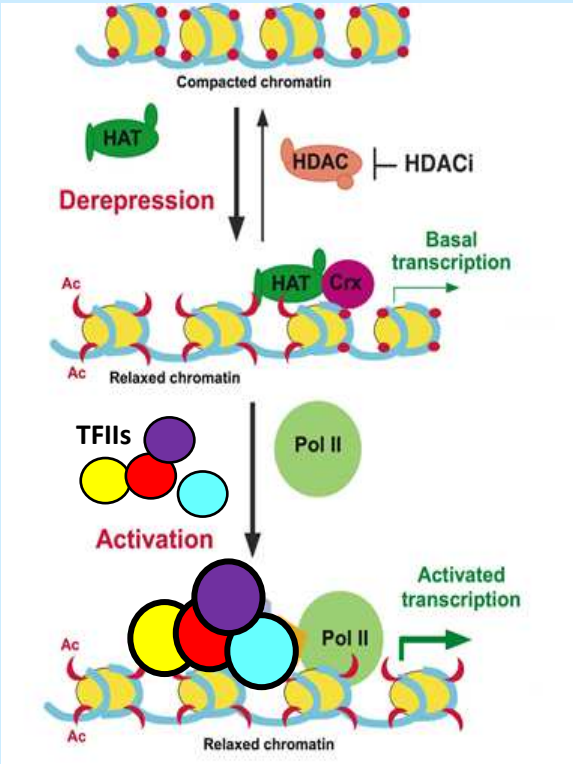


eucrochromatin

Histone modifications: major regulators of alternative splicing

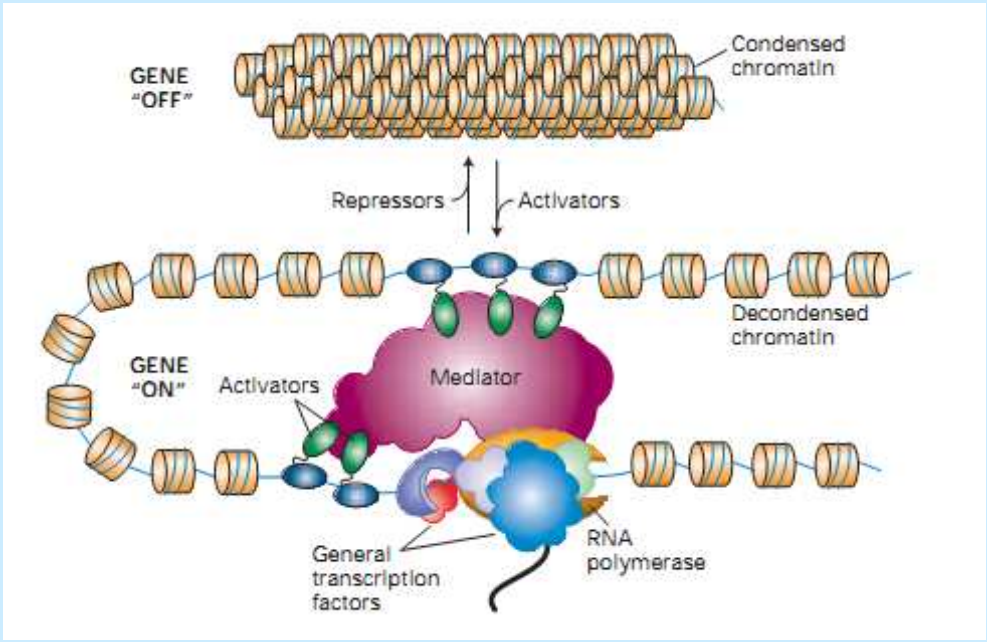
Chromatin structure and transcription

eterochromatin



eucrochromatin

General overview

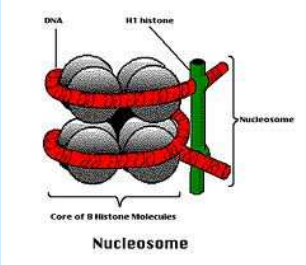


Histone modifications: major regulators of alternative splicing

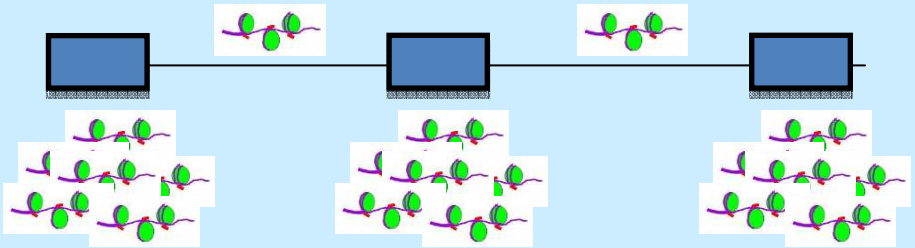
Exons are marked by increased nucleosome occupancy, distinct histone modifications and elevated DNA methylation relative to introns.

Nucleosome position:

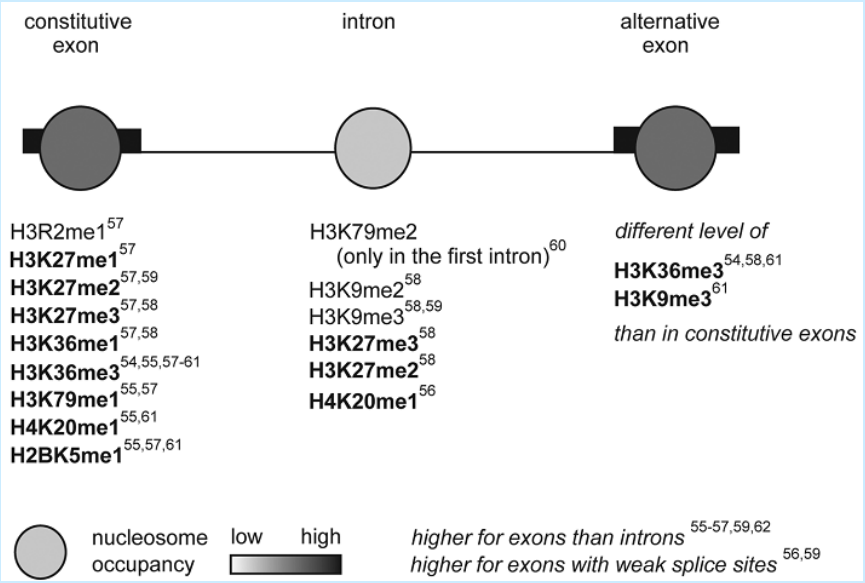
Nucleosome: stretch of ~147bp of DNA wrapped around an octamer of histone proteins



Average size of mammalian exons: 145bp



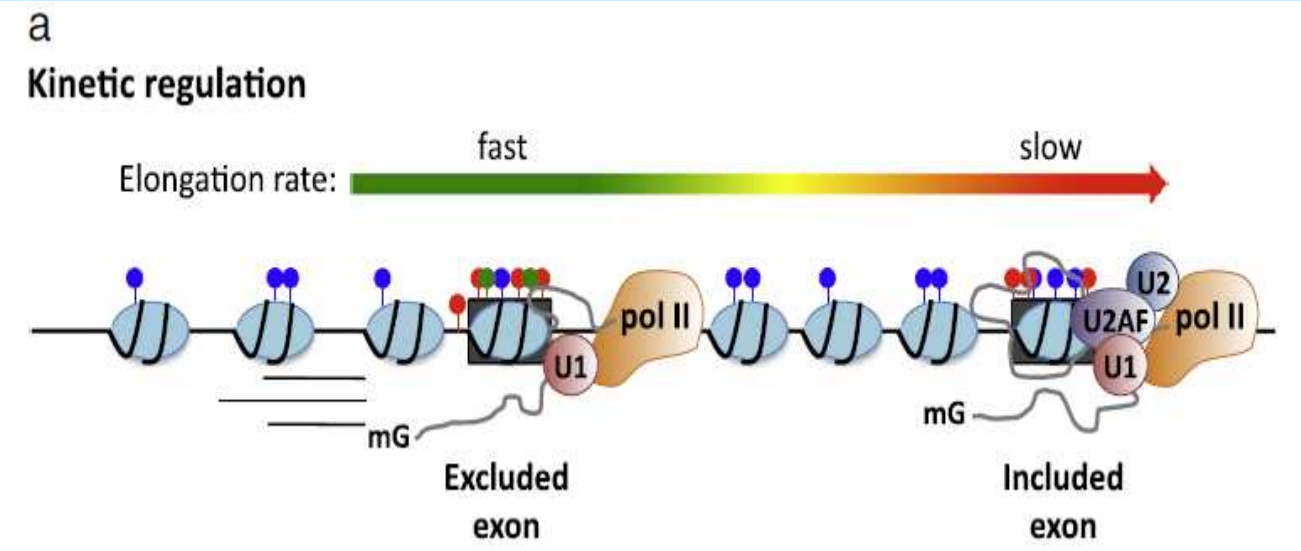
Histone modifications:



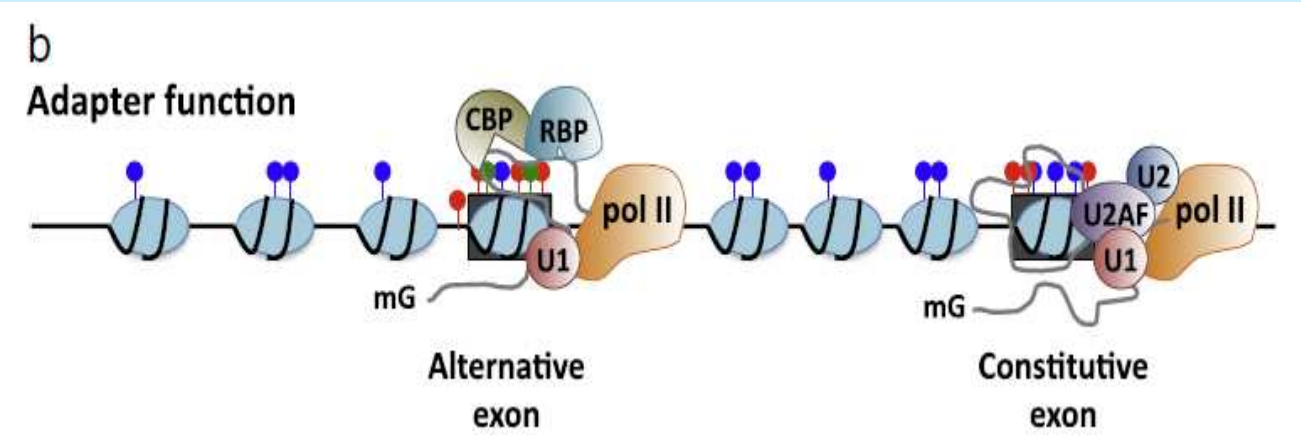
Nucleosomes behave as barrier that slowing down the elongation rate of Pol II

Histone modifications: major regulators of alternative splicing

Histone modifications are not randomly distribuited among genome:

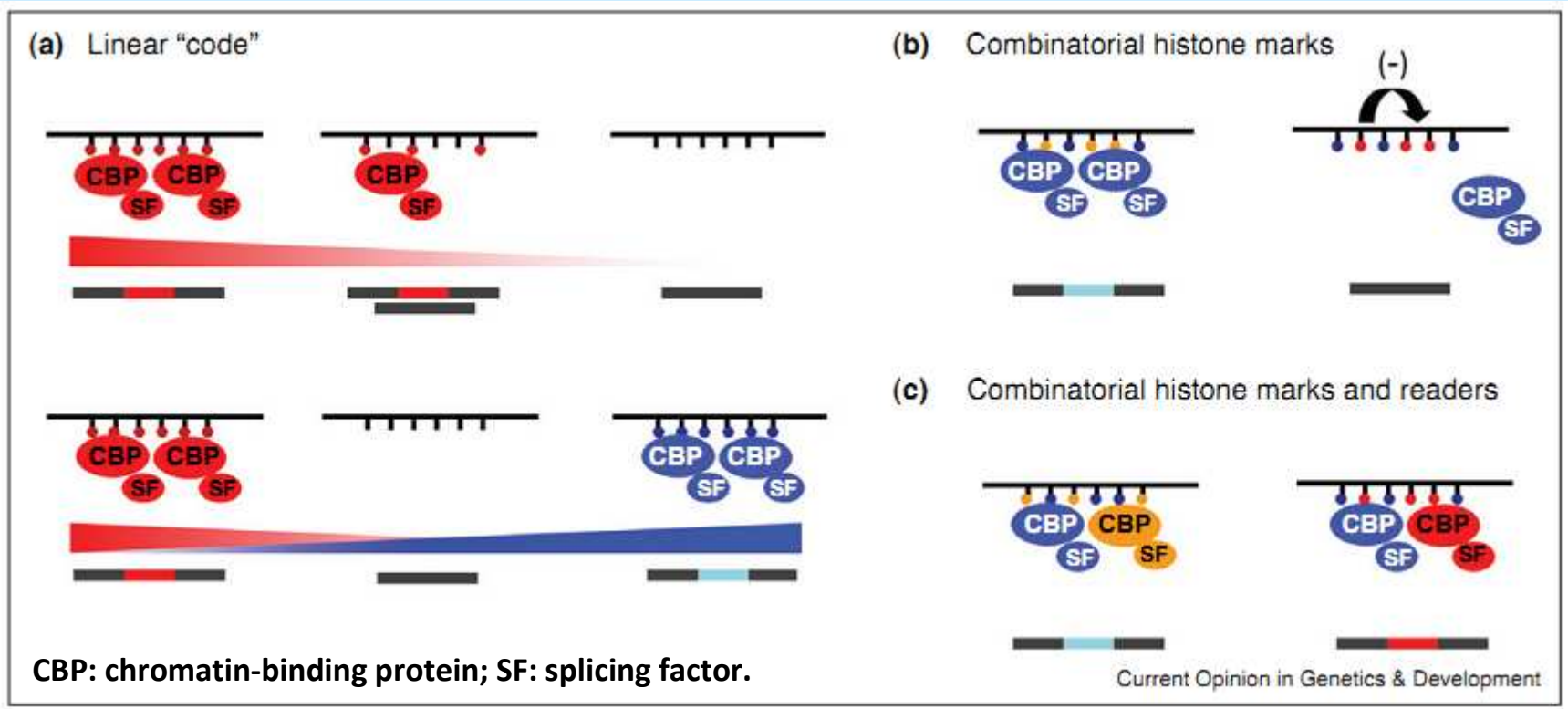


Facilitate the recruitment of splicing regulators at weak exons



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

"Histone code"



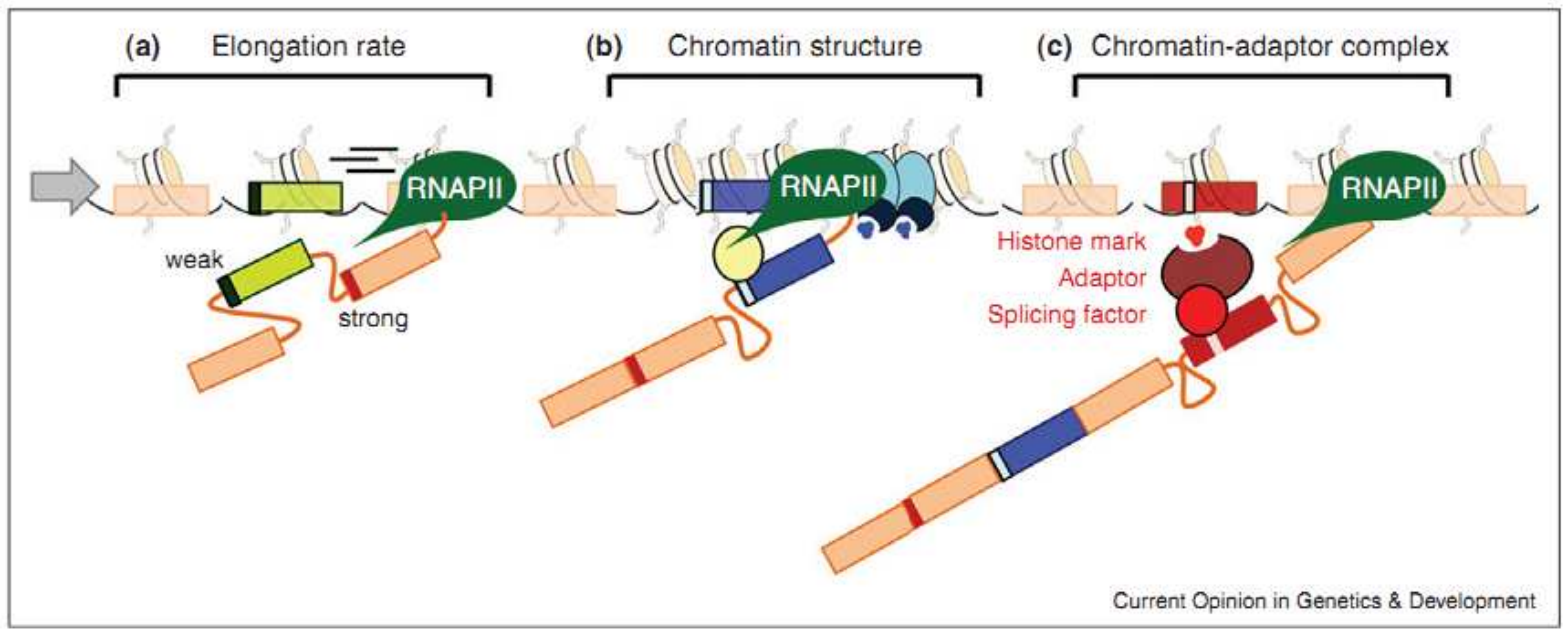
CBP: chromatin-binding protein; SF: splicing factor.

(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:

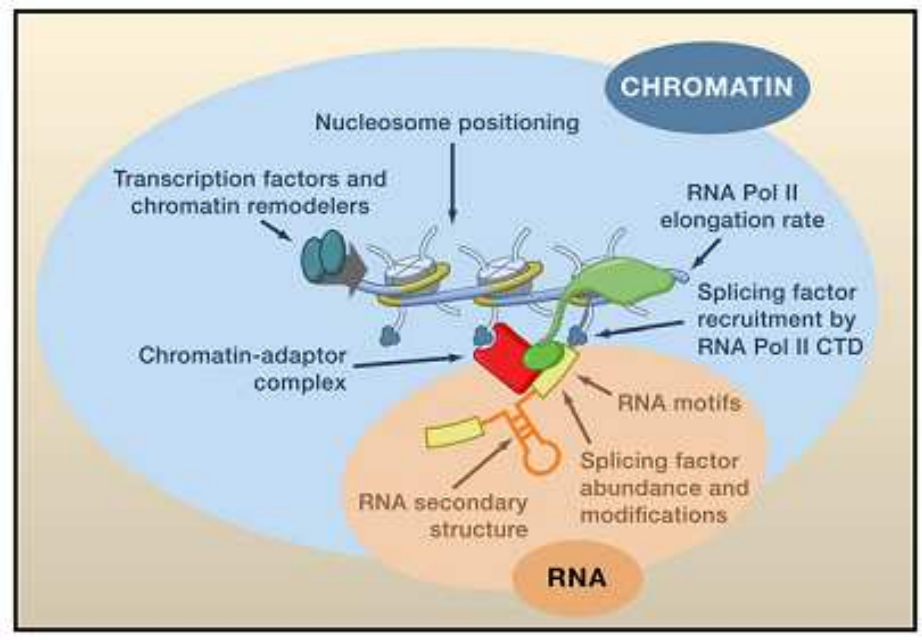


(a) RNAP II elongation rate affects recruitment of the splicing machinery. Fast elongation favors inclusion of a downstream exon with strong splice sites.

(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.

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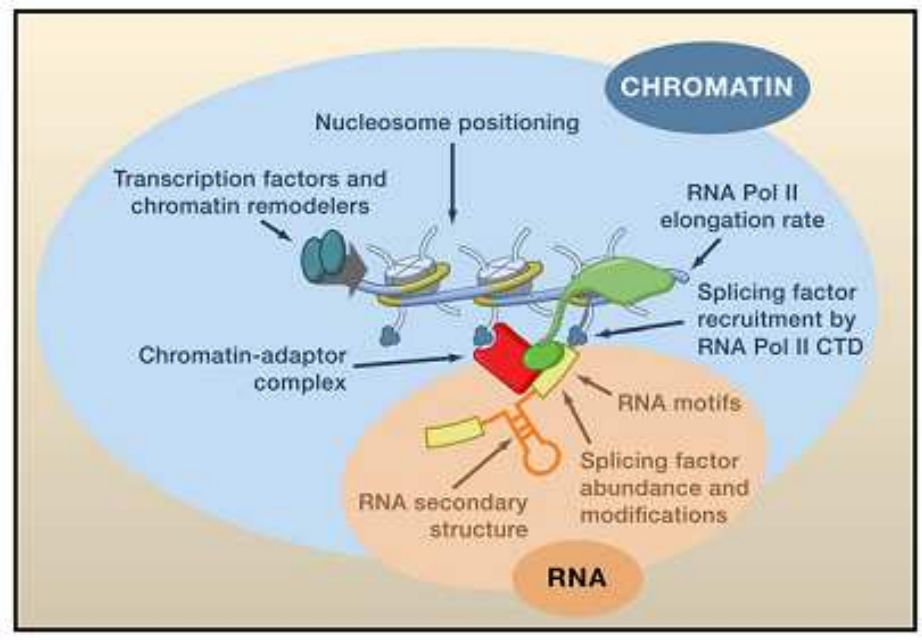

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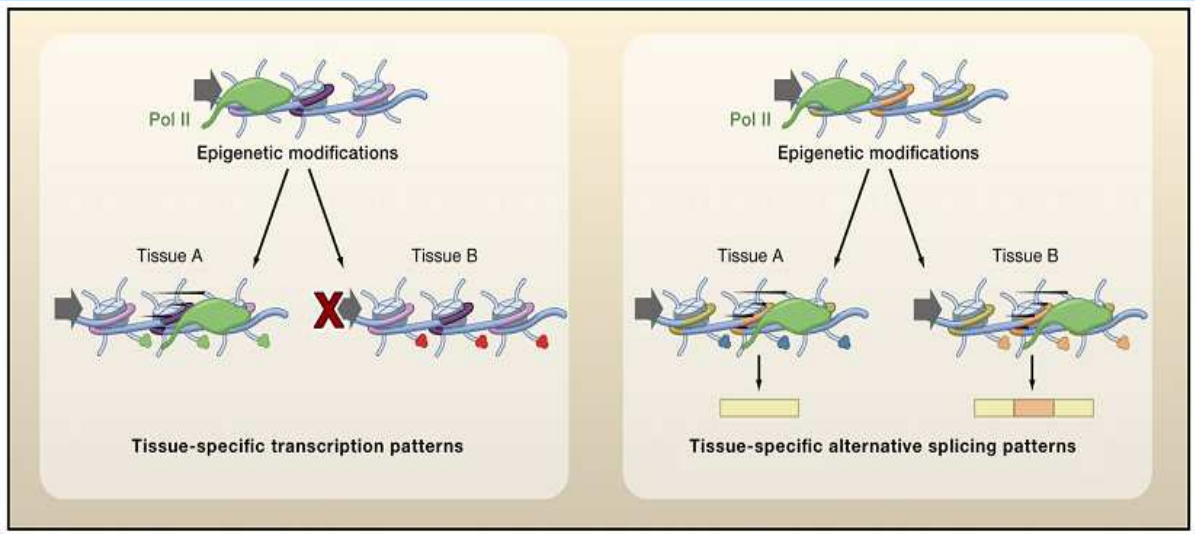

The combination of histone modifications along a gene establishes and maintains tissue-specific transcription patterns (left panel), as well as heritable tissue-specific alternative splicing patterns (right panel)

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

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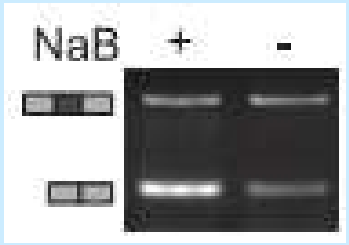
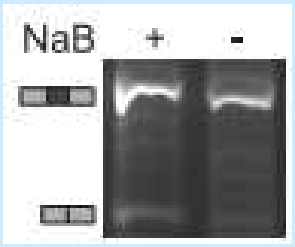
Cells treated with potent HDAC inhibitor – sodium butyrate (NaB)

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

Focus on fibronectin FN1 gene.



FN1
Exon 25 (EDB)



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Exon 33 (EDA)

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

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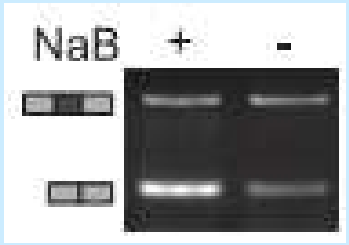

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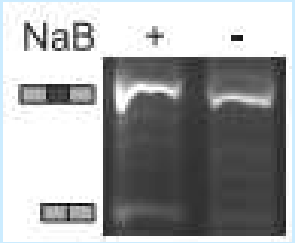

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Exon 33 (EDA)

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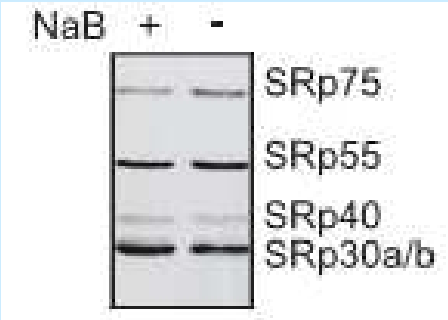
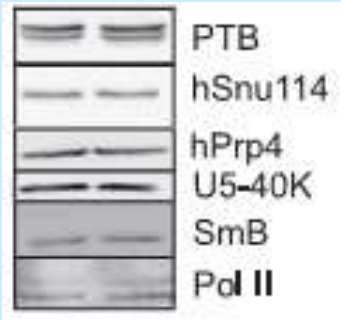
Wich is the relationship between HDAC inhibition and EDB splicing??

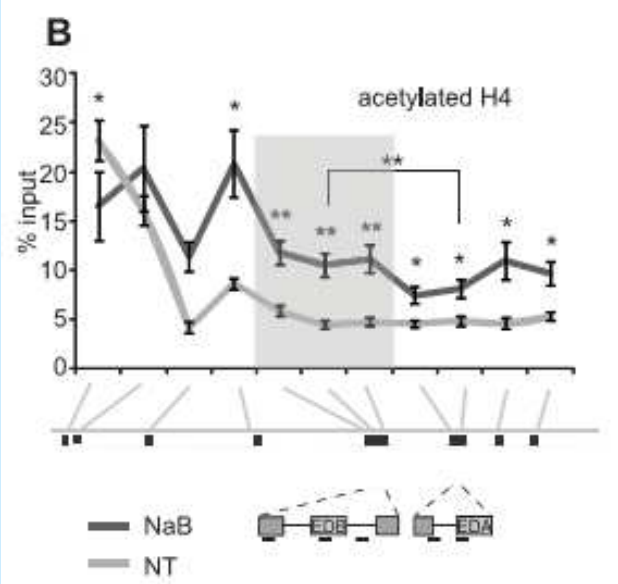
Does the HDAC inhibition affect expression of splicing regulators (SR protein)?





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

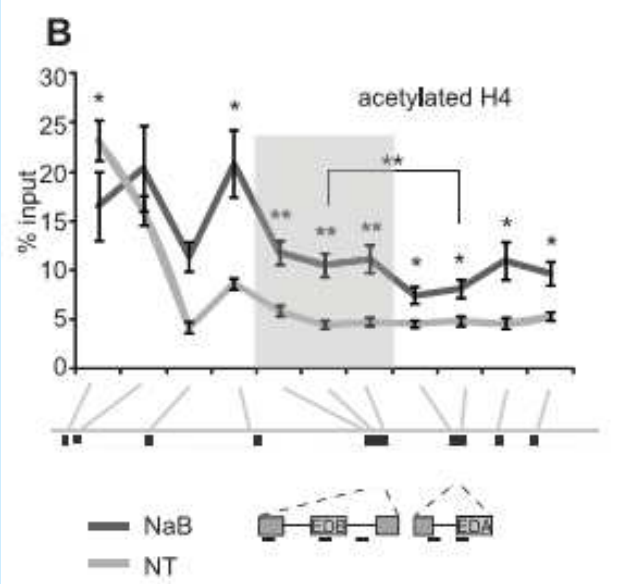
Analysis of chromatin marks along FN1 gene



Higher H4 acetylation levels

H4 acetylation correlates with EDB exon skipping

Analysis of chromatin marks along FN1 gene



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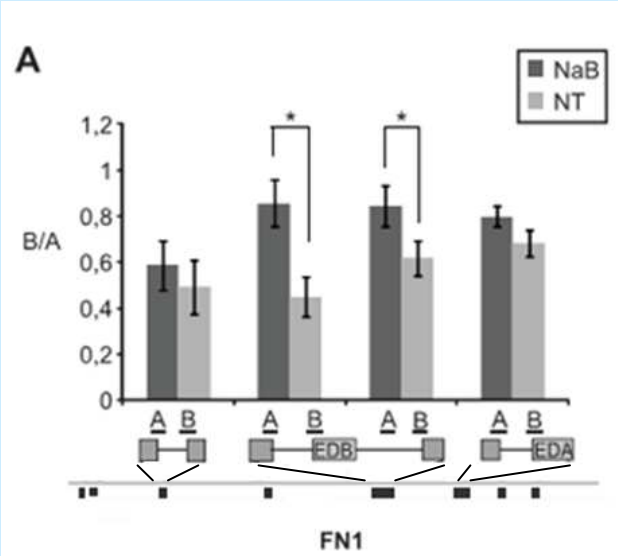
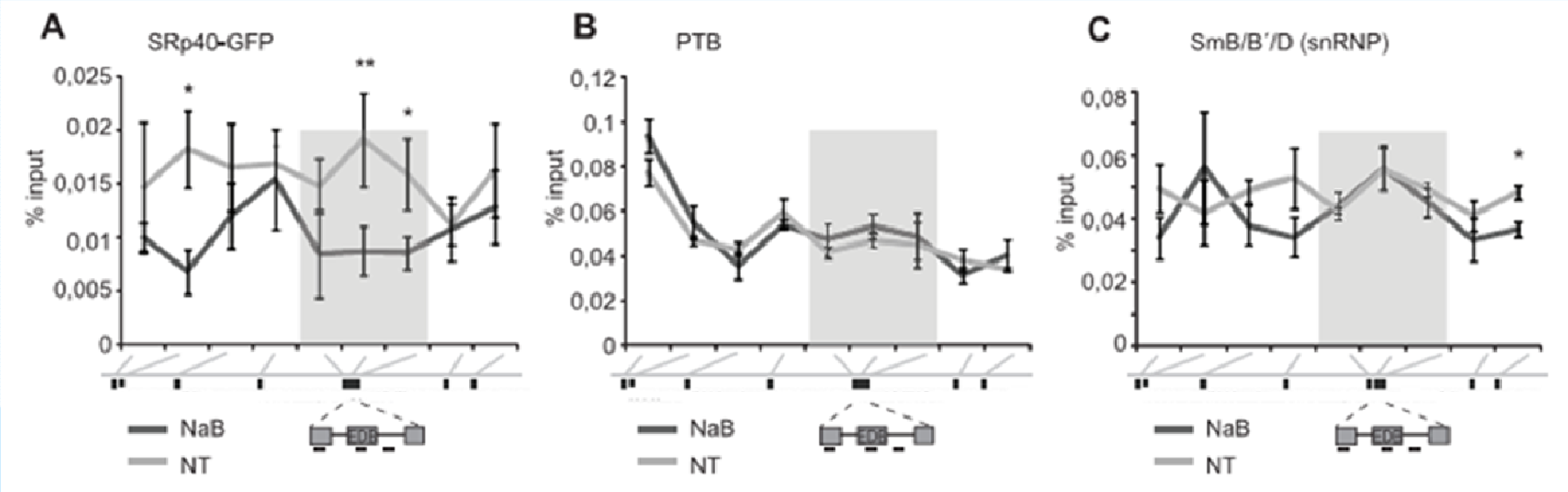
Pol II processivity and HDAC inhibition:

Exist a correlation between H4 acetylation and RNA Pol II processivity



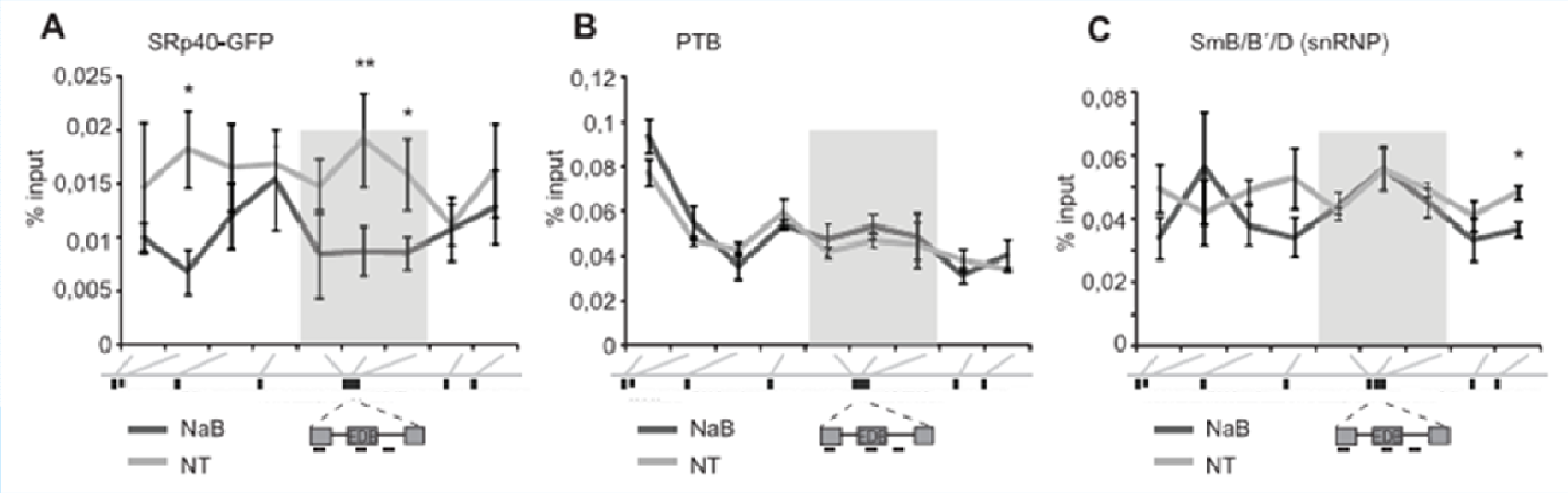
increased Pol II processivity at upstream and downstream introns

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

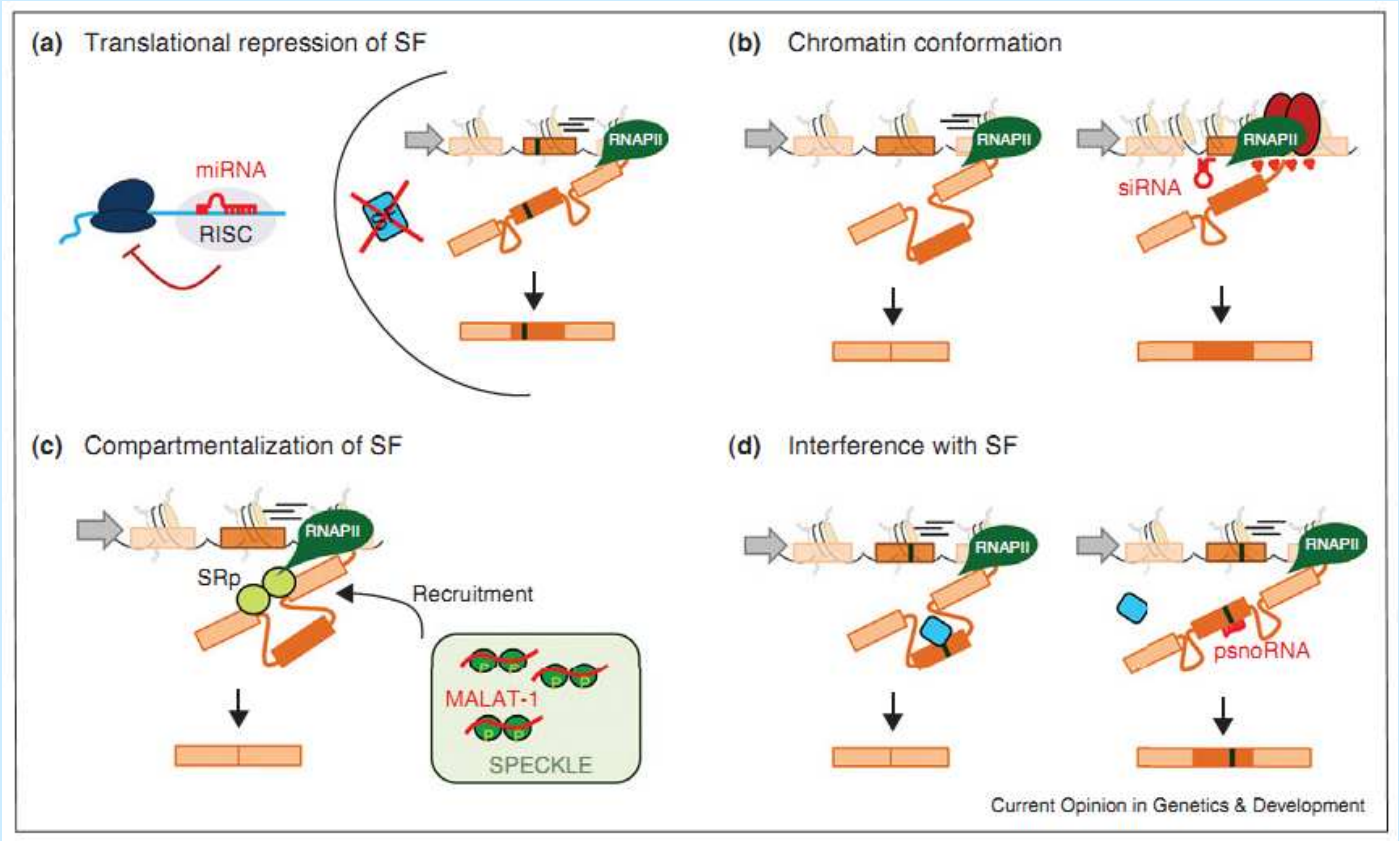
Conclusion:

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

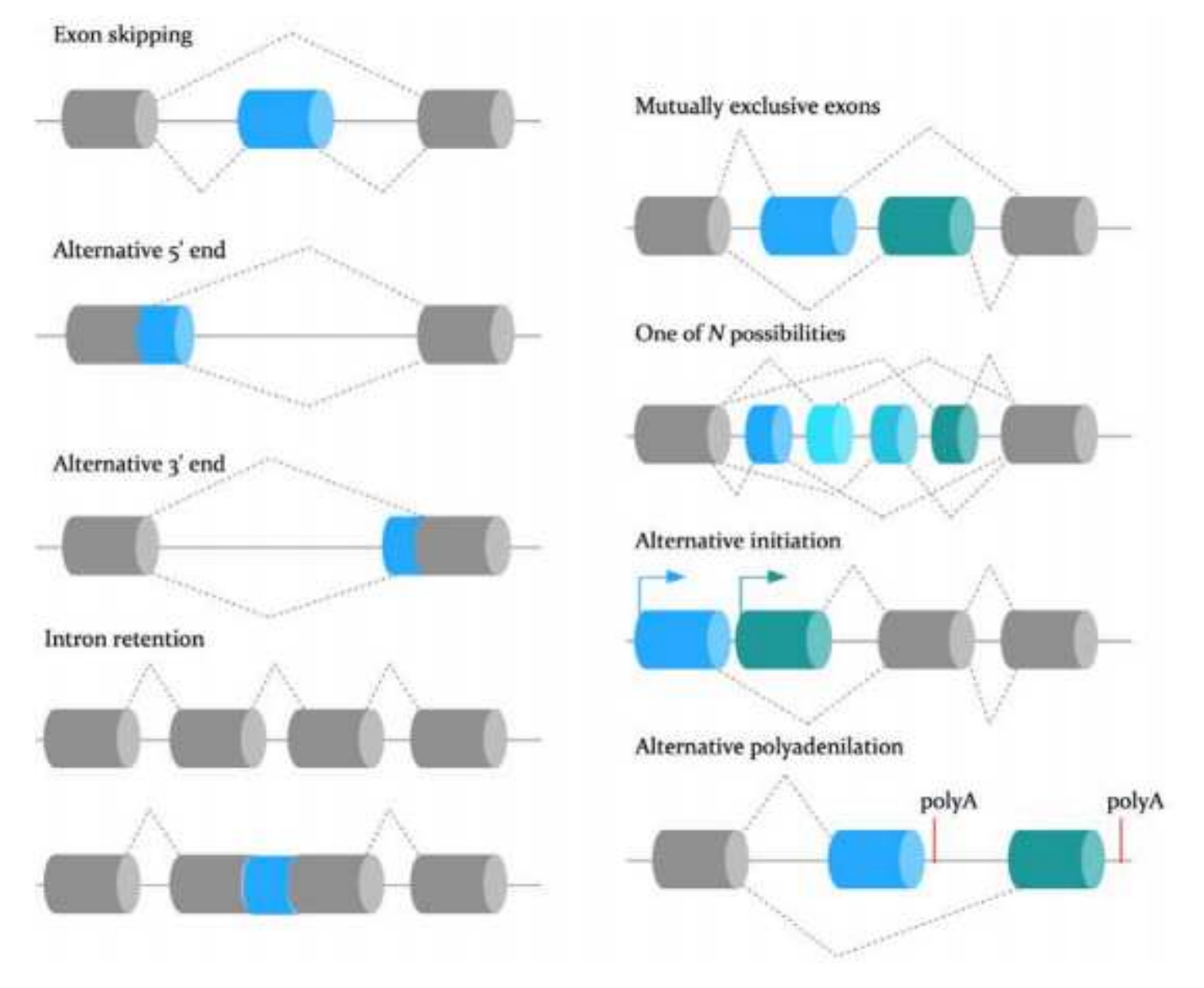
Link between global changes in chromatin structure and local changes within specific genes.

ncRNA and splicing:



(a) MicroRNAs (red hairpin) regulate the protein levels of key developmental splicing factors (SF, blue rectangle).
(b) siRNA-mediate heterochromatinization (red ovals) of a weak exon favors its inclusion.
(c) The long intergenic ncRNA MALAT-1 (red line) maintains a pool of inactive SR proteins (dark green spheres) store d in splicing factor compartments (speck le). Splicing factors are released from speckles when needed.
(d) The binding of a psnoRNA (red line) by sequence complementarity to an RNA silencer in the exon interferes with the recruitment of a splicing factor (blue rectangle) and subsequent exon inclusion.

Alternative Splicing events:



Splicing and transcription