Ch4 – Lesson 2.4

Chromatin effects on splicing

Nucleosome histone modification (epigenetic information)

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Biased Chromatin Signatures around Polyadenylation Sites and Exons

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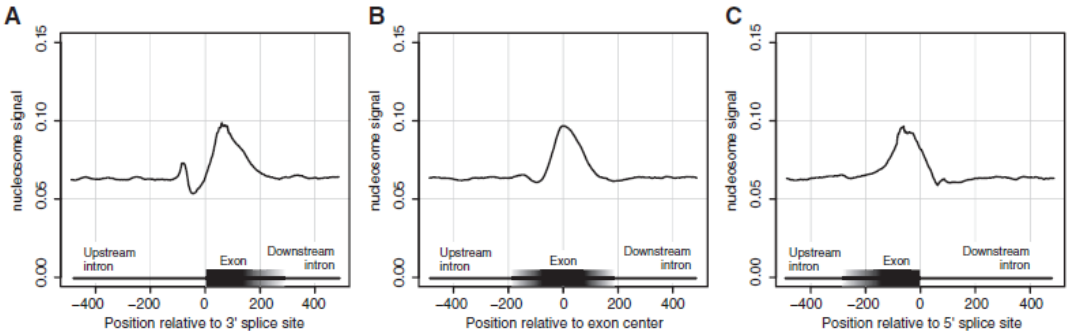
Core RNA-processing reactions in eukaryotic cells occur cotranscriptionally in a chromatin context, but the relationship between chromatin structure and pre-mRNA processing is poorly understood. We observed strong nucleosome depletion around human polyadenylation sites (PAS) and nucleosome enrichment just downstream of PAS. In genes with multiple alternative PAS, higher downstream nucleosome affinity was associated with higher PAS usage, independently of known PAS motifs that function at the RNA level. Conversely, exons were associated with distinct peaks in nucleosome density. Exons flanked by long introns or weak splice sites exhibited stronger nucleosome enrichment, and incorporation of nucleosome density data improved splicing simulation accuracy. Certain histone modifications, including H3K36me3 and H3K27me2, were specifically enriched on exons, suggesting active marking of exon locations at the chromatin level. Together, these findings provide evidence for extensive functional connections between chromatin structure and RNA processing.



nucleosomes are positioned at exons
(data from MNase-Seq experiments)

ChIP-Seq experiments allow measurement of histone modification frequency on exons / introns

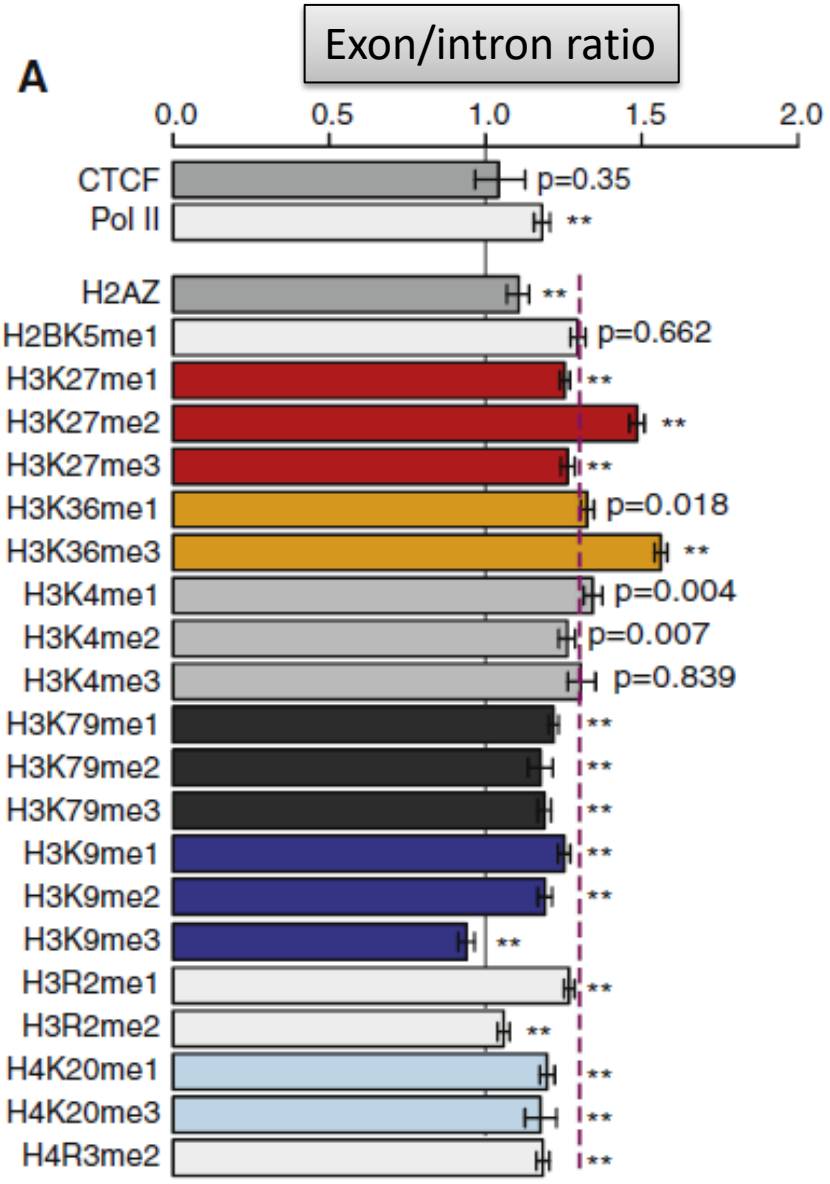
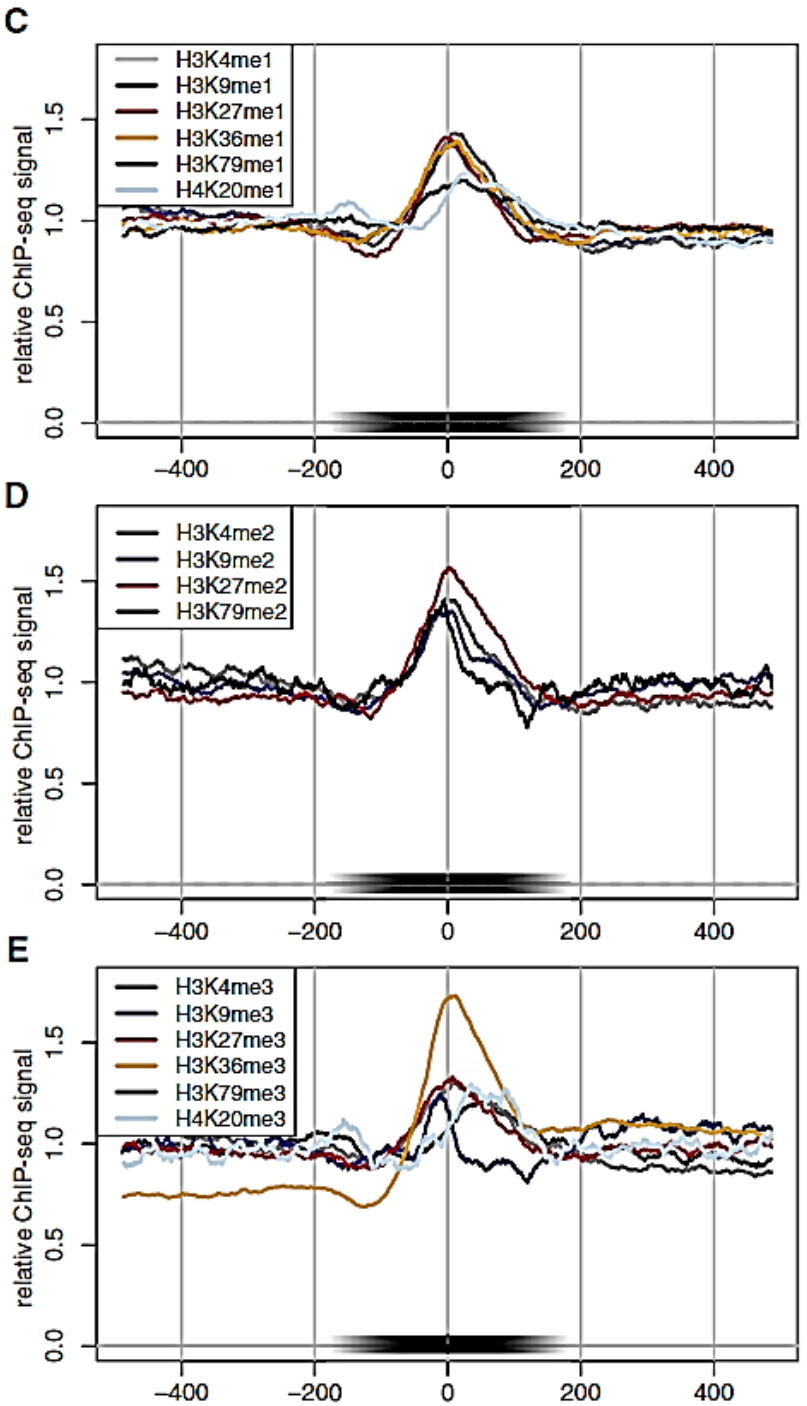


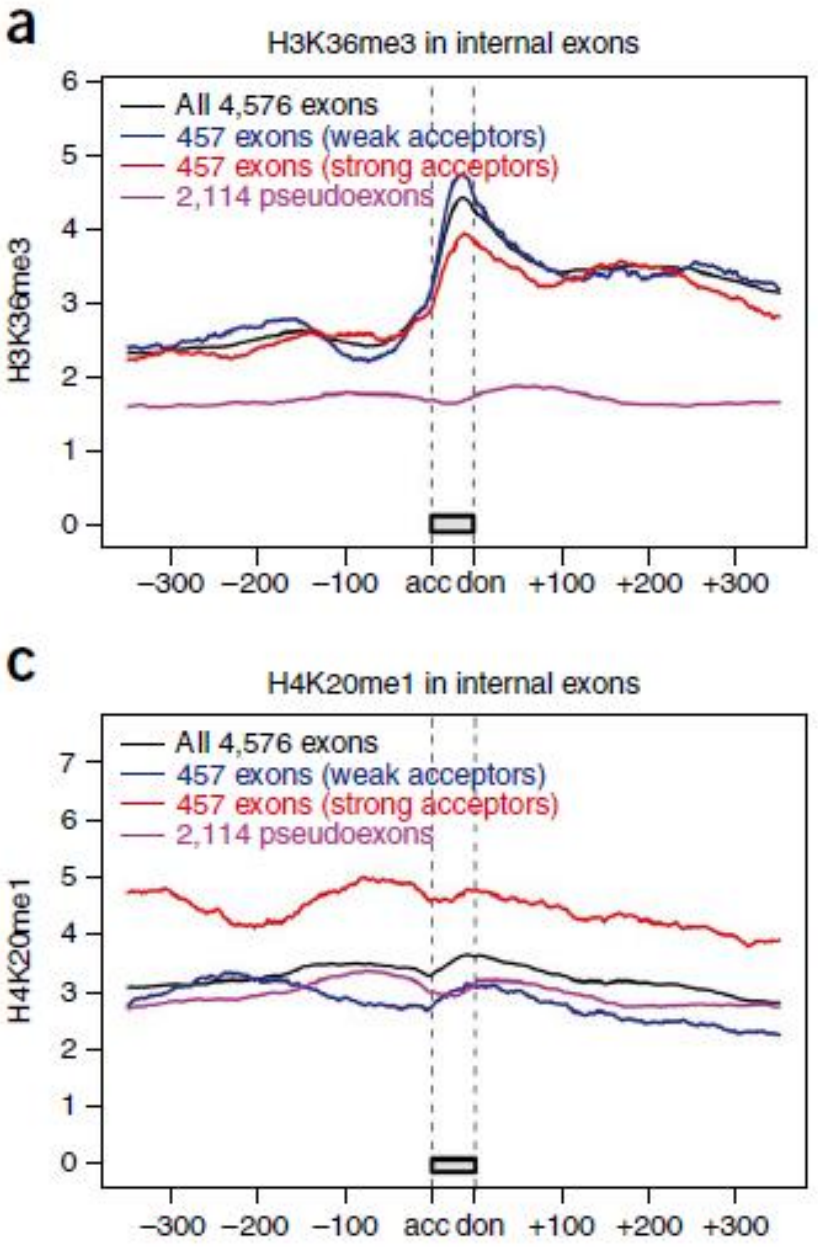
Figure 2. Exon-Biased Distribution of Specific Histone H3 Methylation Marks. (A) ChIP enrichment for exons, relative to flanking intronic regions, compared to 1.0 (CTCF and Pol II) or histone overall average of 1.3 (purple dashed line). Error bars are 95% confidence intervals (resampling). **p < 0.01 after correction for multiple testing (resample test, Bonferroni corrected).

Figure 2. Exon-Biased Distribution of Specific Histone H3 Methylation Marks.
Profiles centered on exons for:
(C) monomethyl histone marks,
(D) dimethyl histone marks,
(E) Trimethylated histone marks.
(C)–(F) are normalized to average library ChIP signal across the displayed region.

H3K36me3 is one of the most evident at exons



H3K36me3 is more evident at weak exons



Data from:
Barski et al. (2007) Cell 129, 823–837.

Regulation of Alternative Splicing by Histone Modifications

Reini F. Luco,[1] Qun Pan,[2] Kaoru Tominaga,[3] Benjamin J. Blencowe,[2]
Olivia M. Pereira-Smith,[3] Tom Misteli[1]*

Alternative splicing of pre-mRNA is a prominent mechanism to generate protein diversity, yet its regulation is poorly understood. We demonstrated a direct role for histone modifications in alternative splicing. We found distinctive histone modification signatures that correlate with the splicing outcome in a set of human genes, and modulation of histone modifications causes splice site switching. Histone marks affect splicing outcome by influencing the recruitment of splicing regulators via a chromatin-binding protein. These results outline an adaptor system for the reading of histone marks by the pre-mRNA splicing machinery.

19 FEBRUARY 2010 VOL 327 **SCIENCE** www.sciencemag.org

Differential splicing of FGFR depends on the PTB regulator, which binds to a splicing silencer around exon IIIb and represses its inclusion

FGFR2 pre-mRNA tissue-specific exon IIIb / IIIc alternative splicing was studied in **PNT2** (prostate normal epithelium) and in **hMSC** (human mesenchymal stem) cells.

The level of H3K36 trimethylation was assessed by ChIP-qPCR along the gene in these cells. Cell-specific over-representation in hMSC was observed around exons/introns interested by alternative splicing:

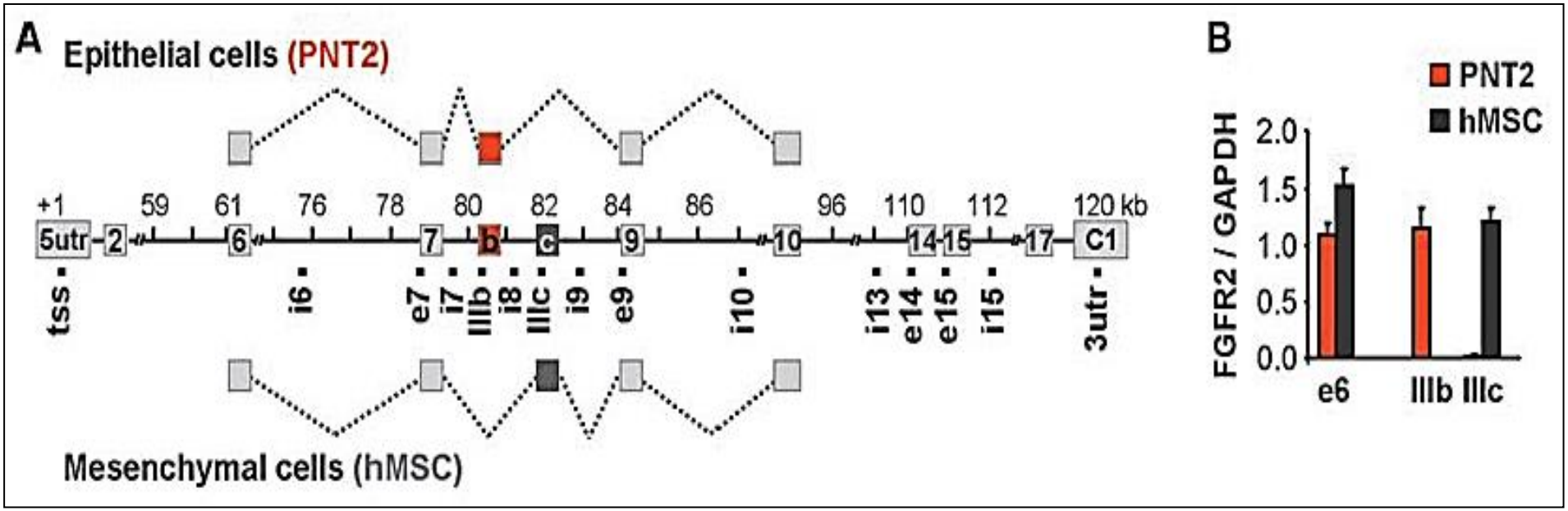
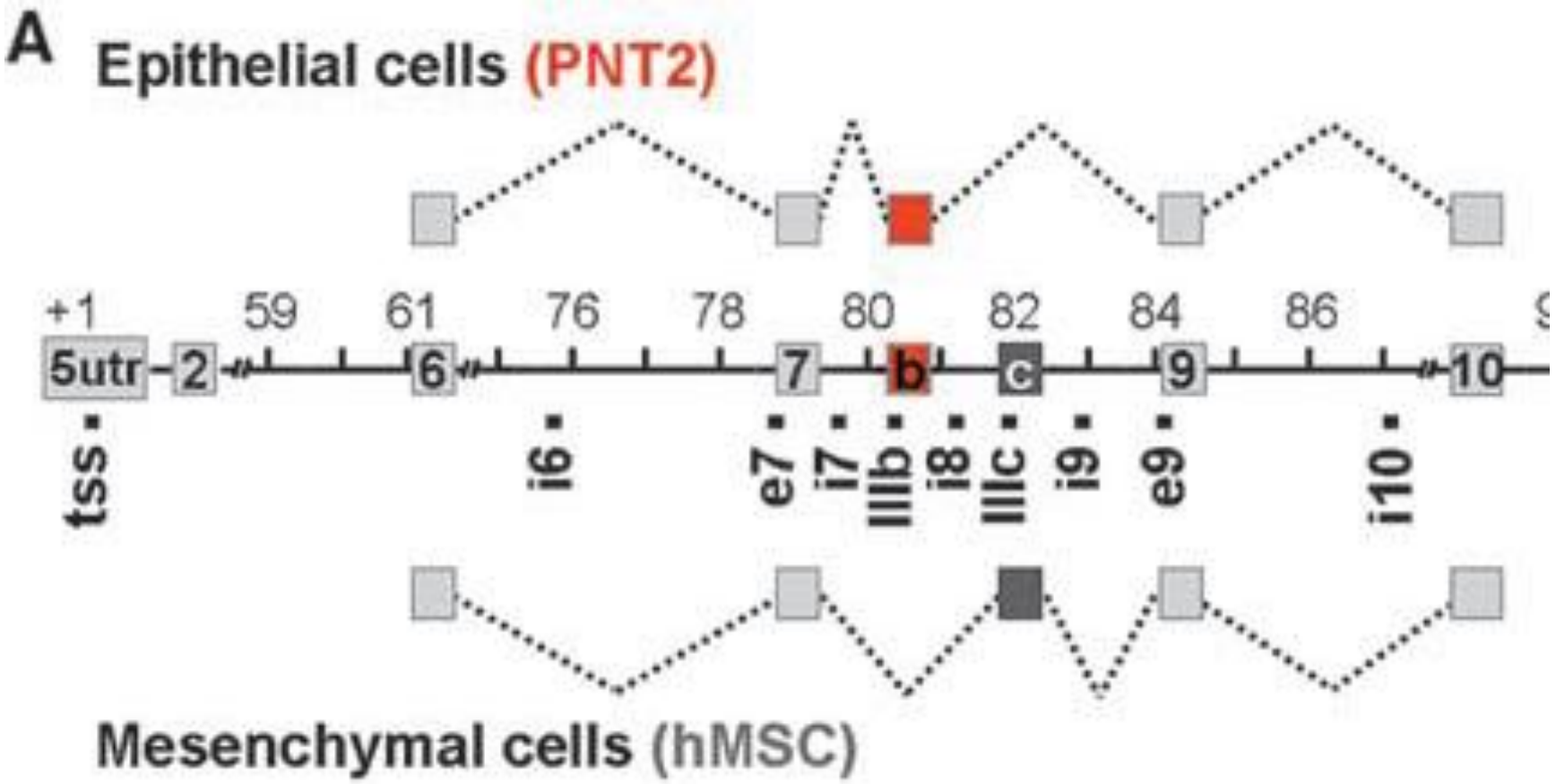


Fig. 1. Splicing-specific histone modifications. (A) Schematic representation of the human FGFR2 gene. Exon IIIb (red) is included in PNT2 epithelial cells, exon IIIc (black) is included in hMSCs. Square dots indicate oligonucleotide pairs used in analysis. (B) Levels of FGFR2 **exon inclusion** relative to GAPDH in PNT2 (red) or hMSCs (black) determined by quantitative polymerase chain reaction (PCR).

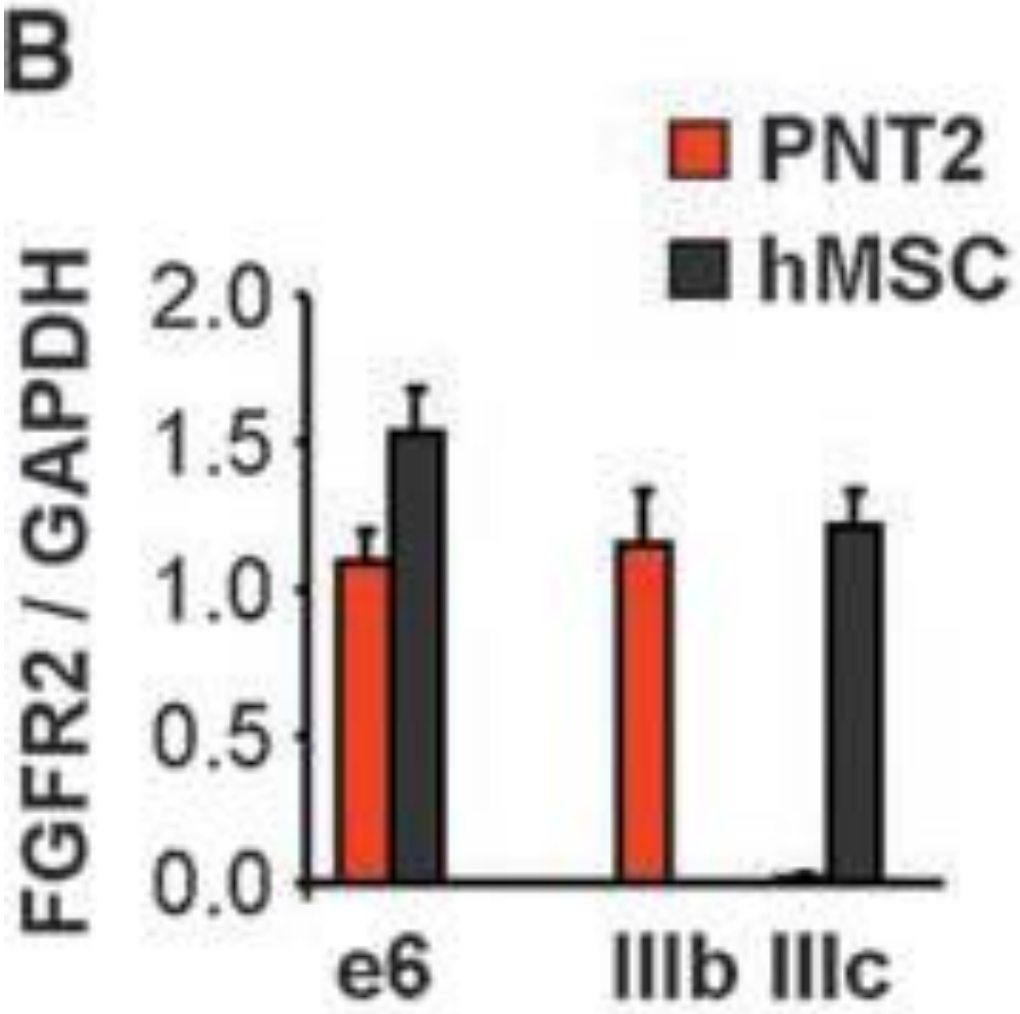


To analyze

e6
IIIb
IIIc

?

Why ratio?



Is there any special histone PTMs at these exons ?

- Chromatin Immunoprecipitation using Antibodies against PTMs

- PCR analysis of single sites as in Figure 1

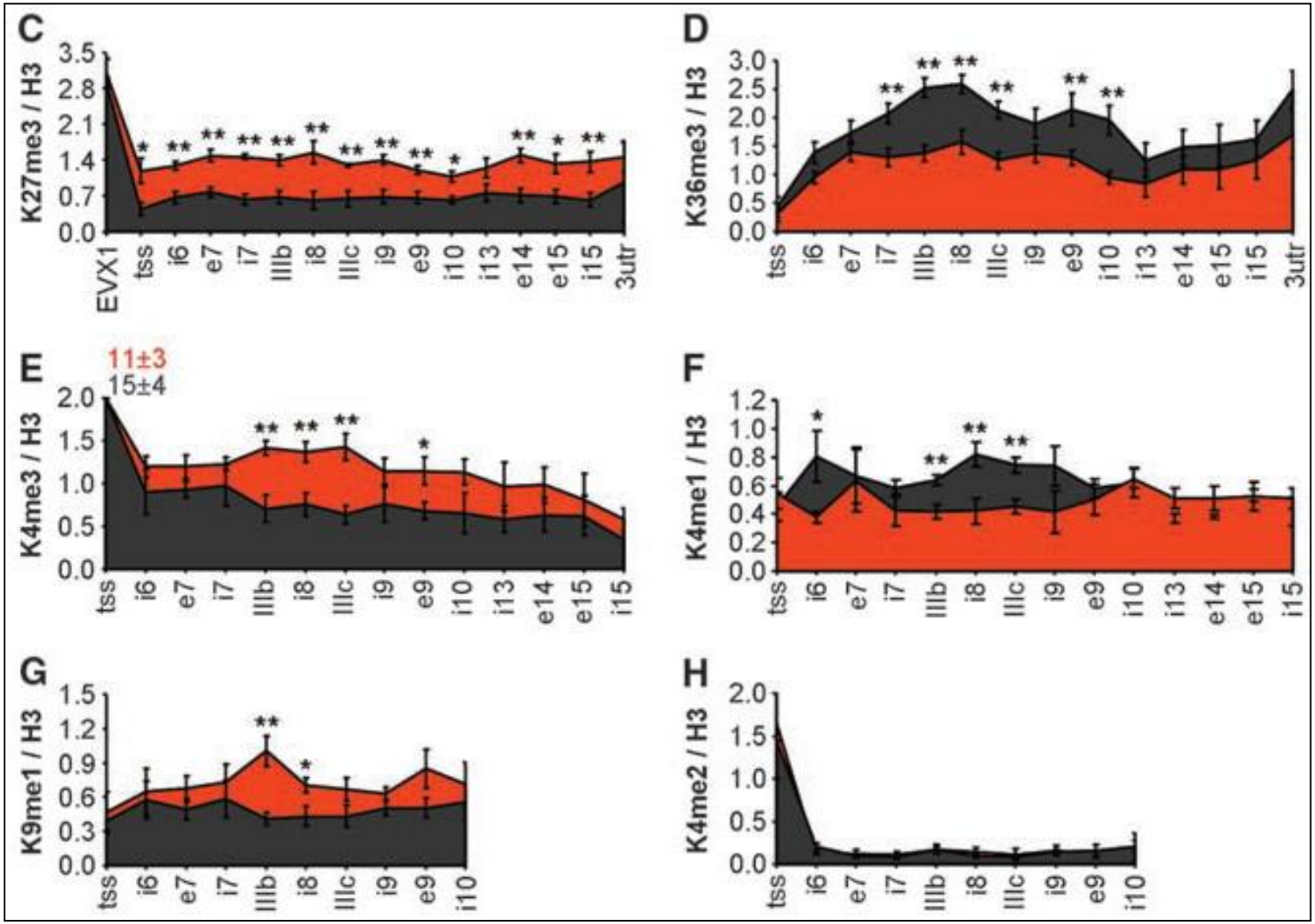
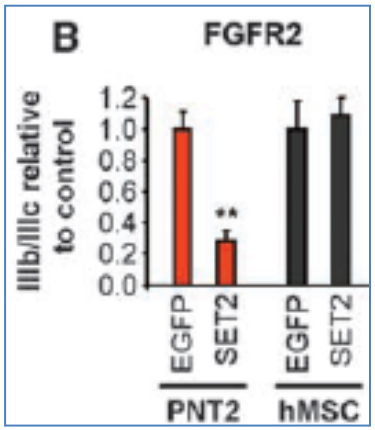


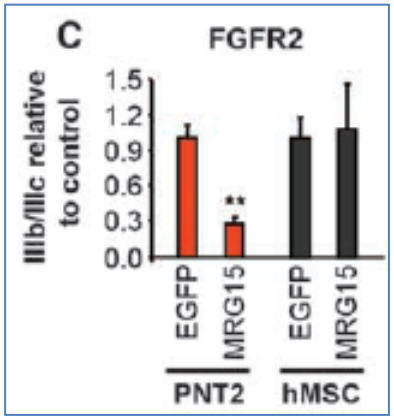
Fig. 1 - (C to H) Mapping of H3-K27me3 (C), H3-K36me3 (D), H3-K4me3 (E), H3-K4me1 (F), H3-K9me1 (G), and H3-K4me2 (H) in FGFR2 in **PNT2 (red)** and **hMSC (black)** cells by **quantitative ChIP.** The percentage of input was normalized to unmodified H3. Values represent means ± SEM from four to six independent experiments. *P <0.05, **P < 0.01, Student's t test.

The HMT specific to H3K36 is SET2.
When SET2 is overexpressed in epithelial cells, IIIb/IIIc ratio falls by 75%:



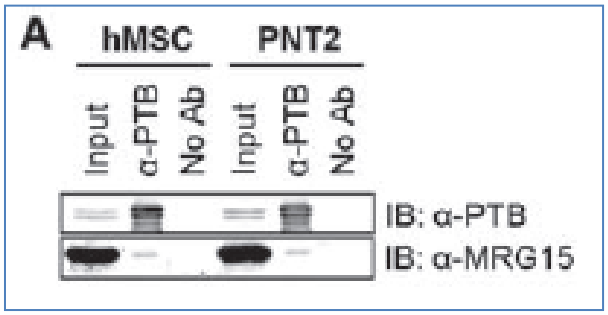
SET2 overexpression

H3K36(me3) is recognized by the bromodomain protein **MRG15 (reader)**
When MRG15 is overexpressed in epithelial cells, IIIb/IIIc raio falls by 75%:

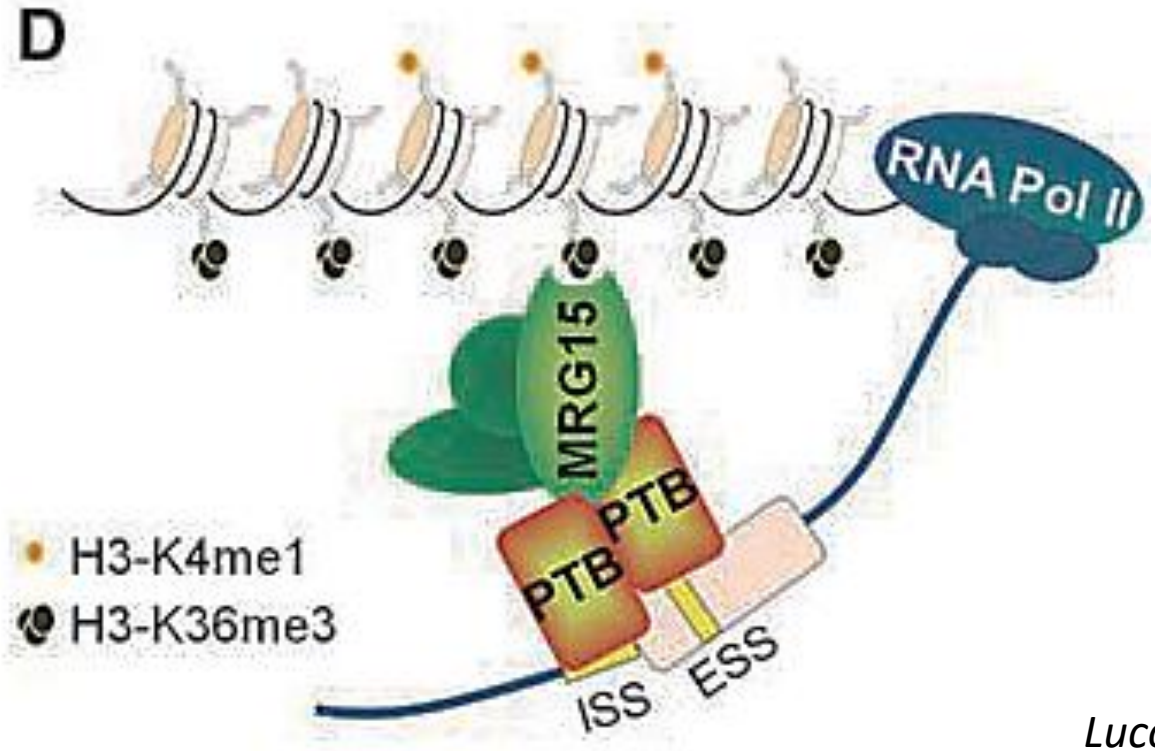


MRG15 overexpression

Exon IIIb has weak site for **PTB**.
MRG15 co-immunoprecipitates with the RNA binding protein PTB →



Co-IP

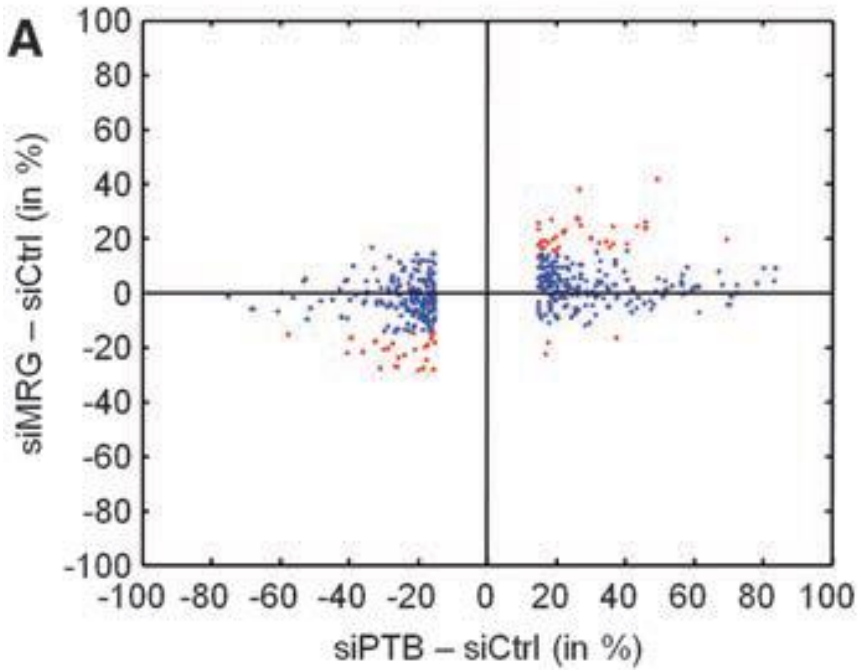


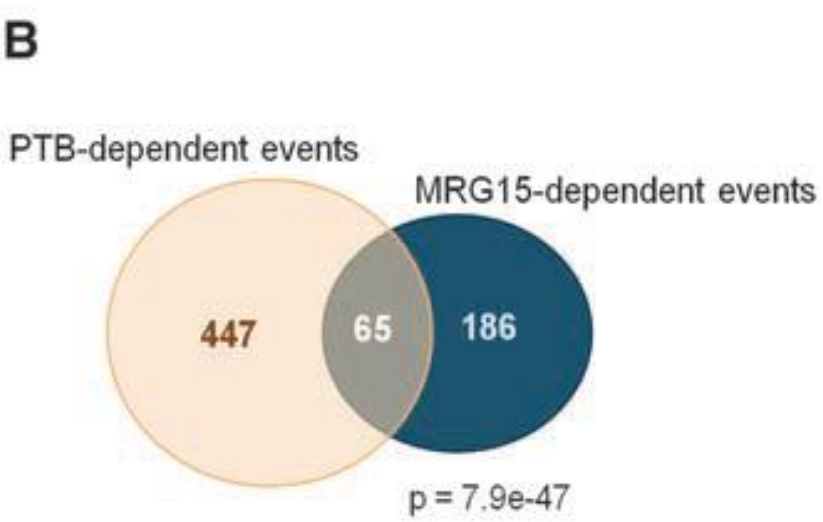
Luco et al, 2011

Are PTB and MRG15 effects limited to FGFR2 exons ?

siRNA-mediated down-regulation of either PTB or MRG2

RNA-seq → splicing read mapped and quantitated for each AS event





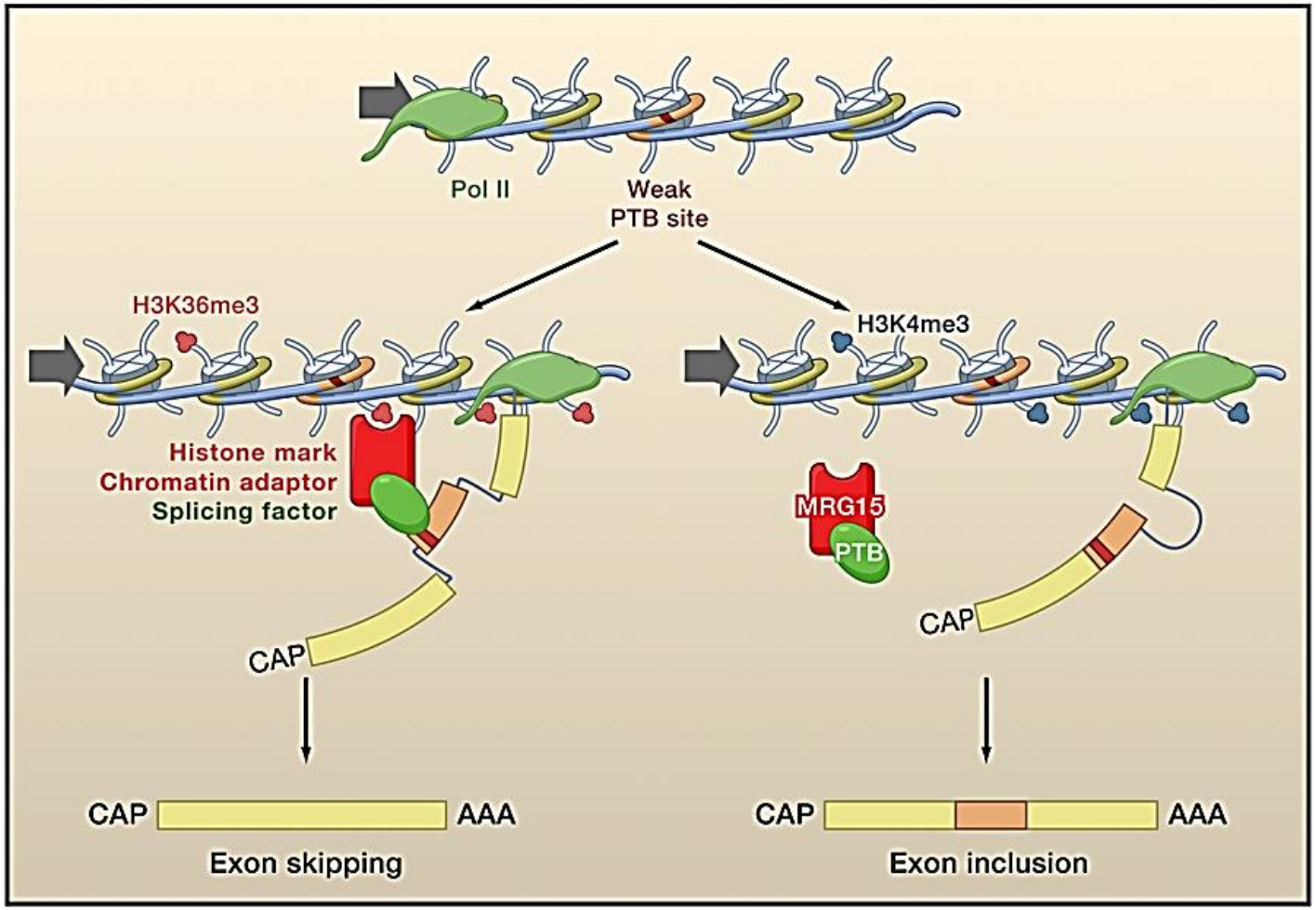


Figure 3. The Chromatin-Adaptor Model of Alternative Splicing. Histone modifications along the gene determine the binding of an adaptor protein that reads specific histone marks and in turn recruits splicing factors. In the case of exons whose alternative splicing is dependent on poly-pyrimidine tractbinding protein (PTB) splicing factor, high levels of trimethylated histone 3 lysine 36 (H3K36me3, red) attract the chromatin-binding factor MRG15 that acts as an adaptor protein and by protein-protein interaction helps to recruit PTB to its weaker binding site inducing exon skipping. If the PTBdependent gene is hypermethylated in H3K4me3 (blue), MRG15 does not accumulate along the gene, and PTB is not recruited to its target premRNA, thus favoring exon inclusion.

This is the first demonstration of a mechanistic link between chromatin and alternative splicing. Other protein-protein interaction between chromatin-competent proteins and RNA binding proteins is present in the literature, however no direct demonstration of a mechanism was given to date. Nonetheless, interactions suggest a possible functional role that should be worked out in the future.

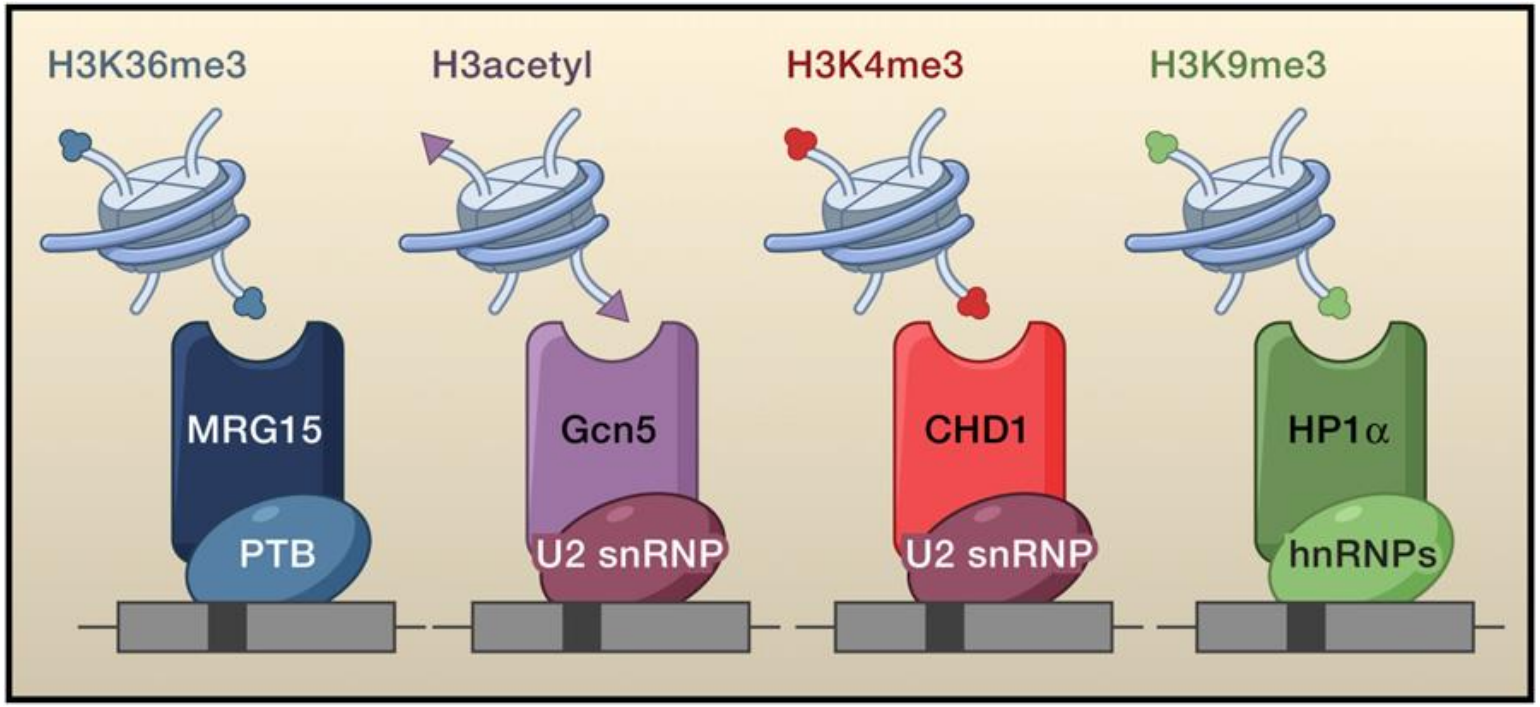


Figure 4. Chromatin-Adaptor Complexes
Several histone modification-binding chromatin proteins interact with splicing factors (Luco et al., 2010; Sims et al., 2007; Gunderson and Johnson, 2009; Piacentini et al., 2009; Loomis et al., 2009).

A model of epigenetic “memory” of alternative splicing in the cells

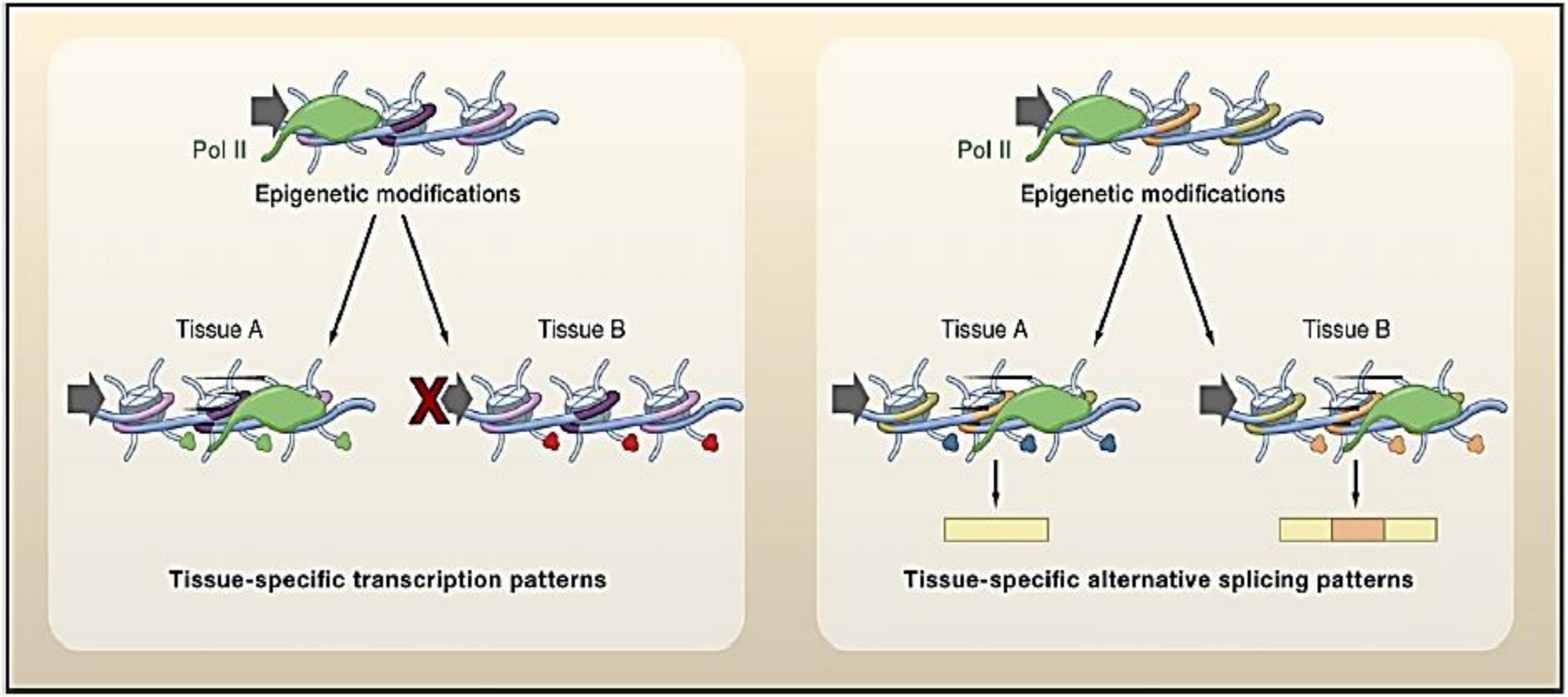


Figure 6. The Epigenetics of Alternative Splicing
The combination of histone modifications along a gene establishes and maintains tissue-specific transcription patterns (left panel), as well as heritable tissue-specific alternative splicing patterns (right panel).

The speed-bump model

After years, this model is now largely accepted, also because it can explain epigenetic effects on the choice of Alternative Splicing patterns

First mechanism

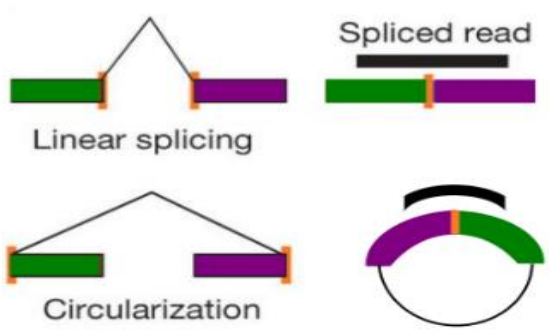
Histone modification can «stabilize» or «destabilize» nucleosomes sat on specific exons, thus modifying the rate of PolII at that specific exon.

Second mechanism

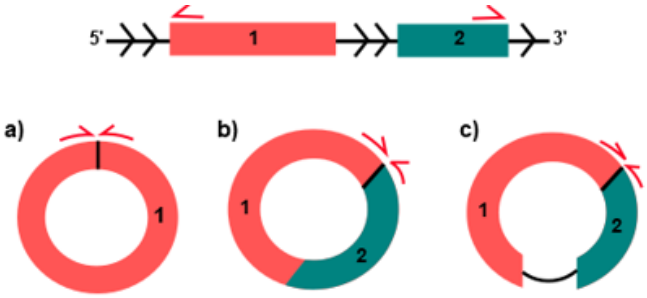
Specific histone PTMs enriched at one exonic nucleosome can bind «readers» associated to Splicing factors, thus increasing the local availability of that Splicing factor, when the exon is transcribed.

circRNAs

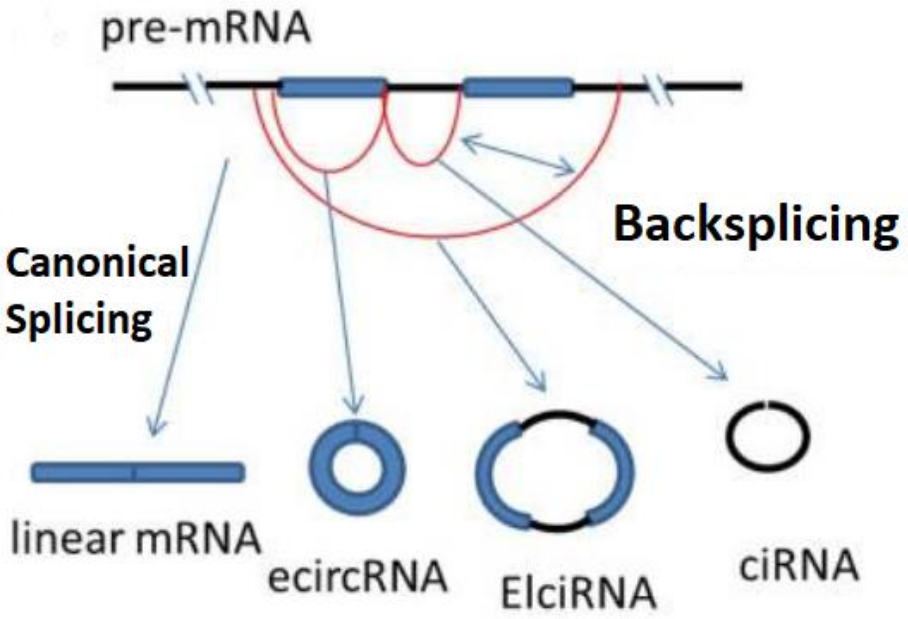
circRNA FORMATION



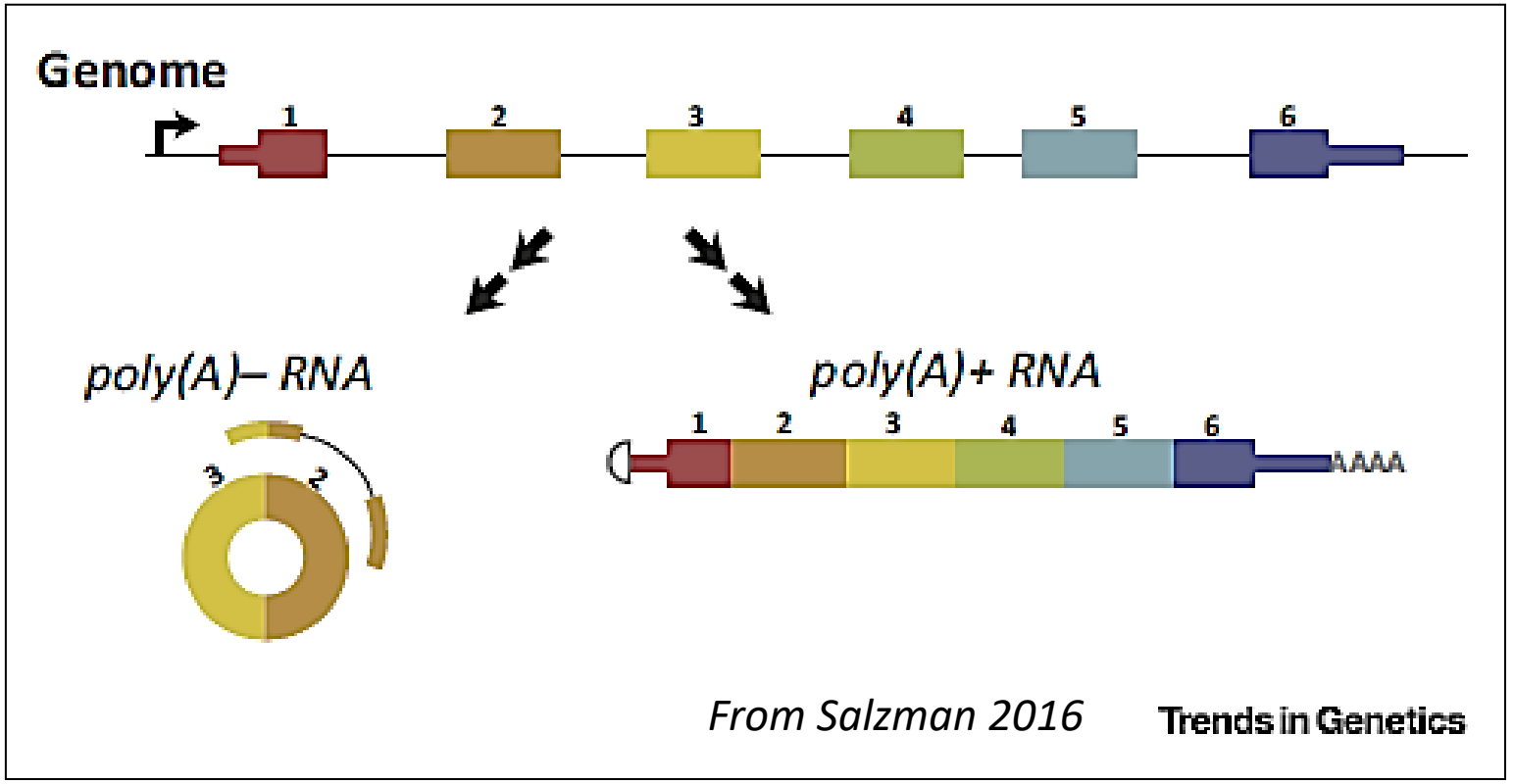
circRNAs can be detected
by backspliced reads



circRNAs can arise from 1 or more Exons
Are detected by divergent primers

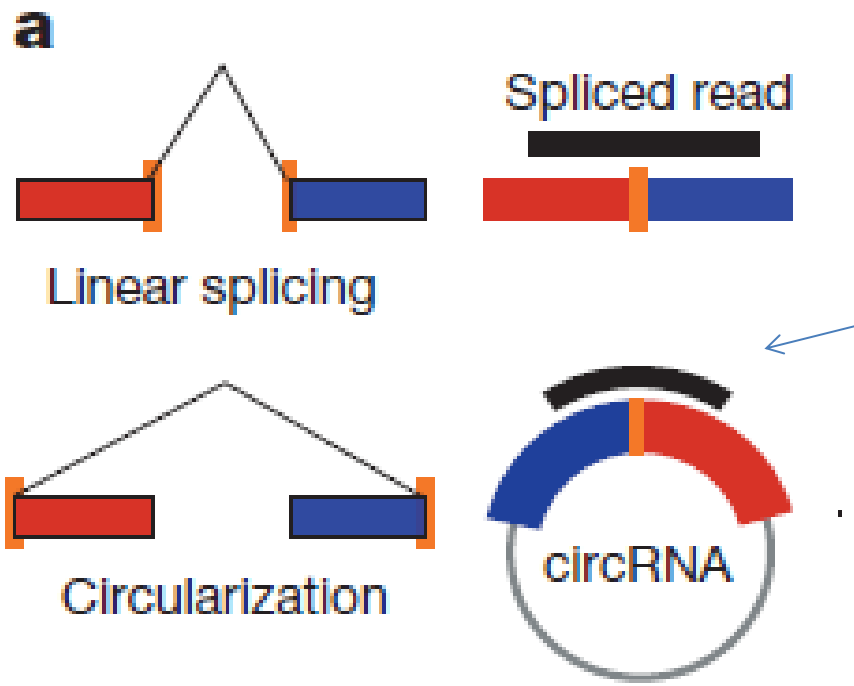


Exon circularization may represent a case of alternative splicing



The sequence of circular RNA comprises exons from protein and noncoding loci, spliced at canonical splice sites. Bi-colored reads represent paired-end RNA-Seq reads used to discover circular RNA that are enriched in polyA-depleted RNAsamples.

circRNAs

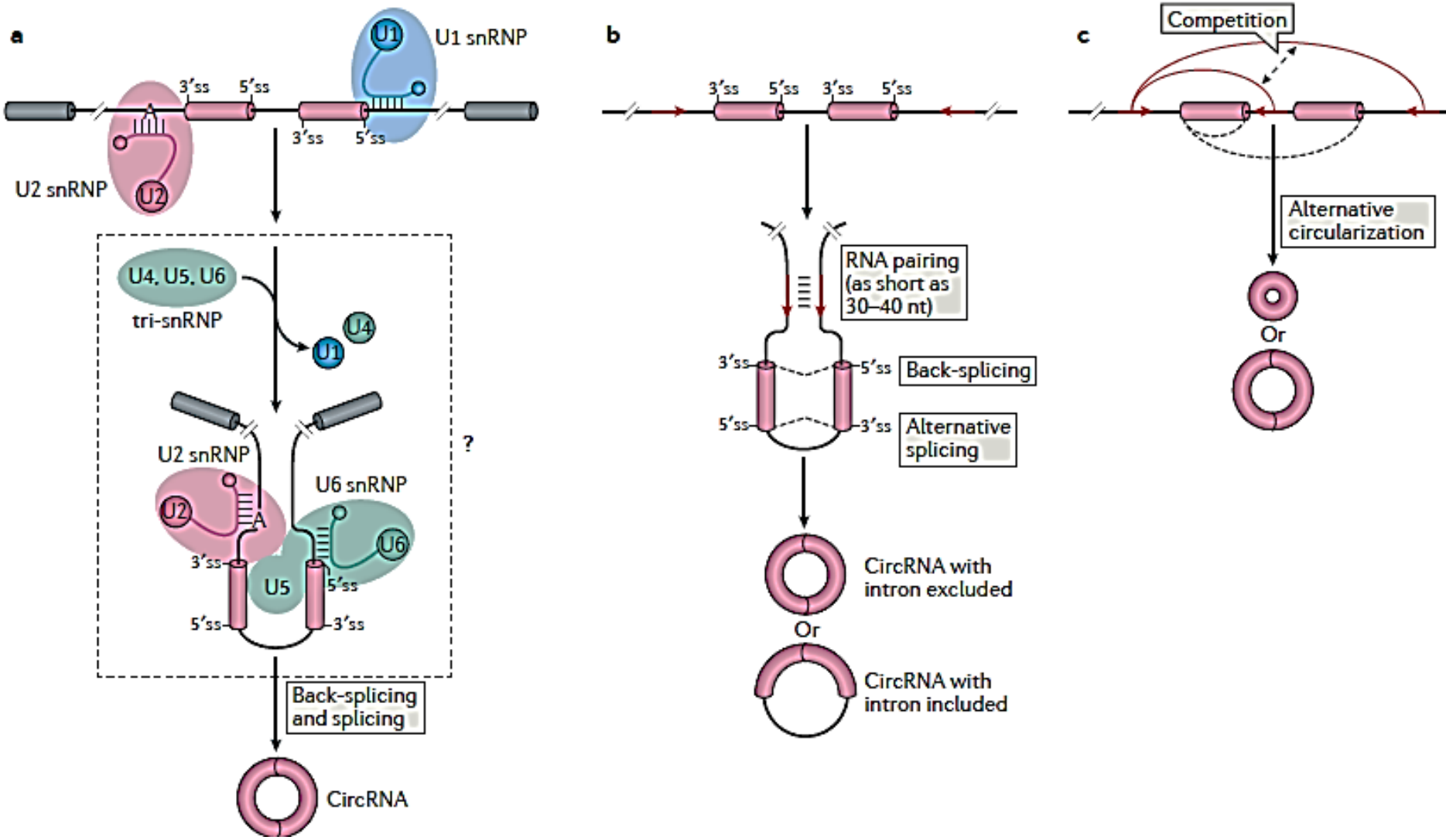


«back-splicing reads»

From Memczak et al. 2013

Possible functions

- Endogenous competing RNAs (ceRNA, see last lesson)
- Activators/repressors of transcription
- Alternative protein-encoding RNAs



From Chen 2016, NRMCB 17:205

circRNA FUNCTIONS

- **nuclear circRNAs enhance host gene transcription**
- **circRNAs can act as miRNA sponges**
- **circRNAs can be translated into proteins**
- **circRNAs as potential biomarkers**