4.3.1

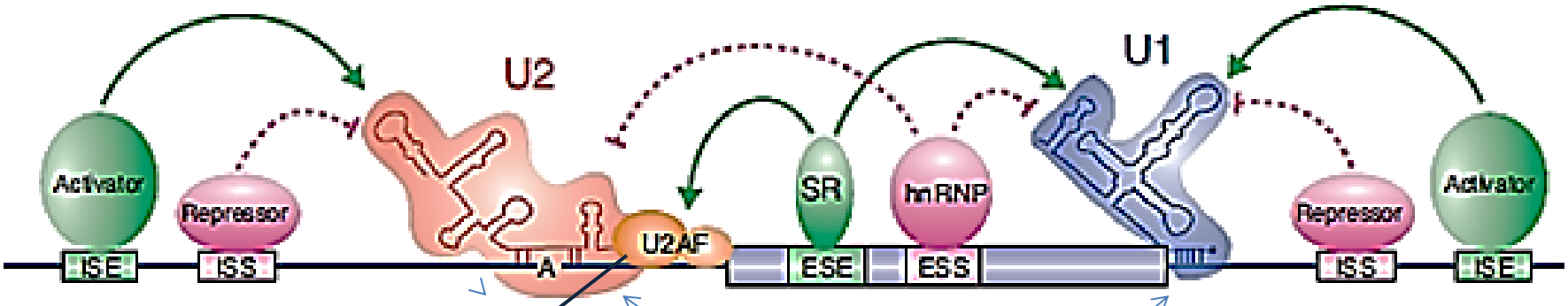
Alternative exon inclusion/exclusion : regulated process

- Exon-intron border sequence (splice-sites)
- *cis*-elements and *trans*-regulatory factors
- SR and hnRNPs factors
- **Tissue-specific Splicing Regulators**
- **Transcriptional speed and pausing**
- **Nucleosome positioning over exons**
- **Histone PTMs and readers-splicing factors association**

Regulatory

- Tissue-specific splicing
- Regulated splicing
- Epigenetic establishment of splicing patterns

1) Tissue-specific splicing factors
2) Signal transduction regulated factors
3) Chromatin effects on splicing choice

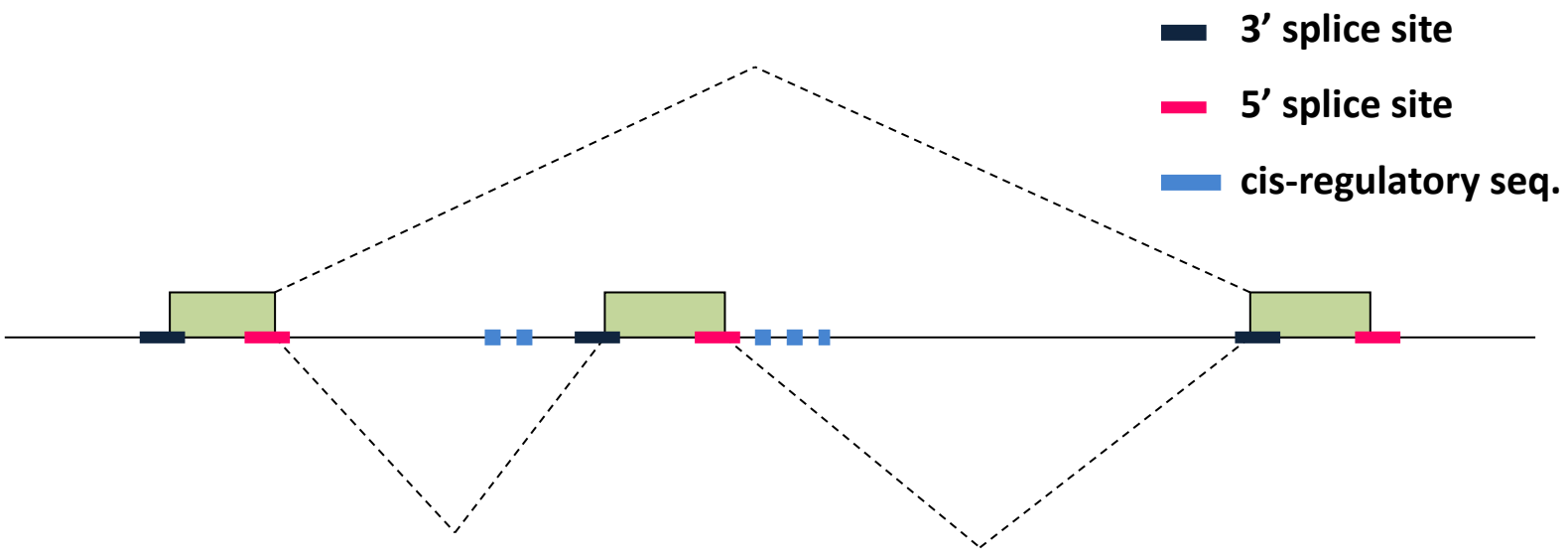


The permanence of U2AF with U2 snRNP is questioned

very poor U-RNA / RNA transcript pairing in higher eukaryotes

The mechanisms of alternative splicing regulation by trans-acting factors has been explained following the **competitive model**

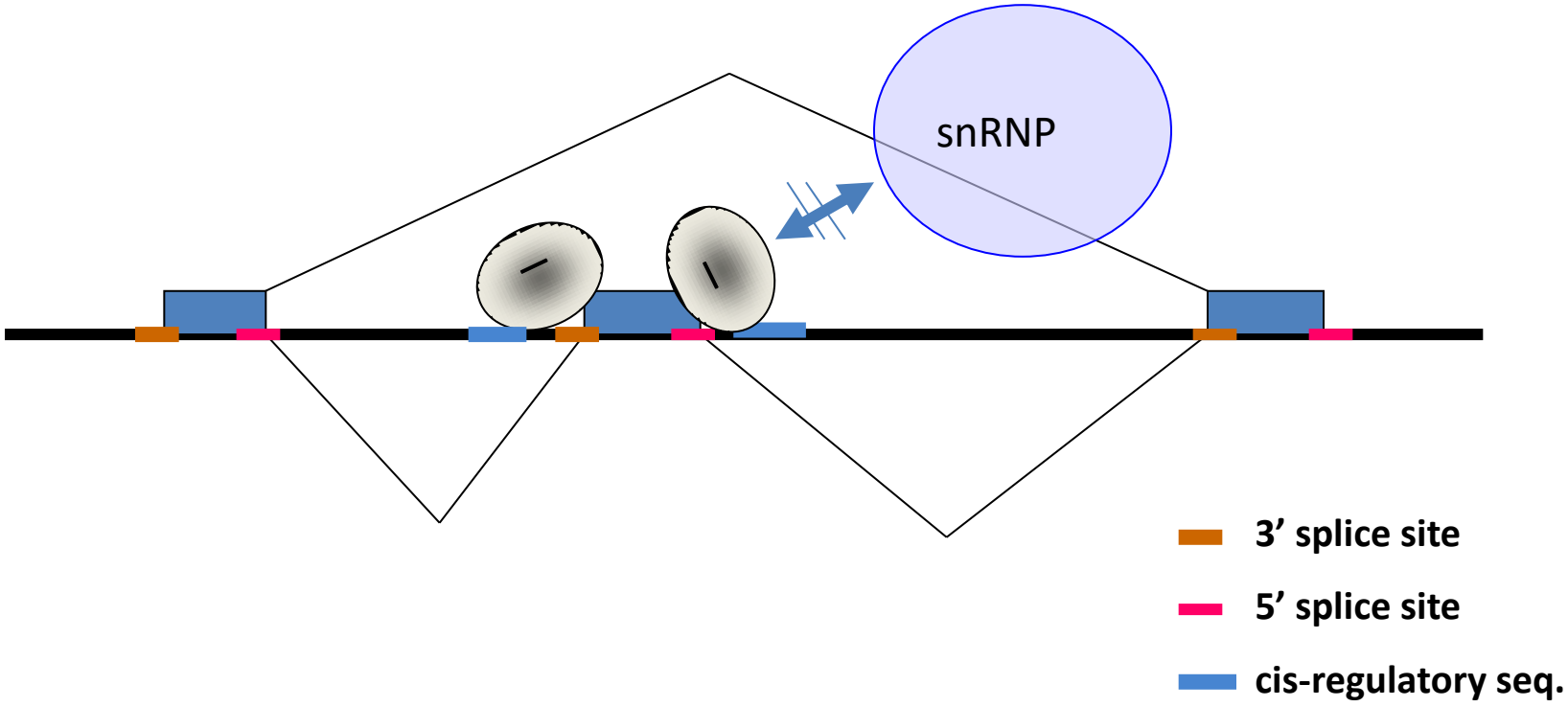
We can consider the older competition model, as exemplified by the case of a «skipping exon»

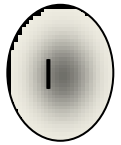


In absence of other regulatory elements (cis-regulatory), only one result is seen: the strongest splice sites will predominate.

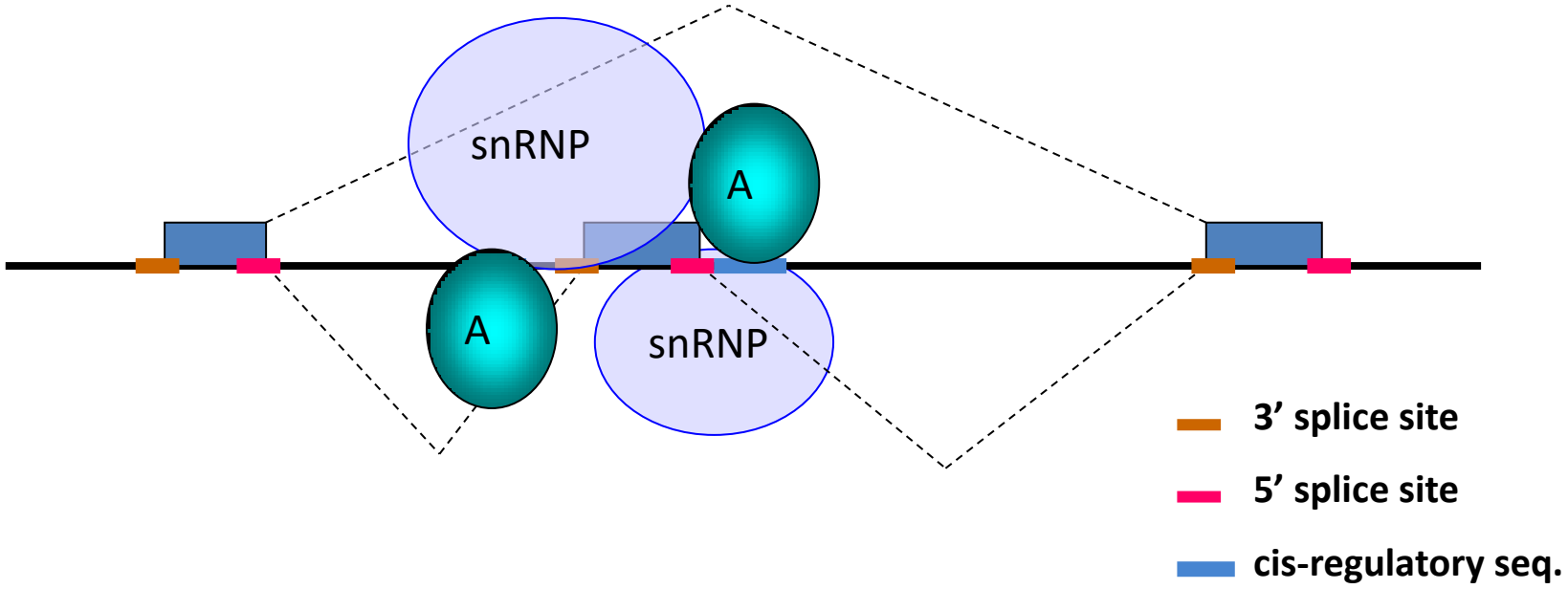
(*Actually, this model did not account for co-transcriptional splicing...discussed below*)

Mechanisms:

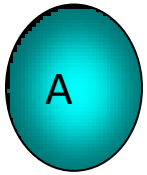


1) Splicing sites are «strong», but they are occluded by inhibiting factor 

Mechanisms:

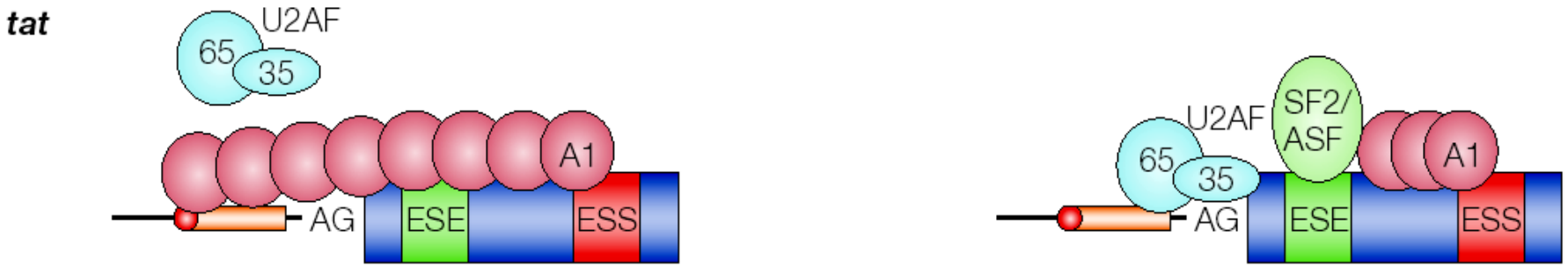


2) sites are «weak» but accessory *cis* sequences + factors will strengthen snRNP interaction.



The balance between SR and hnRNP proteins may explain some cases of alternative splicing

2nd model - competition between SR proteins and hnRNP. (hnRNP A1 multimerizes)

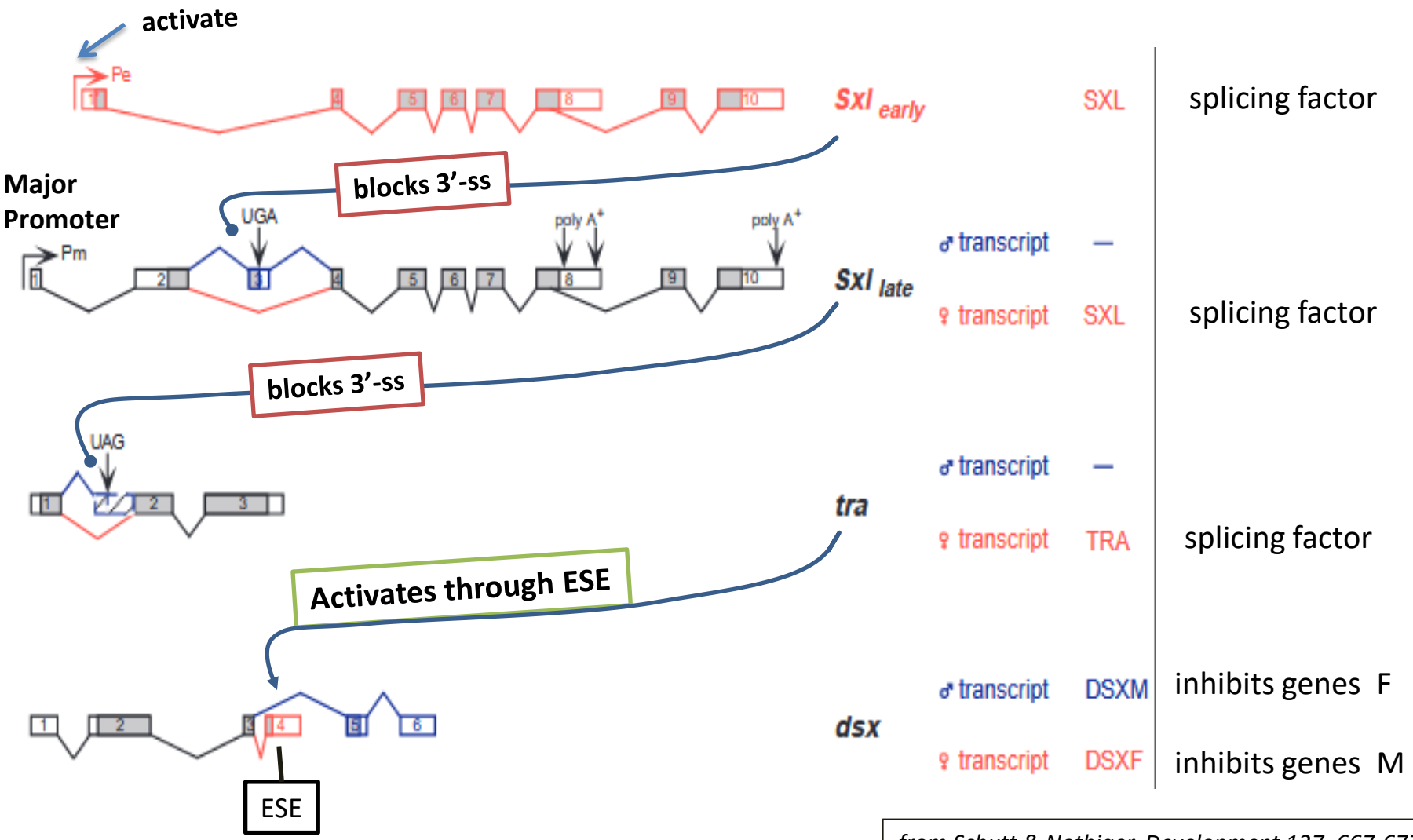


EXAMPLE: Inclusion of exon 3 of HIV1 *tat* pre-mRNA is determined by the nuclear ratio of specific heterogeneous nuclear ribonucleoprotein (hnRNP) and SR proteins. Propagative multimerization of hnRNPA1 from a high-affinity exon splicing silencer (ESS) is sterically blocked by the interaction of SF2/ASF with the upstream ESE.

Note that in this case, ESE function requires the RRM domains but not the RS domain of SF2/ASF.

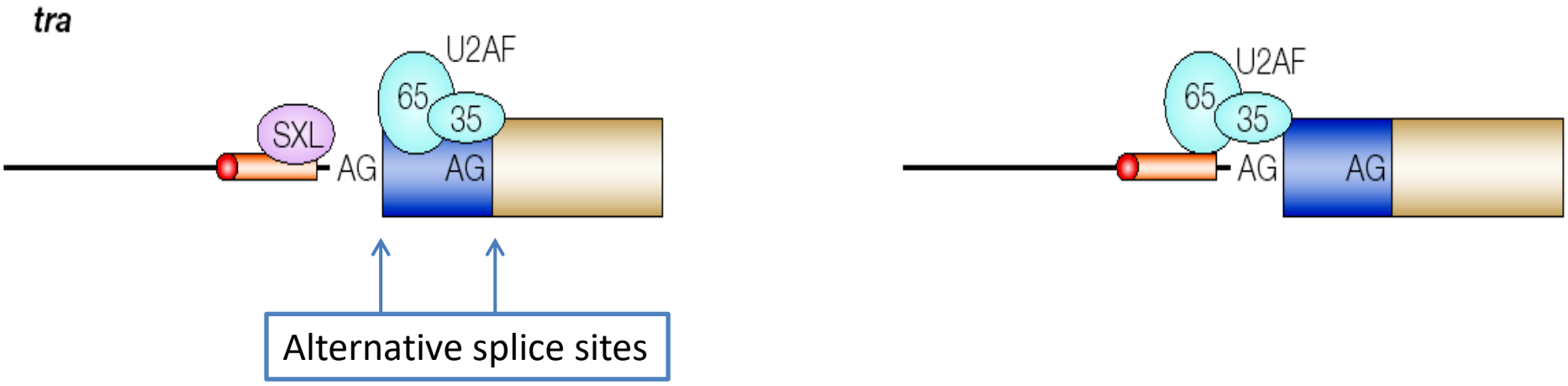
The cascade of AS regulation in sex determination in Drosophila is the most famous, and actually has given the first proven example of an ESE.

In Drosophila, sex is determined by X:autosomal ratio.
In males, the A chr. gene product *dpn* interact with the X chr. gene products *sisA/B* and titrates them out. In female XX, *sisA/B* is double quantity and some remain free, and able to activate the Pe early promoter of Sxl gene. In red what happens in Females.



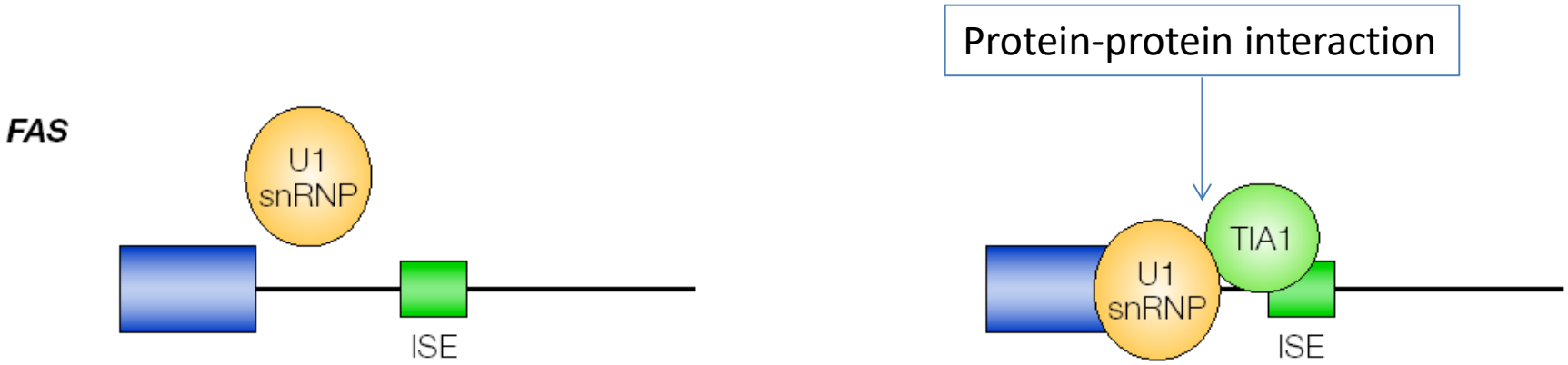
from Schutt & Nothiger, Development 127, 667-677 (2000)

The binding of *Sex lethal* (SXL)a specific RBP to an intronic sequence overlapping poly-pyrimidine competes with U2AF65 binding and inhibits splicing



EXAMPLE: Repression of the non-sex-specific *tra* 3' splice site involves the interaction of SXL with an intron splicing silencer (ISS) embedded in the polypyrimidine tract and the prevention of U2AF binding. This leads to selection of the downstream female-specific 3' splice site.

<u>Another example</u> : an RBP interacts with a small sequence in the pre-mRNA using an RRM domain and interacts with U1snRNP with another domain



EXAMPLE: A weak 5' splice site in the *FAS* transcript is enhanced by TIA1 binding to a down-stream intron splicing enhancer (ISE). TIA1 cooperatively promotes the interaction of U1 small nuclear ribonucleoprotein particles (snRNPs) with the pre-mRNA.

Table 2 | **Tissue-specific alternative splicing factors**

Name	Other names	Binding domain	Binding motif	Tissue expression	Target genes
nPTB	brPTB and PTBP2	RRM	CUCUCU	Neurons, myoblasts and testes	*BIN1*, *GLYRA2*, *ATP2B1*, *MEF2*, *NASP*, *SPAG9* and *SRC*
NOVA1	NA	KH	YCAY	Neurons of the hindbrain and spinal cord	*GABRG2*, *GLYRA2* and *NOVA1*
NOVA2	NA	KH	YCAY	Neurons of the cortex, hippocampus and dorsal spinal cord	*KCNJ*, *APLP2*, *GPHN*, *JNK2*, *NEO*, *GRIN1* and *PLCB4*
FOX1	A2BP1	RRM	(U)GCAUG	Muscle, heart and neurons	*ACTN*, *EWSR1* , *FGFR2*, *FN1* and *SRC*
FOX2	RBM9	RRM	(U)GCAUG	Muscle, heart and neurons	*EWS*, *FGFR2*, *FN1* and *SRC*
RBM35a	ESRP1	RRM	GU rich	Epithelial cells	*FGFR2*, *CD44*, *CTNND1* and *ENAH*
RBM35b	ESRP2	RRM	GU rich	Epithelial cells	*FGFR2*, *CD44*, *CTNND1* and *ENAH*
TIA1	mTIA1	RRM	U rich	Brain, spleen and testes	*MYPT1*, *CD95*, *CALCA*, *FGFR2*, *TIAR*, *IL8*, *VEGF*, *NF1* and *COL2A1*
TIAR	TIAL1 and mTIAR	RRM	U rich	Brain, spleen, lung, liver and testes	*TIA1*, *CALCA* , *TIAR*, *NF1* and *CD95*
SLM2	KHDRBS3 and TSTAR	KH	UAAA	Brain, tests and heart	*CD44* and *VEGFA*
Quaking	QK and QKL	KH	ACUAAY[...]UAAY	Brain	*MAG* and *PLP*
HUB	HUC, HUD and ELAV2	RRM	AU rich	Neurons	*CALCA*, *CD95* and *NF1*

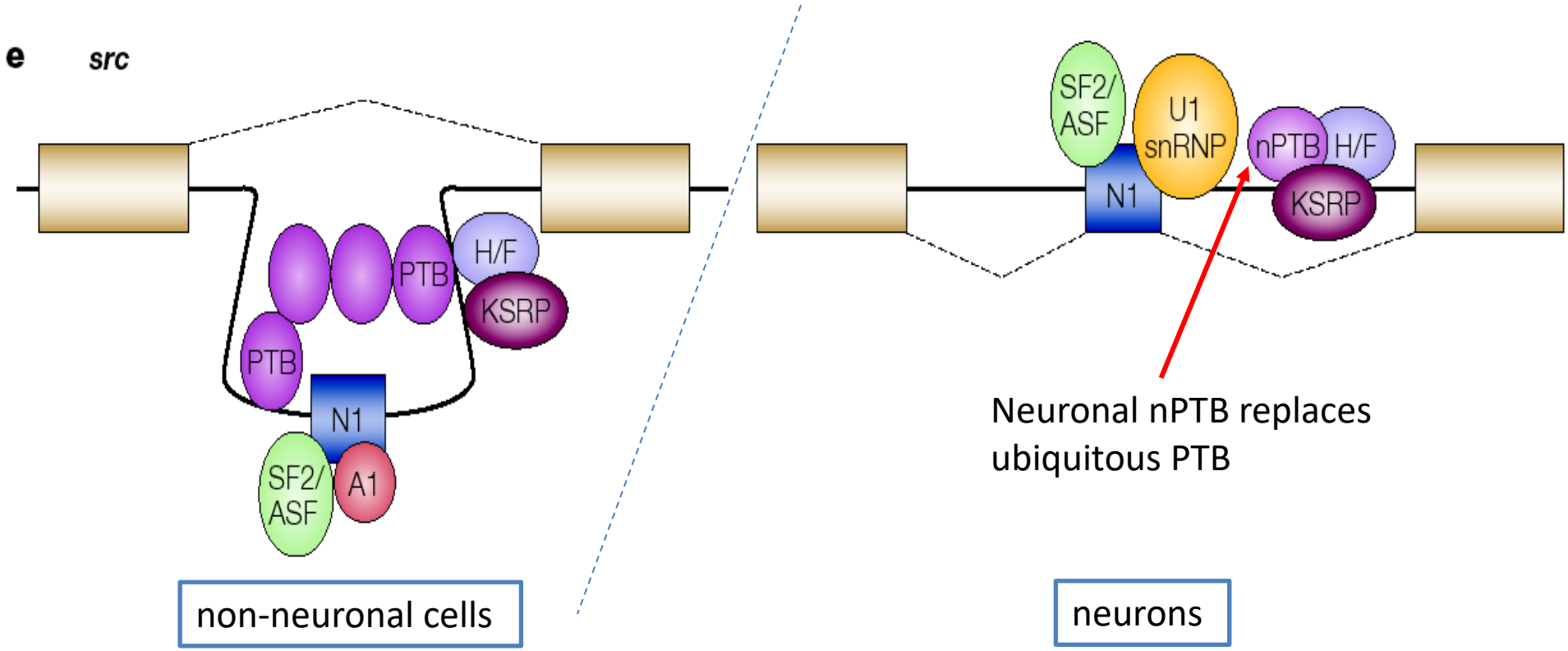
Chen & Manley 2009. Nat Rev Mol Cell Biol., 10:741.

MBNL	NA	CCCH zinc finger domain	YGCU(U/ G)Y	Muscles, uterus and ovaries	*TNTT2*, *INSR*, *CLCN1* and *TNNT3*
CELF1	BRUNOL2	RRM	U and G rich	Brain	*TNTT2* and *INSR*
ETR3	CELF2 and BRUNOL3	RRM	U and G rich	Heart, skeletal muscle and brain	*TNTT2*, *TAU* and *COX2*
CELF4	BRUNOL4	RRM	U and G rich	Muscle	*MTMR1* and *TNTT2*
CELF5	BRUNOL5 and NAPOR	RRM	U and G rich	Heart, skeletal muscle and brain	*ACTN*, *TNTT2* and *GRIN1*
CELF6	BRUNOL6	RRM	U and G rich	Kidney, brain and testes	*TNTT2*

A2BP1, ataxin 2-binding protein 1; *ACTN*, α-actinin; *APLP2*, amyloid-β precursor-like protein 2; *ATP2B1*, ATPase, Ca^{2+} transporting, plasma membrane 1; *BIN1*, bridging integrator 1; *CALCA*, calcitonin-related polypeptide-α; CELF, CUGBP- and ETR3-like factor; *CLCN1*, chloride channel 1; *COL2A1*, collagen, type II, α1; *COX2*, cytochrome *c* oxidase II; *CTNND1*, catenin δ1, *EWSR1*, Ewing sarcoma breakpoint region 1; *FGFR2*, fibroblast growth factor receptor 2; *FN1*, fibronectin 1; *GABRG2*, GABA A receptor, γ2; *GLYRA2*, glycine receptor, α2 subunit; *GPHN*, gephyrin; *GRIN1*, glutamate receptor, ionotropic, NMDA 3B; *IL8*, interleukin-8; *INSR*, insulin receptor; *JNK2*, Jun N-terminal kinase 2; *KCNJ*, potassium inwardly-rectifying channel, subfamily; KHDRBS3, KH domain-containing, RNA-binding, signal transduction-associated protein 3; *MAG*, myelin associated glycoprotein; MBNL, muscleblind; *MEF2*, myocyte enhancing factor 2; *MTMR1*, myotubularin-related protein 1; *NASP*, nuclear autoantigenic sperm protein; *NEO*, neogenin; *NF1*, neurofibromin 1; *NOVA*, neuro-oncological ventral antigen; *PLCB4*, phospholipase C β4; *PLP*, proteolipid protein; PTB, polypyrimidine-tract binding protein; RBM, RNA-binding protein; RRM, RNA recognition motif; *SLM2*, SAM68-like mammalian protein 2; *SPAG9*, sperm associated antigen 9; TIA1, T cell-restricted intracellular antigen 1; TIAR, TIA1-related protein; *TNTT2*, troponin T type 2; *VEGF*, vascular endothelial growth factor.

Chen & Manley 2009. Nat Rev Mol Cell Biol., 10:741.

EXAMPLE : expression of a tissue-specific paralogue of the PTB (polypyrimidine tract binding protein) allows intron definition



e | The regulation of **N1 exon splicing in the *src*** transcript provides an example of combinatorial control by cooperation and antagonism between numerous positively and negatively acting factors. In non-neuronal cells (left), N1 is excluded, whereas in neurons (right), it is included in the mature mRNA. Constitutive exons are shown as beige boxes, whereas alternative exons are shown as blue boxes. KSRP, KH-type splicing regulatory protein; **nPTB**, neural polypyrimidine tract binding protein.

EXAMPLE

nature Vol 444 | 30 November 2006 | doi:10.1038/nature05304

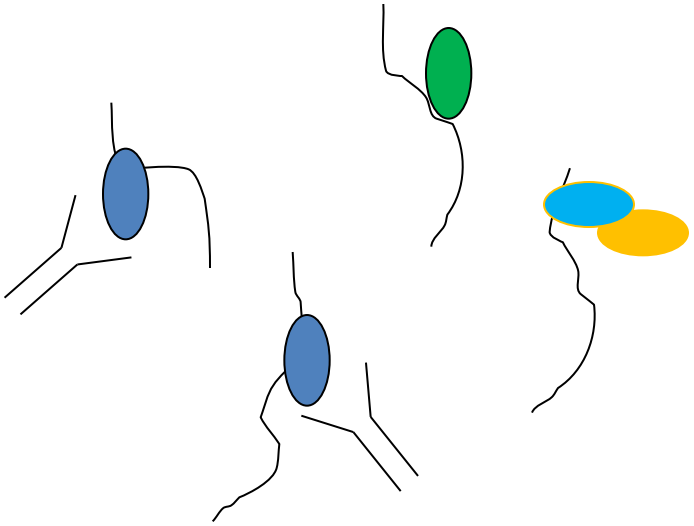
ARTICLES

An RNA map predicting Nova-dependent splicing regulation

Jernej Ule[1,2*†], Giovanni Stefani[1,2*†], Aldo Mele[1,2], Matteo Ruggiu[1,2], Xuning Wang[3], Bahar Taneri[4†], Terry Gaasterland[4†], Benjamin J. Blencowe[5] & Robert B. Darnell[1,2]

Nova proteins are neuron-specific alternative splicing factors. We have combined bioinformatics, biochemistry and genetics to derive an RNA map describing the rules by which Nova proteins regulate alternative splicing. This map revealed that the position of Nova binding sites (YCAY clusters) in a pre-messenger RNA determines the outcome of splicing. The map correctly predicted Nova's effect to inhibit or enhance exon inclusion, which led us to examine the relationship between the map and Nova's mechanism of action. Nova binding to an exonic YCAY cluster changed the protein complexes assembled on pre-mRNA, blocking U1 snRNP (small nuclear ribonucleoprotein) binding and exon inclusion, whereas Nova binding to an intronic YCAY cluster enhanced spliceosome assembly and exon inclusion. Assays of splicing intermediates of Nova-regulated transcripts in mouse brain revealed that Nova preferentially regulates removal of introns harbouring (or closest to) YCAY clusters. These results define a genome-wide map relating the position of a *cis*-acting element to its regulation by an RNA binding protein, namely that Nova binding to YCAY clusters results in a local and asymmetric action to regulate spliceosome assembly and alternative splicing in neurons.

Cross-link (U.V.)



Nova: the first vertebrate tissue-specific splicing factors (neurons) (Nova1 – Nova2)

IMPT

CLIP

Binding sites on mRNAs identified on microarrays

Bioinformatics «YCAY» clusters

48 regulated mRNA targets identified in previous studies

Clustering of “YCAY” Nova recognition sequences in 48 Nova-regulated exons

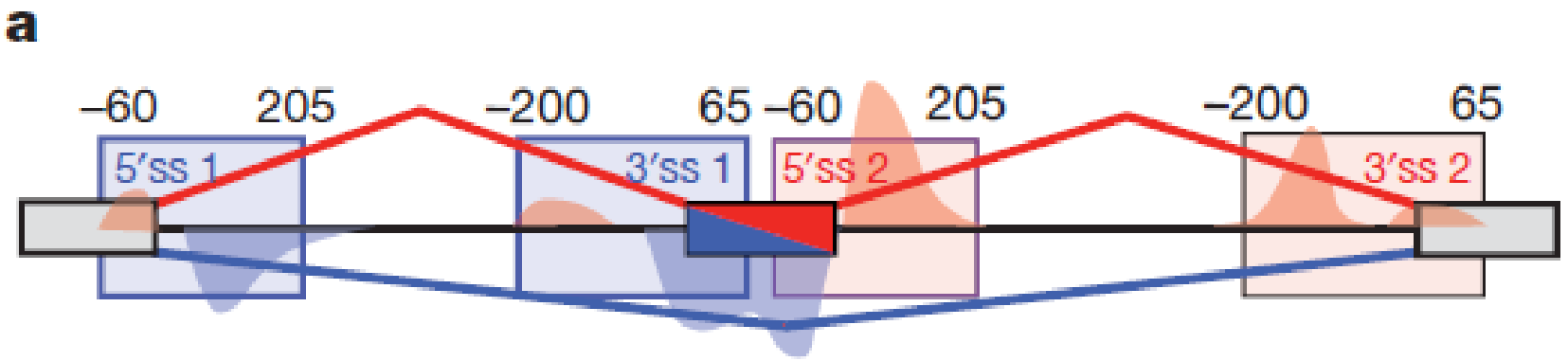
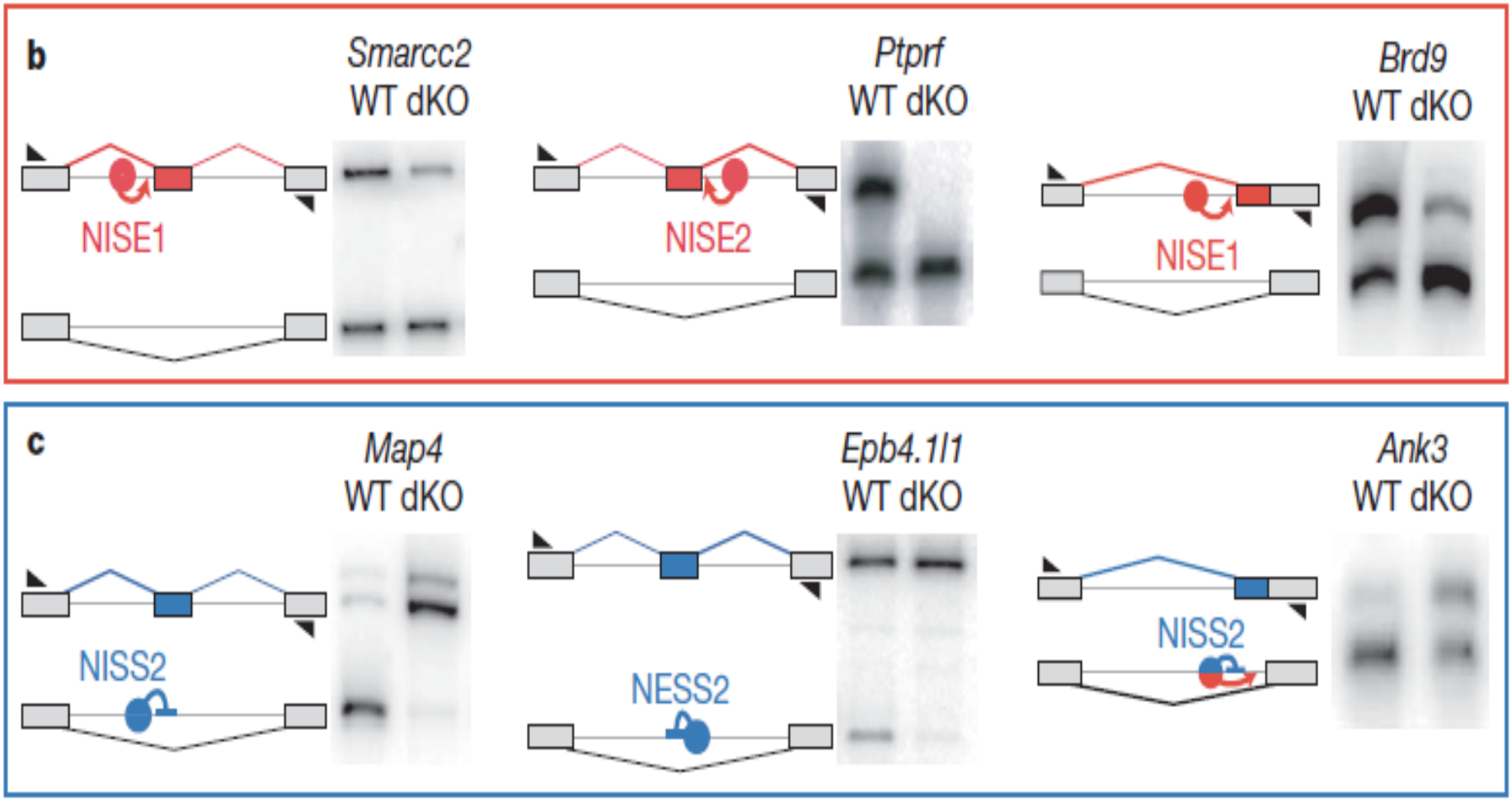


Figure 1 | Definition of the Nova–RNA binding map. a, A generic pre-mRNA showing the four regions that define the Nova–RNA binding map (the start and end of each region is labelled by a nucleotide distance to the splice site). Peaks demonstrate the positions of Nova-dependent **splicing enhancers (red)** or **silencers (blue)**.

The splicing regulatory effect of Nova 1-2 depends on the **position** of its cognate binding site relative to alternative exons.

Examples of predicted Nova-regulated exons: analysis in brain tissues from Nova1$^{-/-}$ / Nova2 $^{-/-}$ double K.O. mice (dKO).



Indicate the primers used for RT-PCR analysis

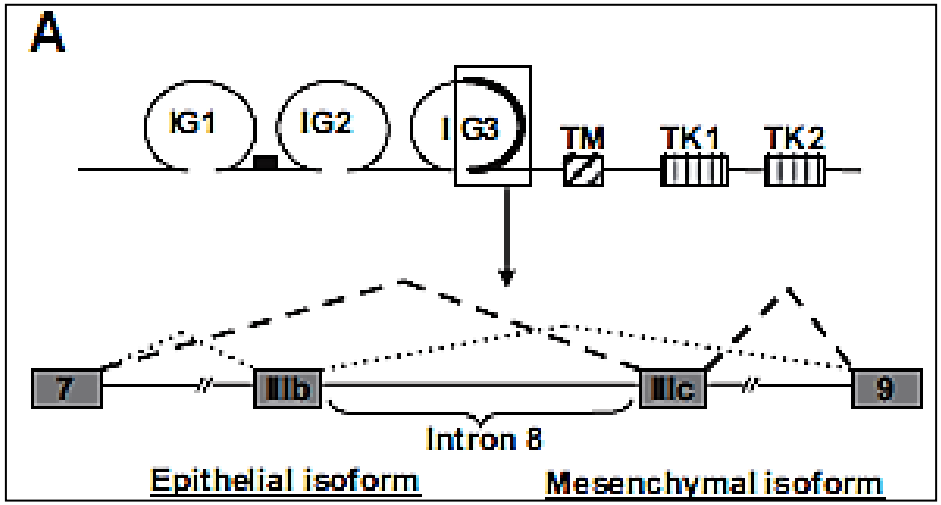
EXAMPLE

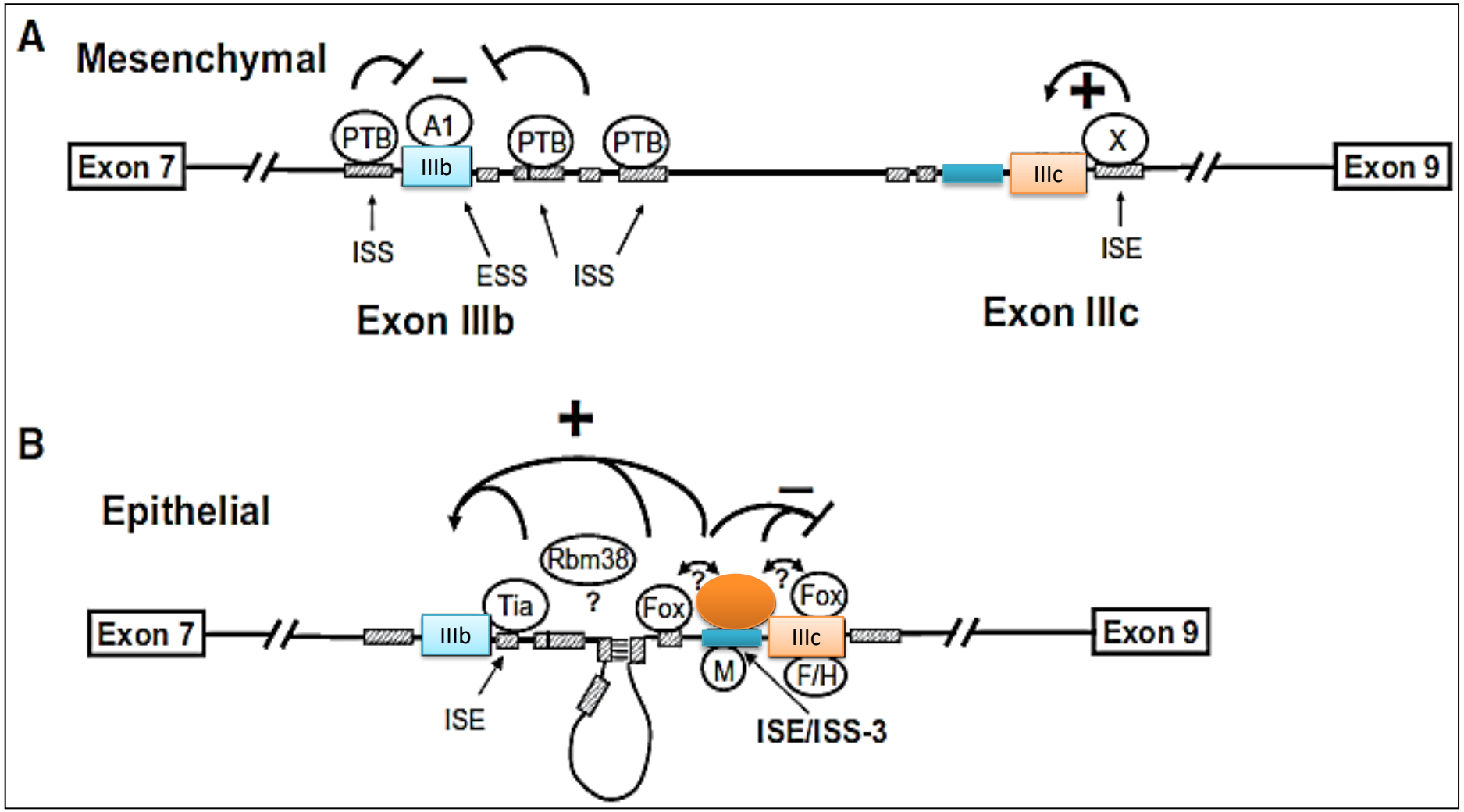
ESRP1 and ESRP2 Are Epithelial Cell-Type-Specific Regulators of FGFR2 Splicing

Claude C. Warzecha,[1,2] Trey K. Sato,[3] Behnam Nabet,[1] John B. Hogenesch,[3,5] and Russ P. Carstens[1,2,4,*]
[1]Renal Division, Department of Medicine
[2]Cell and Molecular Biology Graduate Group
[3]Department of Pharmacology, Institute for Translational Medicine and Therapeutics
[4]Department of Genetics
University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA
[5]Penn Genome Frontiers Institute, University of Pennsylvania, Philadelphia, PA 19104, USA

SUMMARY

Cell-type-specific expression of epithelial and mesenchymal isoforms of Fibroblast Growth Factor Receptor 2 (FGFR2) is achieved through tight regulation of mutually exclusive exons IIIb and IIIc, respectively. Using an application of cell-based cDNA expression screening, we identified two paralogous epithelial cell-type-specific RNA-binding proteins that are essential regulators of *FGFR2* splicing. Ectopic expression of either protein in cells that express FGFR2-IIIc caused a switch in endogenous FGFR2 splicing to the epithelial isoform. Conversely, knockdown of both factors in cells that express FGFR2-IIIb by RNA interference caused a switch from the epithelial to mesenchymal isoform. These factors also regulate splicing of *CD44*, *p120-Catenin* (*CTNND1*), and *hMena* (*ENAH*), three transcripts that undergo changes in splicing during the epithelial-to-mesenchymal transition (EMT). These studies suggest that Epithelial Splicing Regulatory Proteins 1 and 2 (ESRP1 and ESRP2) are coordinators of an epithelial cell-type-specific splicing program.





IIIb

ESS

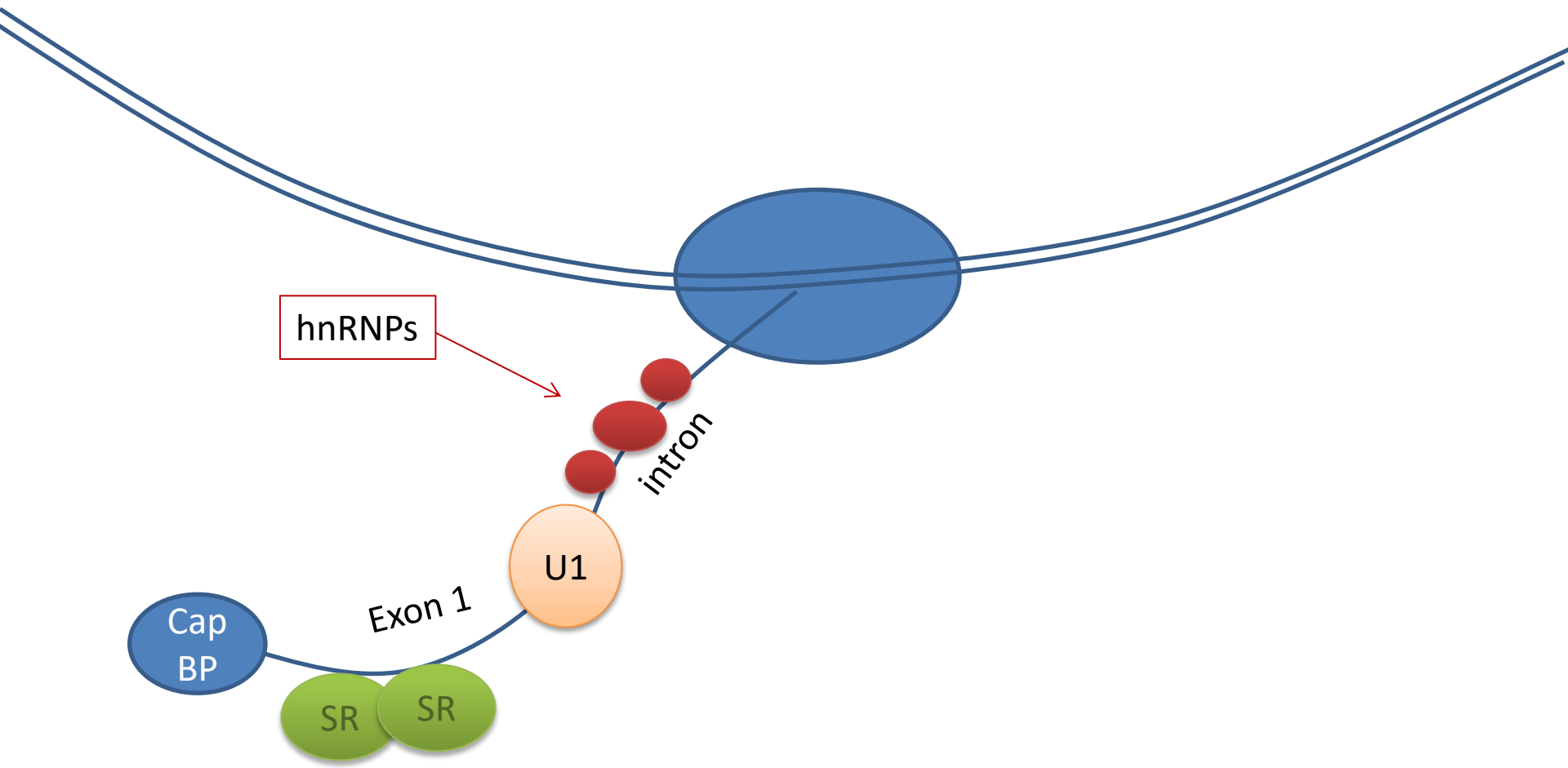
Espr

IIIc

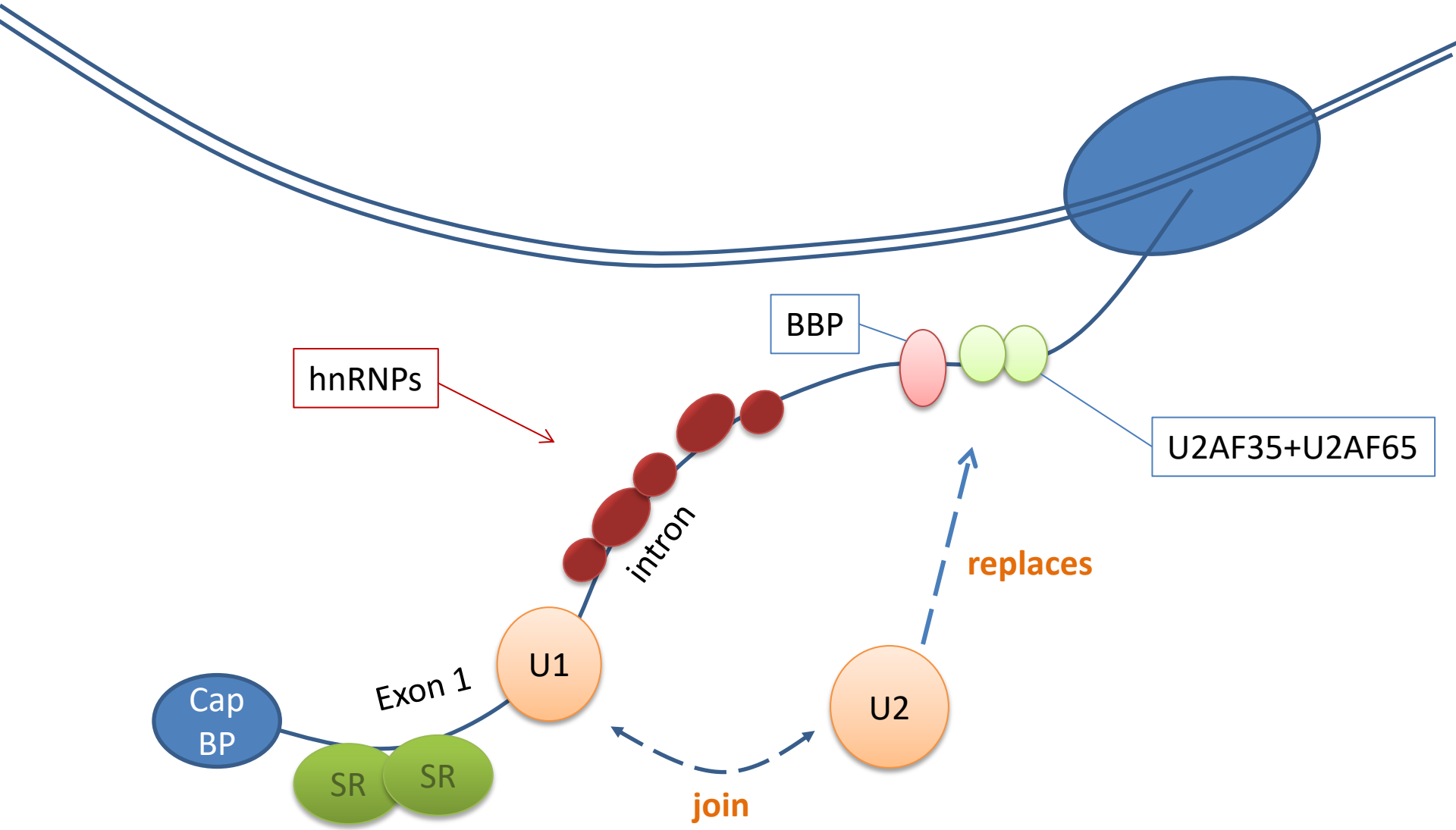
2xESE

The kinetic competition model

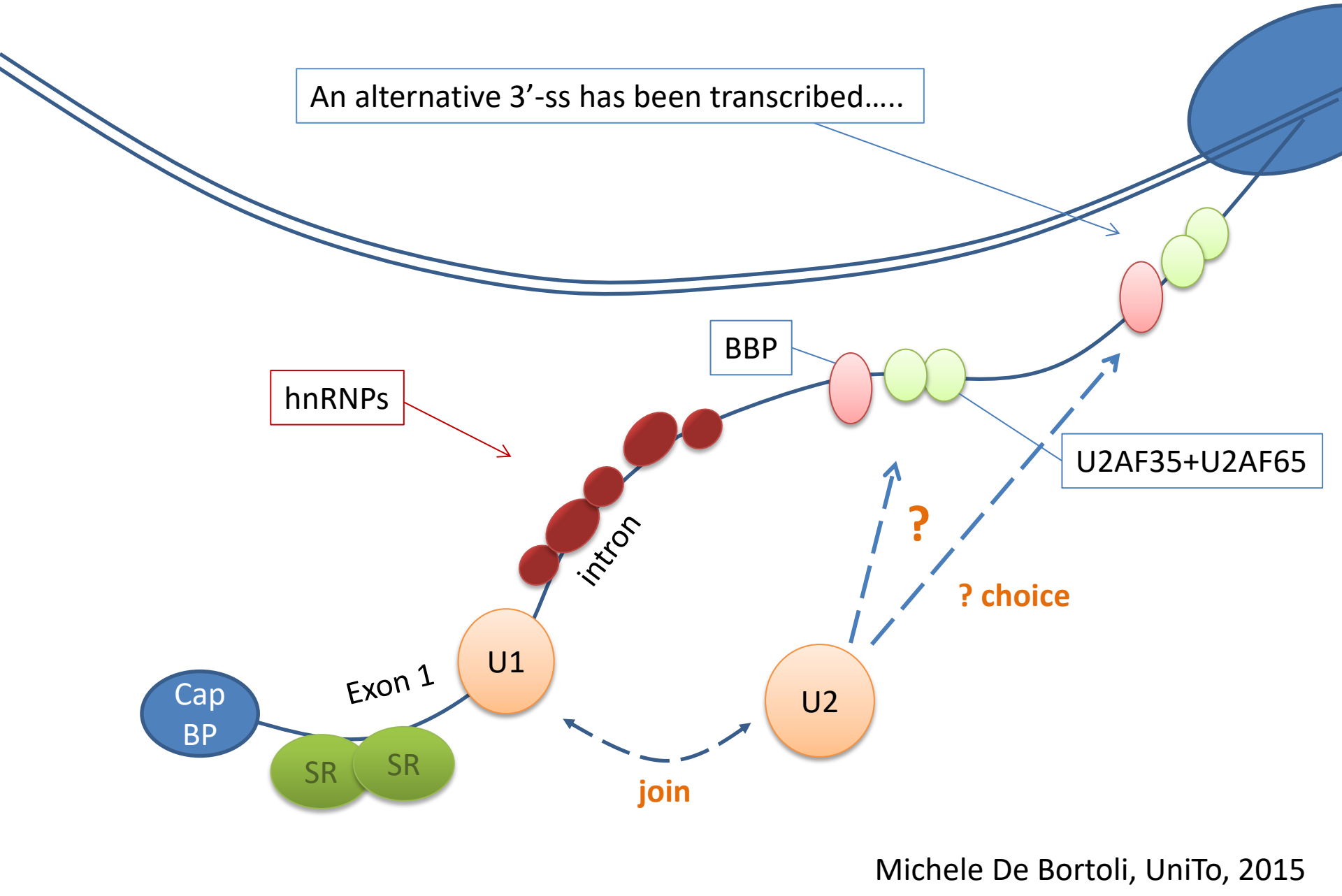
Exon definition



Intron definition



Alternative Splicing



Michele De Bortoli, UniTo, 2015

The « first arrived, first served » model

Is compatible with the fact that, in general, splicing is well ordered and that Alternative Splicing normally (even though not always) concerns a choice between two consecutive splicing sites, only very rarely separated by multiple exons/introns

This model is also compatible with the observed cases in which specific intervention of a ESE-ISE/SR or ISS-ESS/hnRNP takes place (see above).

There is a clear effect of the **speed** of RNA Polymerase

- ✓ the “speed” of RNA Polymerase II (the rate at which Pol II synthesizes RNA is variable and discontinuous, and may depend on elongation factors that are «charged» on RNA Pol II by different promoters/enhancers)

- ✓ the «loading» of RNA Polymerase II CTD with specific Splicing Factors (interaction of several splicing factors with Pol II CTD identified by co-IP)

Regulation of alternative splicing by a transcriptional enhancer through RNA pol II elongation

Sebastián Kadener*, Juan Pablo Fededa*, Michael Rosbash†, and Alberto R. Kornblihtt*‡

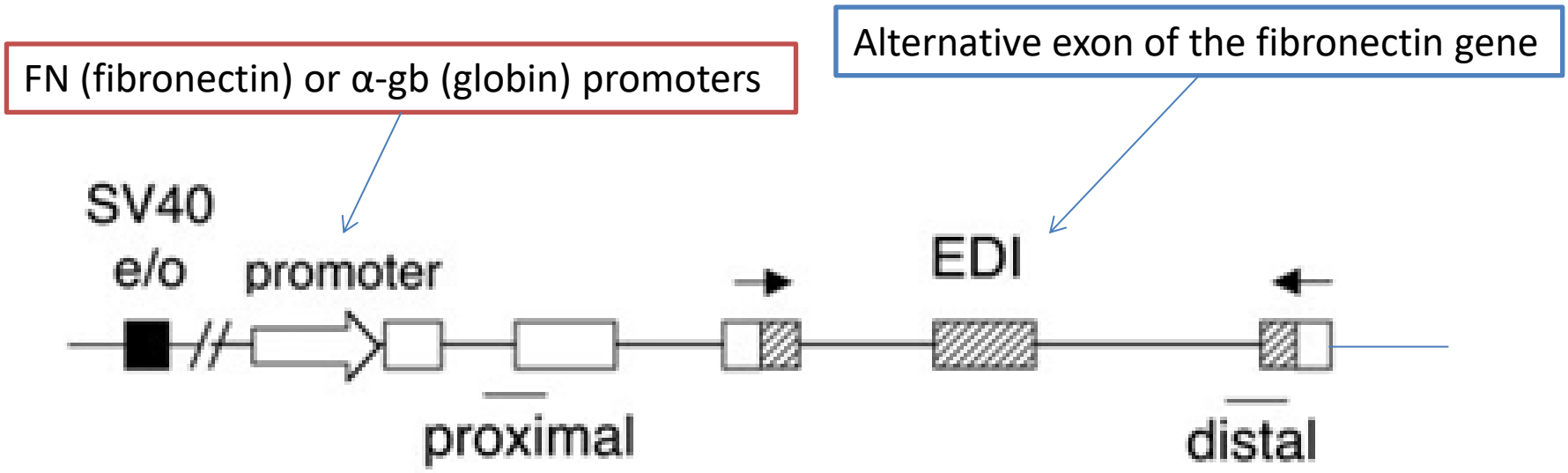
*Laboratorio de Fisiología y Biología Molecular, Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II (C1428EHA), Buenos Aires, Argentina; and †Howard Hughes Medical Institute, Brandeis University, Waltham, MA 02454

Communicated by César Milstein§, Medical Research Council, Cambridge, United Kingdom, April 24, 2002¶ (received for review January 18, 2002)

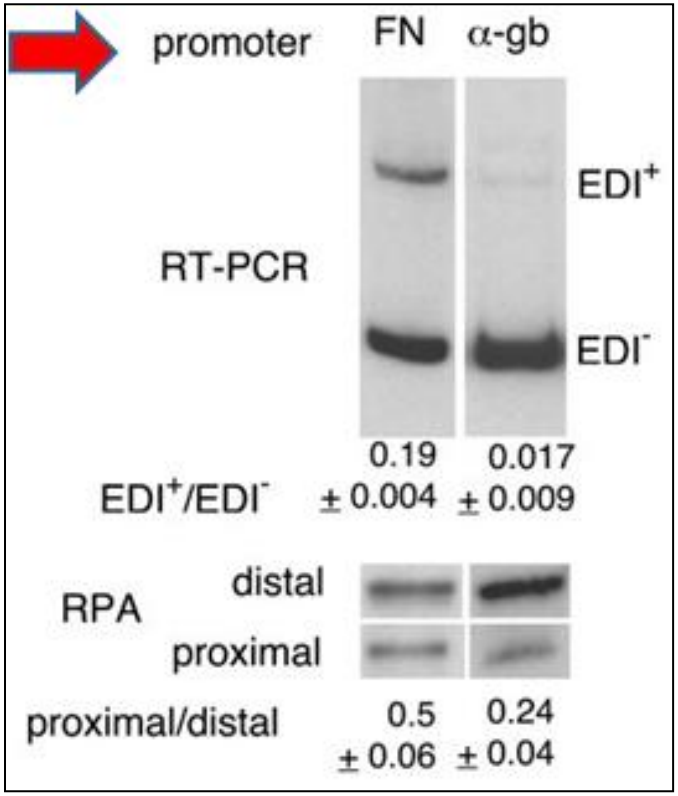
Promoters and enhancers are cis-acting elements that control gene transcription via complex networks of protein–DNA and protein–protein interactions. Whereas promoters deal with putting in place the RNA polymerase, both enhancers and promoters can control transcriptional initiation and elongation. We have previously shown that promoter structure modulates alternative splicing, strengthening the concept of a physical and functional coupling between transcription and splicing. Here we report that the promoter effect is due to the control of RNA pol II elongation. We found that the simian virus 40 (SV40) transcriptional enhancer, inserted in fibronectin (FN) minigene constructs transfected into mammalian cells, controls alternative splicing by inhibiting inclusion of the FN extra domain I (EDI) exon into mature mRNA. Deletion analysis of enhancer subdomains and competitions *in vivo* with excess of specific enhancer DNA subfragments demonstrate that the "minimal" enhancer, consisting of two 72-bp repeats, is responsible for the splicing effect. The 72-bp repeat region has been reported to promote RNA pol II elongation. When transcription is driven by the α-globin promoter linked to the SV40 enhancer, basal EDI inclusion and activation by the SR (Ser–Arg-rich) protein SF2/ASF are much lower than with other promoters. Deletion of only one of the two 72-bp repeats not only provokes higher EDI inclusion levels but allows responsiveness to SF2/ASF. These effects are the consequence of a decrease in RNA pol II elongation evidenced both by an increase in the proportions of shorter proximal over full length transcripts and by higher pol II densities upstream of the alternative exon detected by chromatin immunoprecipitation.

The Speed Bump model was born in Kornblihtt lab more than a decade ago.

Creating a splicing reporter with a weak exon



A) Scheme of the minigenes transfected to assess alternative splicing. Open exons, human-gb; dashed exons, human FN; black box, SV40 e/o; arrows, primers used to amplify the mRNA splicing variants by RT-PCR, and lines, proximal and distal probes used for RPA.



Hep3B cells were transfected with 600 ng of pSVEDAFN (FN promoter) or pSVEDATot (-gb promoter) plus 400 ng of pCMVgal. RNA splicing variants were detected by radioactive RT-PCR and analyzed in 6% native polyacrylamide gels. Ratios between radioactivity in EDI bands and radioactivity in EDI bands are shown under each lane. (*Lower*) RPA with proximal and distal probes shown in *A*, to measure levels of short and long transcripts of transfected Hep3B cells. RT-PCR and RPA ratios correspond to at least three independent transfection experiments.

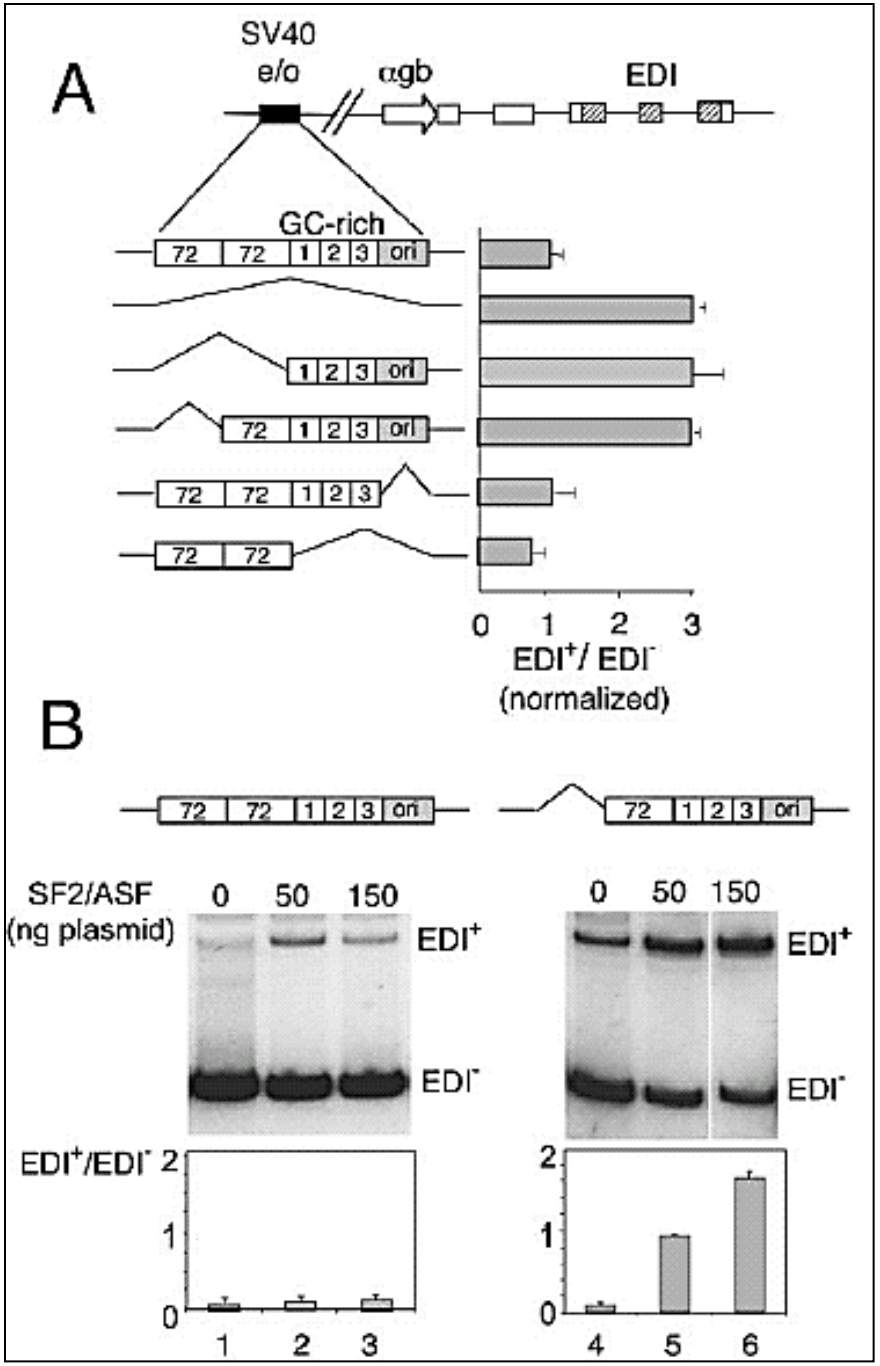
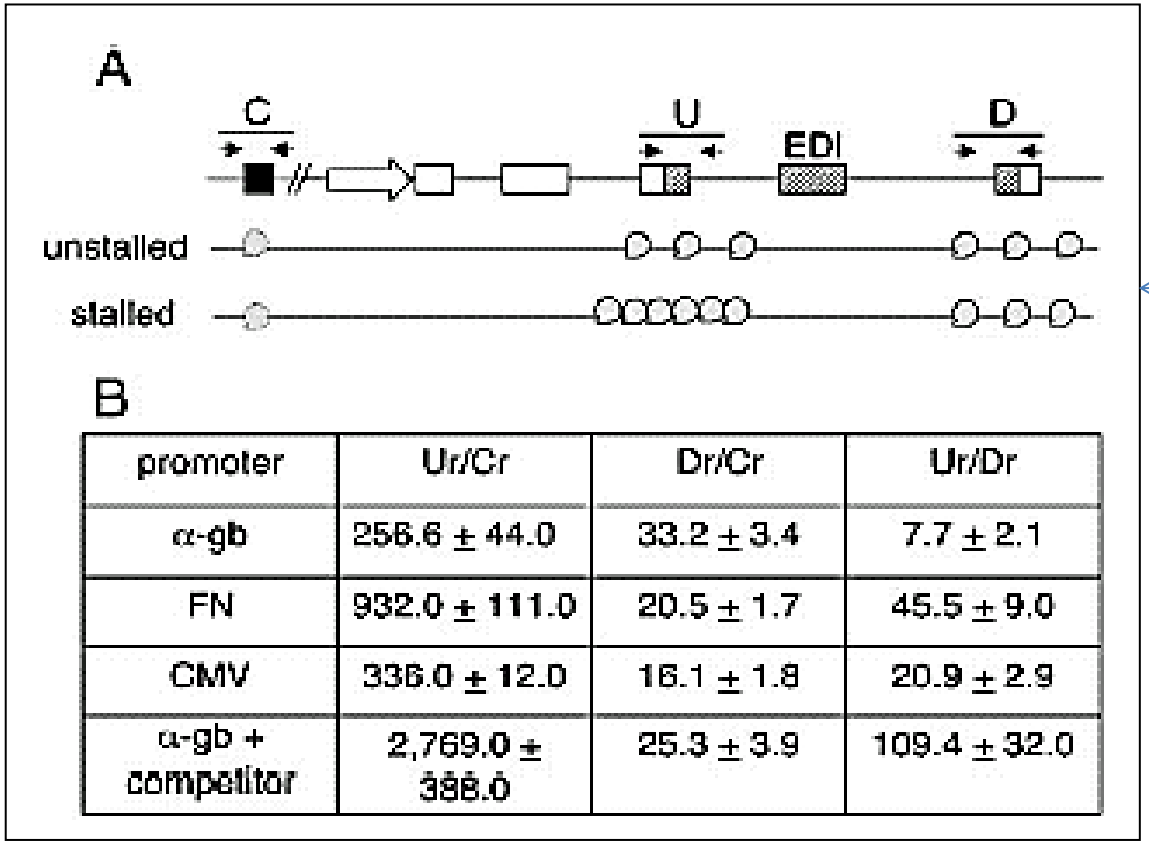


Fig. 3. (*A*) Deletion analysis of the SV40 eo with respect to alternative splicing of the EDI exon. Horizontal bars indicate normalized EDIEDI ratios of Hep3B cells transfected with a series of α–gb promoter constructs carrying different internal deletions of the SV40 eo. Results correspond to the mean SD of at least three independent transfection experiments.

(*B*) Deletion of only one 72-bp repeat confers responsiveness to SF2ASF to the α-gb promoter construct. Hep3B cells were transfected with pSVEDATot (lanes 1–3) or a variant lacking the distal 72-bp repeat of the SV40 enhancer (lanes 4–6) and cotransfected with the indicated amounts of a plasmid expressing SF2ASF (13). Transfections in lanes 1 and 4 contained 150 ng of empty DNA vector.

Similar results were obtained in Cos-7 and HeLa cells.



B

promoter	Ur/Cr	Dr/Cr	Ur/Dr
α-gb	256.6 ± 44.0	33.2 ± 3.4	7.7 ± 2.1
FN	932.0 ± 111.0	20.5 ± 1.7	45.5 ± 9.0
CMV	336.0 ± 12.0	16.1 ± 1.8	20.9 ± 2.9
α-gb + competitor	2,769.0 ± 388.0	25.3 ± 3.9	109.4 ± 32.0

Fig. 5. ChIP with an Ab to RNA pol II. (*A*) Scheme of the minigenes transfected to assess pol II densities. Arrows indicate the pairs of primers used in real time PCRs to quantitatively amplify DNA that is bound to the immunoprecipitated pol II, at two regions mapping U, D of the EDI alternative exon, and at a third C region outside of the transcription unit. (*B*) Cells were transfected with -gb, FN, or CMV promoter constructs and, where indicated, co-transfected with a 10-fold molar excess of a competitor plasmid carrying the SV40 e/o. After 48 h, cells were fixed with formaldehyde and treated for ChIP and real time PCR analysis as described in *Experimental Procedures*. UrUimUin; CrCimCin; DrDimDin where Uim, Cim, and Dim are the template DNA amounts recovered after *im*munoprecipitation by anti-pol II, and Uin, Cin, and Din are the *in*put DNA amounts, all estimated by real time PCR at regions U, C, and D, respectively. Results correspond to a representative transfection experiment of Cos-7 cells and show the mean SD of three real time PCR determinations.

This study demonstrated that:

1. Promoters/enhancers can influence the rate of inclusion of weak exons

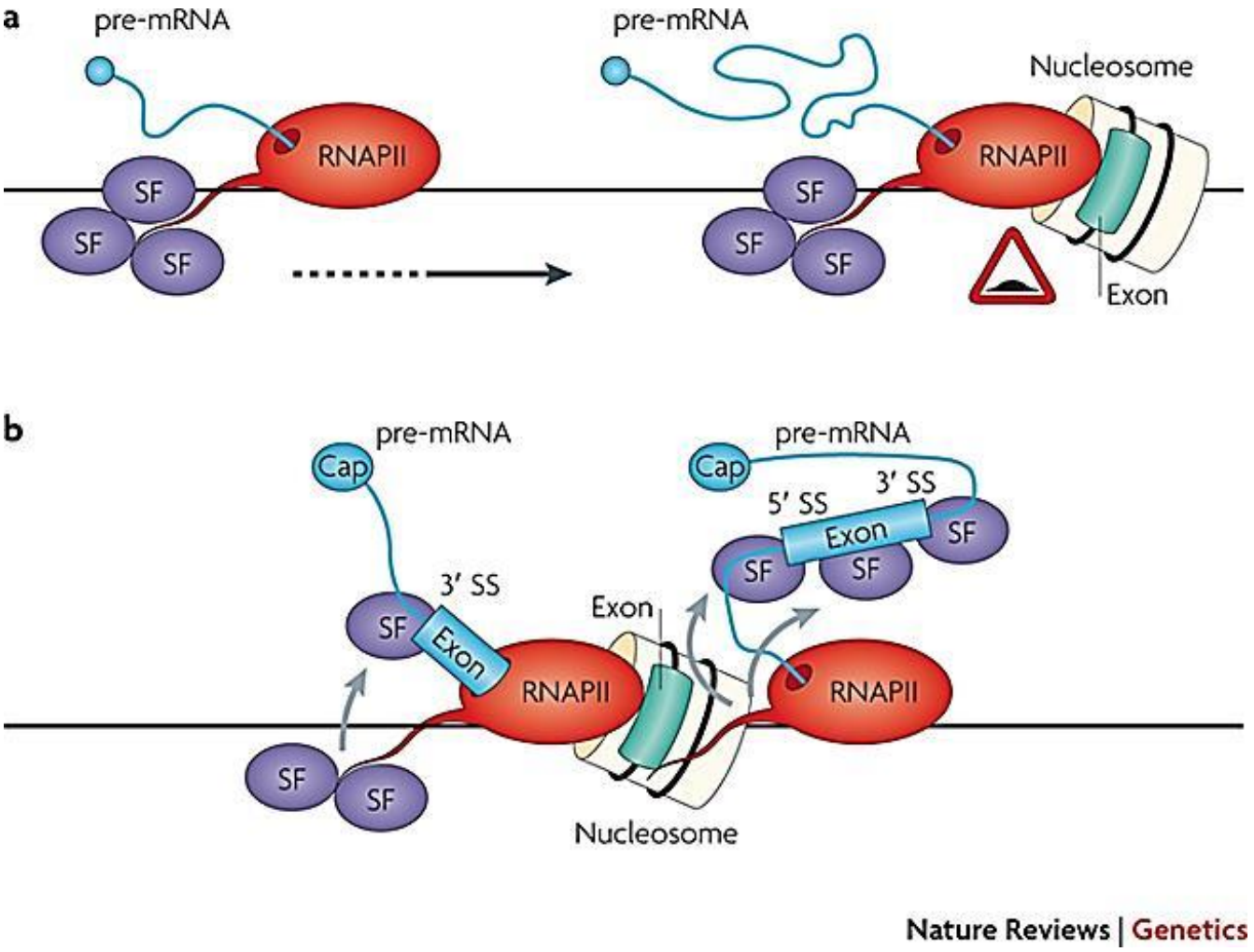
2. They do so in part by influencing the speed of RNA Pol II

3. When a weak exon is included, RNA PolII «slows down» in proximity of that exon (*remember that weak exons have more strongly positioned nucleosomes !*)

… and RNA Pol II CTD carries splicing factors.

the speed-bump model

the speed-bump model



Nature Reviews | Genetics

Nucleosome occupancy marks exons and is coupled to transcription.
a | RNA polymerase II (RNAPII), associated with different splicing factors (SFs), travels along the gene and transcribes it. When RNAPII reaches an area with high nucleosome occupancy and encounters specific histone modifications that mark an exon, it is slowed down.
b | This panel shows RNAPII and the nucleosome at the point at which their coupling marks the exon boundaries for the splicing machinery. RNAPII transcribes the exon and SFs detach from the carboxy-terminal domain of RNAPII and bind to the 3′ splice site (3′ SS) region of the precursor mRNA (pre-mRNA). During transcription elongation, additional SFs bind intronic and exonic splicing regulatory elements and the 5′ SS.

nature
structural &
molecular biology

Nucleosomes are best positioned on exons

Nucleosome positioning as a determinant of exon recognition

Hagen Tilgner[1,3], Christoforos Nikolaou[1,3], Sonja Althammer[1], Michael Sammeth[1], Miguel Beato[1], Juan Valcárcel[1,2] & Roderic Guigó[1]

Chromatin structure influences transcription, but its role in subsequent RNA processing is unclear. Here we present analyses of high-throughput data that imply a relationship between nucleosome positioning and exon definition. First, we have found stable nucleosome occupancy within human and *Caenorhabditis elegans* exons that is stronger in exons with weak splice sites. Conversely, we have found that pseudoexons—intronic sequences that are not included in mRNAs but are flanked by strong splice sites—show nucleosome depletion. Second, the ratio between nucleosome occupancy within and upstream from the exons correlates with exon-inclusion levels. Third, nucleosomes are positioned central to exons rather than proximal to splice sites. These exonic nucleosomal patterns are also observed in non-expressed genes, suggesting that nucleosome marking of exons exists in the absence of transcription. Our analysis provides a framework that contributes to the understanding of splicing on the basis of chromatin architecture.

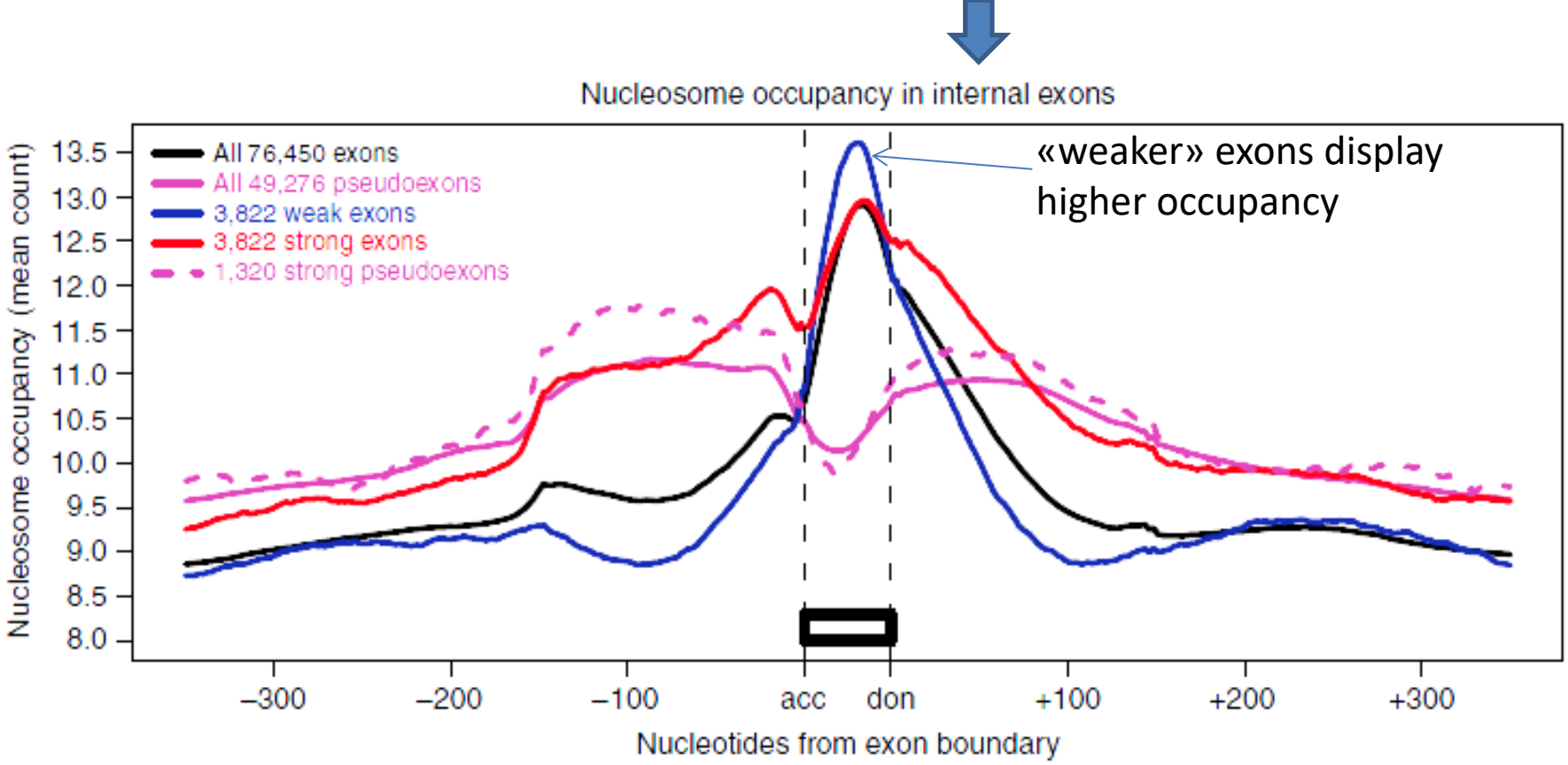


Figure 1 Observed and predicted nucleosome occupancy. (**a**) Nucleosome-occupancy profile across human internal constitutive exons in resting CD4+ T cells. We have computed the number of extended nucleosome reads overlapping each nucleotide. Upstream and downstream of an **idealized internal exon**, we plot the average number of nucleosome reads per nucleotide position, with negative positions relative to the acceptor (acc) site and positive positions relative the donor (don) site. Within the exon, reads have been mapped to 50 identically spaced intervals, irrespective of the length of the exon (see Online Methods). Strong exons are exons with a combined donor and acceptor score among the highest 5%; weak exons are the exons with a combined score among the lowset 5%; pseudoexons are intronic sequences bounded by splice sites; strong pseudoexons are exons with a combined score higher than the 90% percentile of real exons.

Tilgner et al., 2009

Reduction during trascription is less evident at weak exons

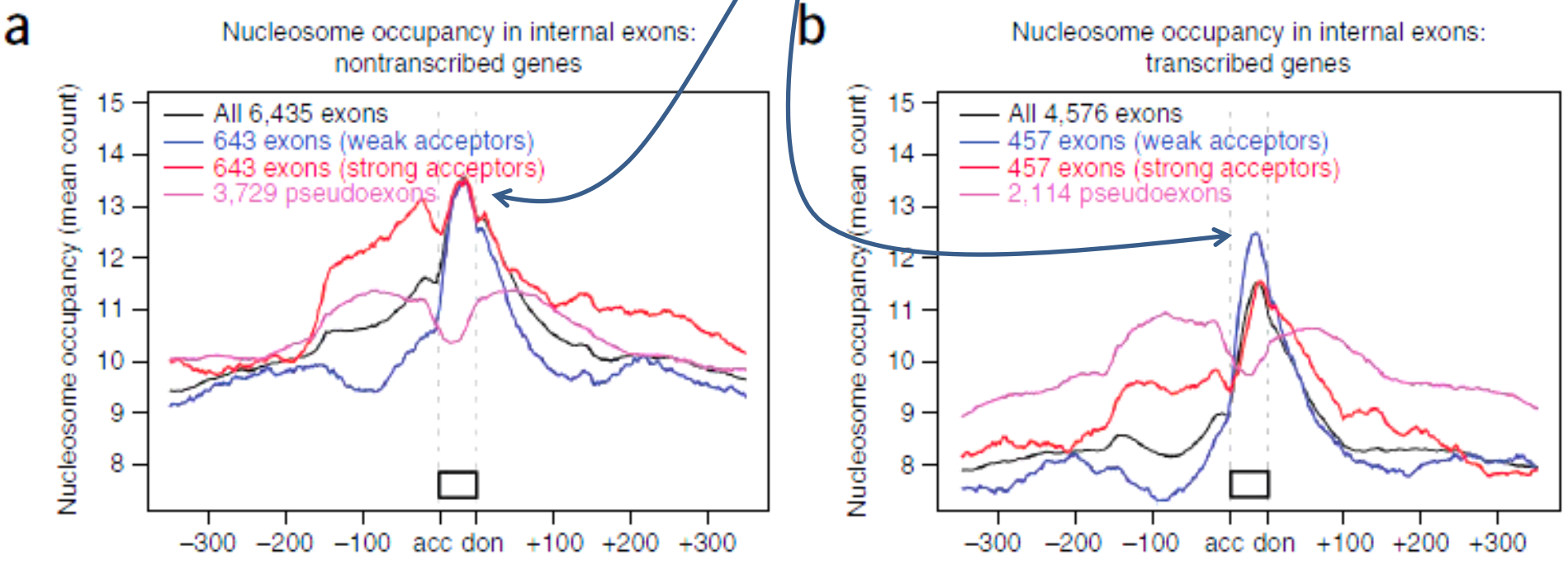


Figure 2 Nucleosome occupancy and expression of genes and exons. (**a**) Nucleosome-occupancy profile across internal acceptor sites from genes that are not expressed in resting CD4+ T cells. Gene expression has been determined using the Affymetrix platform. We plot the average number of nucleosome reads per position in all exons considered together (black), only in exons with strong (red) and weak (blue) acceptor sites, and in intronic pseudoexons. (**b**) Nucleosome-occupancy profile across internal acceptor sites from genes expressed in resting CD4+ T cells, shown as in **a.**

Tilgner et al., 2009

Nucleosome histone modification (epigenetic information)

Molecular Cell 36, 245–254, October 23, 2009

Biased Chromatin Signatures around Polyadenylation Sites and Exons

Noah Spies,[1,2,5] Cydney B. Nielsen,[1,5,6] Richard A. Padgett,[3] and Christopher B. Burge[1,4,*]
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[2]Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA
[3]Cleveland Clinic Foundation, Cleveland, OH 44195, USA
[4]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02142, USA
[5]These authors contributed equally to this work
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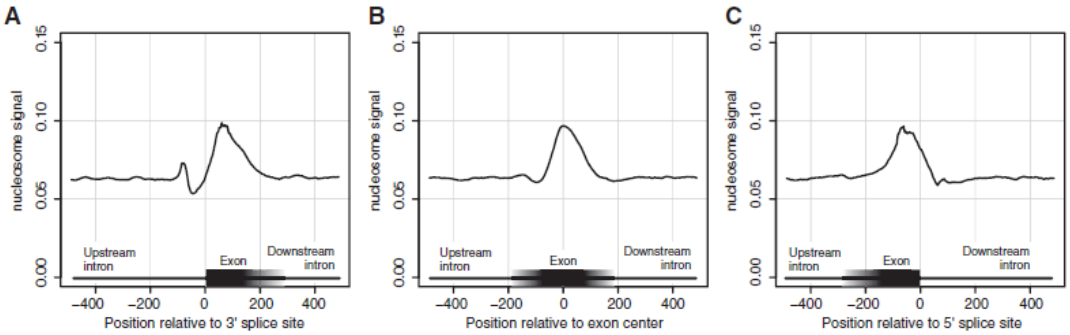
Core RNA-processing reactions in eukaryotic cells occur cotranscriptionally in a chromatin context, but the relationship between chromatin structure and pre-mRNA processing is poorly understood. We observed strong nucleosome depletion around human polyadenylation sites (PAS) and nucleosome enrichment 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

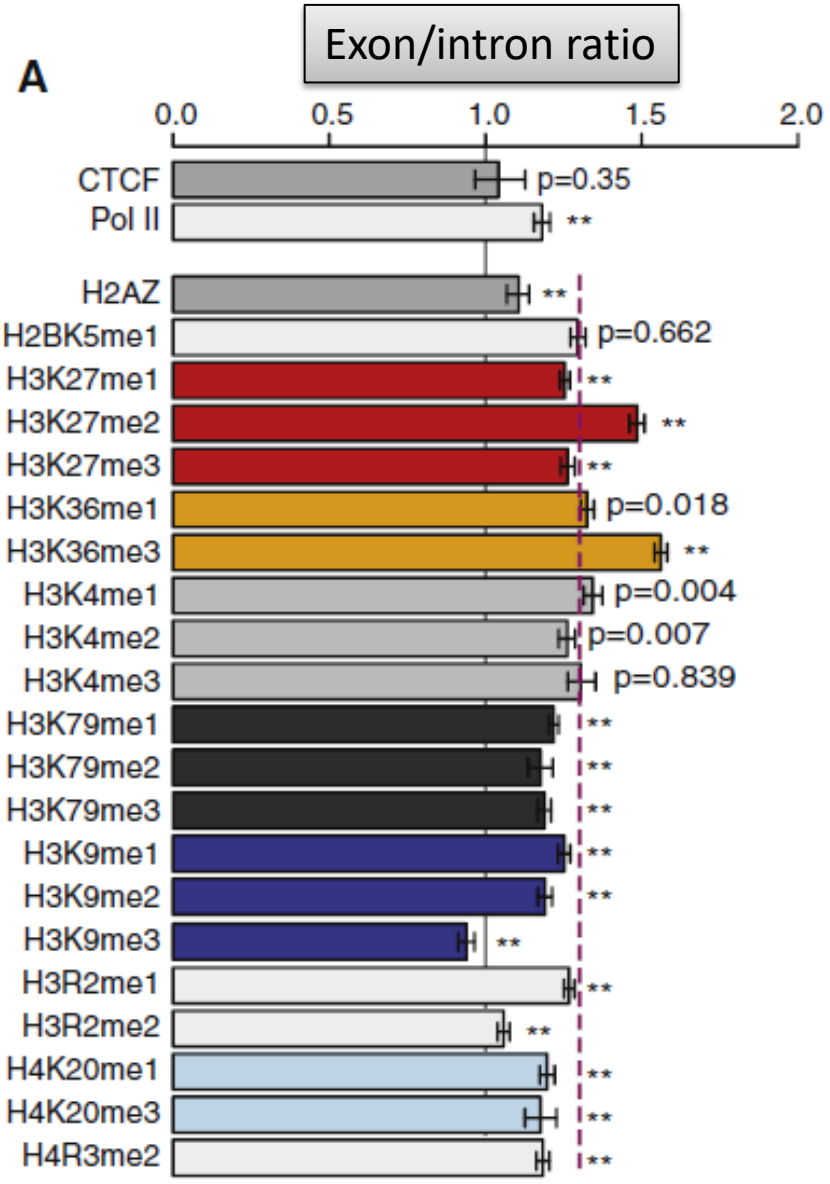
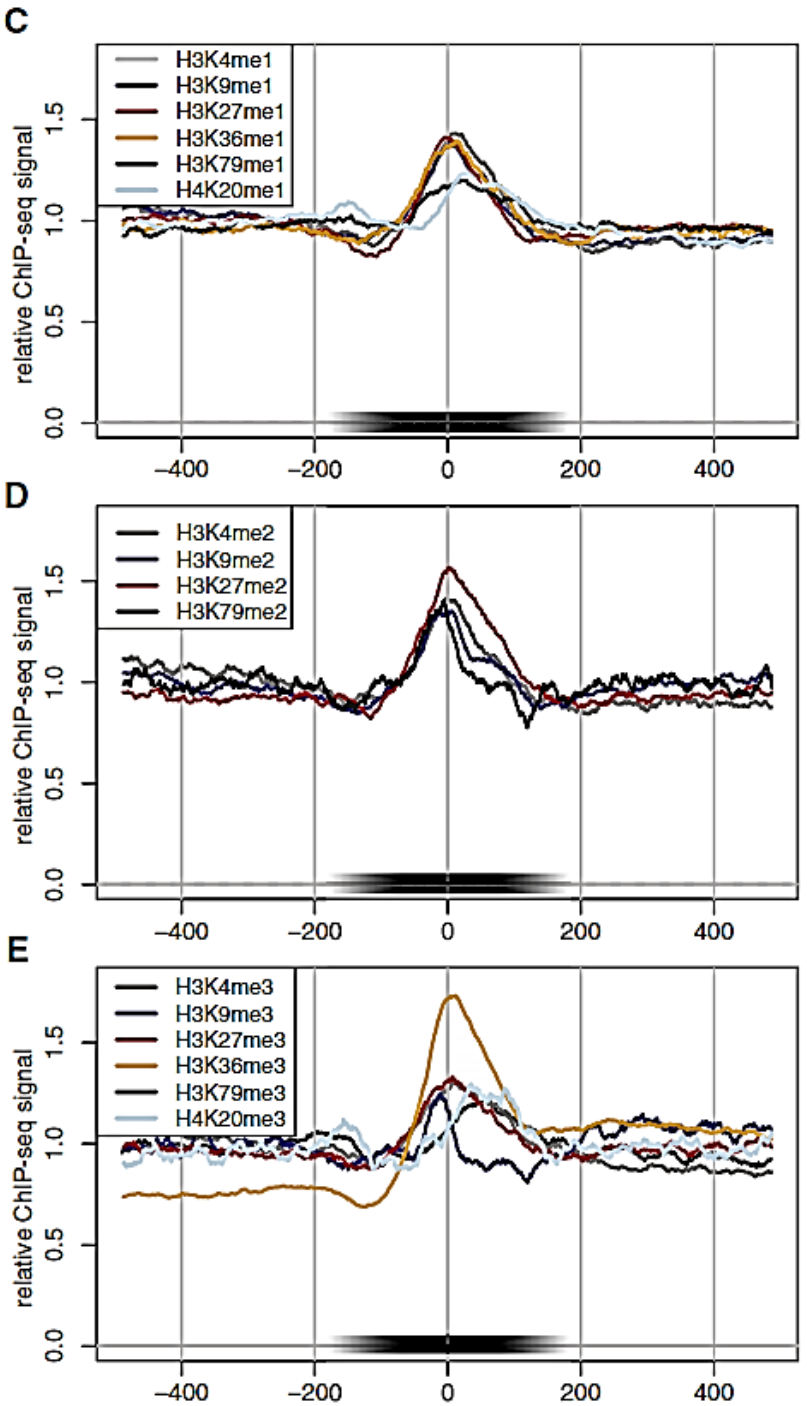


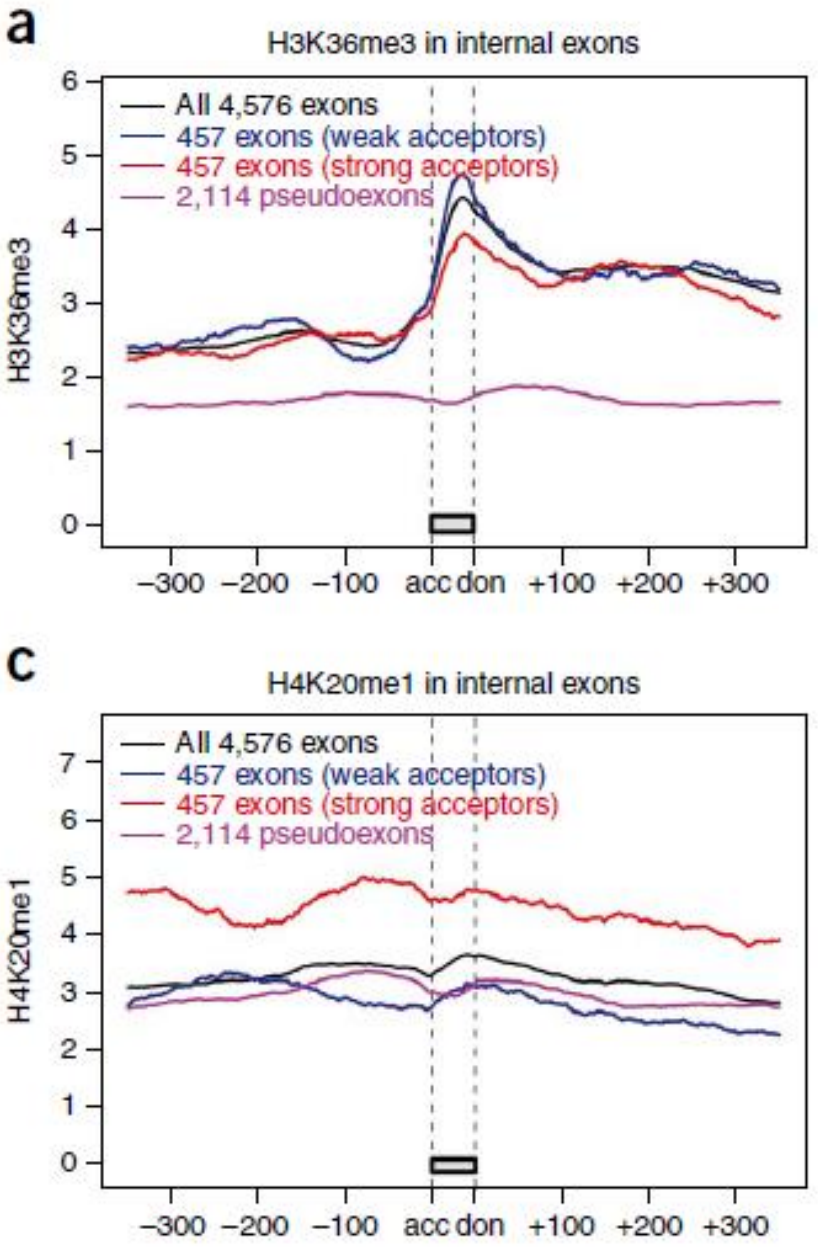
Figure 2. Exon-Biased Distribution of Specific Histone H3 Methylation Marks. (A) ChIP enrichment for exons, relative to flanking intronic regions, compared to 1.0 (CTCF and Pol II) or histone overall average of 1.3 (purple dashed line). Error bars are 95% confidence intervals (resampling). **p < 0.01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

4.3.2 Post-transcriptional regulation

Co-transcriptional processes:
5’-capping
Exon splicing
3’-polyadenylation

(editing – methylation)
Transport to cytoplasm (for a fraction of RNAs)
Quality control
Localization to cellular subcompartments
Regulated translation
Regulated degradation
RNA interference

Other post-transcriptional modifications to RNAs

- N^6-methyladenosine (m^6A) (the most studied today – the most frequent)
- N^1-methyladenosine (m^1A)
- 5-methylcytosine (m^5C)
- Pseudo-uridine (most abundant, mostly in rRNA and tRNA, but several mRNAs)
- 2'-O-methylnucleosides (methylation of 2'-OH of ribose)

Mainly in the nucleus, methylation is frequently co-transcriptional

RNA adenine methylation

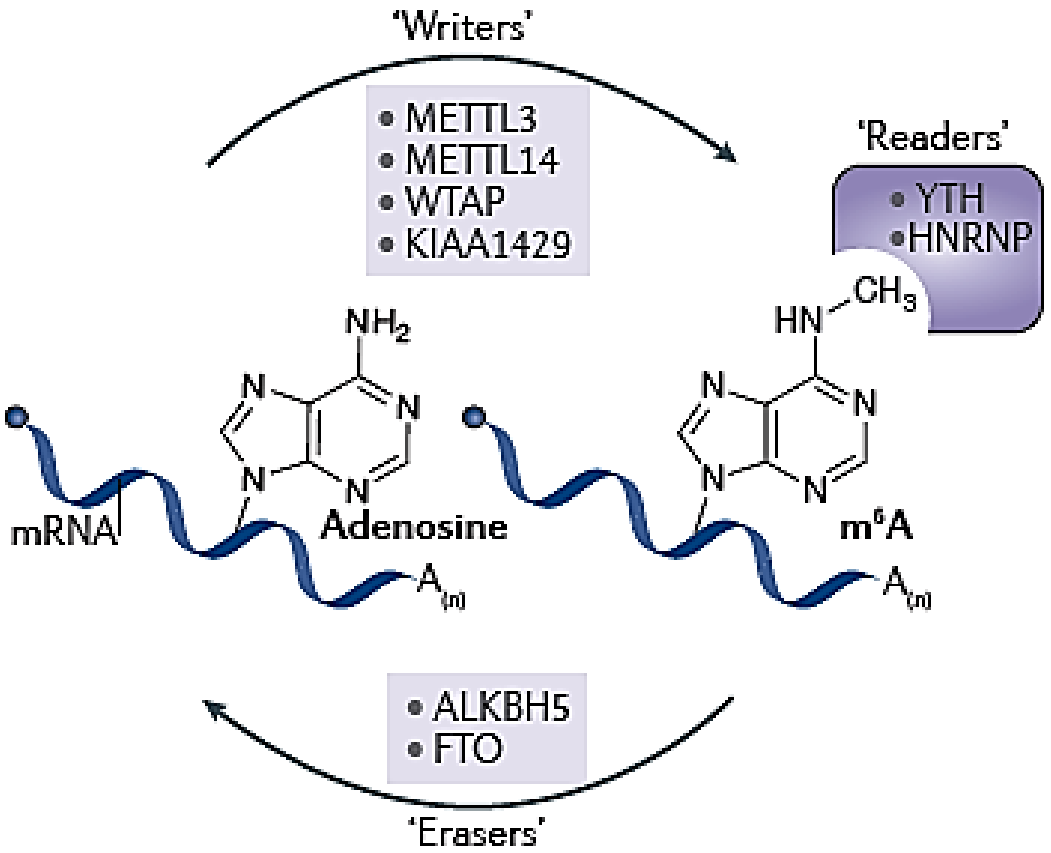


Figure 1 | **The writer, eraser and reader proteins of m^{6}A.**

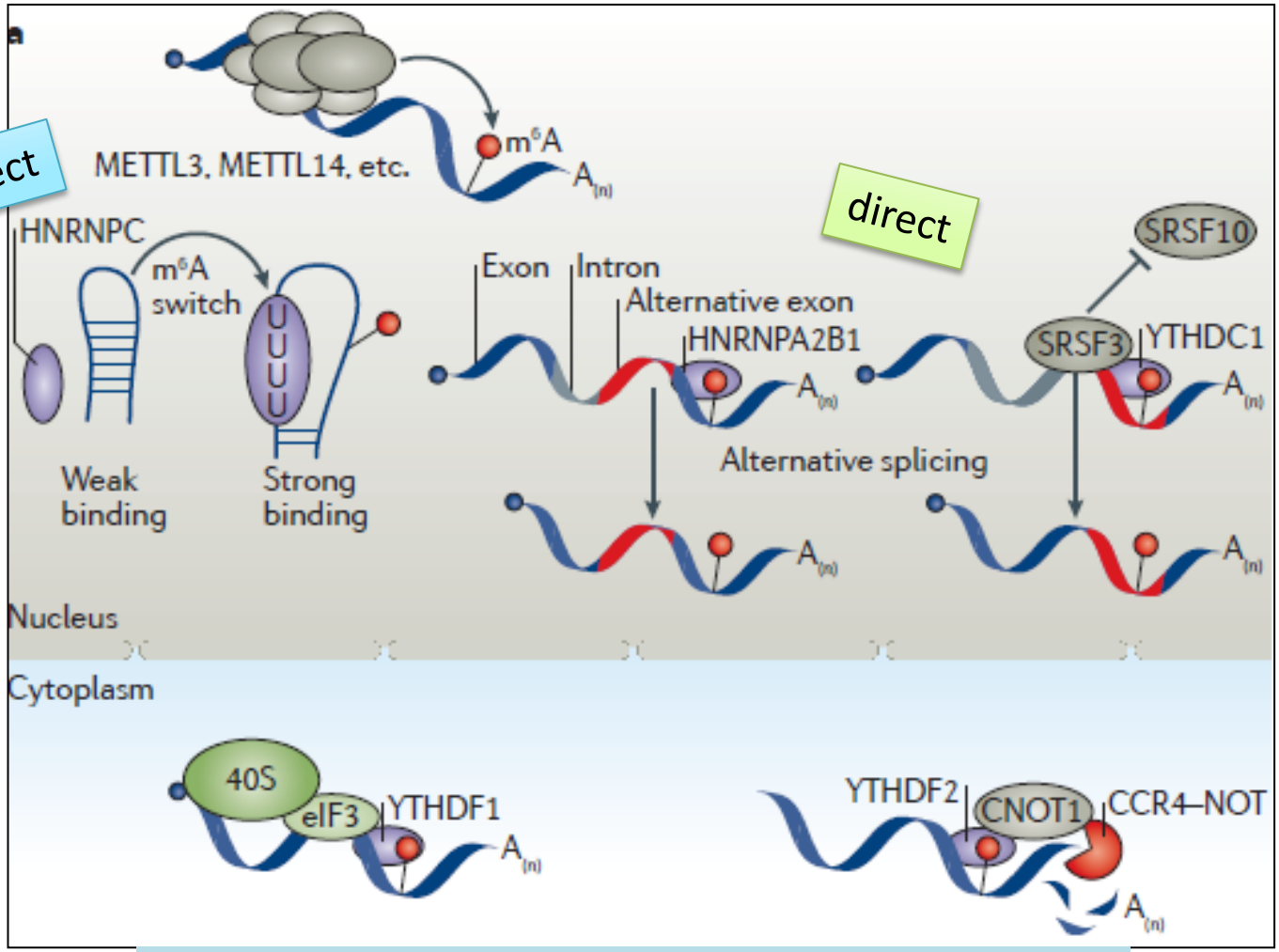
The YT521-B homology (YTH) domain family of proteins (YTHDF1, YTHDF2, YTHDF3 and YTHDC1) are direct readers of m^{6}A and have a conserved m^{6}A-binding pocket.

The heterogeneous nuclear ribonucleoprotein (HNRNP) proteins HNRNPA2B1 and HNRNPC selectively bind m6A-containing mRNAs.

Localized primarily in nuclear speckles

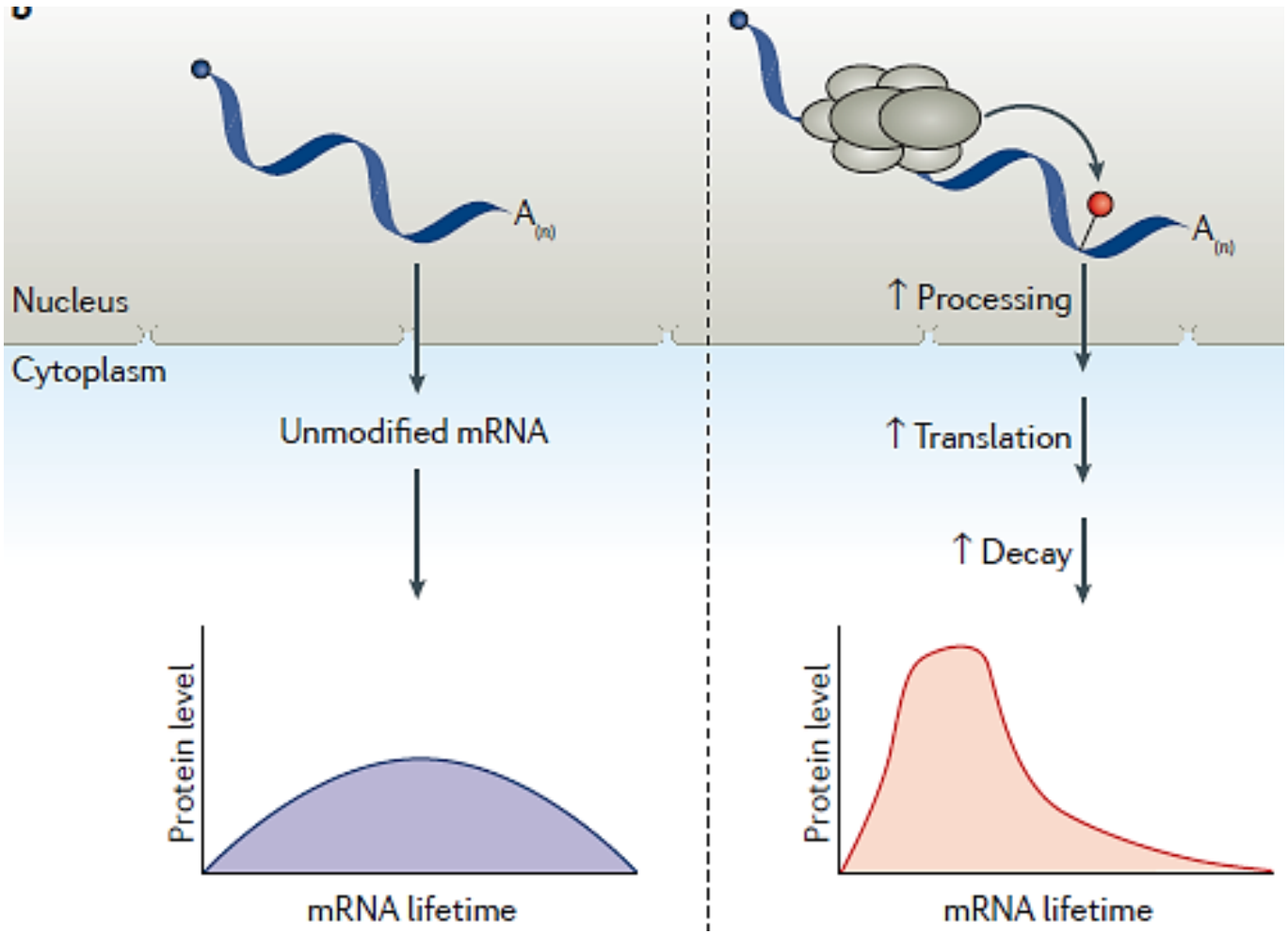
Consensus sequence:
« **RRACH** »

The methyl group at the *N*6 position of m^{6}A does not change Watson–Crick A•U base pairing but weakens duplex RNA by up to 1.4 kcal per mol
Consequence: “indirect” or “direct” effects on protein binding.

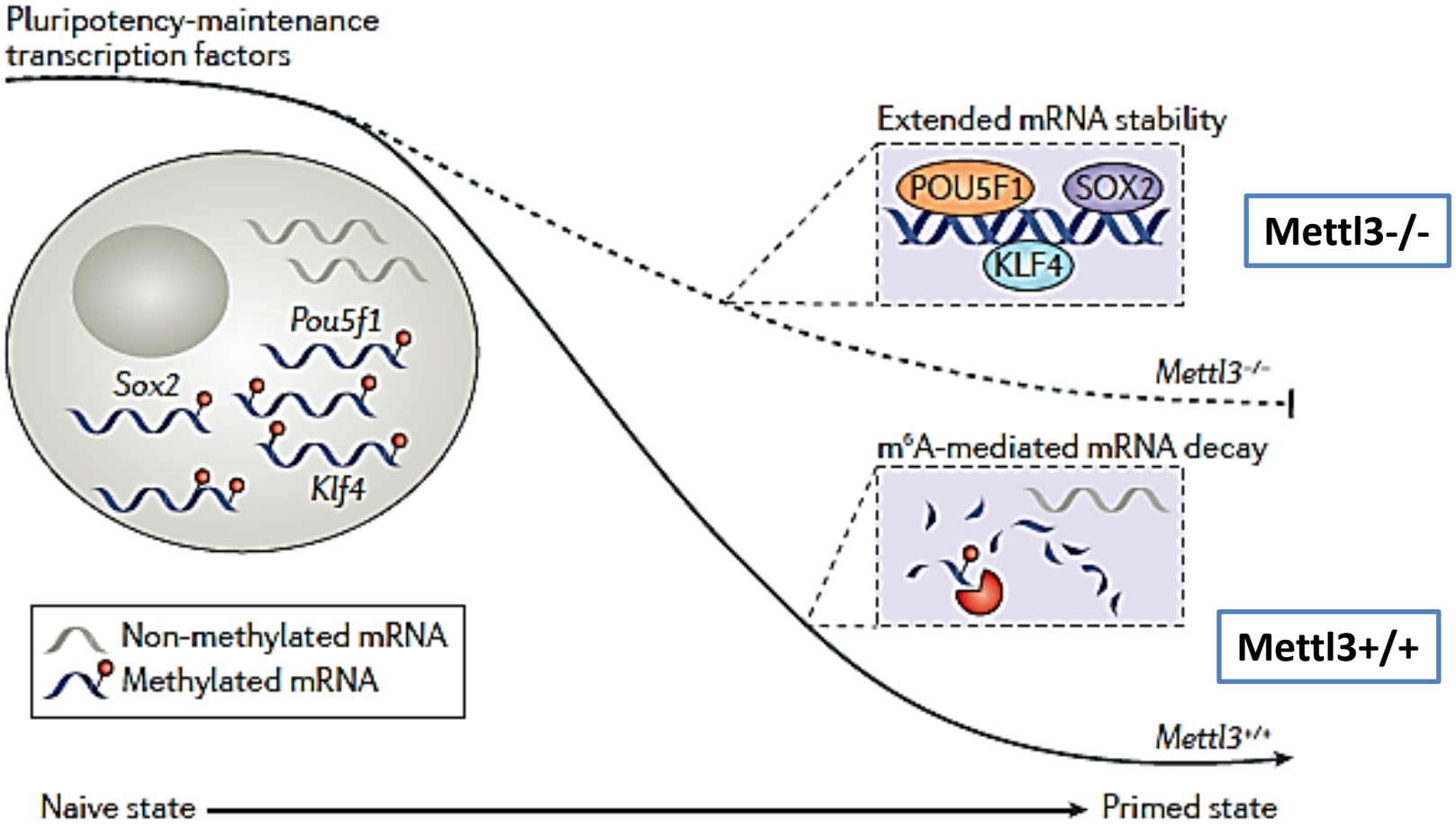


Apparently opposing effects of YTHDF1 and YTHDF2

All steps of RNA metabolism were found to be accelerated by m^6A



A general effect therefore is «sharpening» the window of expression



METTL3 is required for the transition of mouse ES cells **from a naive to the primed state**. During this process, the key pluripotency factor *Pou5f1*, *Klf4* and *Sox2* must be cleared. In mouse ES cells lacking *Mettl3*, this clearance is defective because non-methylated mRNAs are less subjected to decay, which prevents or delays the establishment of a differentiated transcriptome required to achieve a primed mouse ES cell state. *(from Zhao et al., 2017, mod.)*

For those of you who are interested in knowing more:

RNA PROCESSING AND MODIFICATIONS

Post-transcriptional gene regulation by mRNA modifications

Boxuan Simen Zhao, Ian A. Roundtree and Chuan He

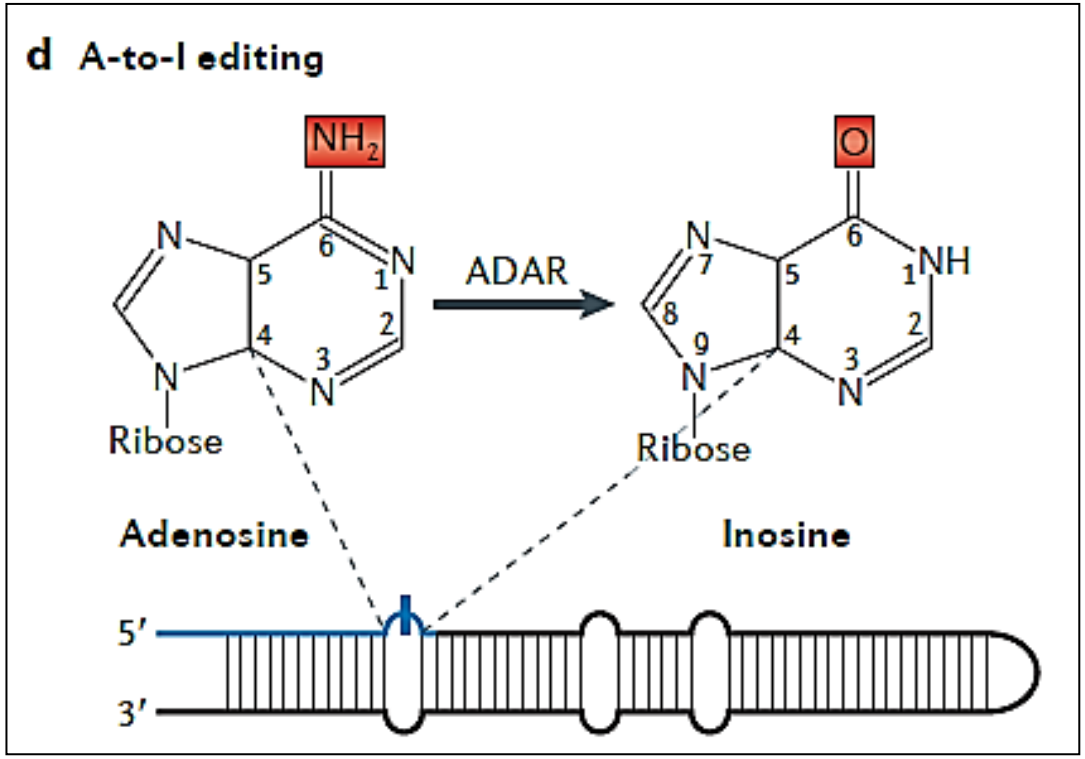
Abstract | The recent discovery of reversible mRNA methylation has opened a new realm of post-transcriptional gene regulation in eukaryotes. The identification and functional characterization of proteins that specifically recognize RNA N^6-methyladenosine (m^6A) unveiled it as a modification that cells utilize to accelerate mRNA metabolism and translation. N^6-adenosine methylation directs mRNAs to distinct fates by grouping them for differential processing, translation and decay in processes such as cell differentiation, embryonic development and stress responses. Other mRNA modifications, including N^1-methyladenosine (m^1A), 5-methylcytosine (m^5C) and pseudouridine, together with m^6A form the epitranscriptome and collectively code a new layer of information that controls protein synthesis.

REVIEWS

RNA Editing

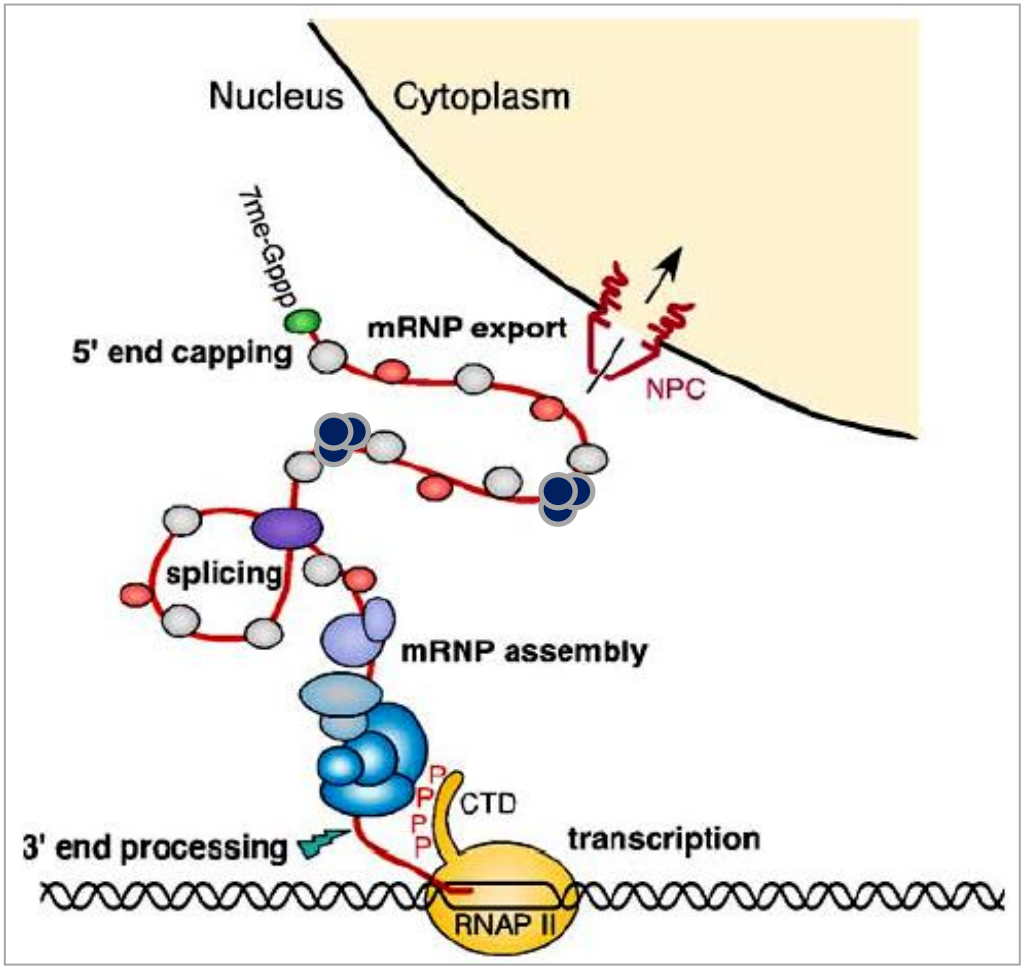
Inosine behaves as a «**G**» in translation and secondary structures

Adenosine-to-inosine (A-to-I) editing is carried out by adenosine deaminases acting on RNA (**ADAR**), which deaminate adenosines into inosines.
The folded *GLUR-2* pre-mRNA substrate is shown with the exon in blue and intron in grey.



Bentley, 2014

Transport to the cytoplasm



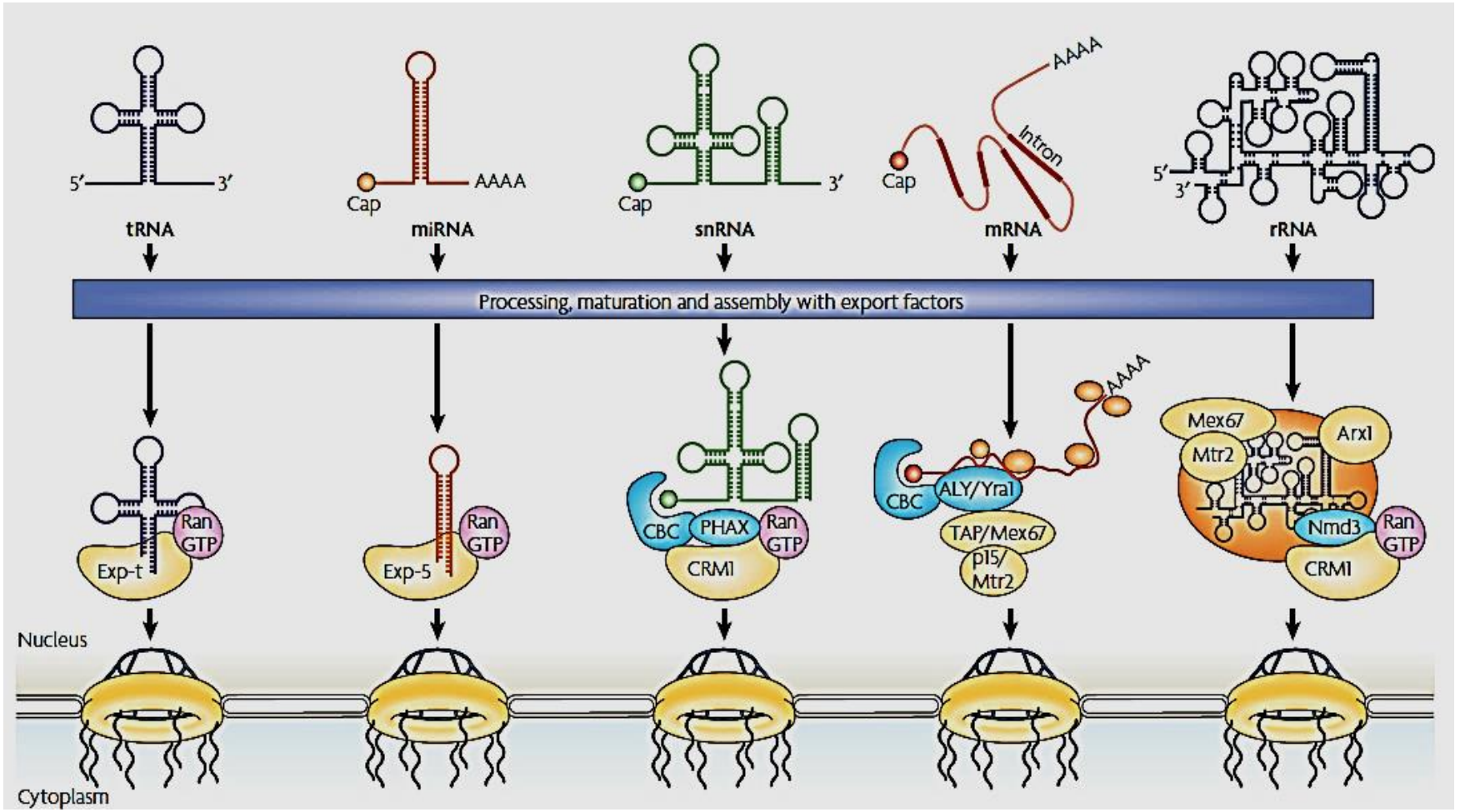
Nucleo-cytoplasmatic transport

- dedicated **exportins**
- accompanying proteins are loaded co-transcriptionally
- some accompanying proteins kept, other exchanged during transport

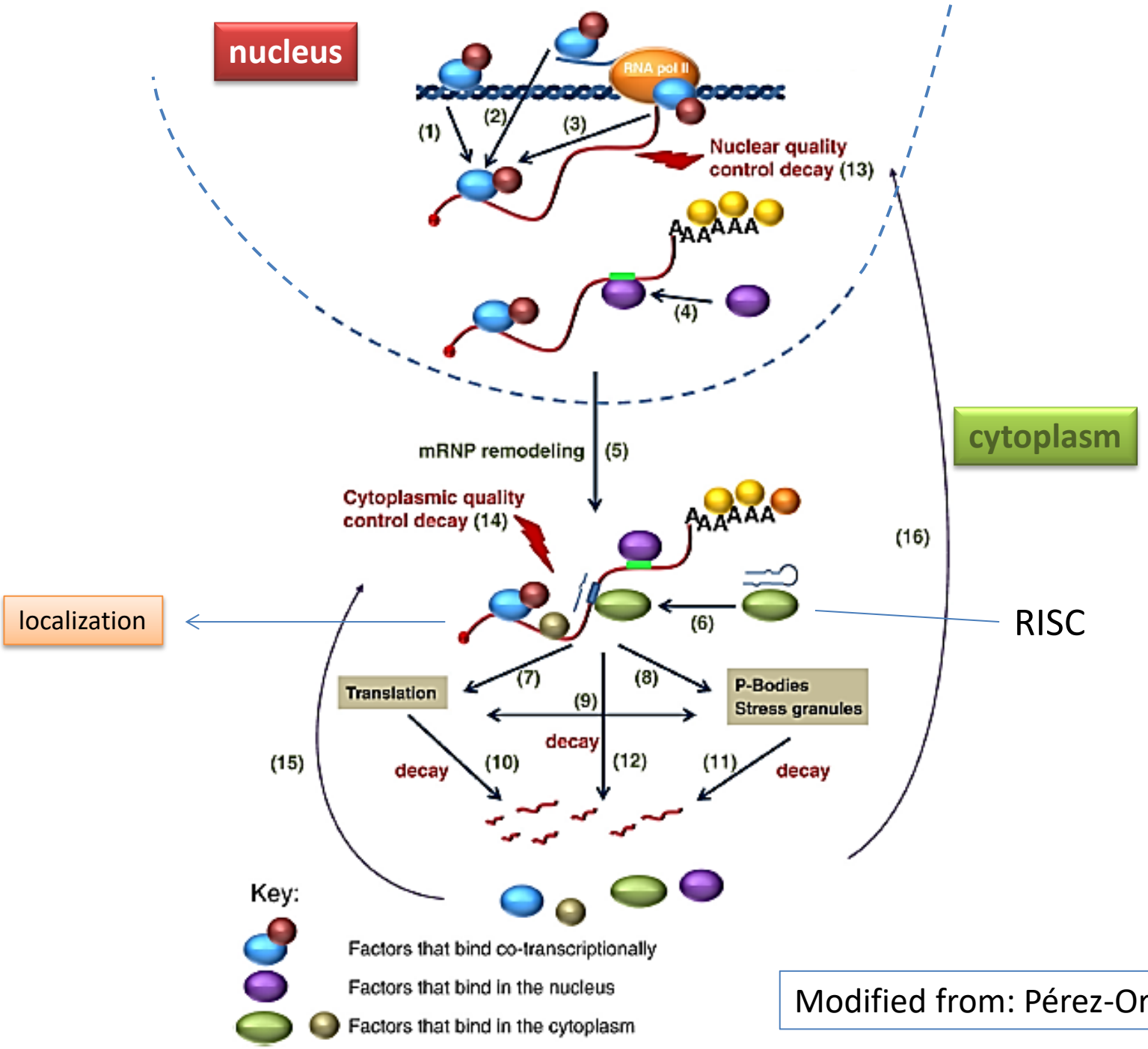
exon junction complex (EJC)

During the second step of splicing, the EJC is deposited approximately 20-24 nt from the 5' end upstream of the splice junction. The EJC is made up of several key proteins: RNPS1, Y14, SRm160, Aly/REF and Magoh, among others. These proteins have functions in **splicing** and **transport**. Important function in **NMD**.

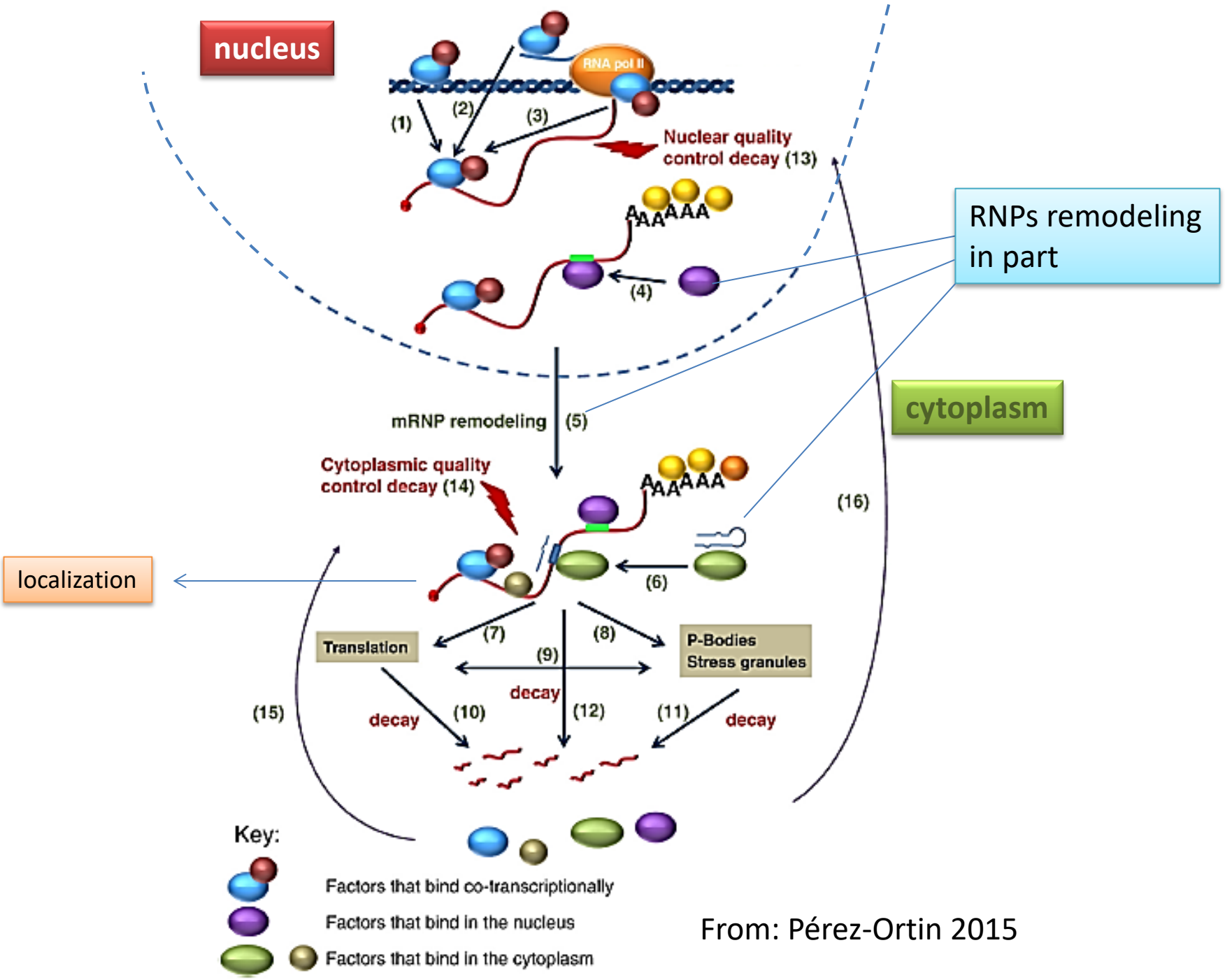
Schematic view of the nuclear side of eukaryotic gene expression, from transcription to nuclear export. NPC Nuclear pore complex, CTD C-terminal domain of Rpb1, RNAPII RNA polymerase II.



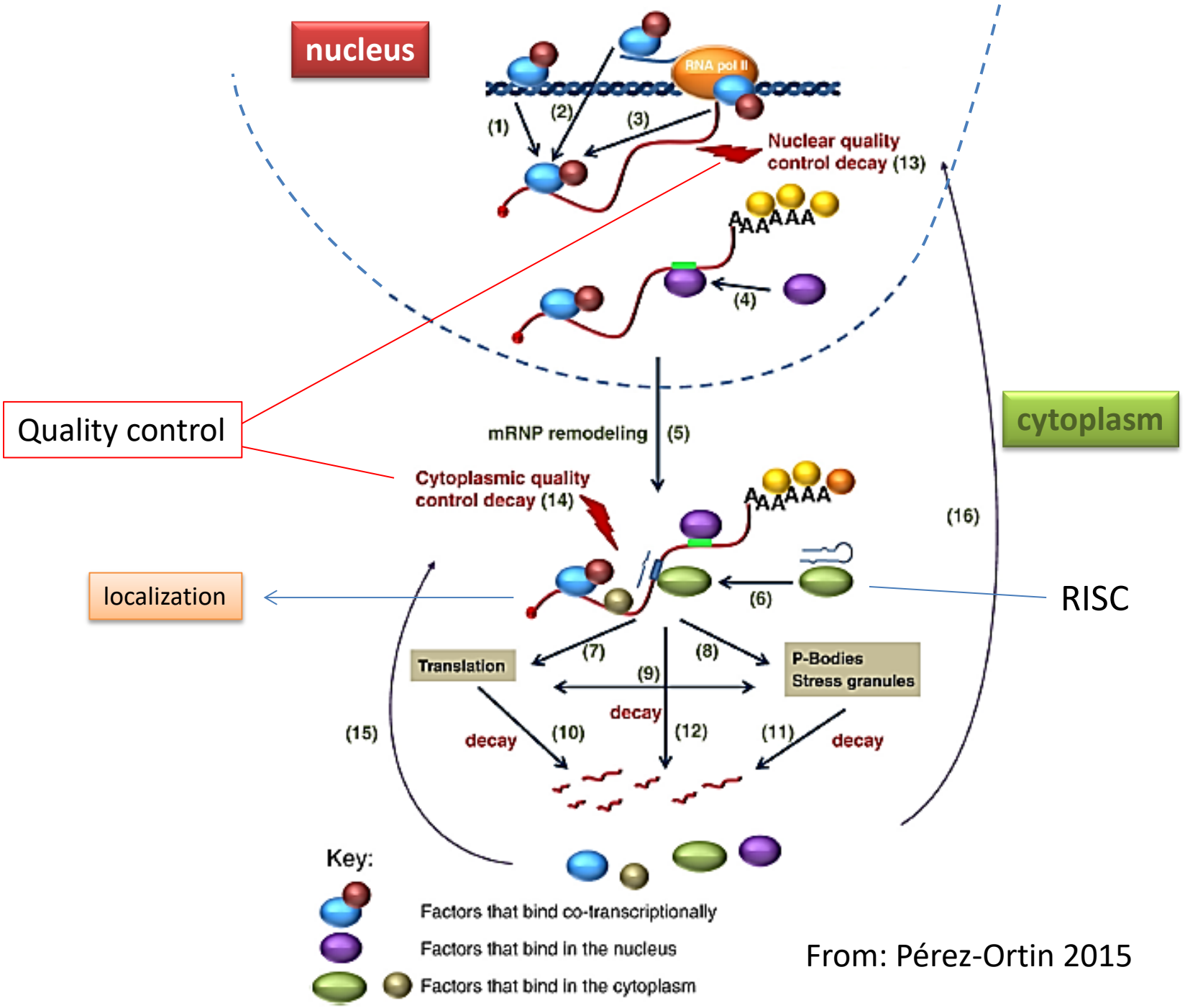
Different RNA export pathways. **export adaptors** and **export receptors**
The names of both metazoan and yeast proteins are indicated.
CBC, cap-binding complex; Exp, exportin.
(*From Kohler & Hurt, Nat Rev Mol Cell Biol, 8:761, 2007*).



Modified from: Pérez-Ortin 2015

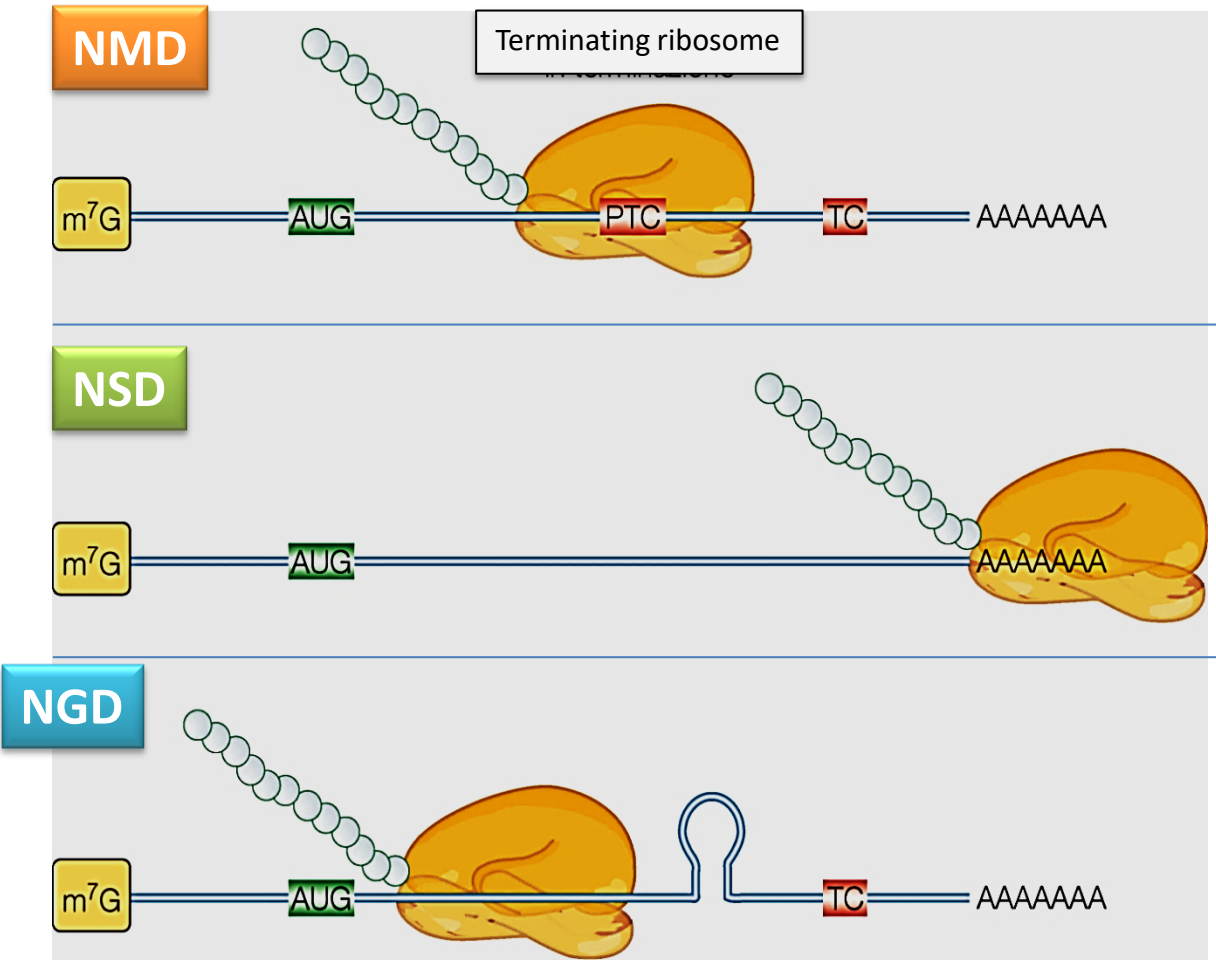


From: Pérez-Ortin 2015



From: Pérez-Ortin 2015

A regulatory role for RNA Quality Control pathways ?



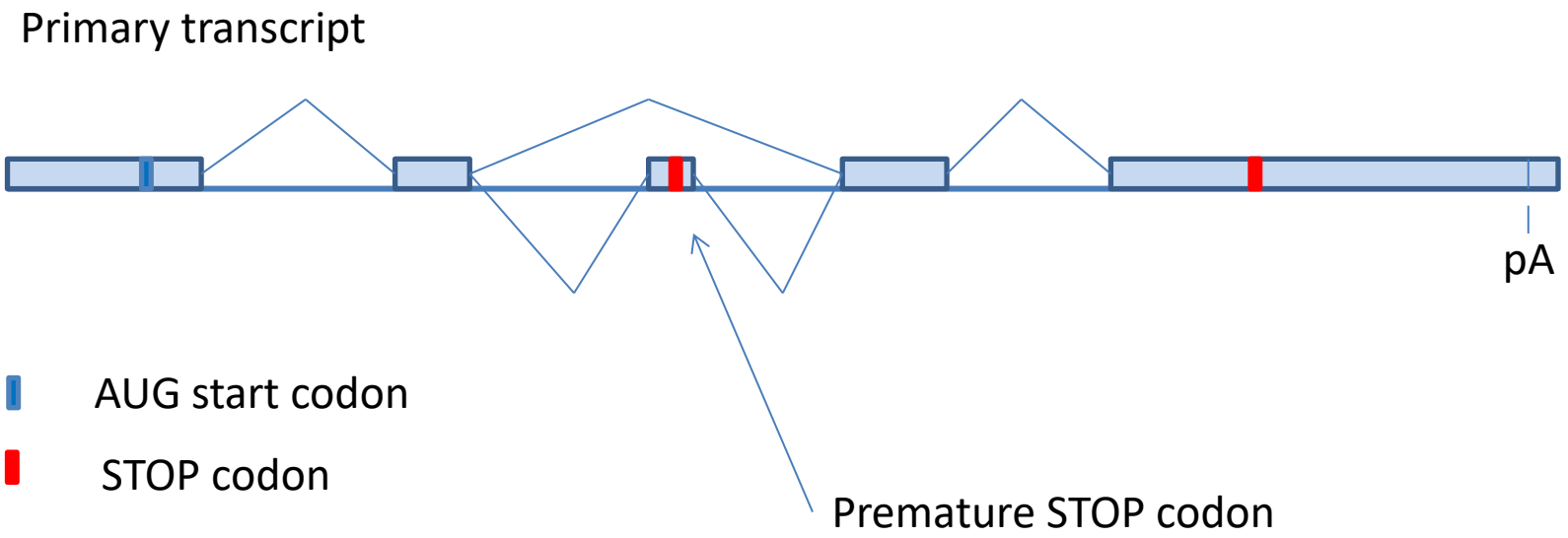
1. Nonsense Mediated Decay
Premature termination codon

2. Non Stop Decay
Absence of termination codon

3. Non-Go Decay
Pausing by structure before TC

What is the function of NMD ? Protection ? Regulatory ?

In Mammals, a number of Alternative Splicing events introduce a premature termination codon into the processed mRNA, not followed immediately by pA.



This induces NMD. It should be considered a silencing AS event.

Next: regulation of RNA stability and decay