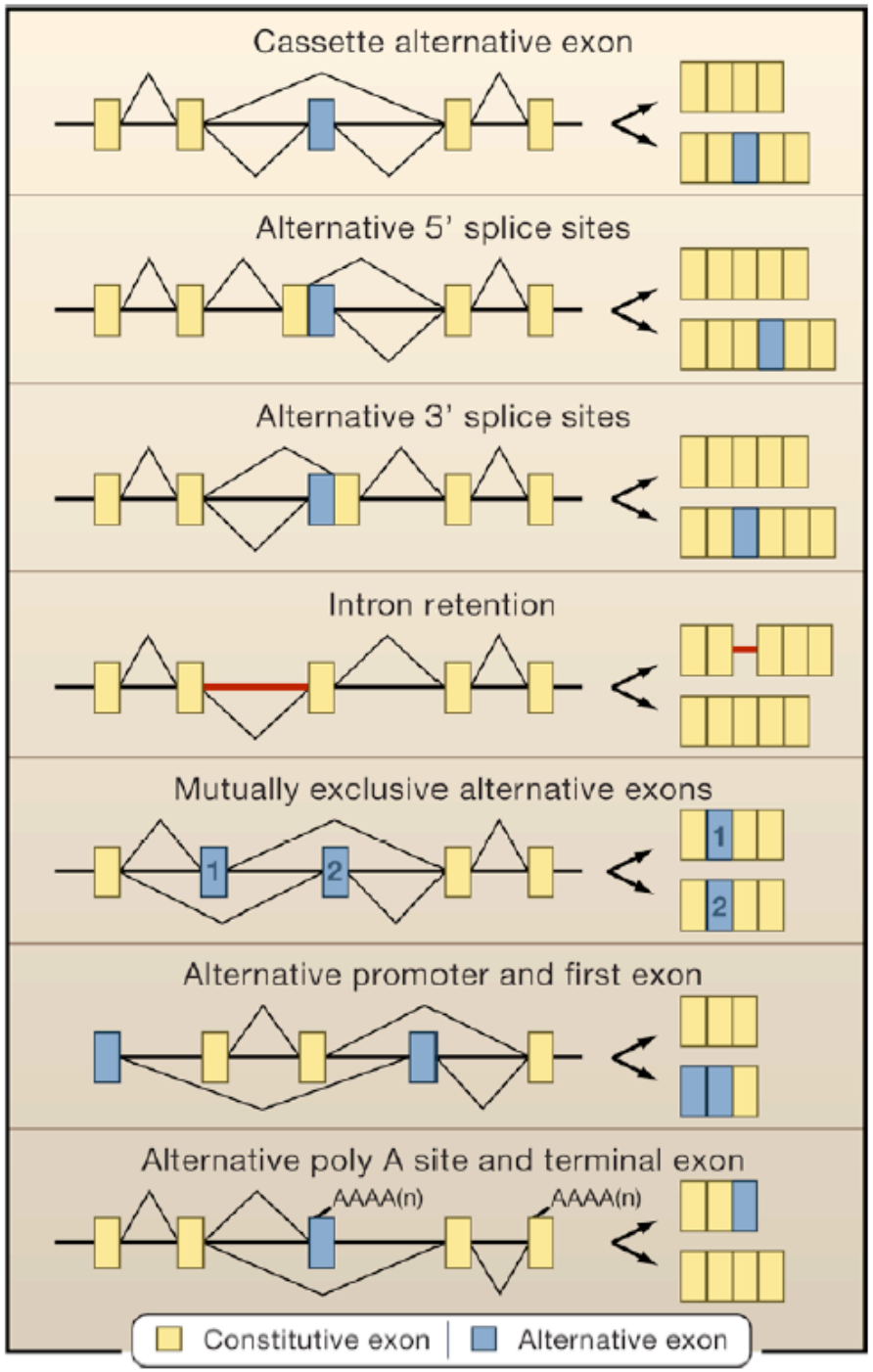
Mechanisms of alternative splicing regulation

The number of mechanisms that are known to be involved in splicing regulation approximates the number of splicing decisions that have been analyzed in detail.

Nilsen TW, Graveley BR. Expansion of the eukaryotic proteome by alternative splicing. Nature. 2010 Jan 28;463(7280):457-63.

Types of Alternative Splicing



38%

18%

8%

3%

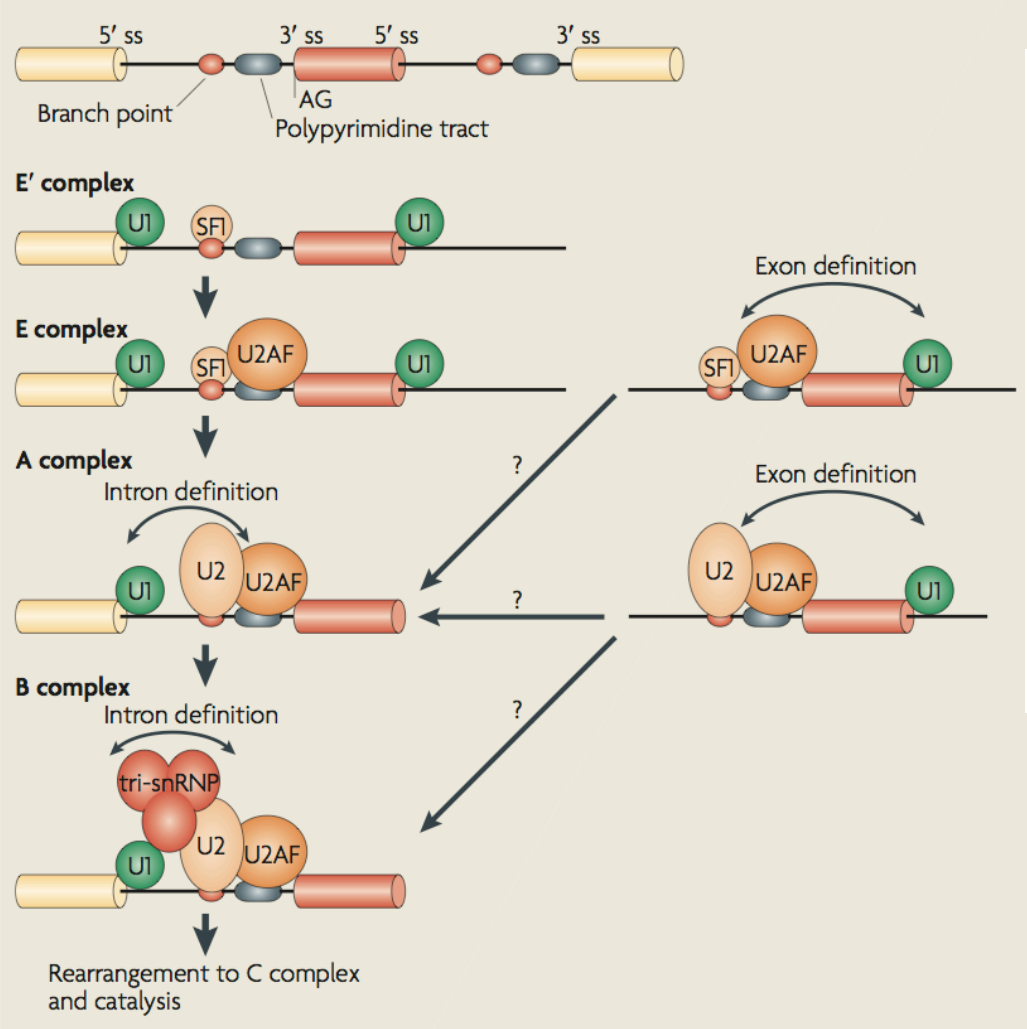
Remaining 33%

Cell 126:37 (2006)
Nature Reviews: Genetics 5:773 (2004)

Mechanisms of alternative splicing regulation

- facilitating splice site recognition
- inhibiting splice site recognition
  - direct interference
  - multimerization and looping out model
- position-dependent splicing regulation
  - some splicing factors act as activators or repressor depending on the location of their binding sites, the CLIP assay (NOVA, FOX and PTB)
- transcription coupled alternative splicing regulation and the kinetic model

Splicing and spliceosomal assembly



Pre-mRNA splicing is a process in which intervening sequences (introns) are removed from an mRNA precursor. Splicing consists of two transesterification steps, each involving a nucleophilic attack on terminal phosphodiester bonds of the intron. In the first step this is carried out by the 2′ hydroxyl of the branch point (usually adenosine) and in the second step by the 3′ hydroxyl of the upstream (5′) exon[1,2]. This process is carried out in the spliceosome, a dynamic molecular machine the assembly of which involves sequential binding and release of small nuclear ribonucleoprotein particles (snRNPs) and numerous protein factors as well as the formation and disruption of RNA–RNA, protein–RNA and protein–protein interactions.

The basic mechanics of spliceosome assembly are well known. Briefly, the process begins with the base pairing of U1 snRNA to the 5′ splice site (ss) and the binding of splicing factor 1 (SF1) to the branch point[3] in an ATP-independent manner to form the E′ complex (see the figure; double-headed arrows indicate an interaction). The E′ complex can be converted into the E complex by the recruitment of U2 auxiliary factor (U2AF) heterodimer (comprising U2AF65 and U2AF35) to the polypyrimidine tract and 3′ terminal AG[158]. The ATP-independent E complex is converted into the ATP-dependent pre-spliceosome A complex by the replacement of SF1 by U2 snRNP at the branch point. Further recruitment of the U4/U6–U5 tri-snRNP leads to the formation of the B complex, which contains all spliceosomal subunits that carry out pre-mRNA splicing. This is followed by extensive conformational changes and remodelling, including the loss of U1 and U4 snRNPs, ultimately resulting in the formation of the C complex, which is the catalytically active spliceosome.

Mechanisms of alternative splicing regulation: insights from molecular and genomics approaches Mo Chen and James L. Manley
NATURE REVIEwS | Molecular cell Biology VOLUME 10 | NOVEMBER 2009 | 741

Mechanisms of alternative splicing regulation

•facilitating splice site recognition

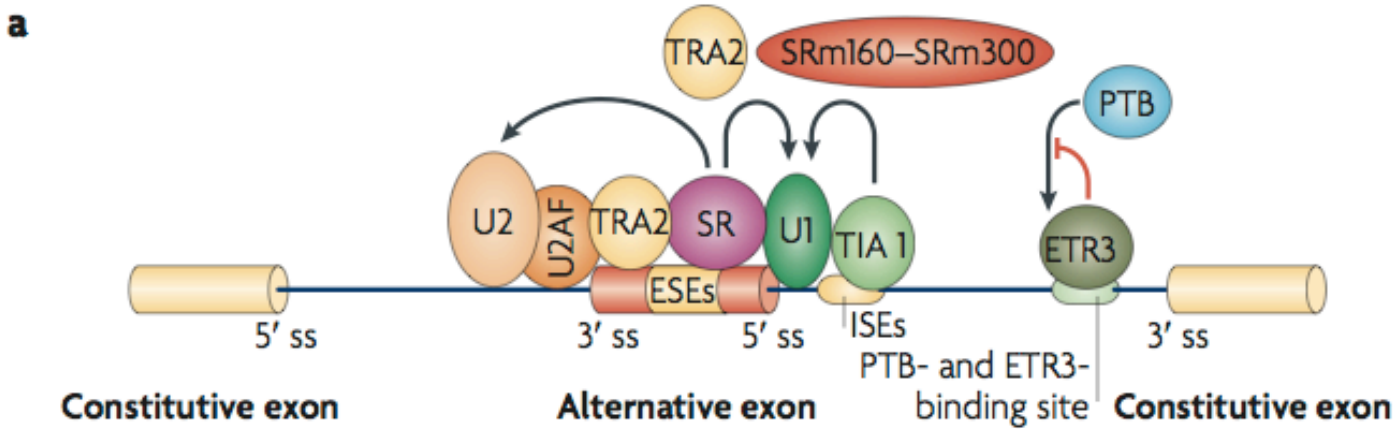
Exonic elements :SR proteins binds ESE and facilitate

recruitment of U2 at the 3'ss

recruitment of U1 at the 5'ss

recruitment of cofactors (Tra2 or SRm160)

Intronic ISE (AU-rich seq) binds TIA1 and facilitate recruitment of U1snRNP



Mechanisms of alternative splicing regulation

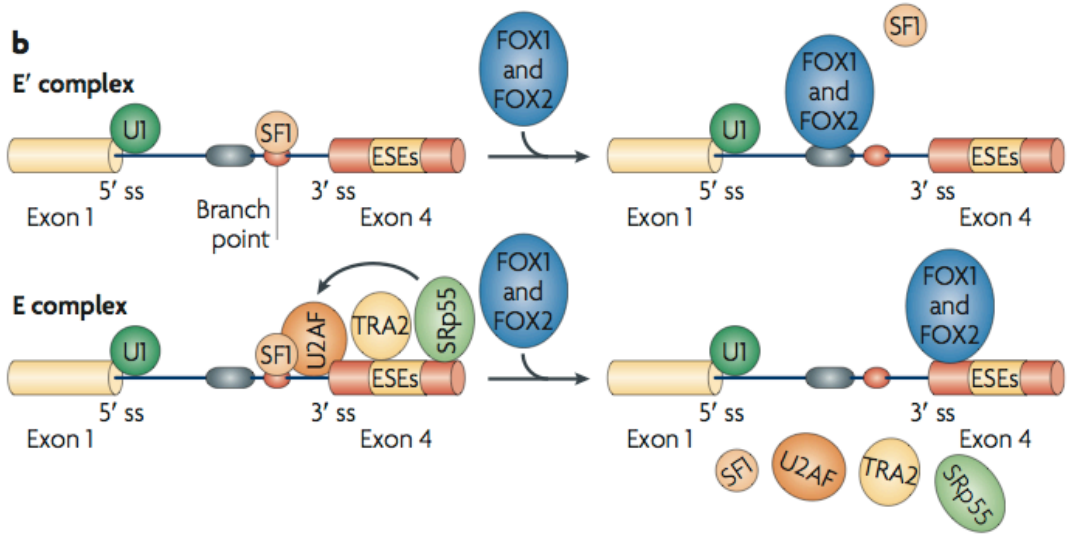
- inhibiting splice site recognition
  - direct interference

Direct steric interference blocking the access of the snRNP or of a positive splicing factor

-Polypyrimidine Tract Binding proteins directly binds to polyY and blocks U2AF

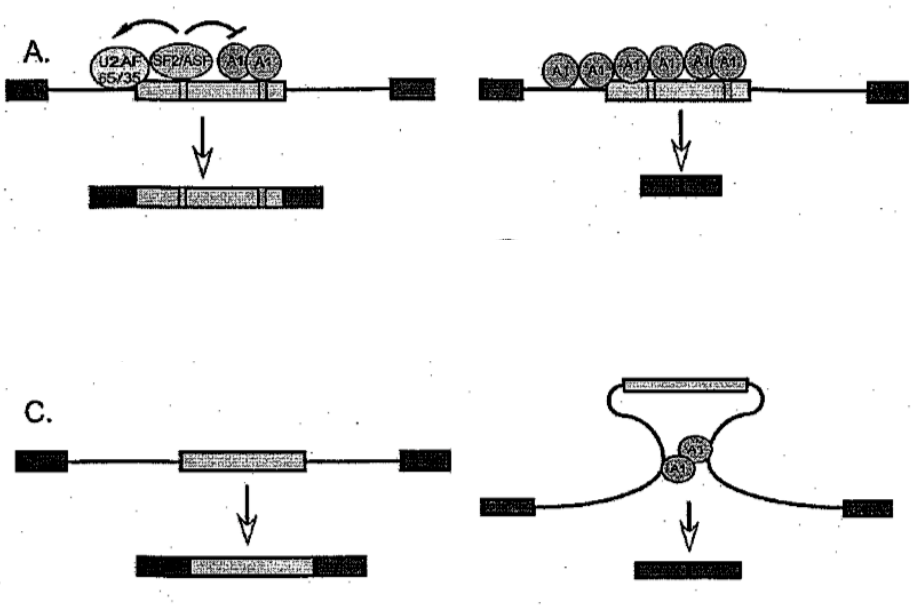
-hnRNPA1 binds to an ISS near U2 site (HIV)

-the tissue specific splicing factors FOX1 and FOX2 interfere with the Branch Point or with an ESE



Mechanisms of alternative splicing regulation

- inhibiting splice site recognition
  - multimerization and looping out models



Mechanisms of alternative splicing regulation

- position-dependent splicing regulation
  - some splicing factors act as activators or repressor depending on their location on their binding sites
  - the CLIP example (NOVA, FOX and PTB)

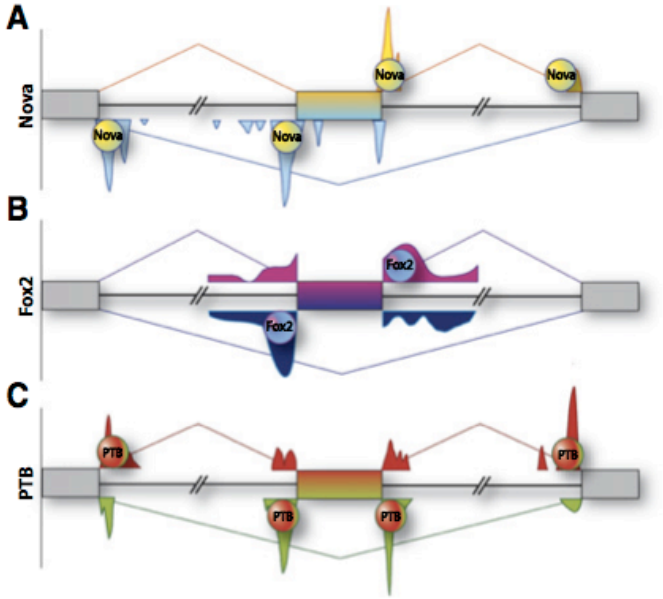


Figure 1. RNA Maps of Three Splicing Regulators
RNA maps depict the correlation between sites of regulatory protein binding (determined by CLIP) relative to a model alternative exon and the outcome of splicing regulation (exon inclusion or skipping). Peaks represent regions with higher density of CLIP reads. Binding of Nova or Fox2 upstream of the exon correlates with exon skipping, whereas binding downstream of the exon correlates with inclusion. For PTB, skipping correlates with binding of the protein near the alternative exon, whereas inclusion correlates with binding near the distal, constitutive sites.

Anna Corrionero and Juan Valcarcel Molecular Cell 36, December 24, 2009

Cross-Linking ImmunoPrecipitation (CLIP)

•A method to identify protein binding site on RNA in living cells.

•After UV irradiation, cells are lysed and the crosslinked RNA-protein complexes immunoprecipitated.

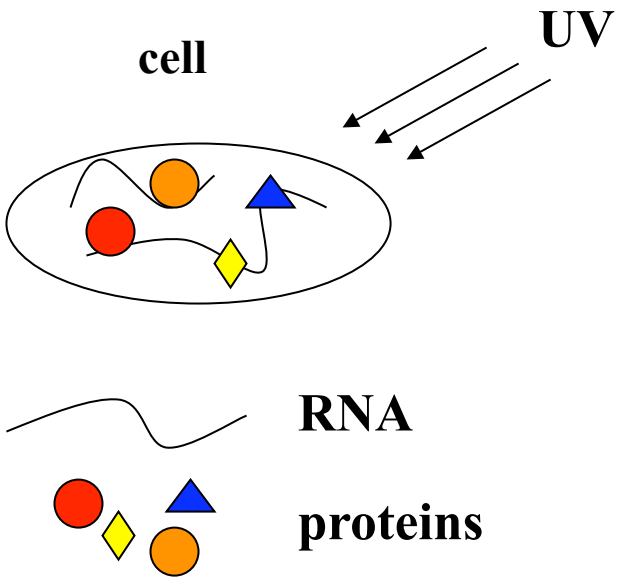
•Treatment with RNase digests away the RNA molecules except for those fragments protected by their interaction with the protein.

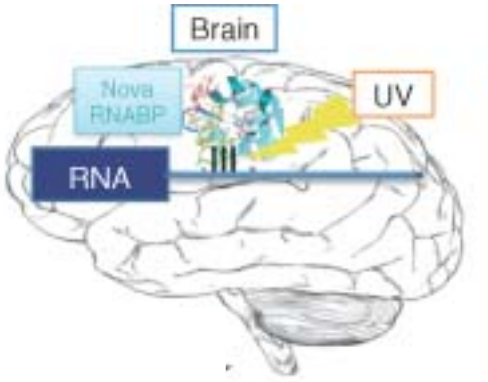
•Sequencing of these RNA tags not only identifies target transcripts, but also informs about the location of protein binding within the transcript.

•When combined with high-throughput sequencing technologies, the method allows one to exhaustively characterize binding sites in whole transcriptomes, thus providing a panoramic view of targets and possible functions of the RNA-binding protein.

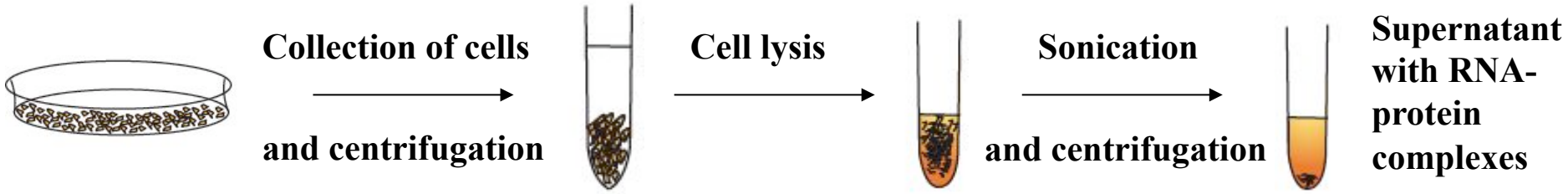
How it works?

- Cells or tissues are treated with UV irradiation that generates a covalent bond between RNA and protein that are in close contact within the complex

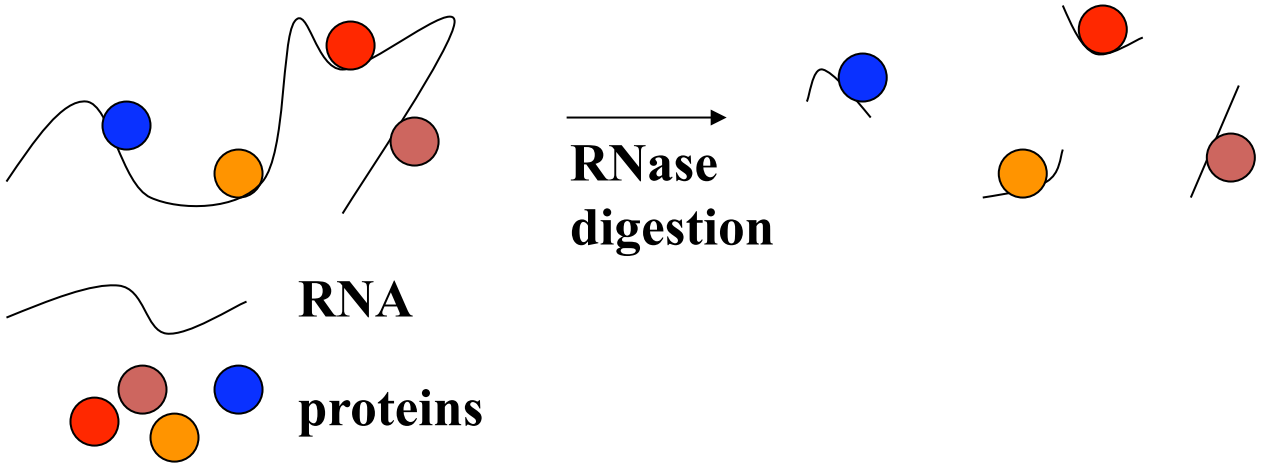




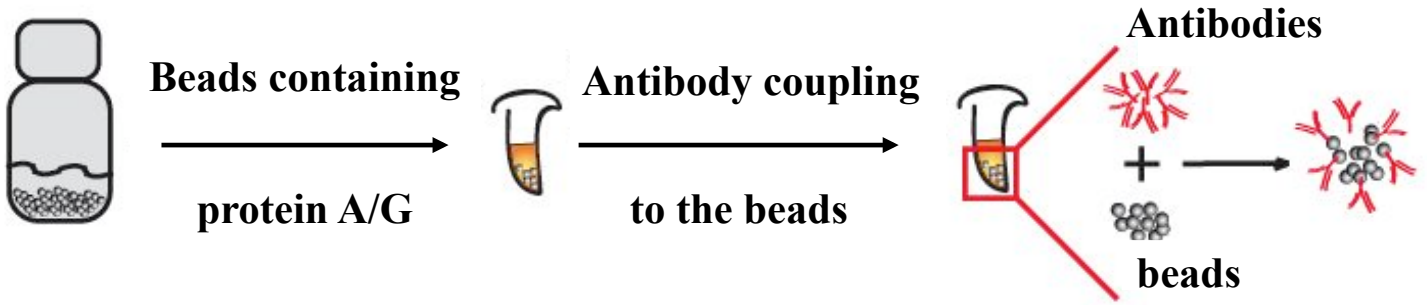
- Cell lysate preparation



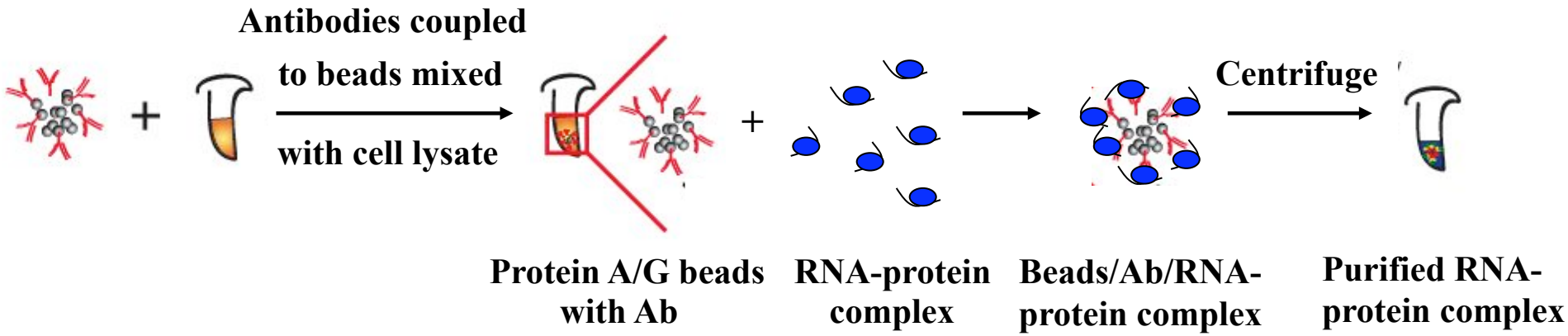
- Partial RNase digestion (size of cross-linked RNA is reduced to approximately 50 nucleotides)



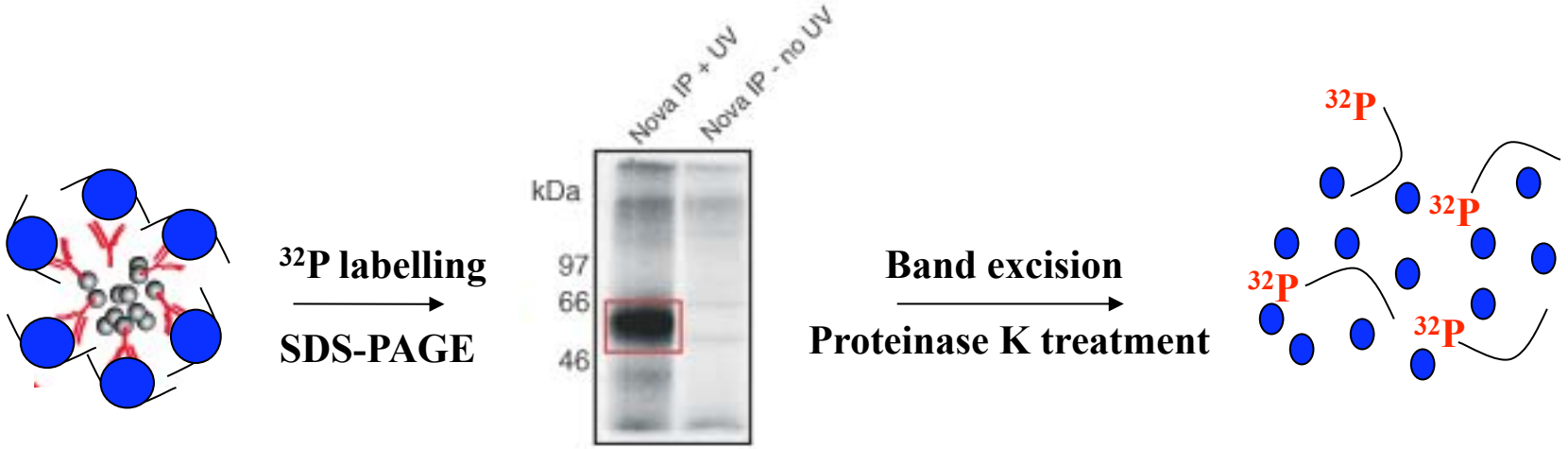
- Antibody and beads preparation



- Immunoprecipitation
  (purification of RNA-protein complexes)



- RNA labelling, SDS-PAGE and protein degradation



- RNA isolation and purification
- RT-PCR and sequencing of RNA tags (HITS-CLIP
- Gene alignment and *in vivo* validation

Cross-Linking ImmunoPrecipitation summary

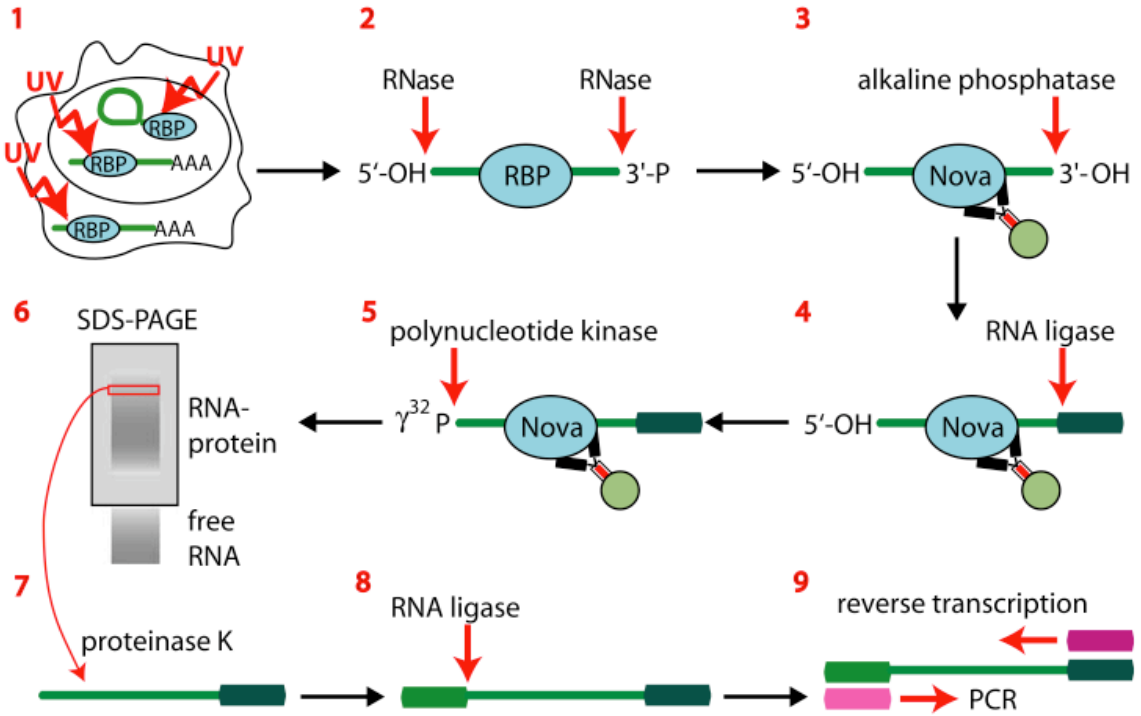


Fig. 1. A schematic representation of CLIP procedure for identification of protein binding sites on RNAs in the context of intact cells. Dissociated cells are UV-irradiated on ice, leading to formation of a covalent bond between protein and RNA. This allows partial digestion of RNA after lysis, followed by stringent protein purification, such as immunoprecipitation, SDS–PAGE electrophoresis and transfer to nitrocellulose. Prior to SDS–PAGE, RNA is dephosphorylated, ligated to 3′ RNA linker and radioactively labeled by 5′ γ^{32}P. After transfer to nitrocellulose, the membrane is exposed to X-ray film, and a thin region of the membrane corresponding to protein–RNA complexes of appropriate size is excised, protein is digested by proteinase K, and 5′ RNA linker is ligated to free RNA. The CLIP tags are then amplified by RT-PCR using the DNA primers with sequence complimentary to the RNA linkers.

CLIP Identifies Nova-Regulated RNA Networks in the Brain

Jernej Ule,[1,2*] Kirk B. Jensen,[1,2*] Matteo Ruggiu,[1,2] Aldo Mele,[1,2] Aljaž Ule,[3] Robert B. Darnell[1,2†]

Nova proteins are neuron-specific antigens targeted in paraneoplastic opsoclonus myoclonus ataxia (POMA), an autoimmune neurologic disease characterized by abnormal motor inhibition. Nova proteins regulate neuronal pre-messenger RNA splicing by directly binding to RNA. To identify Nova RNA targets, we developed a method to purify protein-RNA complexes from mouse brain with the use of ultraviolet cross-linking and immunoprecipitation (CLIP). Thirty-four transcripts were identified multiple times by Nova CLIP. Three-quarters of these encode proteins that function at the neuronal synapse, and one-third are involved in neuronal inhibition. Splicing targets confirmed in $Nova^{-/-}$ mice include c-Jun N-terminal kinase 2, neogenin, and gephyrin; the latter encodes a protein that clusters inhibitory γ-aminobutyric acid and glycine receptors, two previously identified Nova splicing targets. Thus, CLIP reveals that Nova coordinately regulates a biologically coherent set of RNAs encoding multiple components of the inhibitory synapse, an observation that may relate to the cause of abnormal motor inhibition in POMA.

Ule, J., Jensen, K.B., Ruggiu, M., Mele, A., Ule, A., and Darnell, R.B. (2003). Science 302, 1212–1215.

RNA binding proteins studied by CLIP

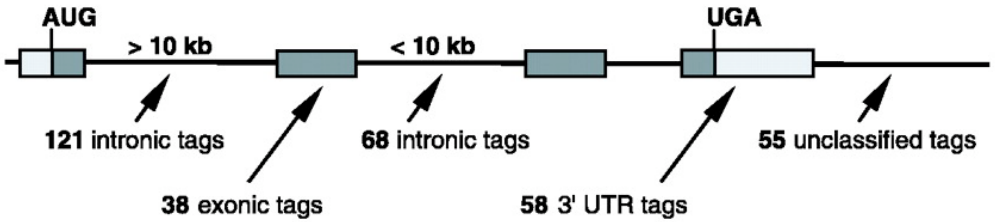
1) NOVA proteins

Ule, J., Jensen, K.B., Ruggiu, M., Mele, A., Ule, A., and Darnell, R.B. (2003). Science 302, 1212–1215.

Neuron-specific antigens

Regulate neuronal pre-mRNA splicing

Target transcripts encode for proteins that function at the neuronal synapse

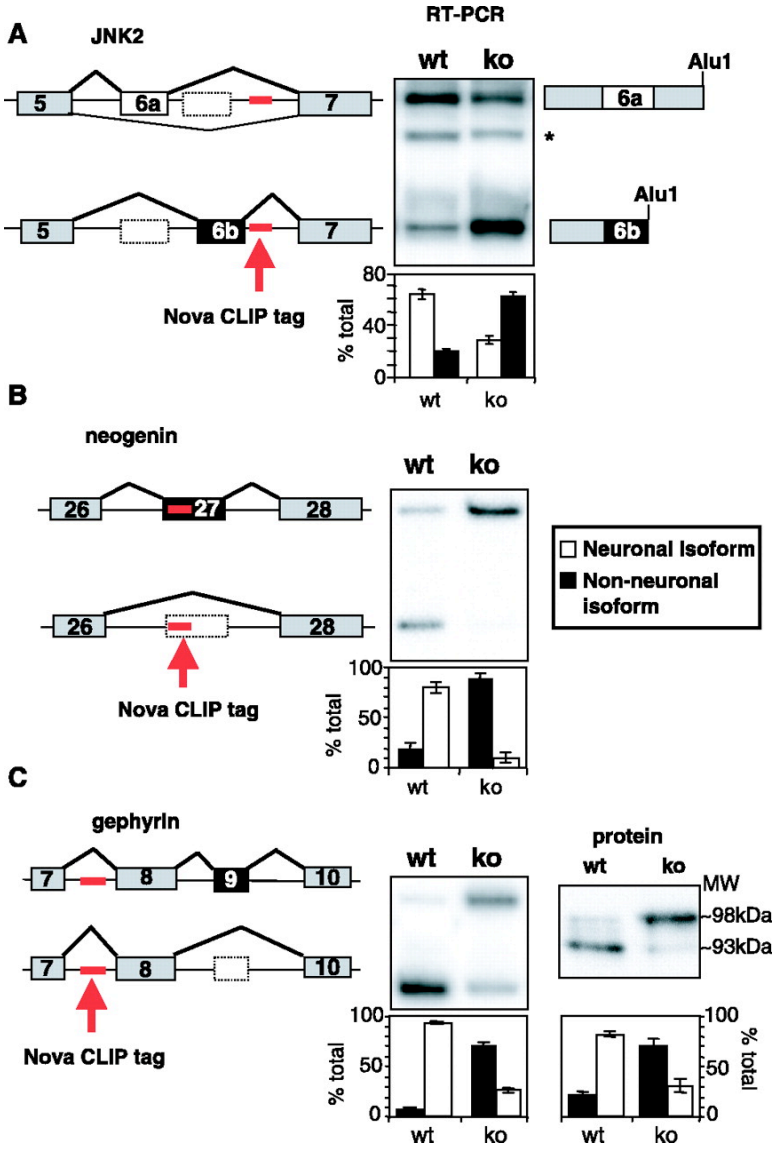


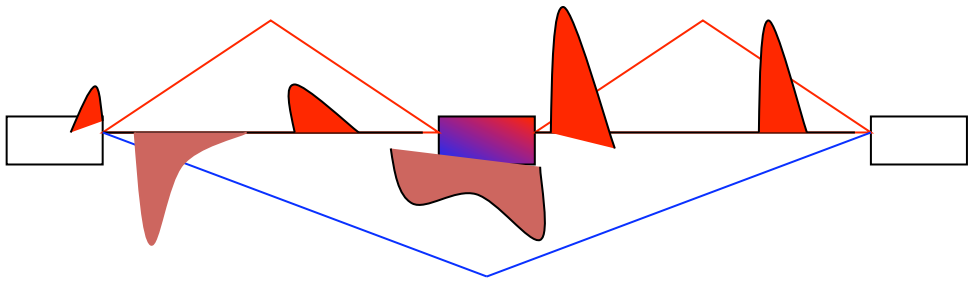
Genomic location of the tags

YCAY tetramer abundance in NOVA tags

RNA tag	transcribed sequence
gephyrin1	GGGCCAGUUCAACCACAGGUCCCCAGCUUCCAUCCAUUGGUUGGGUGCUAGUAUCUGCAUCUGACUCUUUCAGCU
control	GGGAGCUGAUGUAUGCUGCCUGGUUGGUGGCUCAGUGUCCUAGAGAUCUCGGGAUCCAGGUCAGUUGAGACUGCC
gephyrin2	GGGCCAACCACAAAUGCCAGCACCUCUUAAUAACAAUCAGCAUGACCUCUGCCUAAGUCUUGGCUUCUUCCUCAGAA
control	GGGGAGGAUGCCCUGCACCAGUACAGUAAAUGAGUGCUUGGAGGAAGAUCAGUGUGGCACUGUAAAGCCUAGUGUGC
JNK2	GGGGUGUUCUUCCAUUUUCCACAUUCUUCACACUAACAUGCGUCUUCAUGCU
control	GGGGUUUUGUAAAGGGAGCCGACGGCAUAGGGCACUAGCUCUGUCCCCUCGU
neogenin	GGGCUGUGAUUAGUGCCCAUCCCAUCCAUUCCCUCGAUAACCCUCACCAUCAUUUCCACUCCAG
control	GGGGUCUACACUGGCUGGAAGGAGGGGAAUGAGACCAAAAAUGAUGAUGCCCUUUGACUCUCAG

NOVA-dependent regulation of alternative splicing



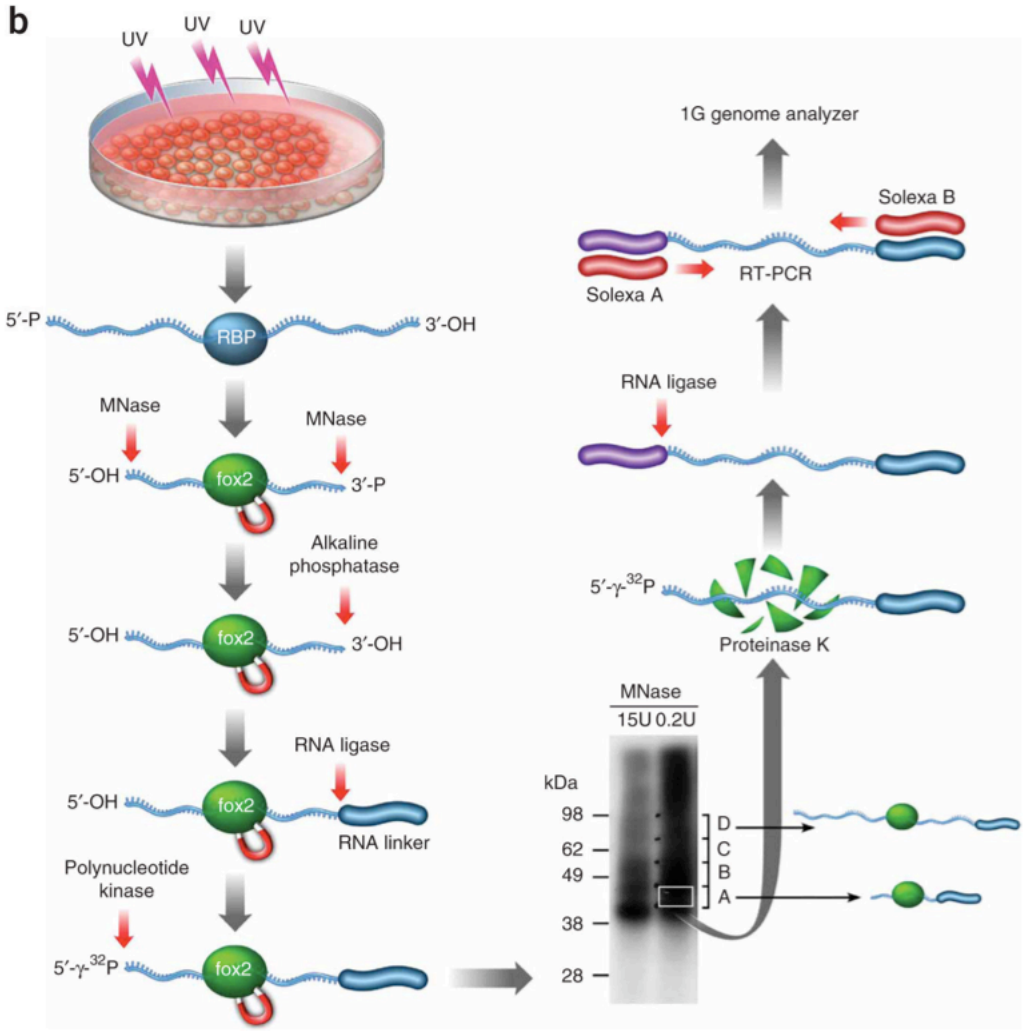


RNA map predicts NOVA-dependent splicing regulation

2) Fox 2 protein

tissue-specific expression in muscle and neuronal cells

Regulates alternative splicing through binding to UGCAUG sequence



stem cells

Yeo et al Nat Struct Mol Biol. 2009 16(2): 130–137

2) Fox 2 protein

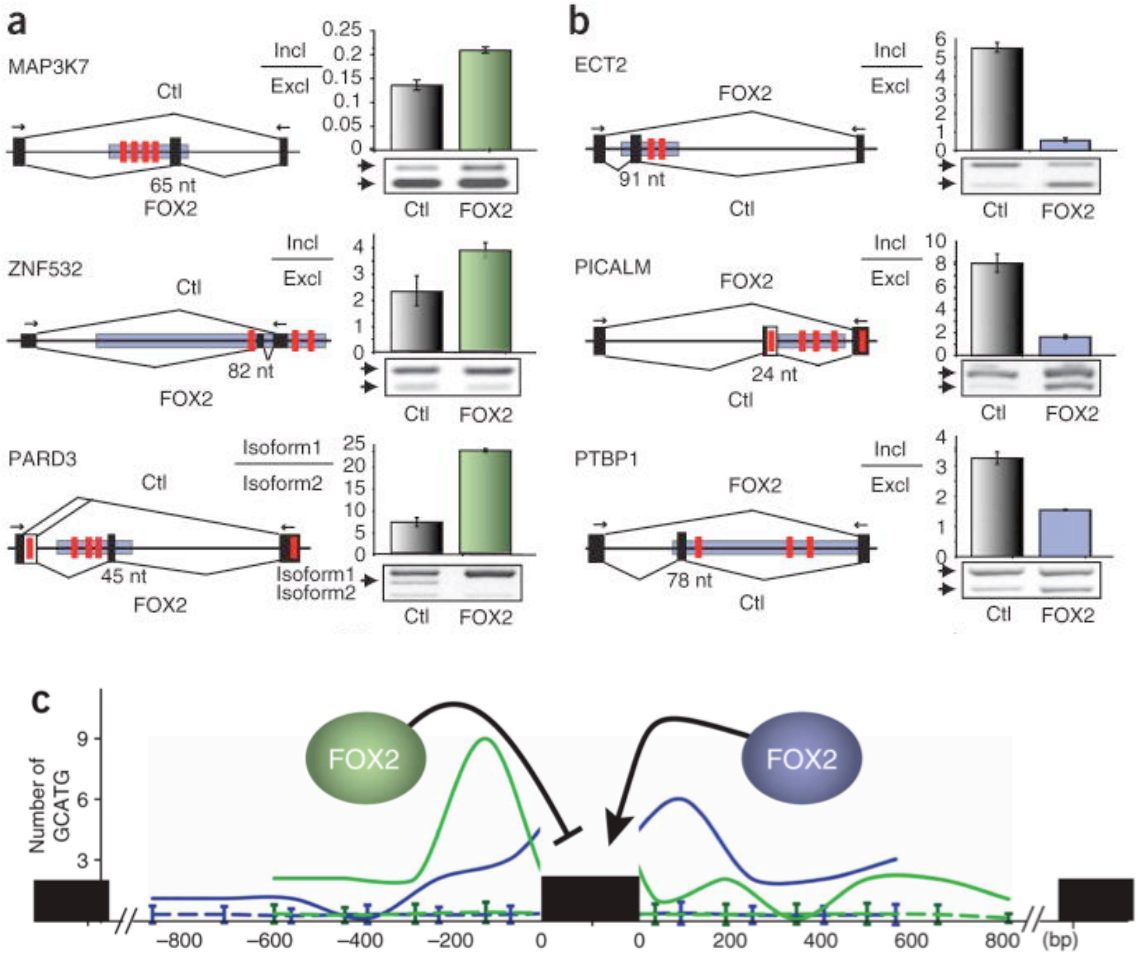


Figure 4.
RNA map of FOX2-regulated alternative splicing. (**a**) FOX2-dependent exon skipping. (**b**) FOX2-dependent exon inclusion. Each gene is diagrammed by vertical black bars (exons) and thin horizontal lines (introns) with arrows representing specific RT-PCR primers. The conserved GCAUG FOX2 binding motifs (red vertical bars) generally overlap with mapped FOX2 binding sites by CLIP-seq (blue horizontal bars). Regulated splicing in control (Ctl) shRNA–and FOX2 (FOX2) shRNA–treated hESCs was analyzed by RT-PCR in triplicate, and s.d. is indicated by error bars. Changes in alternative splicing were significant in all cases, as determined by the Student's *t*-test (*P*-value < 0.05). (**c**) Number of conserved GCAUG sites proximal to the RT-PCR–validated FOX2-regulated alternative splicing, showing that

3) PTB

RNA-binding protein
Generally considered as a splicing repressor

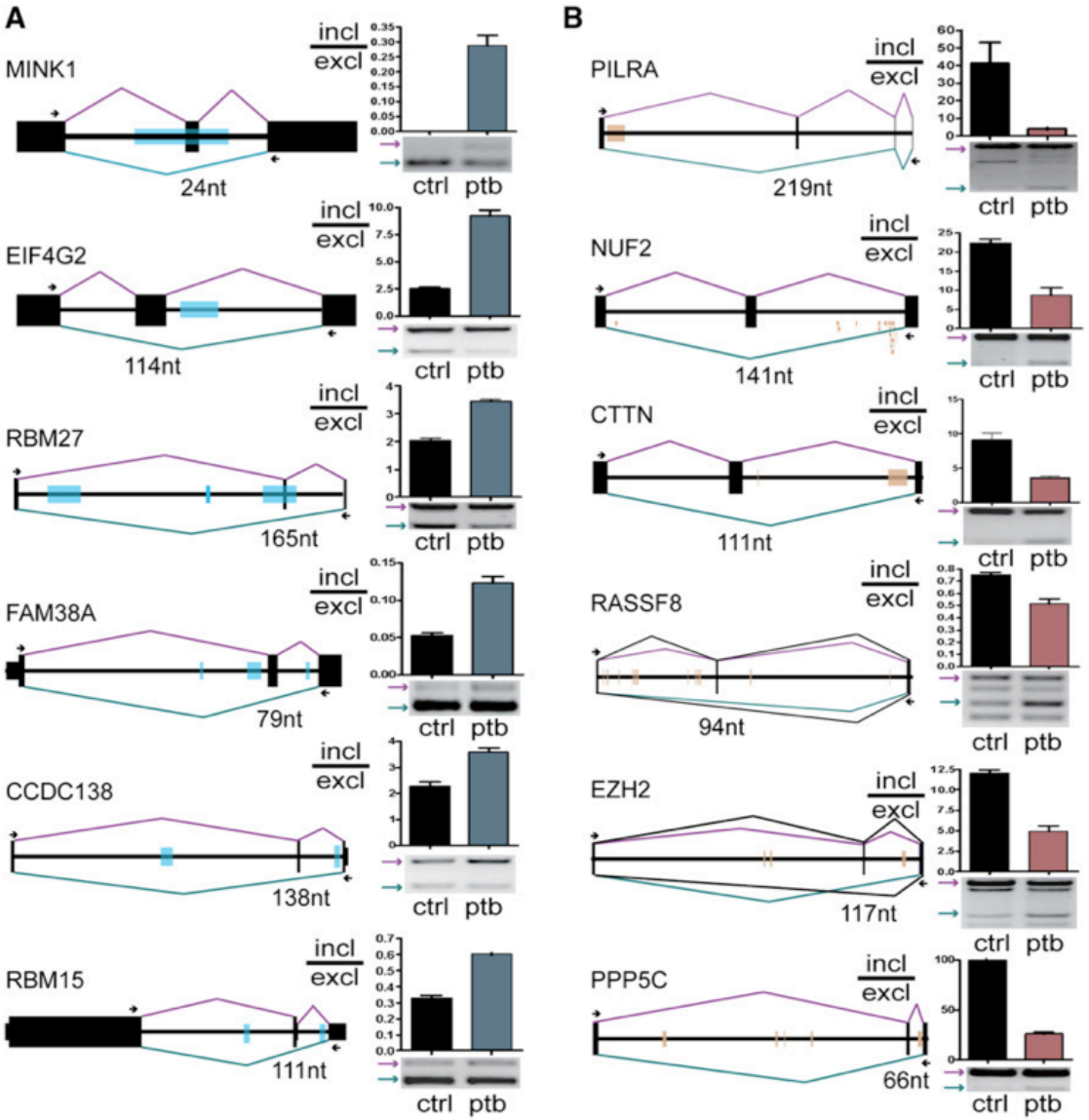


Figure 4. PTB Can Either Represses or Enhance Alternative Splicing In Vivo

(A) Examples of PTB-dependent exon skipping. Each is schematically diagramed (exon, black box; intron, black line) with mapped PTB-binding clusters as marked by blue boxes. PTB RNAi-induced splicing changes are shown on the right. Error bars are based on SEM from three independent experiments. All detected changes are significant as determined by the Student's t test ($p < 0.05$).

(B) Examples of PTB-dependent exon inclusion with mapped PTB-binding clusters marked by brown boxes. For *NUF2*, PTB tags (not clusters) are shown under the intron line.

HeLa cells

Molecular Cell 36, 996–1006 2009

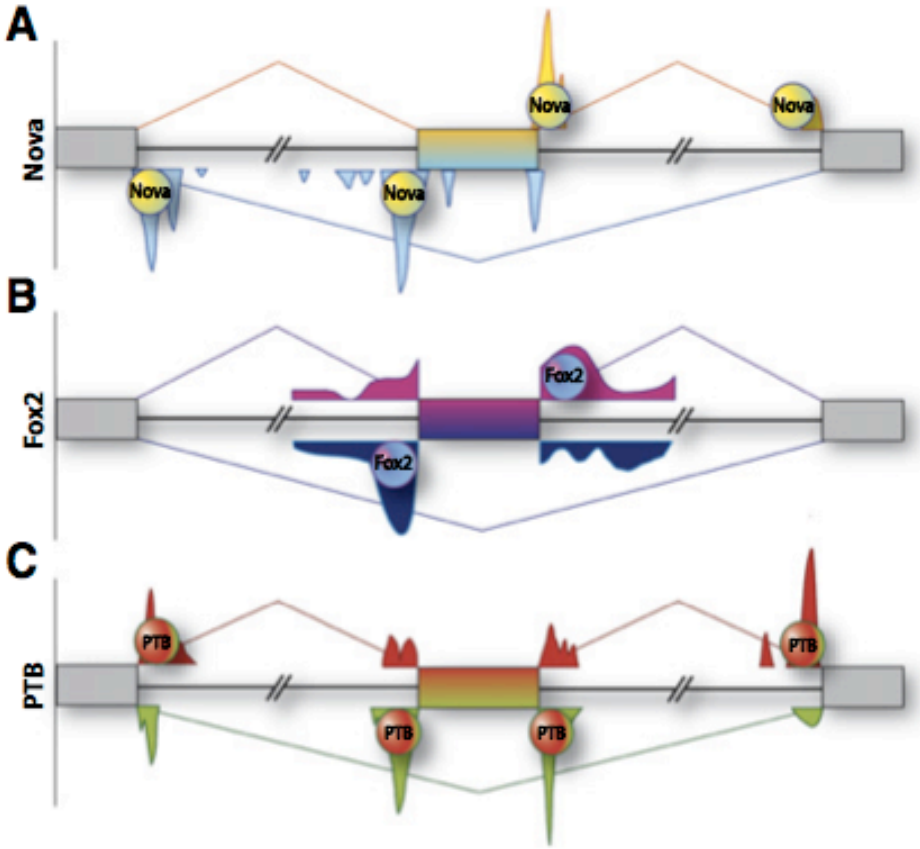


Figure 1. RNA Maps of Three Splicing Regulators

RNA maps depict the correlation between sites of regulatory protein binding (determined by CLIP) relative to a model alternative exon and the outcome of splicing regulation (exon inclusion or skipping). Peaks represent regions with higher density of CLIP reads. Binding of Nova or Fox2 upstream of the exon correlates with exon skipping, whereas binding downstream of the exon correlates with inclusion. For PTB, skipping correlates with binding of the protein near the alternative exon, whereas inclusion correlates with binding near the distal, constitutive sites.

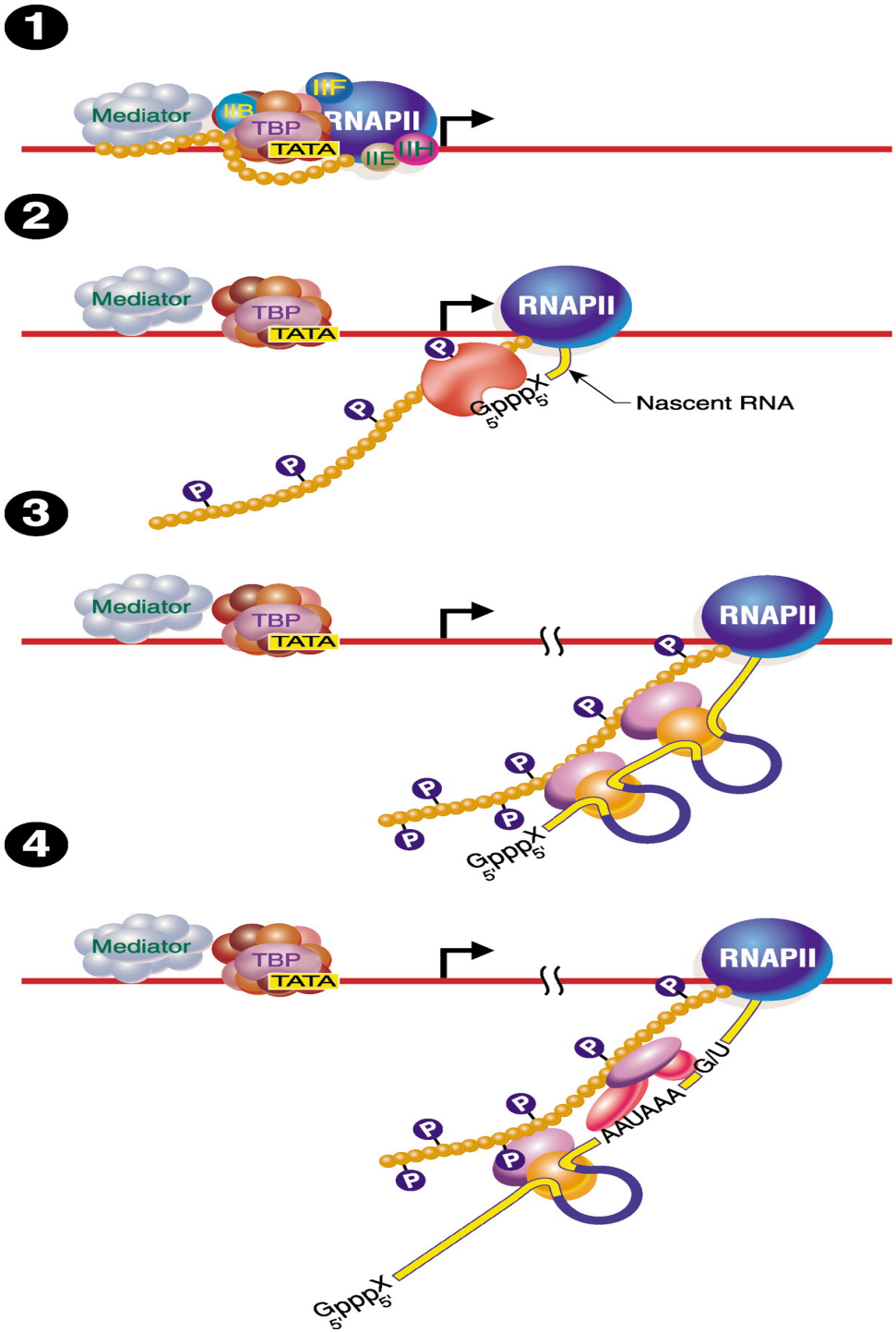
Anna Corrionero and Juan Valcarcel Molecular Cell 36, December 24, 2009

Mechanisms of alternative splicing regulation

- transcription coupled alternative splicing regulation
  - recruitment model
  - kinetic model

Coupling of pre mRNA processing and transcription

- Bentley experiments
  - bacterial Polymerases do not support RNA processing
  - use amanitin-resistant PolII with different length of the tail
  - reducing the PolII tail reduces RNA processing (capping, splicing and polyadenilation)
- promoter and alternative splicing
  - the promoter architecture modulate alternative splicing
- speed of the the polymerase
  - PolII processivity influences alternative splicing



Before transcription initiation, the CTD is unphosphorylated and is associated with the mediator and components of the initiation machinery.

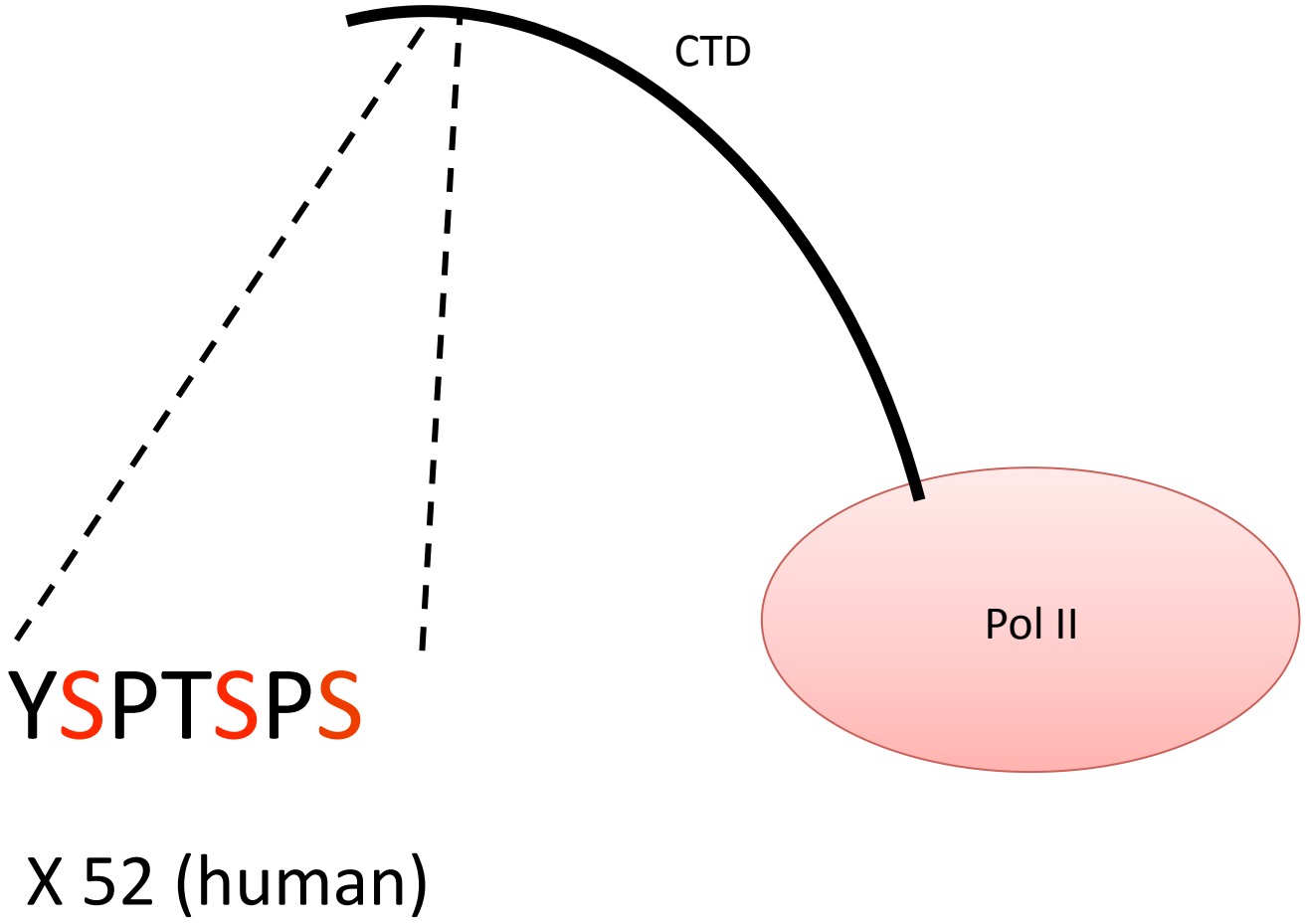
Partial phosphorylation of the CTD during transcription initiation recruits the capping enzymes, which cap the 5' end of the nascent transcript.

Further phosphorylation of the CTD upon promoter escape recruits components of the pre-mRNA splicing machinery.

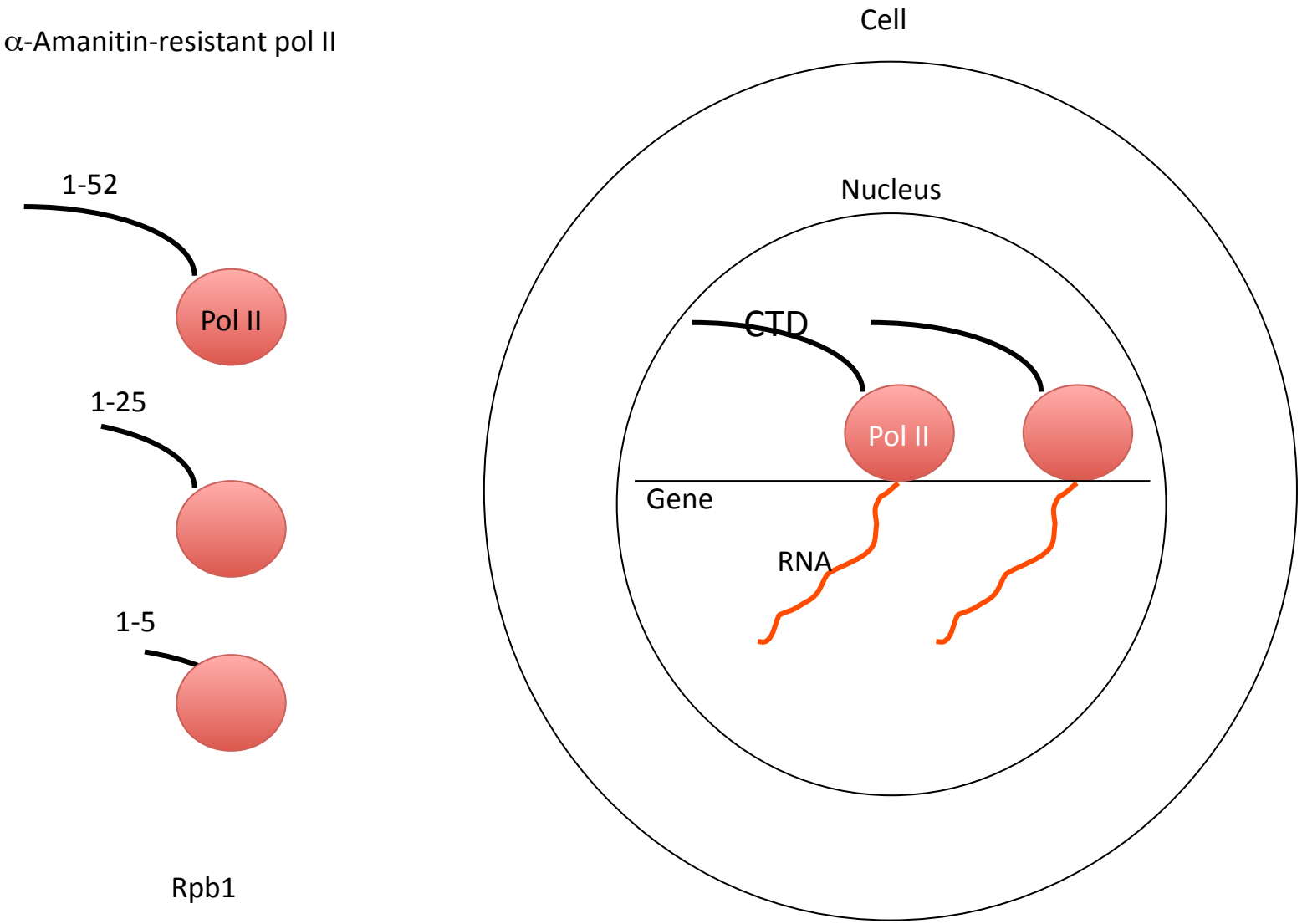
RNAPII reaches termination signals and factors required for cleavage and 3' poly-adenylation of the transcript associate with the CTD and recognize sequences in the RNA. Some of these components may be recruited during initiation (see text for details).

RNA Processing is Coupled with Tx

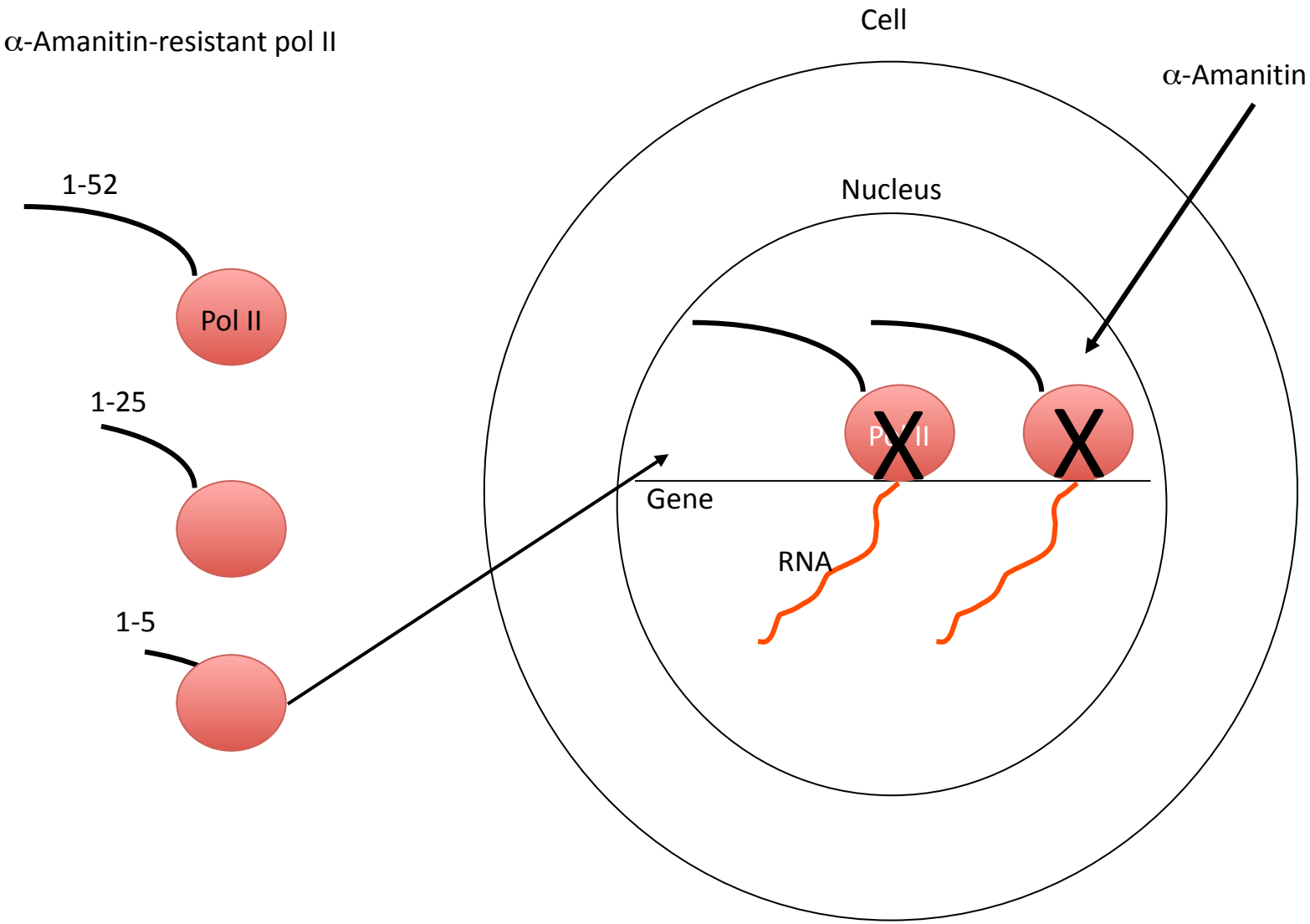
The CTD of the large subunit co-ordinates transcription and RNA processing in protein-coding genes



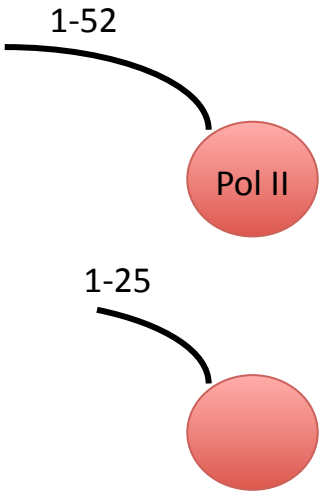
McCracken *et al*. Nature 1997

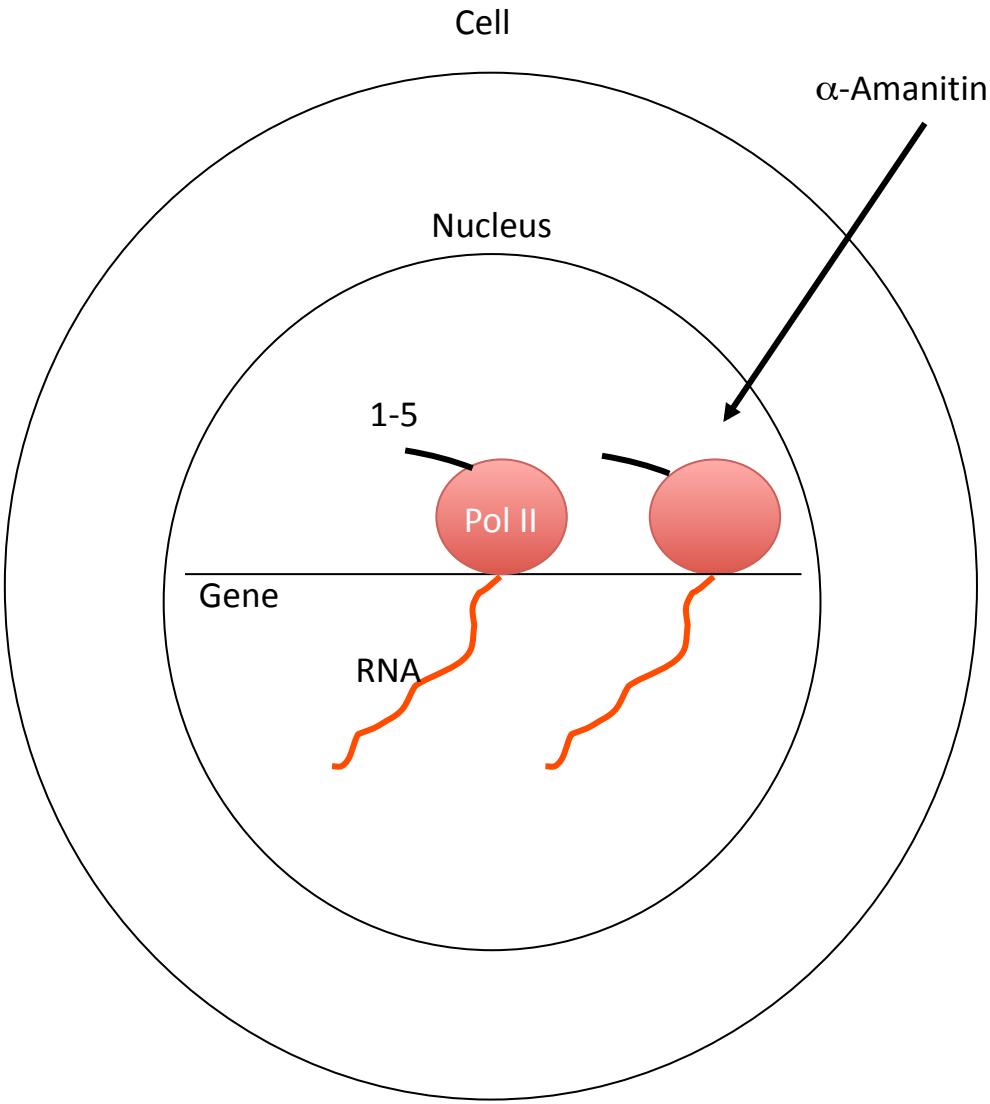


CTD complementation system


α-Amanitin-resistant pol II
Cell
α-Amanitin
1-52
Pol II
1-25
1-5
Nucleus
Gene
RNA

α-Amanitin-resistant pol II





The C-terminal domain of PolII couples RNA processing to transcription
Bentley Nature 1997

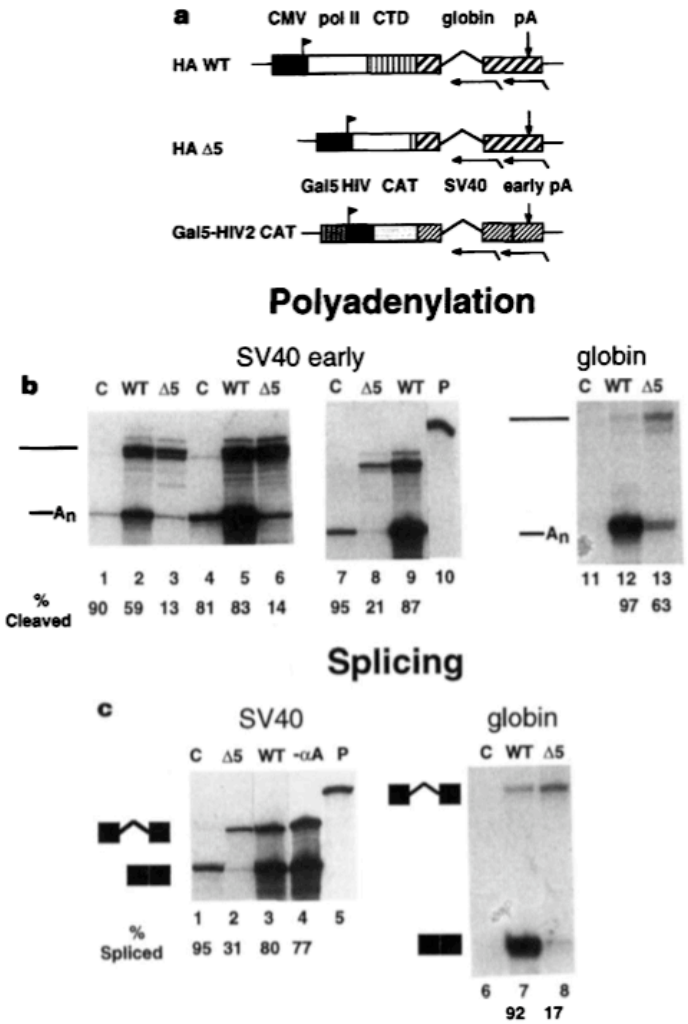


Figure 1 CTD truncation inhibits 3′ processing and splicing. **a**, Wild-type (WT) and CTD-truncated (Δ5) pol II expression plasmids and Gal5–HIV2 CAT reporter. **b**, RNAse protection of cleavage at the SV40 early (lanes 1–9) and rabbit β-globin (lanes 11–13) poly(A) sites. Transcription was activated by Gal4–Sp1 (lanes 1–3) or Gal4–VP16 (lanes 4–9). In lanes 7–9, Gal5–HIV2 CAT was transfected after addition of α-amanitin (see Methods). C, CMV *neo* control; P, probe. **c**, RNAse protection of splicing at the SV40-t intron (lanes 1–4) and rabbit β-globin intron 2 (lanes 6–8). Lanes 1–3 are the same RNAs as in **b**, lanes 7–9. Lanes 1, 2 were exposed for twice as long as lane 3. Lane 4, control without pol II plasmid or α-amanitin.

The promoter architecture modulates alternative splicing

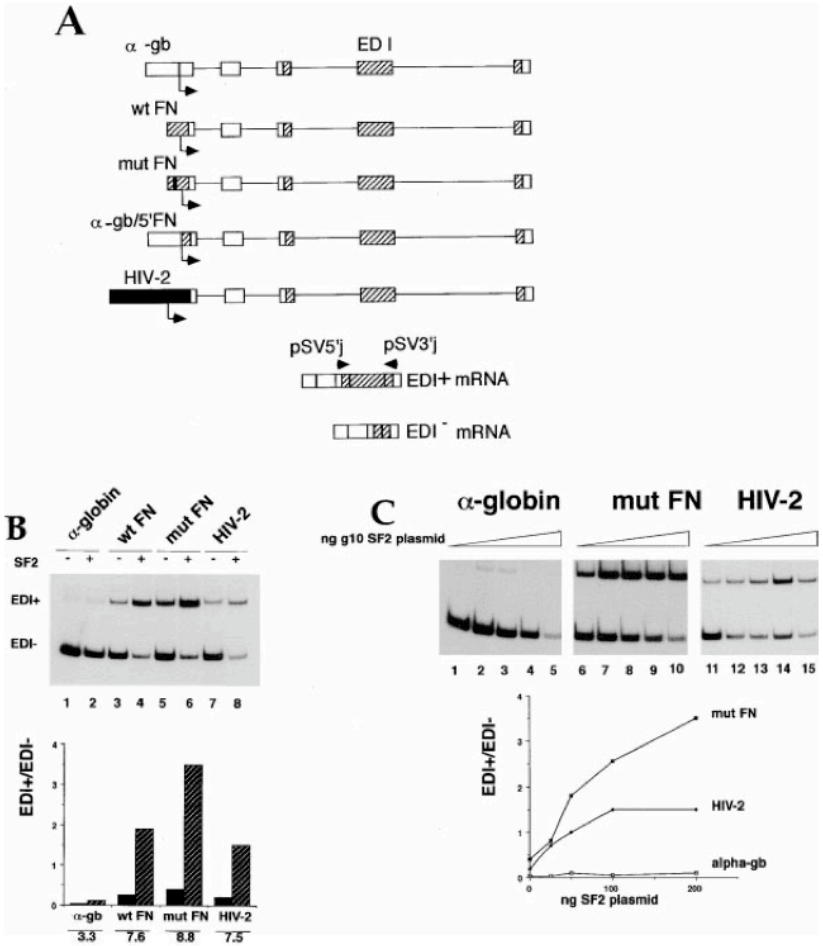


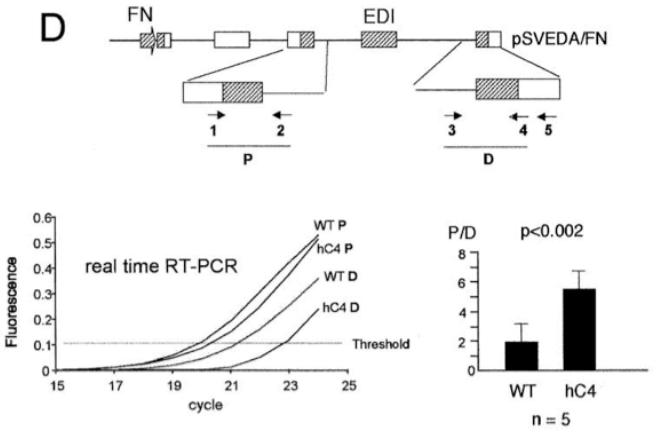
Figure 1. The SF2/ASF Effect on Alternative Splicing Depends on Promoter Structure

(A) Schemes of the plasmid constructs carrying the different promoters. Empty boxes, human globin sequences; dashed boxes, human FN sequences; black box, Gal_5-HIV-2 sequence. Arrows indicate transcription initiation sites.

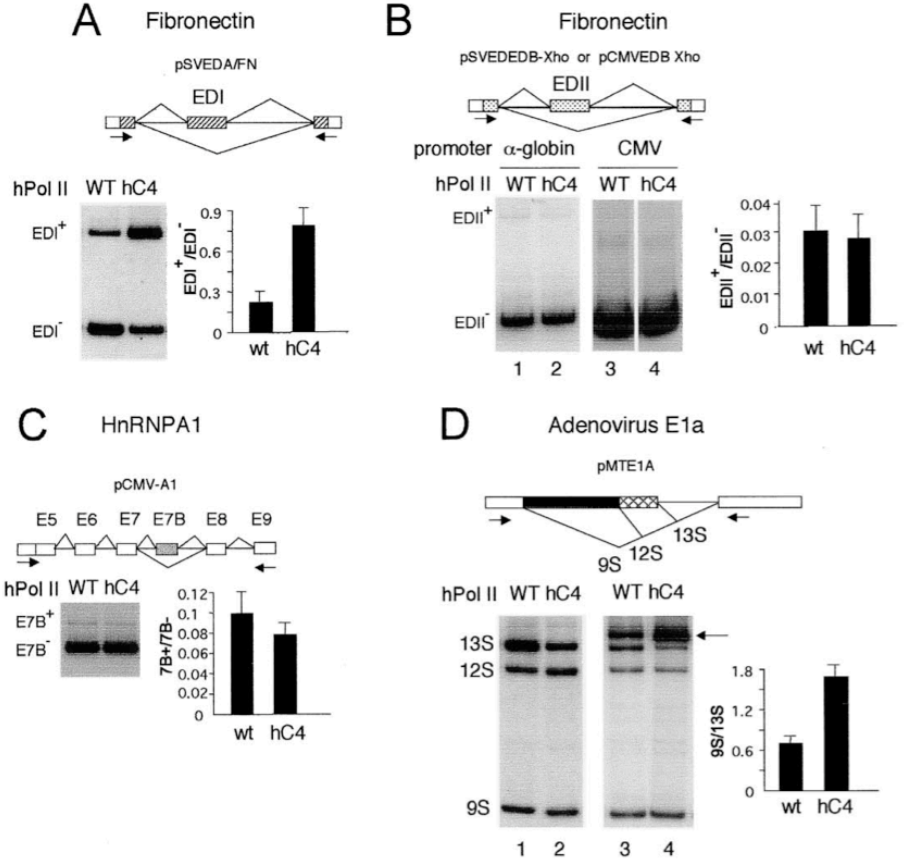
(B) Effect of SF2/ASF on alternative splicing in the context of different promoters. Hep3B cells were transfected with 800 ng of the corresponding minigene plasmid and 200 ng of g10 SF2/ASF wild-type plasmid (even lanes) or pBS SK^+ (odd lanes). alpha-gb, human α-globin promoter; wt FN, −220 fragment of wild-type human FN promoter; mut FN, −220 fragment of mutant (CRE^-/$CCAAT^-$) human FN promoter. RNA splicing variants were detected by radioactive RT-PCR and analyzed in 6% native polyacrylamide gels. Histograms display the ratios between radioactivity in EDI^+ bands and radioactivity in EDI^- bands. Numbers below each pair of bars indicate the fold increase of EDI^+/EDI^- ratios in presence/absence of the SF2/ASF plasmid.

(C) Dose response curve of SF2/ASF on alternative splicing of minigenes driven by the α-globin, mutant FN, and HIV-2 promoters in vivo. Hep3B cells were transfected with 800 ng of pSVEDA/FN mutant plasmid, pSVEDAtot, or pSVEDA/Gal_5-HIV-2 and varying amounts of g10 SF2/ASF wild-type plasmid: 0 ng, lanes 1, 6, and 11; 25 ng, lanes 2, 7, and 12; 50 ng, lanes 3, 8, and 13; 100 ng, lanes 4, 9, and 14; 200 ng, lanes 5, 10, and 15. Cotransfection of varying amounts of pBS SK^+ allowed keeping the same mass of total DNA in every well. Lane 14 has the double amount of both isoforms compared to lanes 11, 12, 13, and 15.

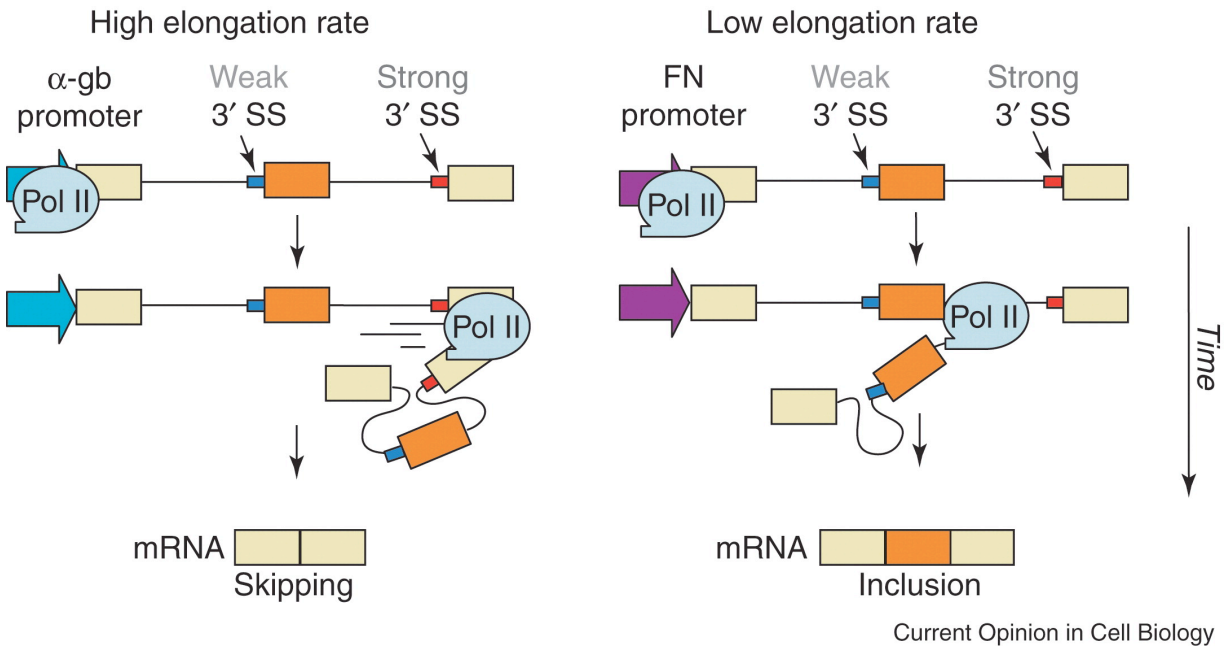
A human PolII carrying a mutation (R749H) is less processive in vivo and affects alternative splicing



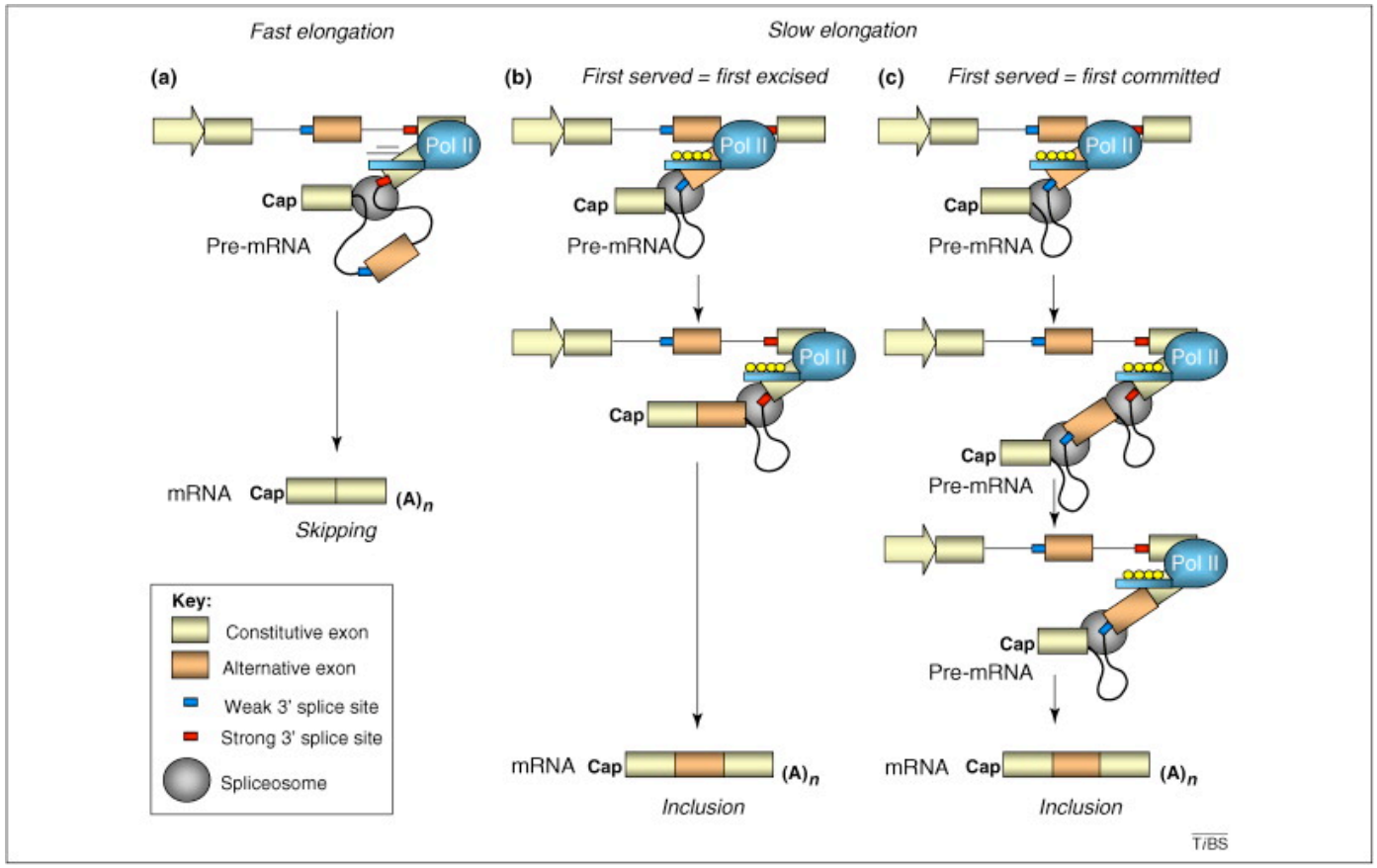
(D) Real time RT-PCR to quantify pre-mRNA accumulation at proximal and distal regions with respect to the transcription start site. Hep3B cells were transfected with pSVEDA/FN (top diagram) and either pAT7Rpb1αAm^r (WT) or pAT7Rpb1αAm^{r}R749H (hC4), and treated with α-amanitin as indicated in Experimental Procedures. 48 hr after transfection, nuclei were isolated, total RNA was prepared, and cDNA was synthesized using primers "2" or "5." PCR reactions containing the fluorescent dye SYBR green were performed in a DNA Engine Opticon System (MJ Research) with primers "1" and "2" for the proximal (P) region and primers "3" and "4" for the distal (D) region. The Opticon screen view (bottom



Kinetic coupling model for the regulation of alternative splicing by pol II elongation

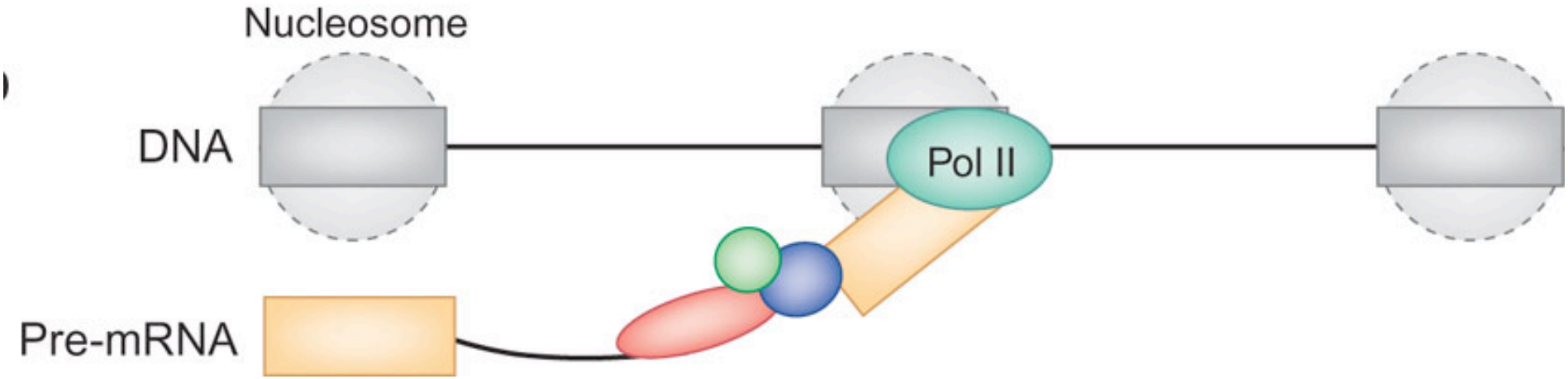


Kinetic coupling model for the regulation of alternative splicing by pol II elongation



Slow elongation is caused by the CTD hyperphosphorylation (yellow circles) that follows for example UV-triggered DNA damage. The 3' splice site by the alternative cassette exon (blue) is weaker than the 3' splice site of the downstream intron (red). High elongation rates (a) favor skipping, whereas low transcriptional elongation rates (b) and (c) favor exon inclusion. (b) and (c) depict two alternative pathways for the "first come, first served" mechanism of splice site selection leading to higher exon inclusion. (b) Slow elongation causes preferential excision of the upstream intron (first served = first excised). (c) Slow elongation causes commitment to inclusion of the alternative exon via recruitment of splicing factors (first served = first committed) independent of the relative order of intron removal

Mean exon size corresponds to nucleosome accommodation on DNA



Nucleosomes (broken circles) are preferentially bound to exons, whereas introns are mostly devoid of nucleosomes. Exons are therefore marked at the DNA level by nucleosome positioning, which may act as 'speed bumps' for RNA polymerase II, helping in the co-transcriptional recruitment of splicing factors to the nascent pre-mRNA and improving exon definition.
As nucleosomes accommodate DNA stretches of approximately 147 nt, their preferential location on exons (mean size 145bp) may act as the selective pressure factor for the conservation in exon length.

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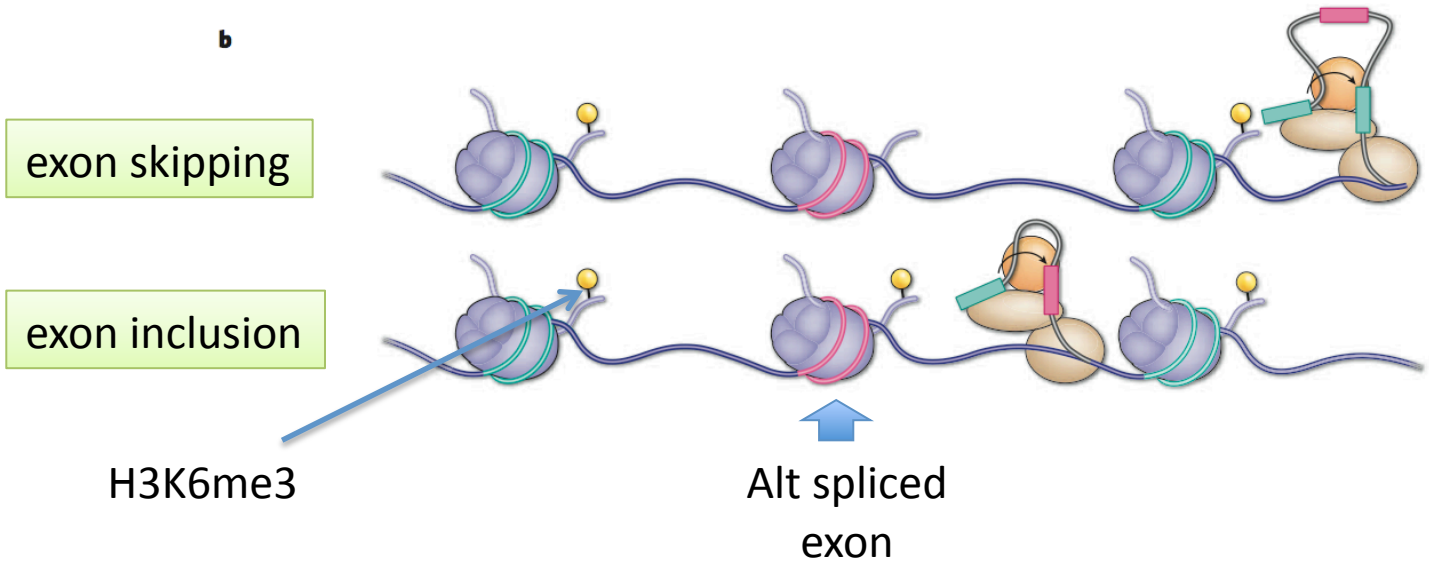

which either splice site is used. **b**, Interplay between transcription elongation rate, chromatin structure and histone modifications, and their impact on alternative splicing. A hypothetical gene is depicted; three exons are shown packaged into nucleosomes. The constitutively transcribed exons (green) are packaged into nucleosomes that constitutively contain histone H3 that is trimethylated (yellow) at lysine residue 36 (H3K36me3). In cells that do not include the alternative exon (pink) in the mRNA, the nucleosomes packaging this exonic DNA do not contain H3K36me3 (top). We propose that when RNA polymerase (brown ovals; spliceosome, orange circle) transcribes a gene with this chromatin configuration (with the pre-mRNA shown here in grey), it traverses the alternative exon rapidly, and this exon is not tethered to the RNA polymerase and, accordingly, sequence corresponding to this exon is not included in the mature mRNA (the splicing of exonic sequences is indicated by curved arrows). By contrast, when the nucleosome that is packaging the alternative exon contains H3K36me3, this slows the progress of the RNA polymerase, allowing it to capture the exon, resulting in the inclusion of sequence corresponding to this sequence in the mature mRNA (bottom).

Alternative splicing and tissue specificity

- AS regulation by constitutive splicing factors

  subtle changes in the concentration of antagonistic splicing factors (hnRNPs and SR proteins)

- AS regulation by post-translational modification of splicing factors (phosphorylation)
- AS regulation by tissue specific splicing factors

Alternative splicing and tissue specificity

- AS regulation by tissue specific splicing factors

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*
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.

Readings

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- Nilsen TW, Graveley BR. Expansion of the eukaryotic proteome by alternative splicing. Nature. 2010 Jan 28;463(7280):457-63.
- Ule J, Jensen KB, Ruggiu M, Mele A, Ule A, Darnell RB. CLIP identifies Nova-regulated RNA networks in the brain. Science. 2003 Nov 14;302(5648):1212-5.
- Anna Corrionero and Juan Valcarcel Molecular. Cell 36, December 24, 2009 (News and Views on CLIP assays)
- Alberto Kornblihtt. Promoter usage and alternative splicing . Current Opinion Cell Biol 2005 17:262-268.
- Nature Structural & Molecular Biology 16, 902 - 903 (2009)
  When chromatin meets splicing Kornblihtt A. et al
- Nature. 2008 Nov 27;456(7221):464-9
  HITS-CLIP yields genome-wide insights into brain alternative RNA processing. Licatalosi DD, et al (The CLIP identify NOVA as regulator of Alterantive PolyAdenilation)

In red those papers that are part of the exam (i.e. one question will be to comment a figure)