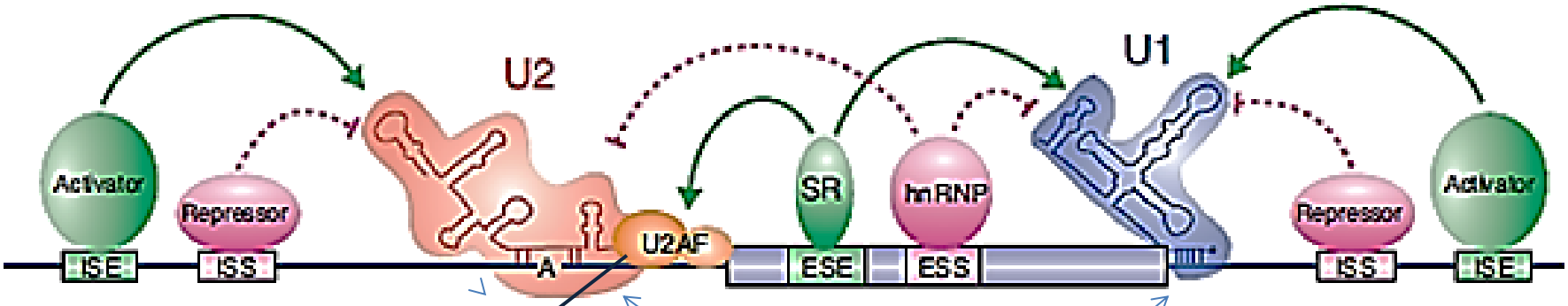
Ch4 - Lesson 2.2

- Tissue-specific Splicing Regulators
- RBPs

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

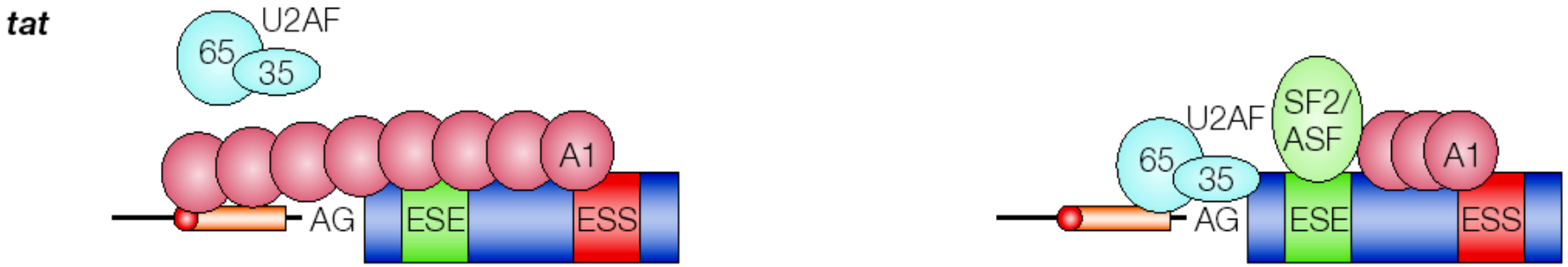


The permanence of U2AF with U2 snRNP is questioned

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

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

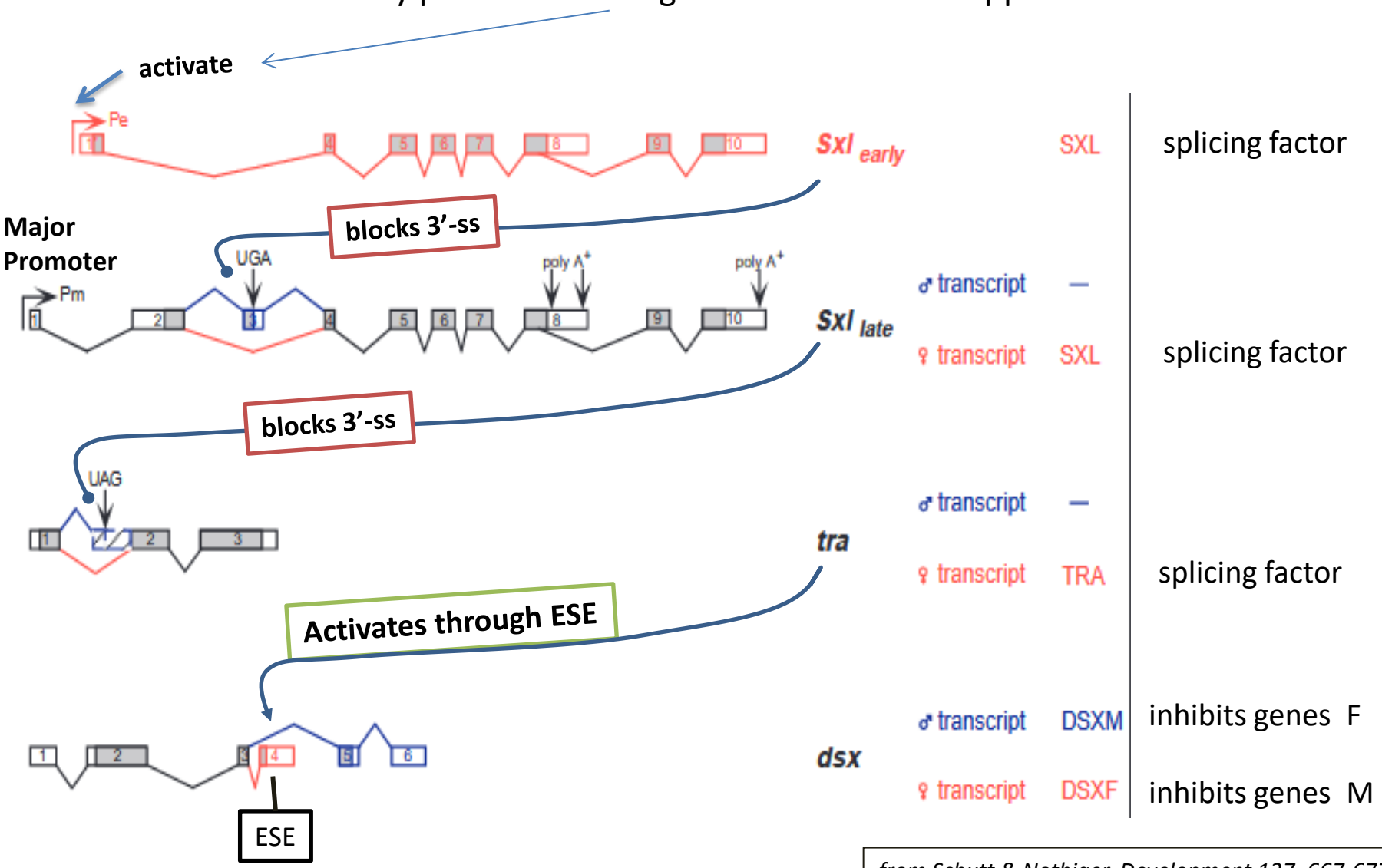
model - competition between SR proteins and hnRNP. (hnRNP A1 multimerizes)



EXAMPLE: Inclusion of **exon 3 of HIV1 *tat*** pre-mRNA is determined by the nuclear ratio of specific (hnRNP) and SR proteins.

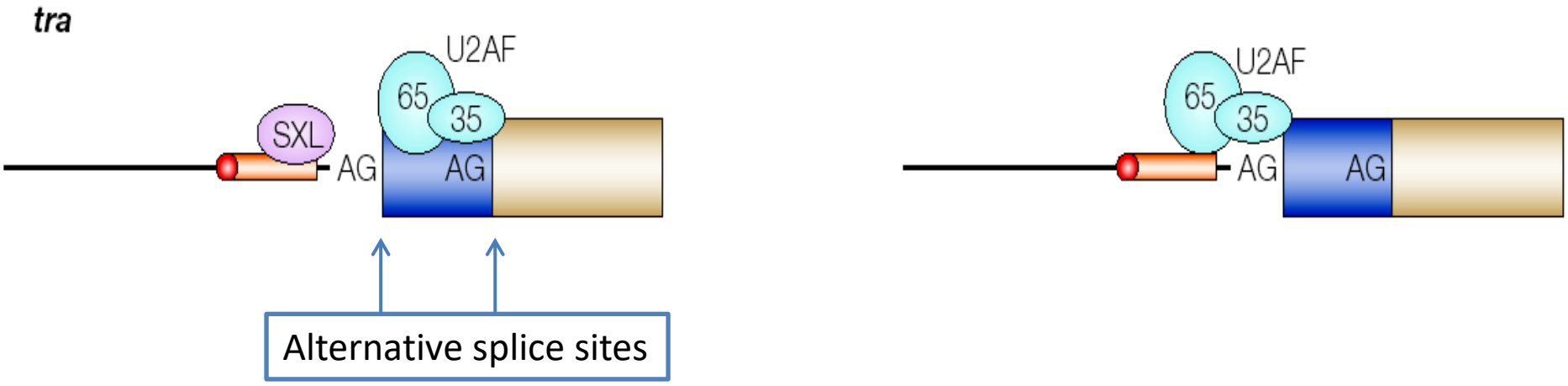
Propagative multimerization of hnRNPA1 from a high-affinity exon splicing silencer (ESS) is sterically blocked by the interaction of SF2/ASF with the upstream ESE.

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.



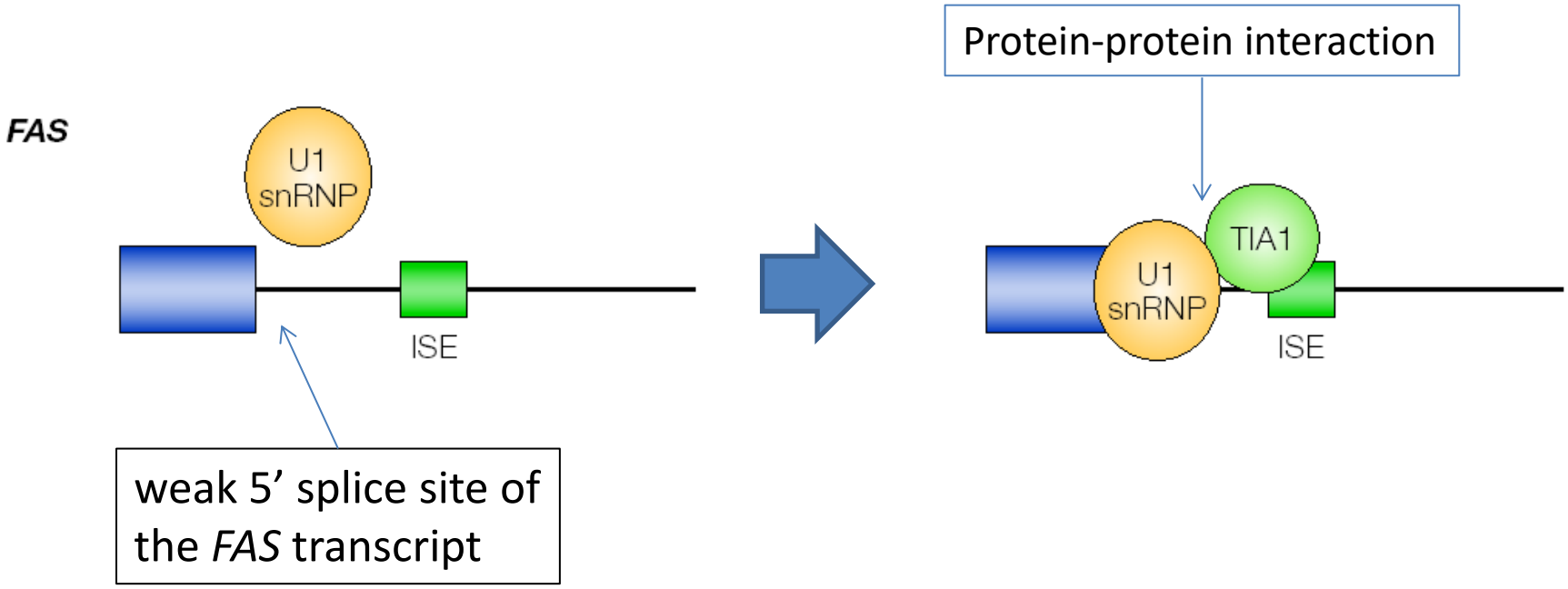
from Schutt & Nothiger, Development 127, 667-677 (2000)

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



Tissue-specific Alternative 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*

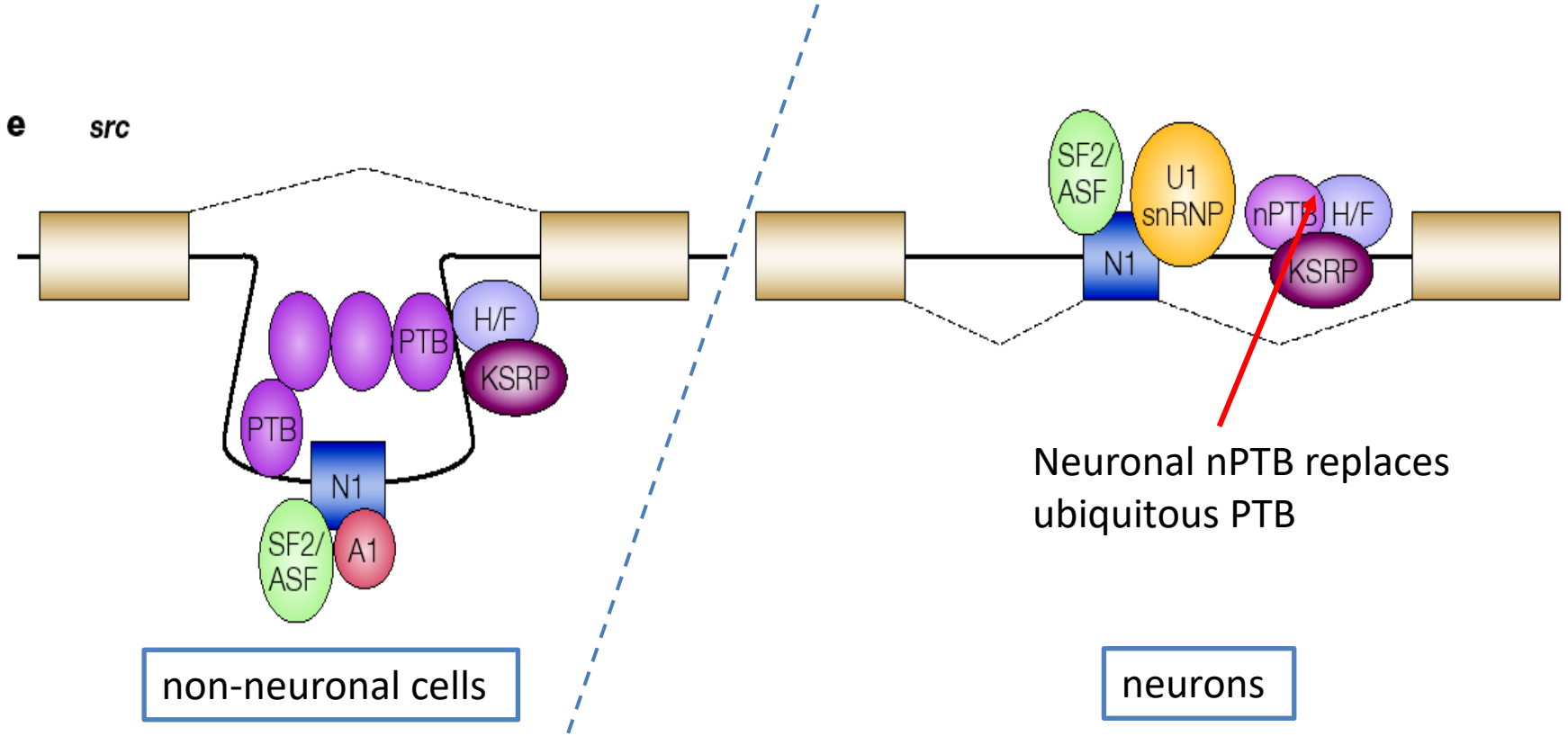
Chen & Manley 2009. Nat Rev Mol Cell Biol., 10:741.

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



N1 exon splicing in the *src* transcript.
combinatorial control by cooperation and antagonism between numerous positively and negatively acting factors.
KSRP, KH-type splicing regulatory protein;
nPTB, neural polypyrimidine tract binding protein.

EXAMPLE

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

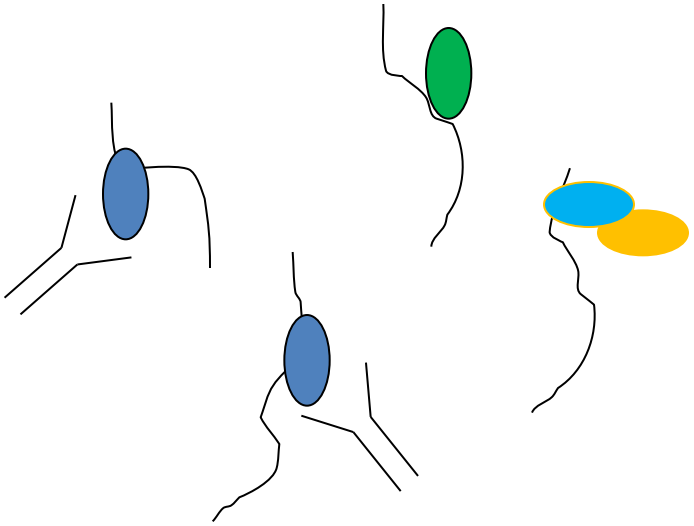
ARTICLES

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

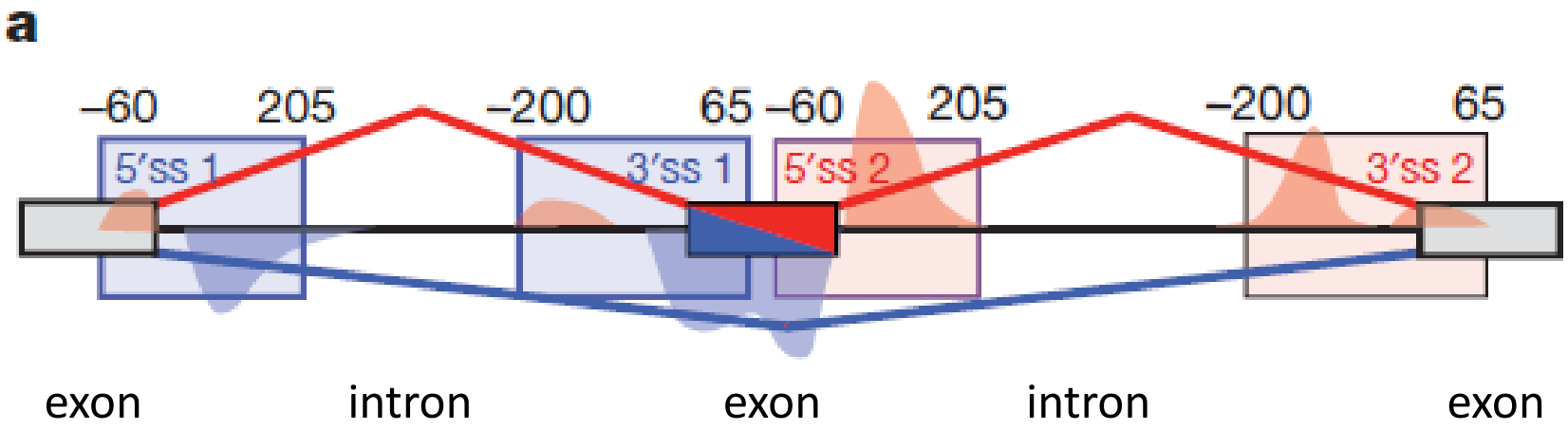
CLIP

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

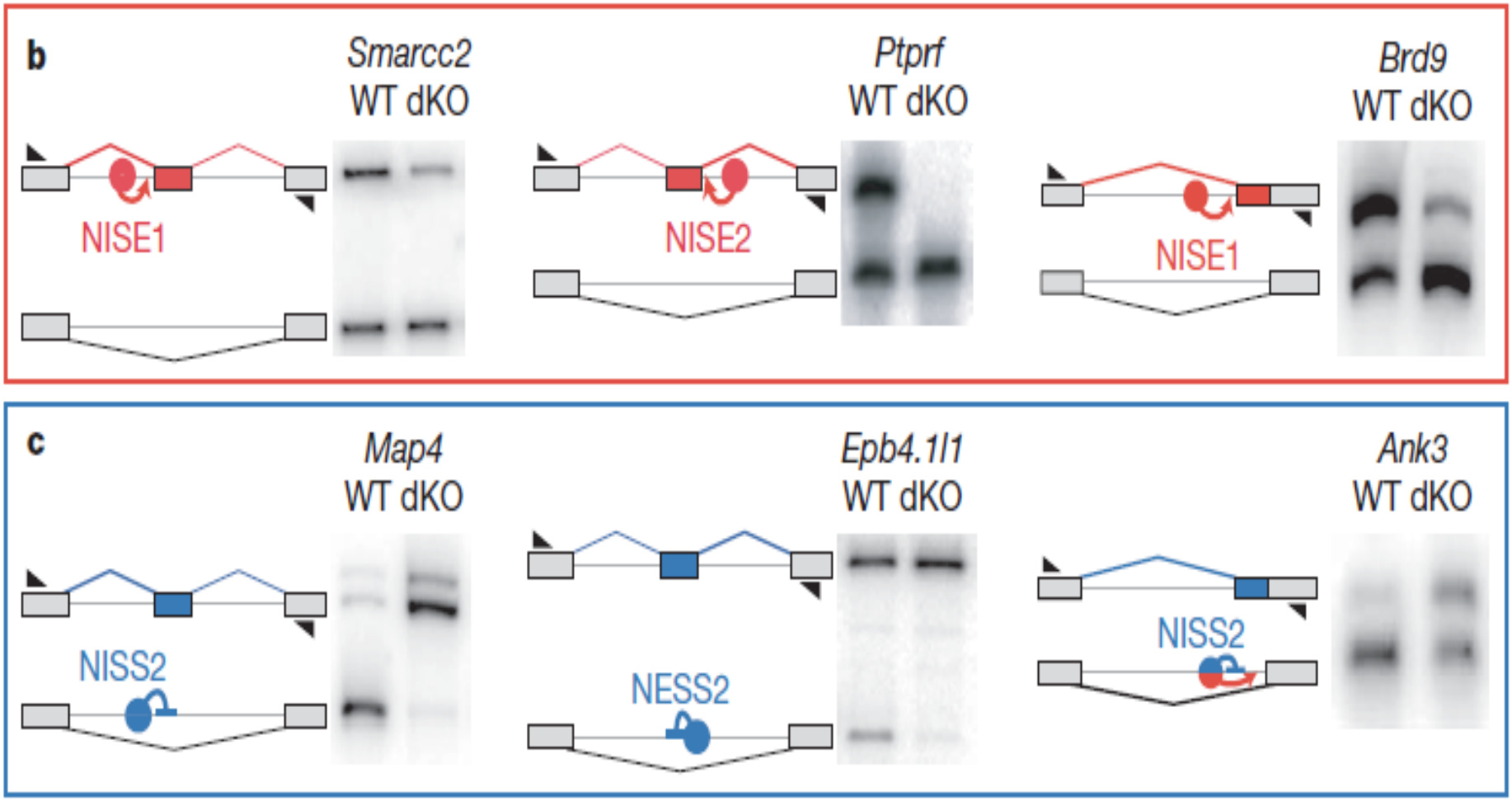


A generic pre-mRNA showing the four regions that define the Nova–RNA binding map (the start and end of each region is labelled by a nucleotide distance to the splice site).

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

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[2]Cell and Molecular Biology Graduate Group
[3]Department of Pharmacology, Institute for Translational Medicine and Therapeutics
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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.

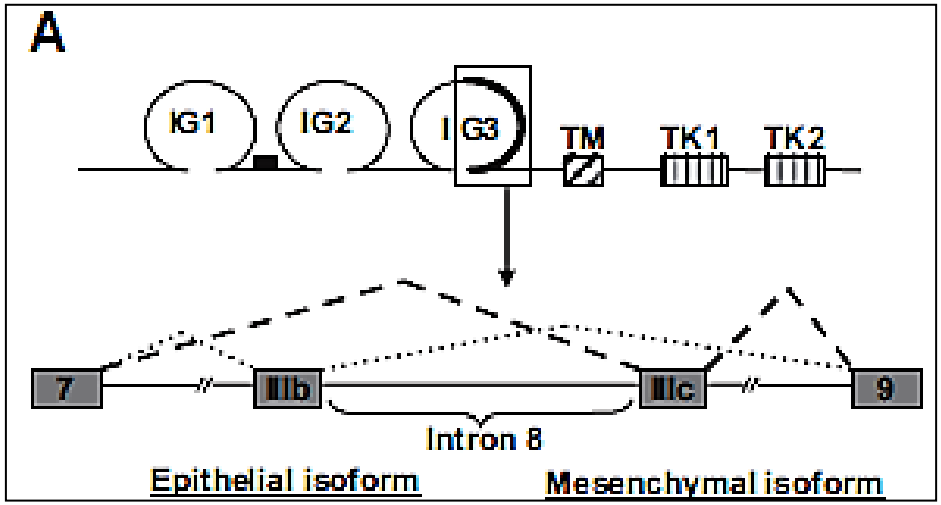
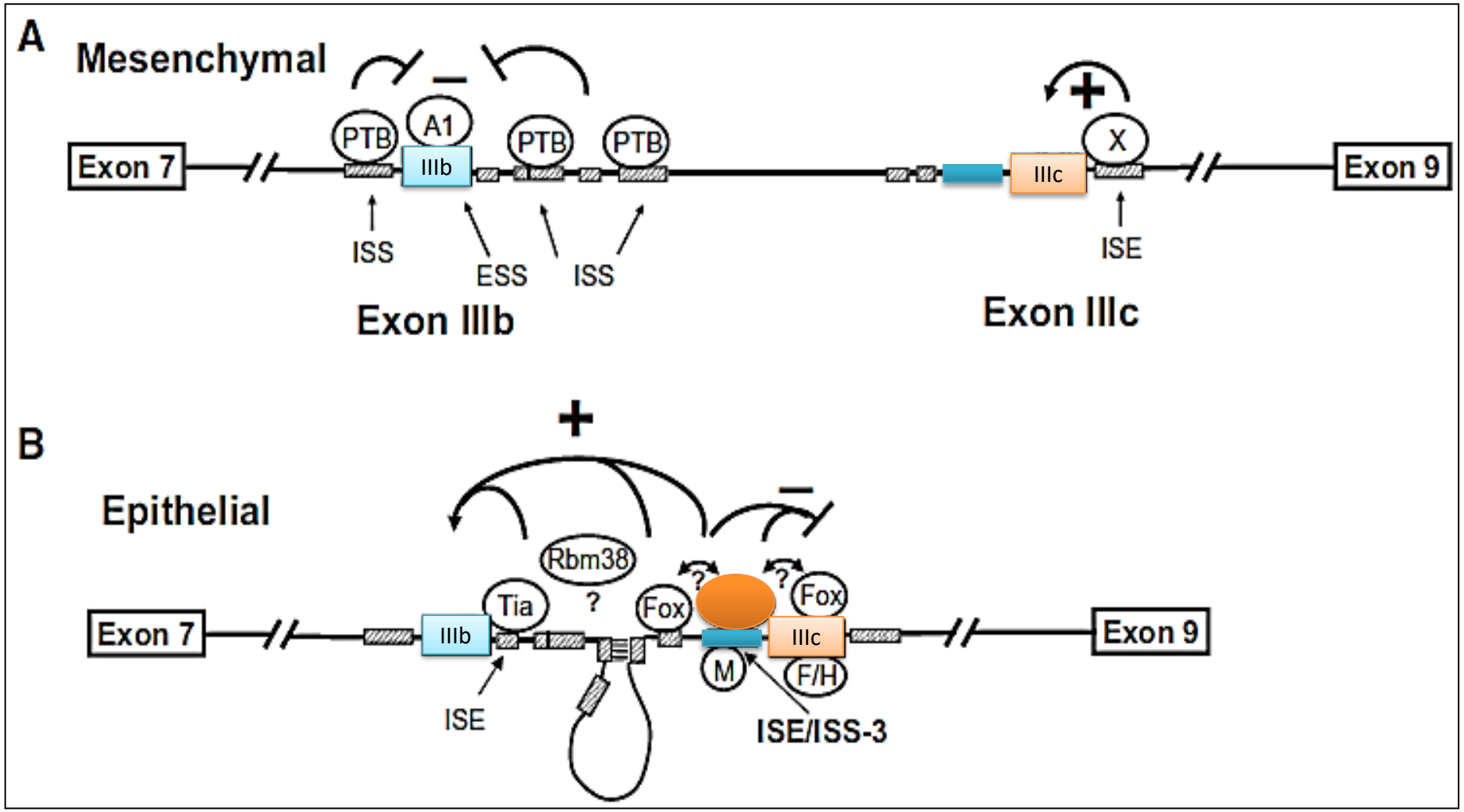


Table 1
Ligand-binding pattern of FGFR IIIb and IIIc isoforms [8].

Receptor	Ligands binding	
	IIIb	IIIc
FGFR2	FGF1, FGF3, FGF7, FGF10, FGF22	FGF1, FGF2, FGF4, FGF5, FGF6, FGF8, FGF9, FGF16, FGF17, FGF18, FGF20, FGF21, FGF23

In epithelial cells (studied here PNT2s are normal epithelial cells from human prostate) **FGFR2 IIIb** is predominant, wheras in mesenchymal cells (here hMSCs) the **IIIc form** is predominant

from Holzmann et al., 2012, modified



IIIb

ESS

Espr

IIIc

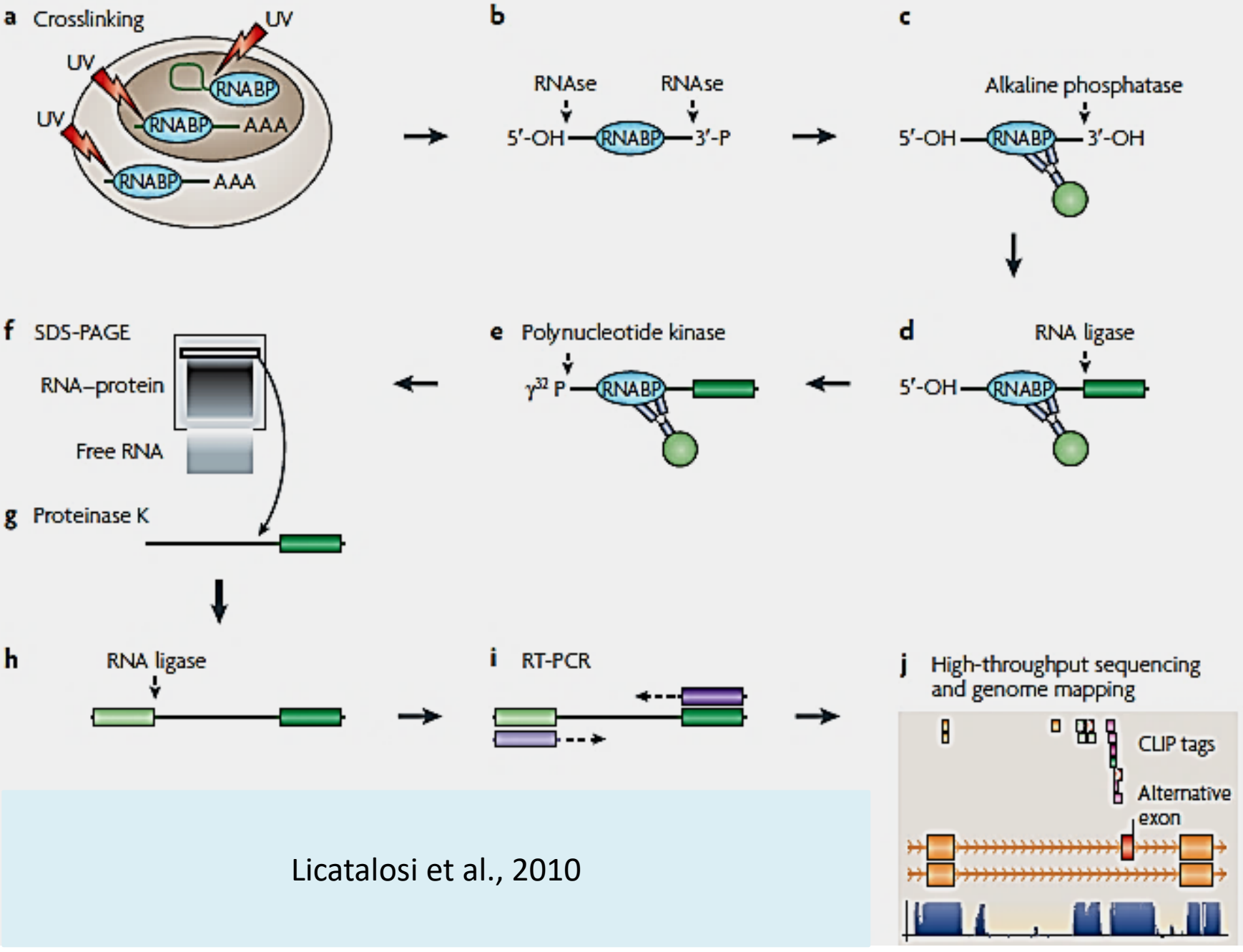
2xESE

RNA binding proteins (RBP) represents one of the most intensive field of research today

(More than 700 proteins annotated to this cathegory)

Once Your Favorite Splicing Regulator is identified, you can look for:

- The RNA transcripts that are bound by YFSR
- the RNA element recognized by YFSR
- the effect of YFSR on the RNA it binds to



Licatalosi et al., 2010