V26 Regular vs. alternative splicing

- **Regular splicing**

  mechanistic steps

  recognition of splice sites

- **Alternative splicing**

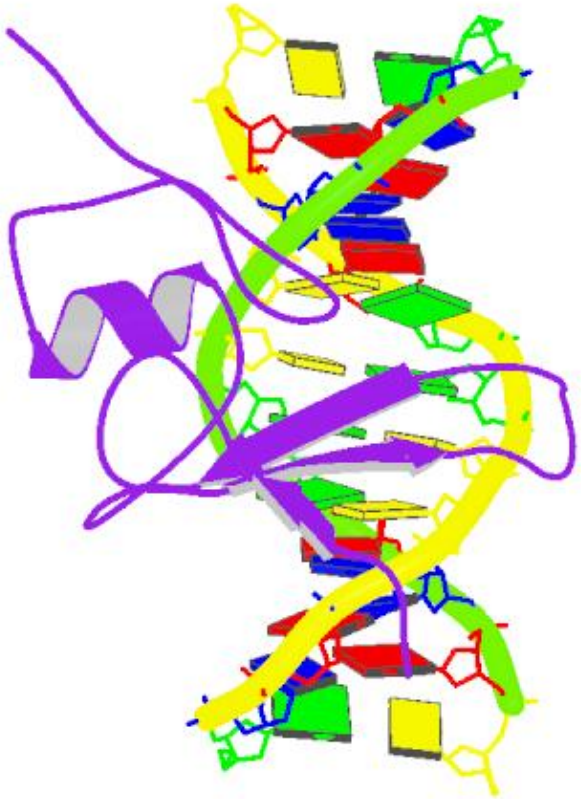
  different mechanisms

  how frequent is alternative splicing?

- Effect of alternative splicing on **protein – protein interactions**

- Interplay of **alternative splicing** and **epigenetic modifications**

Our contact with splicing:
MBD2 recognizes methylated cytosines



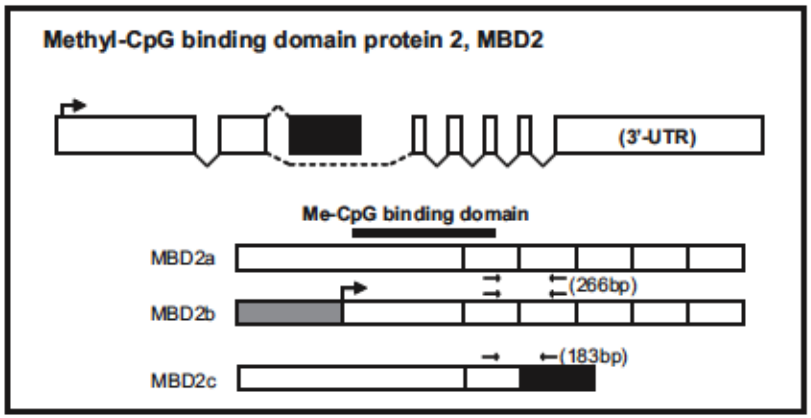
Ohki et al. (2001) Cell **105**: 487-497

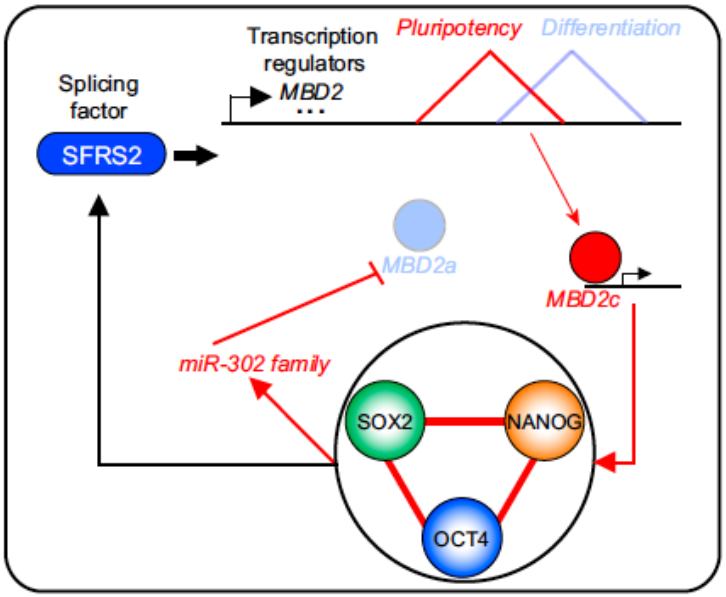
MBD2 is alternatively spliced and then plays a role for maintenance of pluripotency

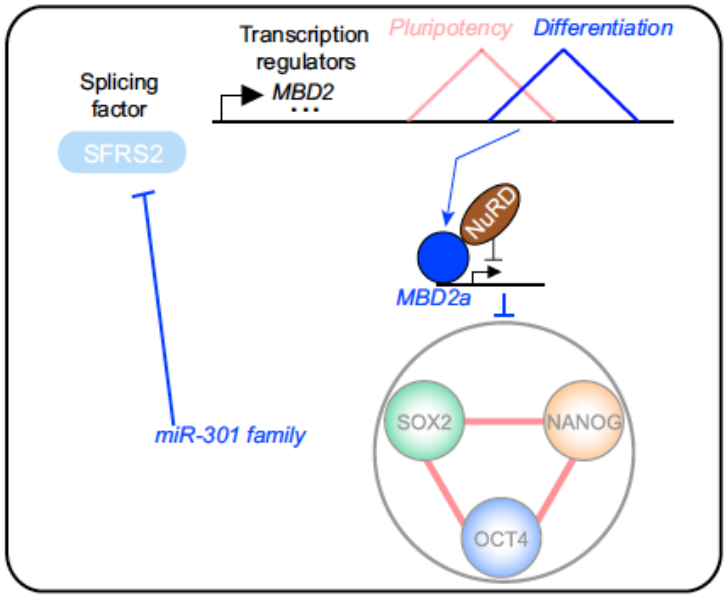
Cell Stem Cell
Short Article

Alternative Splicing of MBD2 Supports Self-Renewal in Human Pluripotent Stem Cells

Yu Lu,[1,2,3,13] Yuin-Han Loh,[2,4,5,13] Hu Li,[6,7,8,13] Marcella Cesana,[2,4] Scott B. Ficarro,[1,2,9] Jignesh R. Parikh,[9,10] Nathan Salomonis,[11] Cheng-Xu Delon Toh,[5] Stelios T. Andreadis,[12] C. John Luckey,[3,14] James J. Collins,[6,7,14] George Q. Daley,[2,4,14,*] and Jarrod A. Marto[1,2,9,14,*]
[1]Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA 02115, USA
[2]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA

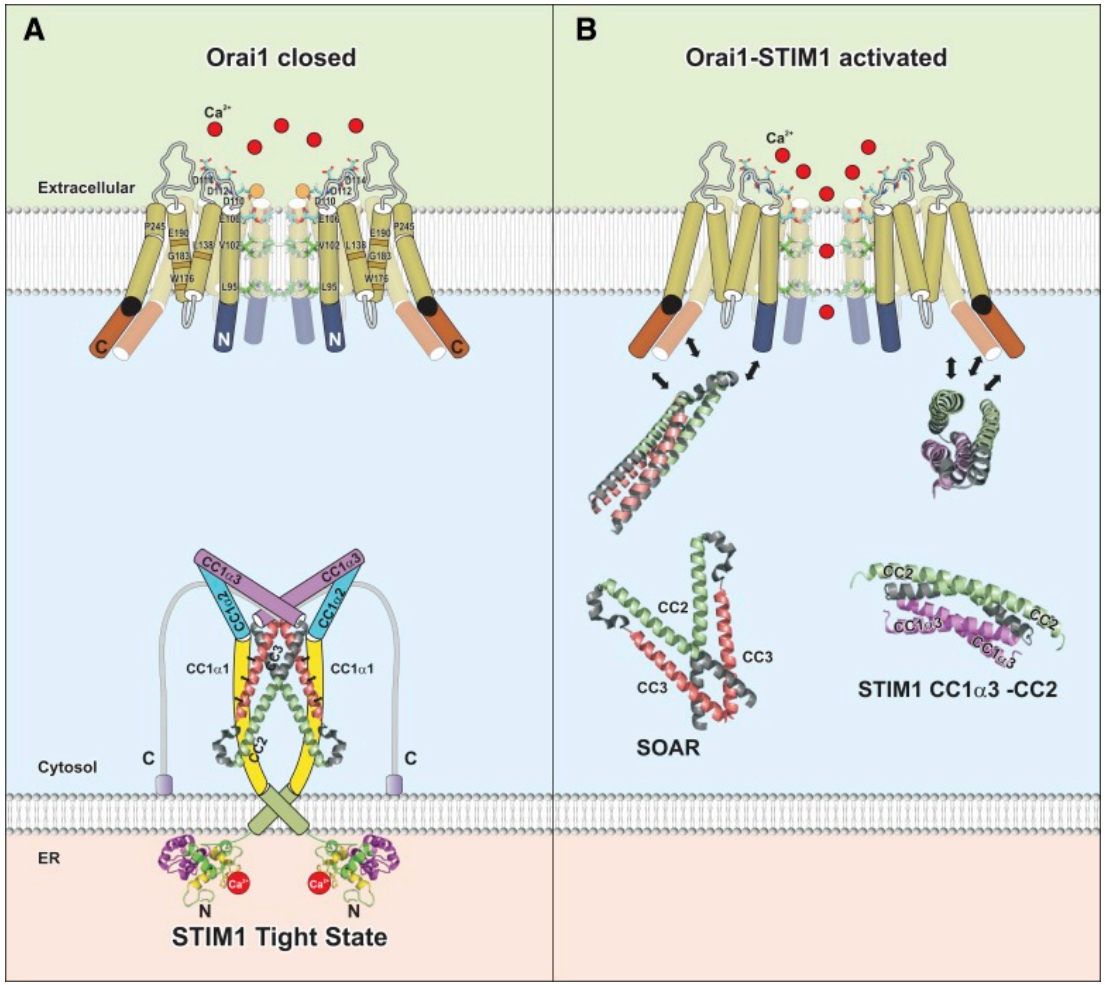






STIM: Orai channels

STIM proteins regulate **store-operated calcium entry** (SOCE) by sensing Ca^{2+} concentration in the ER and forming oligomers to trigger Ca^{2+} entry through plasma membrane-localized Orai1 channels.



Derler et al. Am J Physiol Cell Physiol. (2016) 310: C643–C662.

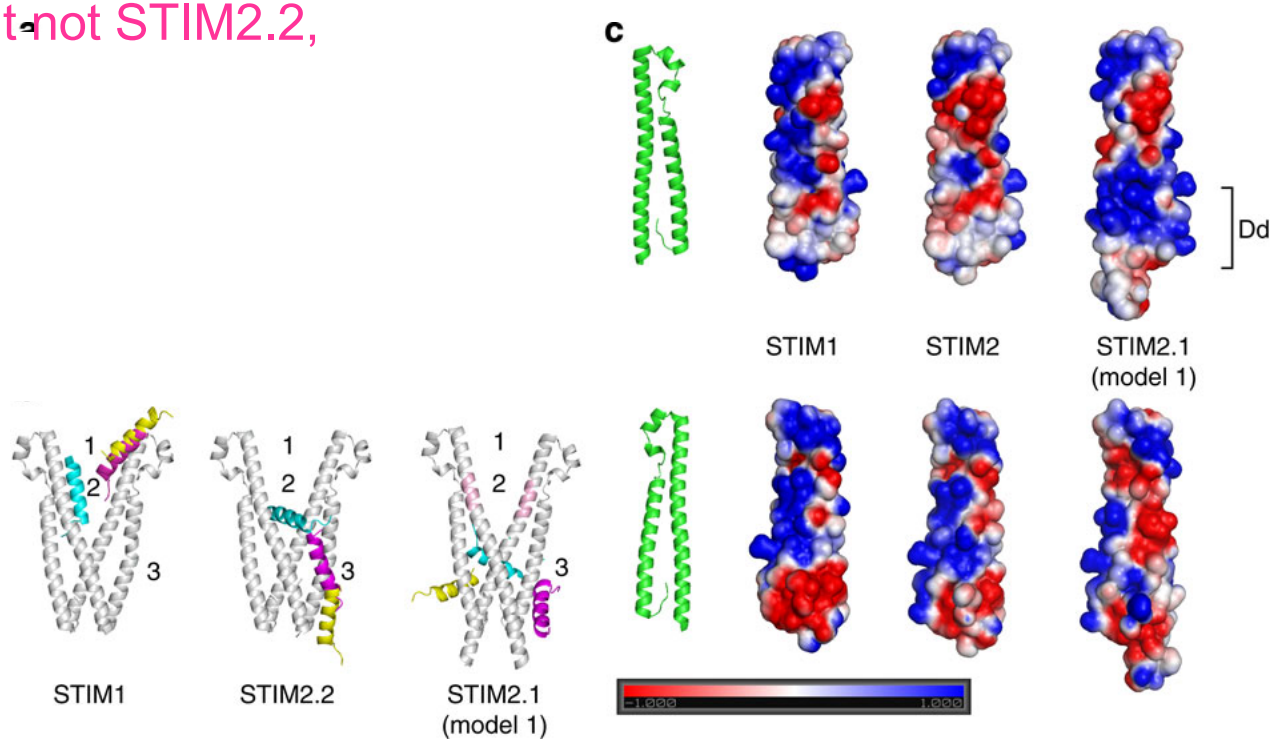
Alternative splicing may affect PP interactions: STIM2 splice variant

Niemeyer and co-workers characterized a *STIM2* splice variant which retains an additional 8-amino acid exon in the region encoding the channel-activating domain.

STIM2.1 knockdown **increases** SOCE in naive CD4$^+$ T cells, whereas knockdown of STIM2.2 **decreases** SOCE.

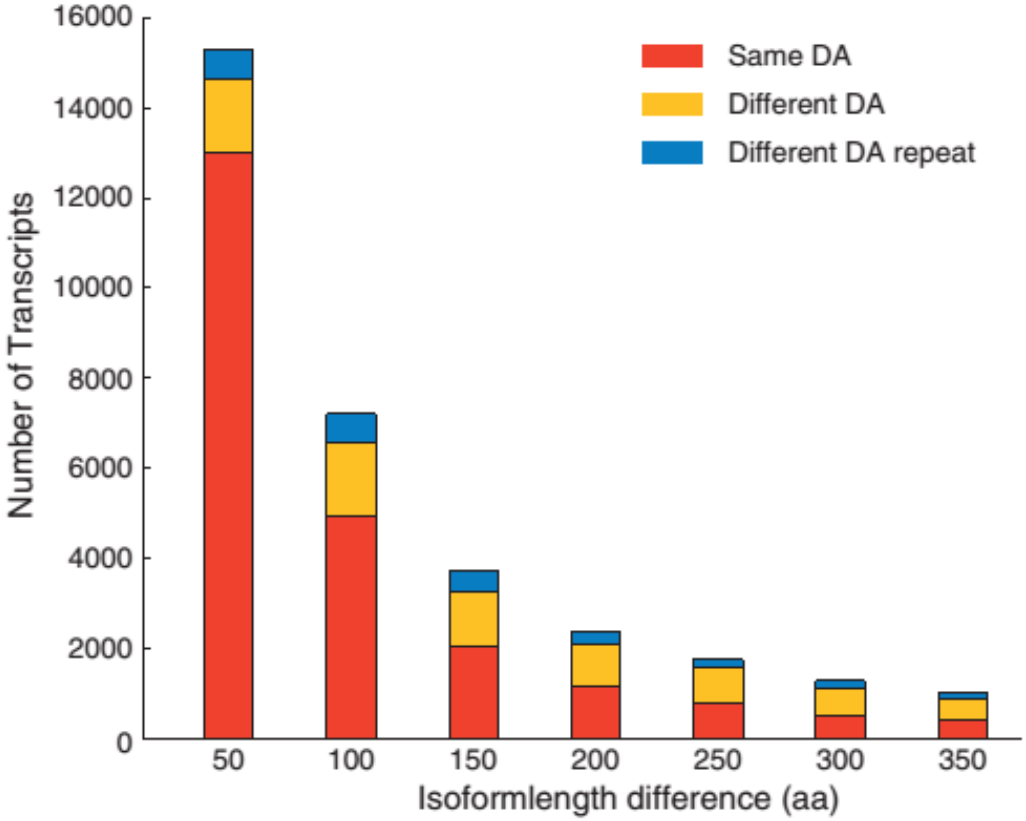
Overexpression of STIM2.1, but not STIM2.2, decreases SOCE.

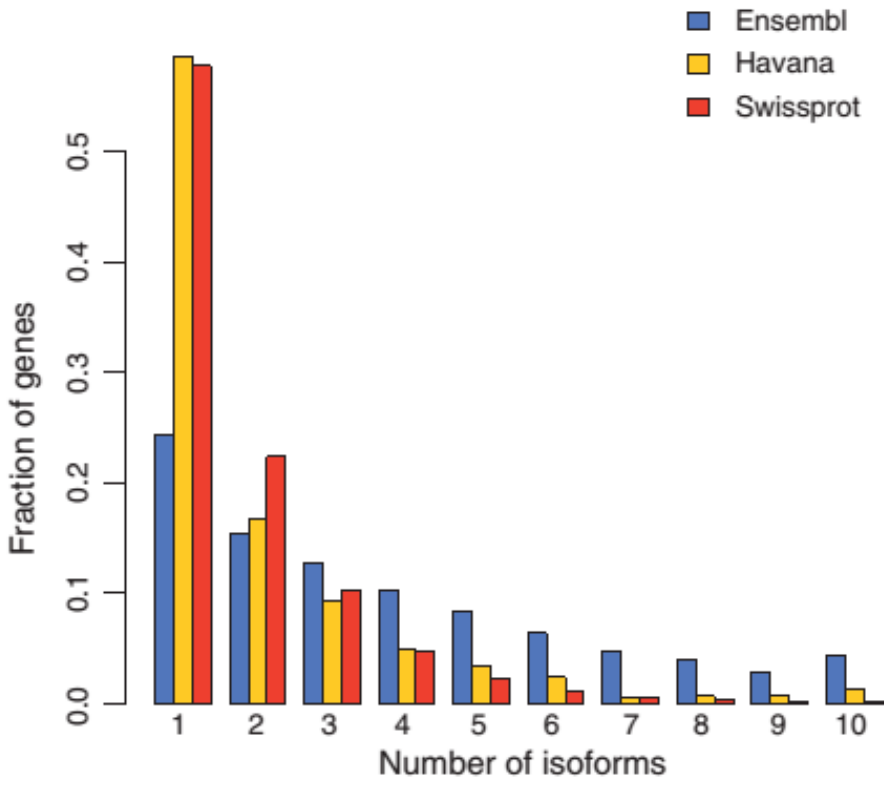
STIM2.1 interaction with Orai1 is impaired and prevents Orai1 activation.



Miederer, ..., Lee, ..., Helms, Barbara Niemeyer, Nature Commun 6, 6899 (2015)

Effect of AS on protein domain architecture



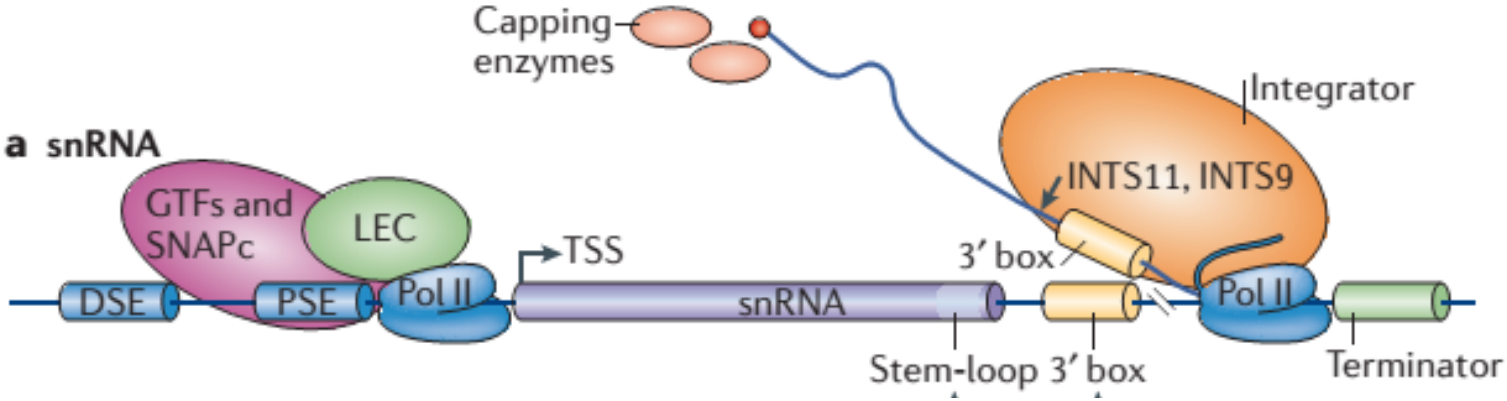


(left) fraction of proteins where the domain architecture (DA) is altered as a result of splicing (based on Swissprot transcripts)

(right) number of isoforms for 3 databases; Ensembl, Vega/ Havana and Swissprot.

Light & Elofsson Curr Opin Struct Biol (2013) 23: 451-458

Transcription + processing of snRNAs and mRNAs



small nuclear RNA (**snRNA**) genes are part of the **spliceosome**.
Shown are *cis*-acting elements and *trans*-acting factors involved in the expression of snRNA genes.

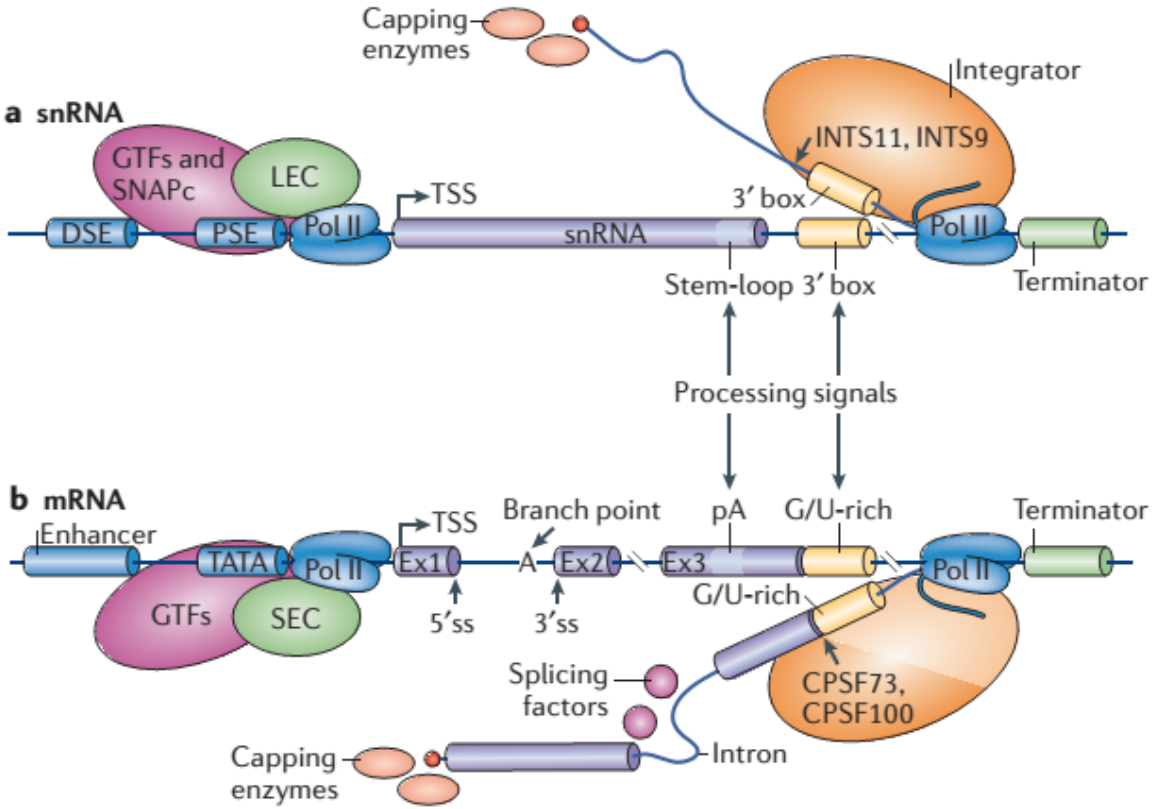
DSE: distal sequence element, and
PSE: proximal sequence element
TSS, transcription start site.

snRNA promoters recruit **the little elongation complex (LEC).**

Initiation of snRNA transcription requires **general transcription factors (GTFs)**, as well as the **snRNA-activating protein complex (SNAPc)**.

Matera & Wang, Nature Rev Mol Cell Biol 15, 108–121 (2014)

Transcription + processing of snRNAs and mRNAs



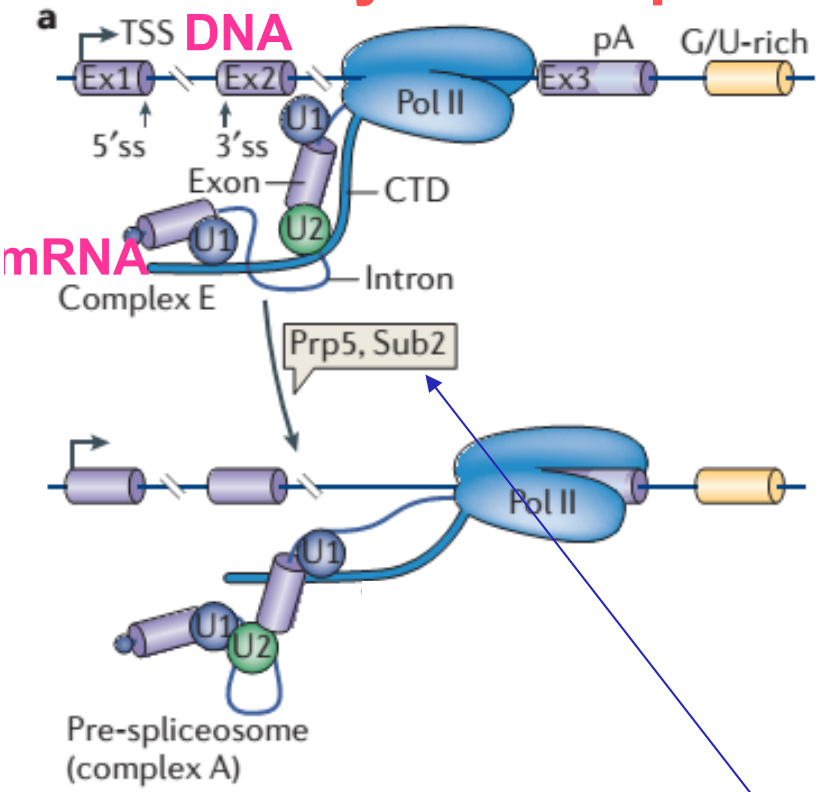
Shown in **(b)** are *cis*-acting elements and *trans*-acting factors involved in the expression of protein-coding mRNA genes.
DSE and **PSE** of snRNAs are roughly equivalent to the **enhancer** and **TATA** box elements, respectively, of mRNA genes.
Ex, exon
pA, polyA signal
ss, splice site.

While snRNA promoters recruit the **LEC**, mRNA promoters recruit the **super elongation complex (SEC)**.

Integrator subunit 11 (INTS11) and **INTS9** have sequence similarities to the mRNA 3´-processing factors **cleavage and polyadenylation specificity factor** 73 kDa subunit (**CPSF73**) and **CPSF100**, respectively.

Matera & Wang, Nature Rev Mol Cell Biol 15, 108–121 (2014)

Assembly of the splicesome + splicing steps of pre-mRNA



Spliceosome assembly takes place at sites of transcription.

The U1 and U2 small nuclear ribonucleoproteins (**snRNPs**) assemble onto the pre-mRNA in a co-transcriptional manner through recognition of the 5´ splice site (5´ss) and 3´ss.

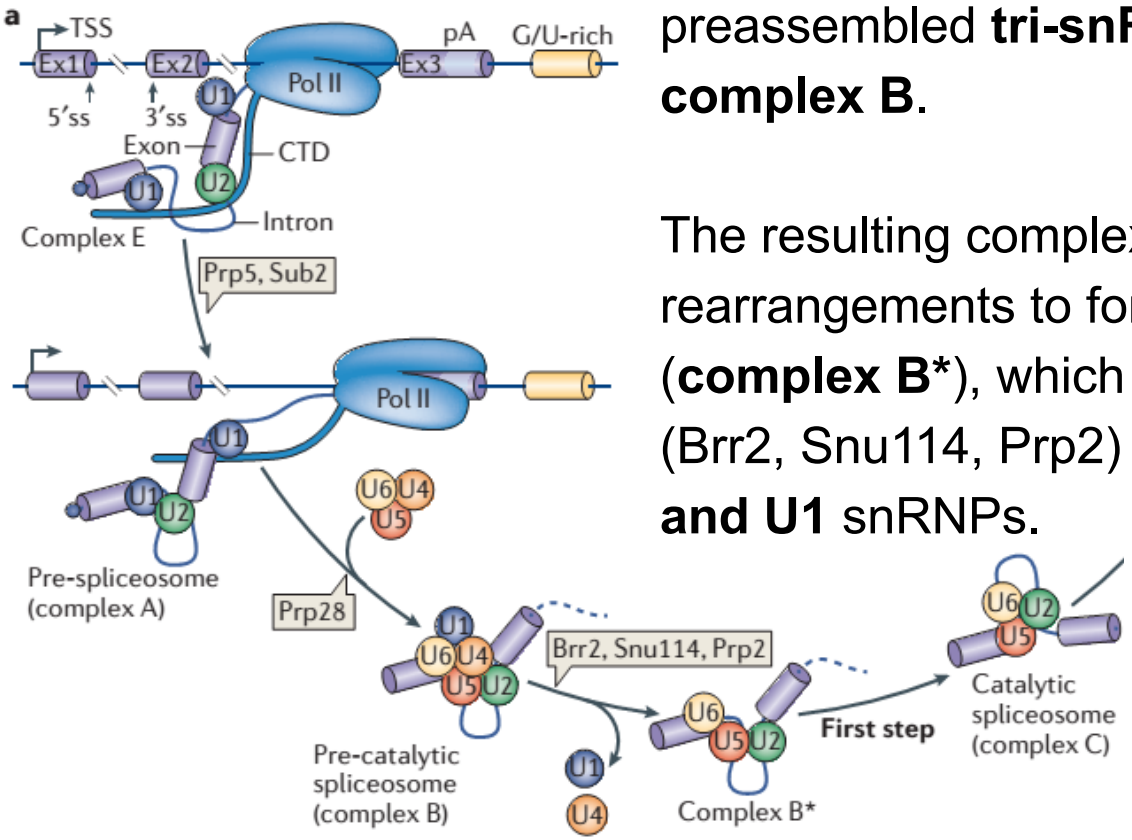
Recognition is mediated by the carboxy-terminal domain (CTD) of polymerase II.

The U1 and U2 snRNPs of different exons then interact with each other to form the pre-spliceosome (**complex A**).

This process is dependent on DExD/H helicases pre-mRNA-processing 5 (**Prp5**) and **Sub2**.

Matera & Wang,
Nature Rev Mol Cell Biol
15, 108–121 (2014)

Assembly of the splicesome + splicing steps



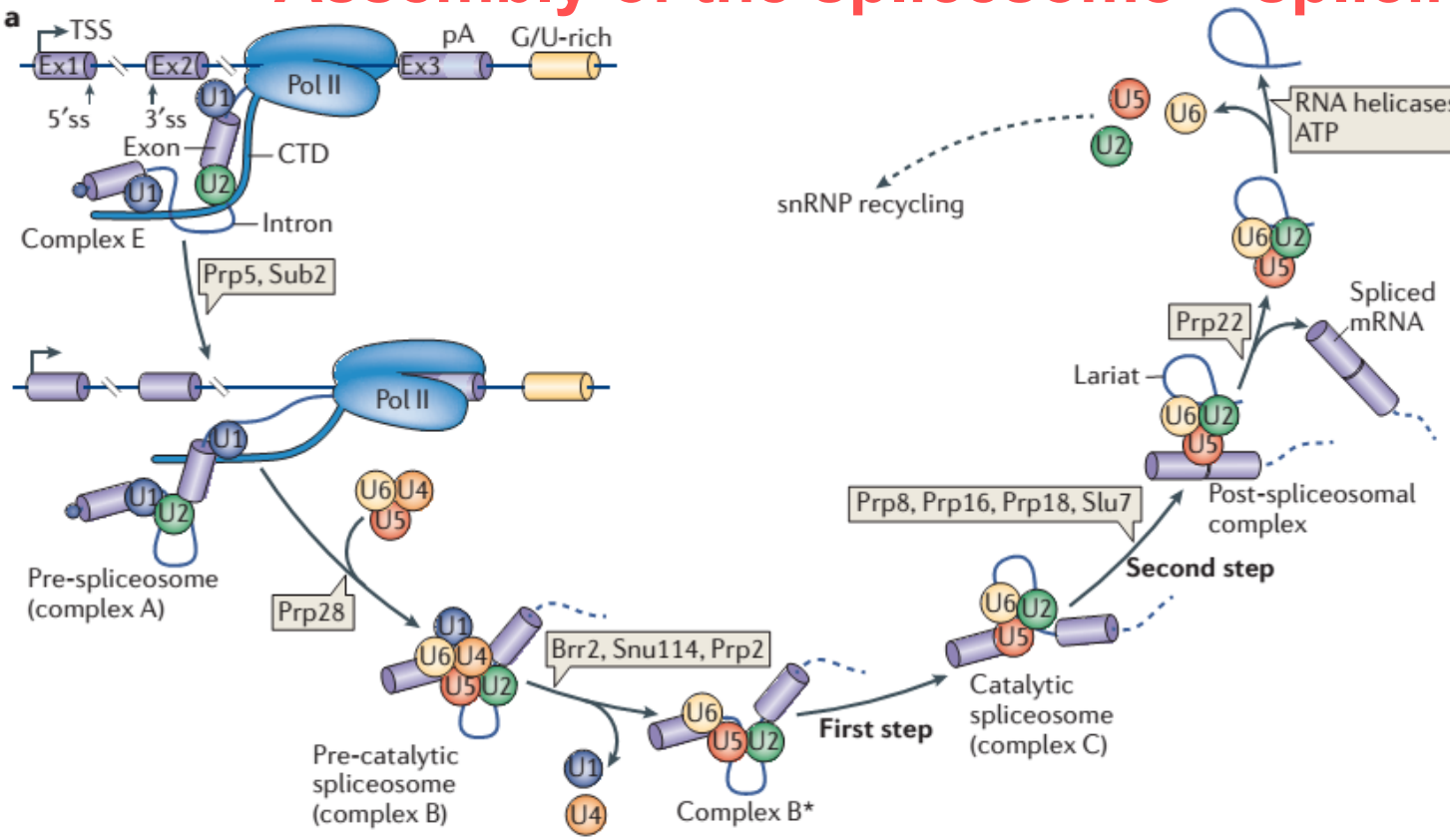
In a subsequent reaction catalysed by Prp28, the preassembled **tri-snRNP U4–U6•U5** is **recruited** to form **complex B**.

The resulting complex B undergoes a series of rearrangements to form a catalytically active complex B (**complex B***), which requires multiple RNA helicases (Brr2, Snu114, Prp2) and results in the **release of U4 and U1** snRNPs.

Complex B* then carries out the first catalytic step of splicing, generating **complex C**, which contains free exon 1 (Ex1) and the intron–exon 2 “lariat intermediate”.

Matera & Wang,
Nature Rev Mol Cell Biol
15, 108–121 (2014)

Assembly of the splicesome + splicing steps



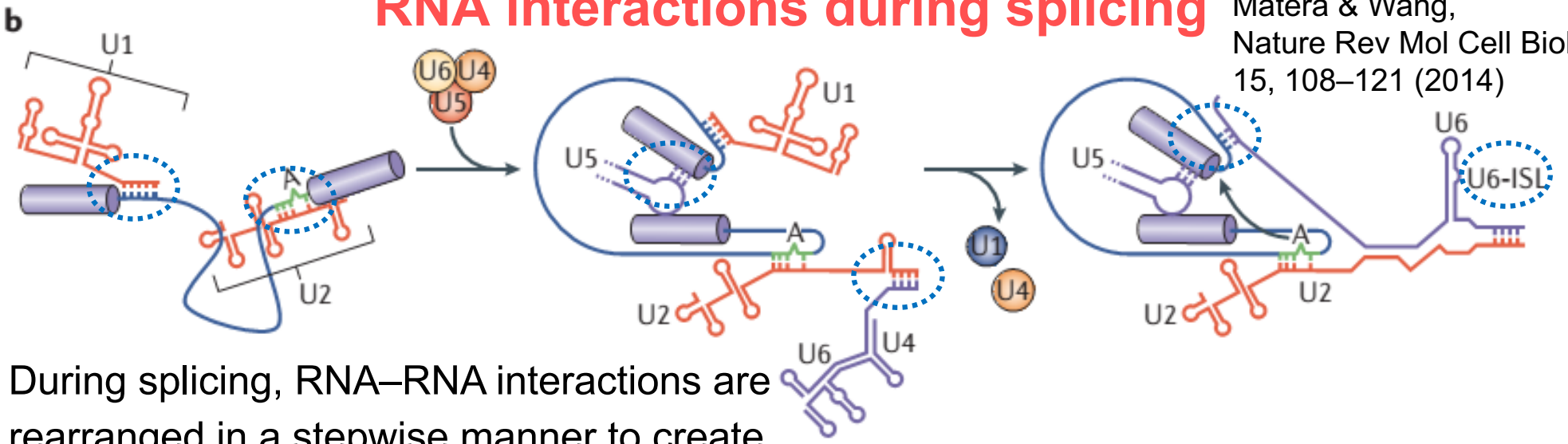
Matera & Wang,
Nature Rev Mol Cell Biol
15, 108–121 (2014)

Complex C undergoes additional rearrangements and then carries out the second catalytic step, resulting in a post-spliceosomal complex that contains the lariat intron and spliced exons.

Finally, the U2, U5 and U6 snRNPs are released from the mRNP particle and recycled for additional rounds of splicing. Release of the spliced product from the spliceosome is catalysed by the DExD/H helicase Prp22.

RNA interactions during splicing

Matera & Wang,
Nature Rev Mol Cell Biol
15, 108–121 (2014)

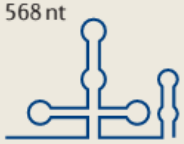
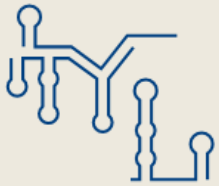


During splicing, RNA–RNA interactions are rearranged in a stepwise manner to create the **catalytic centre** of the spliceosome.

- Initially, U1 and U2 small nuclear RNA (snRNA) pair with the 5´ss and the branch point sequence within **complex A** (the branch point adenosine is indicated by the letter A).
- Subsequently, complex A associates with the U4–U6•U5 tri-snRNP, leading to new base pairs between U2 and U6 snRNA and between U5 snRNA and exonic sequences near the 5´ss.
- The U4 snRNA is disassociated from U6 to expose the 5´ end of U6, which then base pairs with the 5´ss to displace U1 snRNA.
- In the end, an extensive network of base-pairing interactions is formed between U6 and U2, **juxtaposing** the 5´ss and branch-point adenosine for the first catalytic step of splicing. The central region of U6 snRNA forms an intramolecular stem-loop (the U6-ISL), which is essential for splicing catalysis.

Composition of spliceosomal snRNPs

Table 1 | **Composition of major spliceosomal snRNPs***

snRNP	RNA secondary structure‡	Sm proteins	Other core proteins associated with snRNA	Associated proteins
U1	568 nt 	B, D3, G, E, F, D2 and D1	Snp1 (U1-70K), Mud1 (U1A) and Yhc (U1C)	Prp39, Prp40, Prp42, Snu71, Nam8, Snu56 and Urn1
U2	1,175 nt 	B, D3, G, E, F, D2 and D1	Lea1 (U2A′), Msl1 (U2B″), Prp9 (SF3B60), Prp11 (SF3A66), Prp21 (SF3A120), Rds3 (SF3B14B), Snu17 (SF3B14A; also known as p14), Hsh155 (SF3B155), Cus1 (SF3B145), Rse1 (SF3B130), Hsh49 (SF3B49) and Ysf3 (SF3B10)	U2AF35, Mud2 (U2AF65) and Msl5 (SF1; also known as BBP)
U4–U6	160 nt and 112 nt, respectively 	U4: B, D3, G, E, F, D2 and D1; U6: Lsm2–8	Prp3, Prp31, Prp4 and Snu13	
U5	179 nt for short form; 214 nt for long form 	B, D3, G, E, F, D2 and D1	Prp8, Prp6, Prp28, Brr2, Snu114, U5-40K and Dib1	Snu23, Prp38, Prp2, Spp2, Yju2 and Cbc2 (52K)
U4–U6•U5 	U4 and U5: B, D3, G, E, F, D2 and D1 (i.e. two sets); U6: Lsm2–8	Prp3, Prp31, Prp4, Snu13/15.5K, Prp8, Prp6, Prp28, Brr2, Snu114, U5–40K, snRNP27 and Dib1	Snu23, Prp38, Prp2, Spp2, Yju2, Snu66 and Sad1	

Cus1, cold-sensitive U2 snRNA suppressor 1; Lea1, looks exceptionally like U2A 1; Lsm, Sm-like; Msl, Male-specific lethal homologue; nt, nucleotides; Prp, pre-mRNA-processing; Rds3, regulator of drug sensitivity 3; Rse1, RNA splicing and ER to Golgi transport factor 1; Sad1, snRNP assembly-defective 1; SF, splicing factor; Spp2, suppressor of PRP; snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein; Snu, U5 small nuclear ribonucleoprotein component; U2AF, U2 auxiliary factor; Urn1, U2-U5-U6 snRNP, RES complex and NTC-interacting pre-mRNA-splicing factor 1. *The protein composition is primarily based on a mass spectrometric analysis of the yeast spliceosome[203]; certain regulatory factors that are closely associated with the core spliceosome (such as SR proteins) are not included. Proteins are listed using the budding yeast nomenclature unless there is no known yeast homologue. In certain cases, the common name of a metazoan homologue is also included in brackets. ‡The snRNA lengths are based on yeast transcripts.

Matera & Wang,
Nature Rev Mol Cell Biol
15, 108–121 (2014)

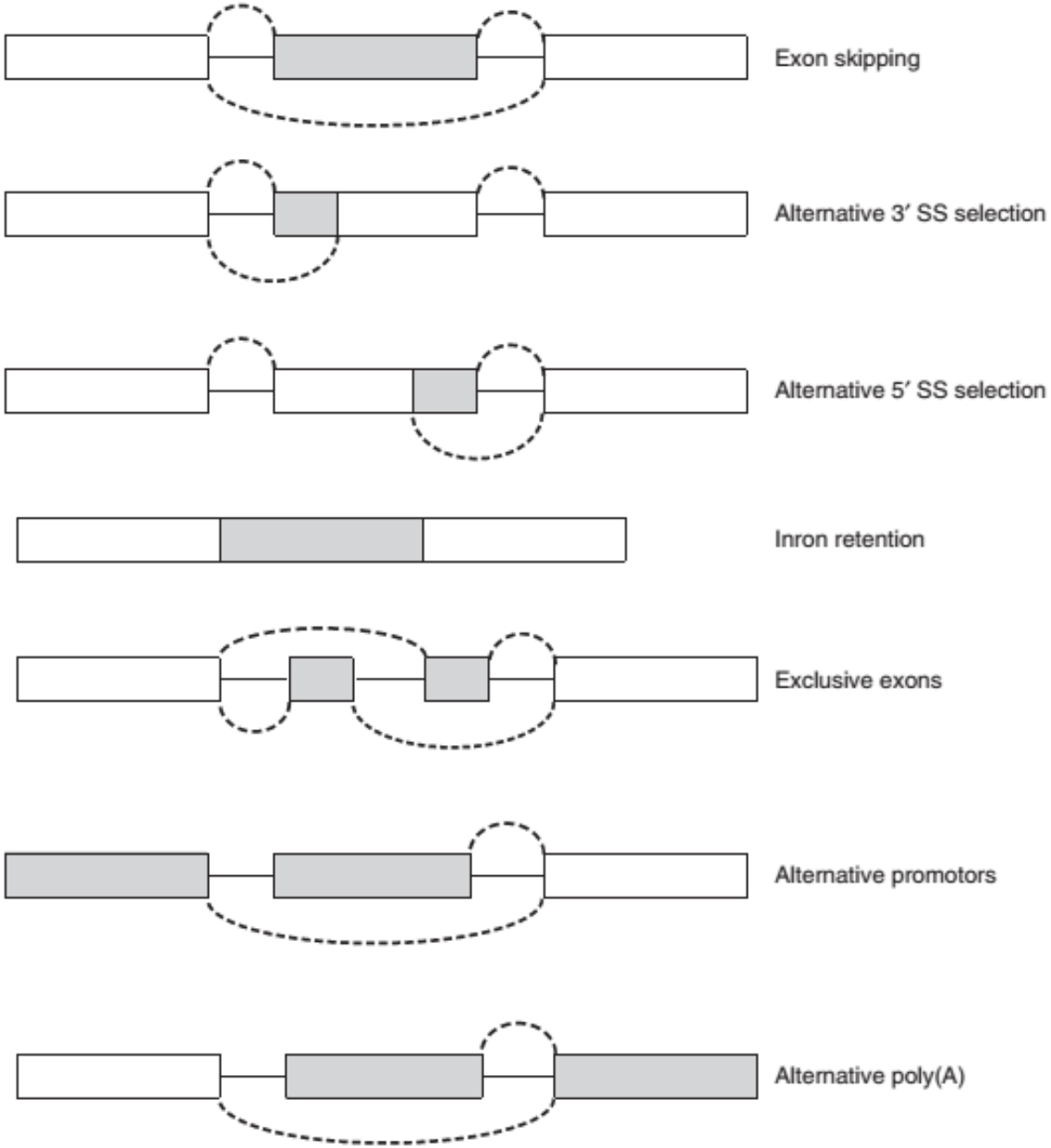
most important sequence patterns related to a splicing

The splicing starts with an **AG site** and is preceded with a non-AG (**pyrimidine rich**) region preceded by the branch point that includes an **Adenosine** residue.

The 5’ end of the intron contains an almost invariant **GU sequence**.

Light & Elofsson Curr Opin Struct Biol (2013) 23: 451-458

Mechanisms of alternative splicing



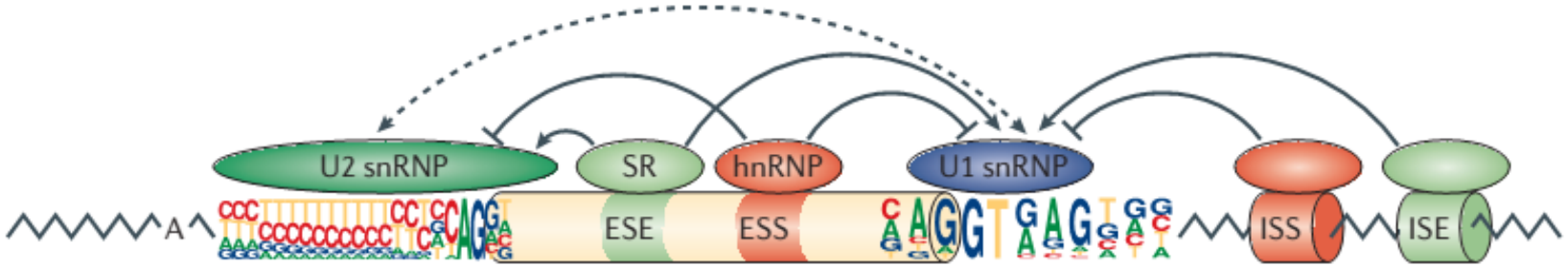
Gray boxes: exons

White boxes: introns

The (gray) protein coding regions are excluded/included in different transcripts.

Light & Elofsson Curr Opin Struct Biol (2013) 23: 451-458

Regulation of alternative splicing



Splice site choice is regulated through ***cis*-acting splicing regulatory elements** (SREs) and ***trans*-acting splicing factors**.

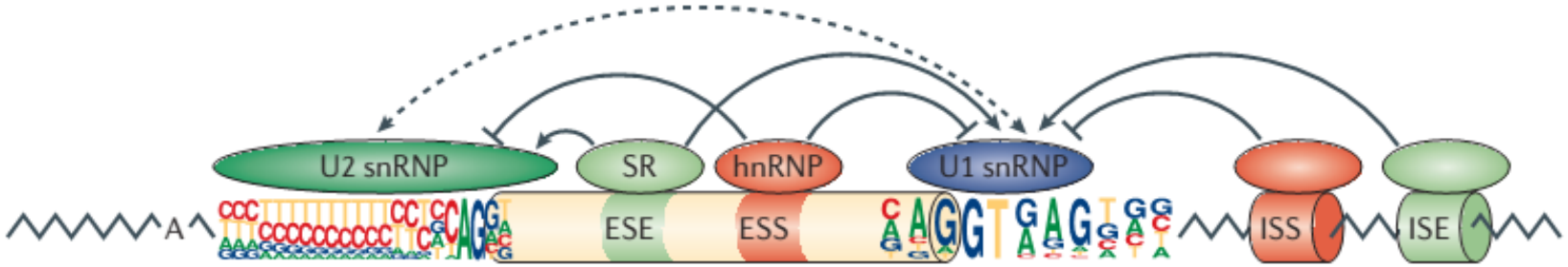
Shown **sequence motifs** are the consensus motifs of splice sites.

The height of each letter represents the nucleotide frequency in each position.

The dashed arrow represents the formation of the exon definition complex.

Matera & Wang,
Nature Rev Mol Cell Biol
15, 108–121 (2014)

Regulation of alternative splicing



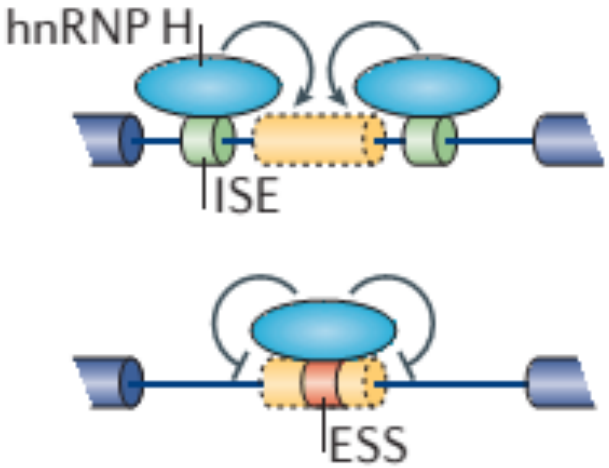
On the basis of their relative locations and activities, **splicing regulatory elements** are classified as

- exonic splicing enhancers (**ESEs**),
- intronic splicing enhancers (**ISEs**),
- exonic splicing silencers (**ESSs**) or
- intronic splicing silencers (**ISSs**).

Sequence motifs cannot exert their effects directly → these SREs specifically recruit **splicing factors** to promote or inhibit recognition of nearby splice sites:
- **SR proteins** recognize ESEs to **promote splicing**,
- heterogeneous nuclear ribonucleoproteins (**hnRNPs**) typically recognize ESSs to **inhibit splicing**.

Matera & Wang,
Nature Rev Mol Cell Biol
15, 108–121 (2014)

Activity of splicing factors and SREs



The activity of splicing factors and *cis*-acting SREs is **context-dependent.**

Oligo-G tracts are recognized by hnRNP H.

(Top) When the oligo-G tracts are located inside an intron, they function as intronic splicing enhancers (ISE) to promote splicing.

(Bottom) When they are located within exons, they function as exonic splicing silencers (ESSs).

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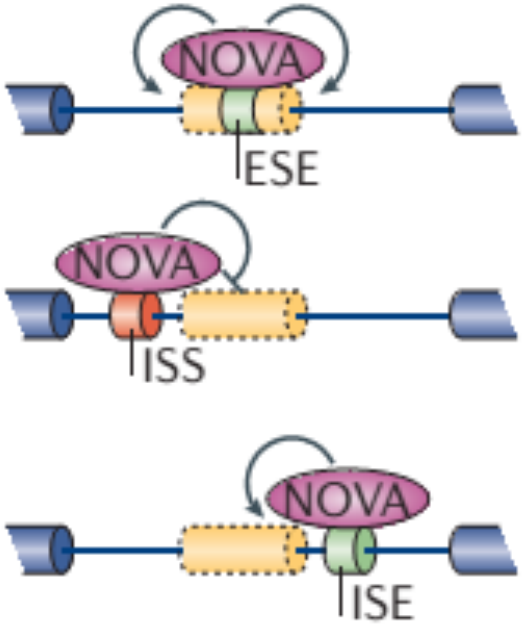
15, 108–121 (2014)

Activity of splicing factors and SREs



YCAY motifs are recognized by neuro-oncological ventral antigen (NOVA).

Y stands for pyrimidine (C/T).

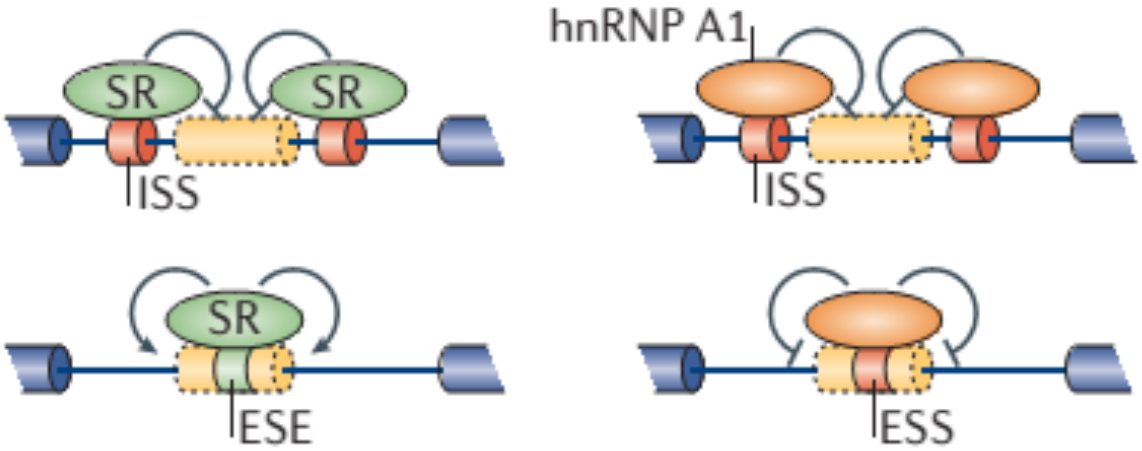
(Top) When YCAY motifs are located inside an exon, they act as ESEs,

(Middle) When they are located in the upstream intron of an alternative exon, they act as ISSs,

(Bottom) When they are located inside an intron, they act as ISEs.

Matera & Wang,
Nature Rev Mol Cell Biol
15, 108–121 (2014)

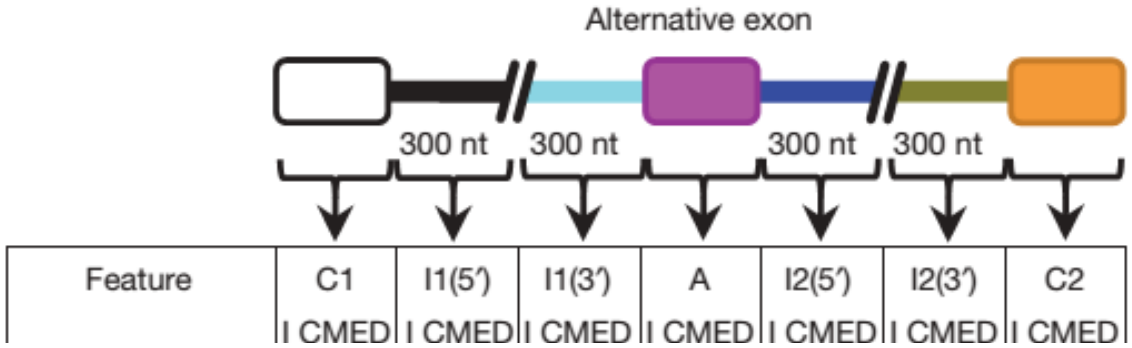
Activity of splicing factors and SREs



Binding sites for SR proteins and hnRNP A1 also have distinct activities when located at different regions on the pre-mRNA.

Matera & Wang,
Nature Rev Mol Cell Biol
15, 108–121 (2014)

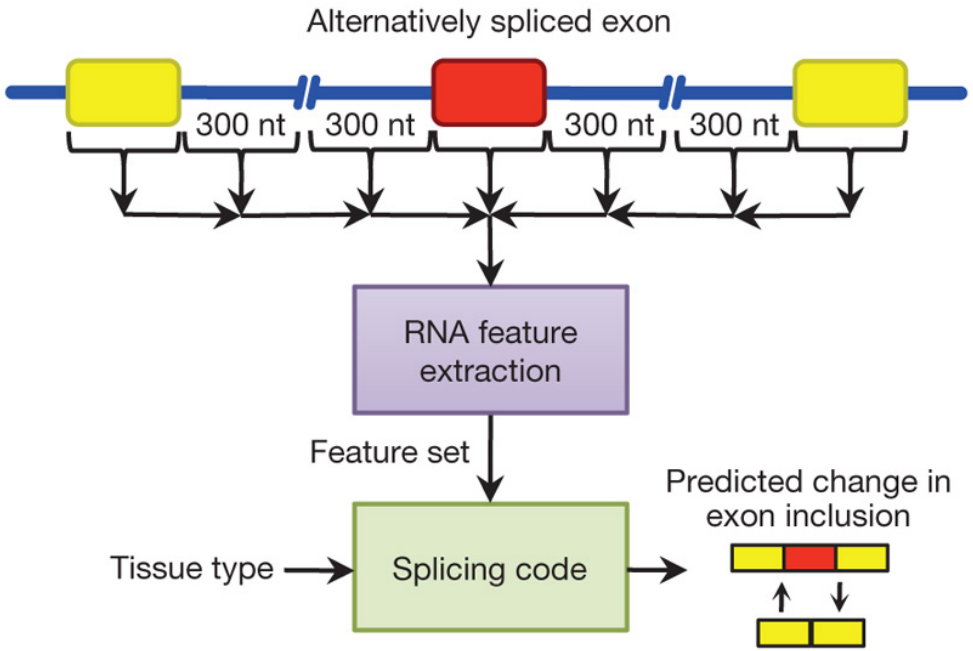
Sequence motifs of the splicing code

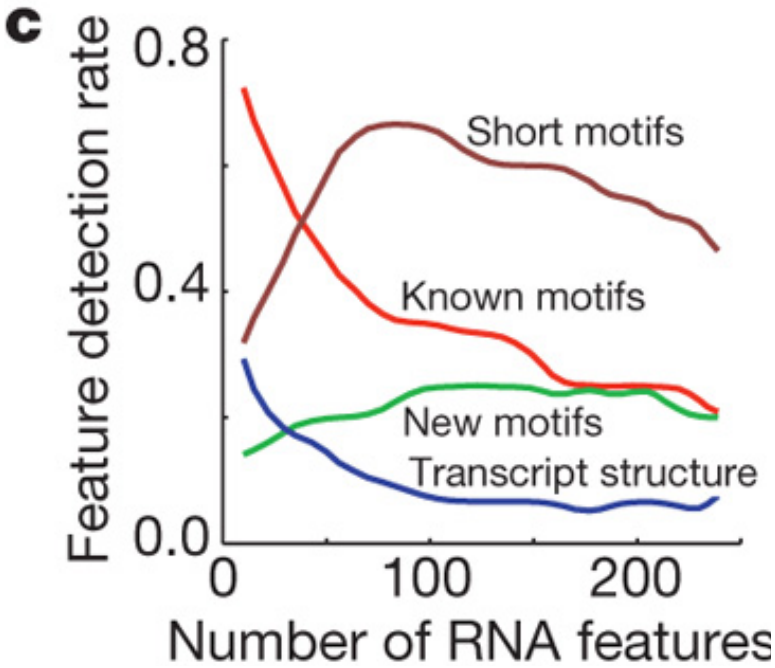


Define 3 regions **C1/I1(5‘)/I1(3‘)** before an „alternative exon“ (**A**) and 3 regions **I2(5‘)/I2(3‘)/C2** behind the alternative exon.

Barash et al. Nature
465, 53- (2010)

Approach to extract RNA features



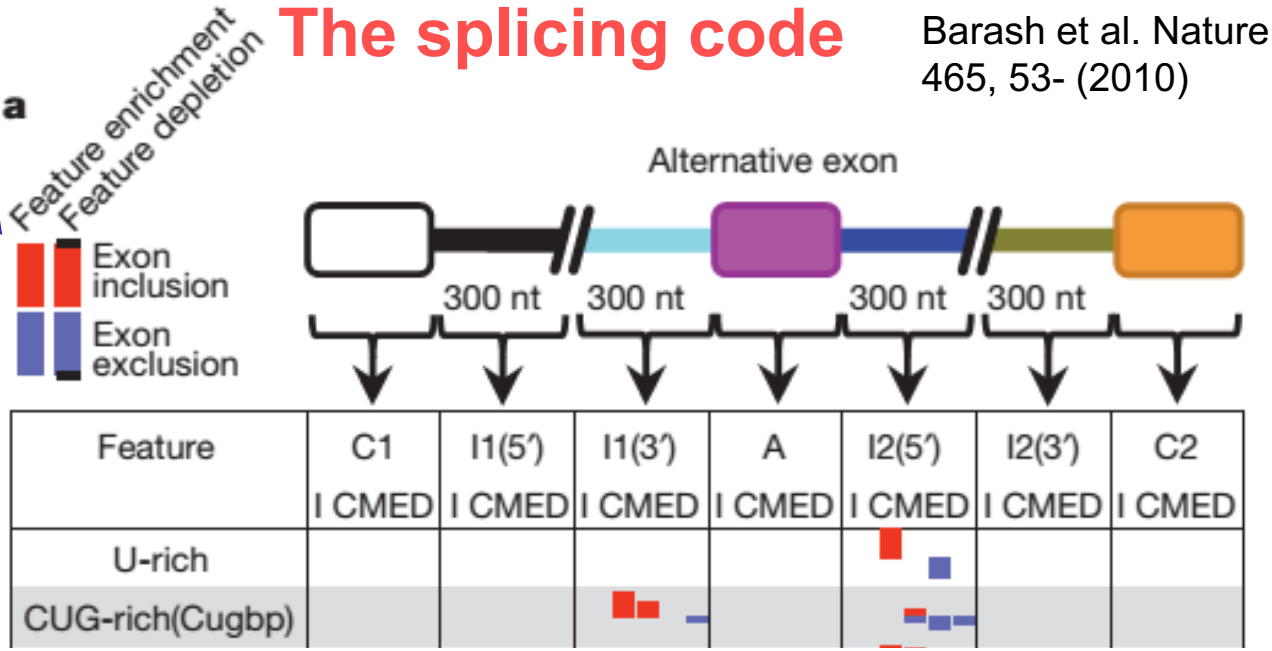


Barash et al. Nature
465, 53- (2010)

The splicing code

Barash et al. Nature 465, 53- (2010)

Bars without black line denote feature enrichment, bars with **black line** feature depletion.



Bar size conveys enrichment P-value; $P < 0.005$ in all cases.

Column “Feature” lists sequence motifs.

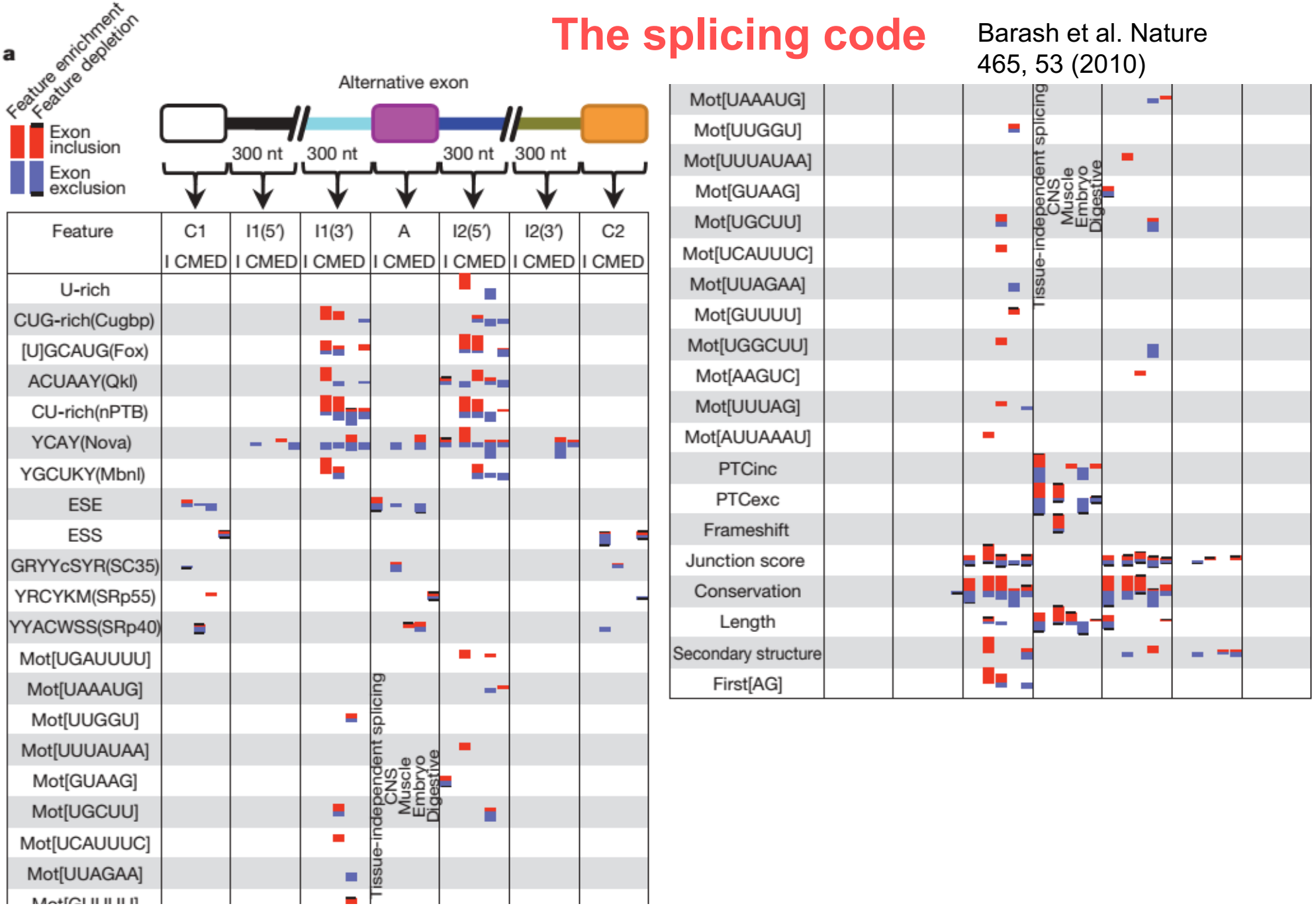
E.g. the CUG-rich motif of the second row recruits the binding protein Cugbp.

Each table cell contains 5 bars. They show the region-specific activity of each feature for increased exon inclusion (**red bar**) or exclusion (**blue bar**) in 5 different mouse tissues:

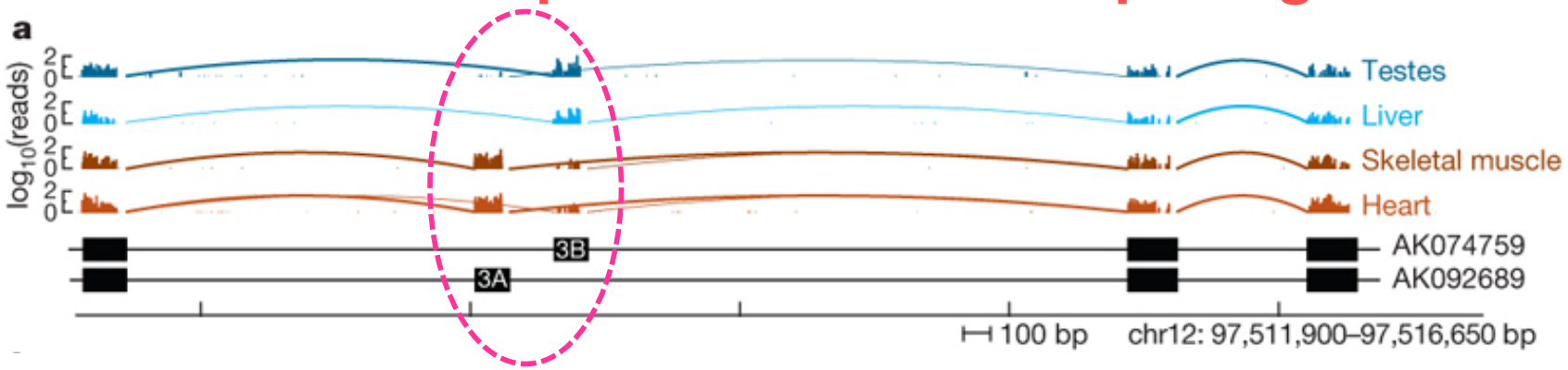
- CNS (C),
- muscle (M),
- embryo (E) and
- digestive (D) tissues, plus a
- tissue-independent mixture (I).

The splicing code

Barash et al. Nature 465, 53 (2010)



Tissue-specific alternative splicing



Shown are mRNA-Seq reads mapping to a portion of the *SLC25A3* gene locus. SLC25a3 is a mitochondrial phosphate carrier.

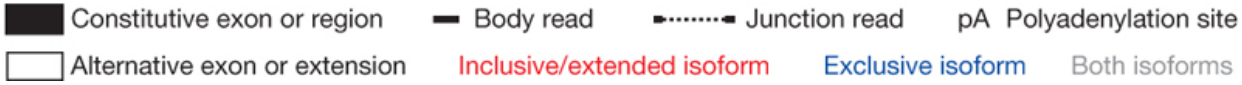
The number of mapped reads starting at each nucleotide position is displayed ($\log_{10}$) for the tissues listed at the right.

Bottom: exon/intron structures of representative transcripts containing **mutually exclusive exons** 3A and 3B (GenBank accession numbers AK074759 and AK092689).

Wang et al. Nature (2008) 456: 470-6

tissue-specific regulation of alternative mRNA isoforms

Alternative transcript events	Total events ($\times 10^3$)	Number detected ($\times 10^3$)	Both isoforms detected	Number tissue-regulated
Skipped exon	37	35	10,436	6,822
Retained intron	1	1	167	96
Alternative 5′ splice site (A5SS)	15	15	2,168	1,386
Alternative 3′ splice site (A3SS)	17	16	4,181	2,655
Mutually exclusive exon (MXE)	4	4	167	95
Alternative first exon (AFE)	14	13	10,281	5,311
Alternative last exon (ALE)	9	8	5,246	2,491
Tandem 3′ UTRs	7	7	5,136	3,801
Total	105	100	37,782	22,657

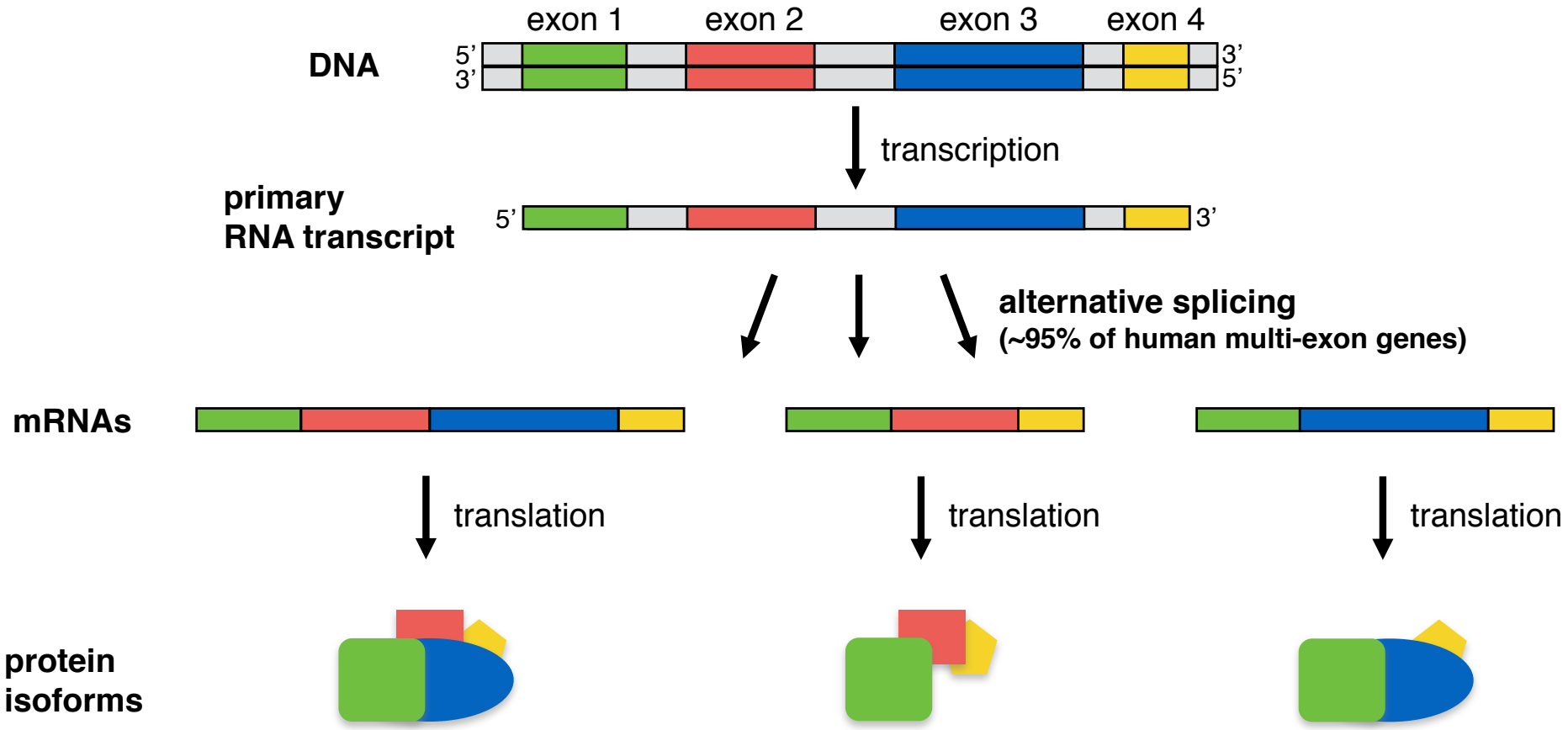


Blue, **red**, **grey**: mapped reads supporting expression of upper isoform, lower isoform or both isoforms.

The four columns to the right show the numbers of events of each type:
(1) supported by cDNA and/or EST data;
(2) 1 isoform supported by mRNA-Seq reads;
(3) both isoforms supported by reads;
(4) events detected as tissue-regulated (difference significant under Fisher's exact test).

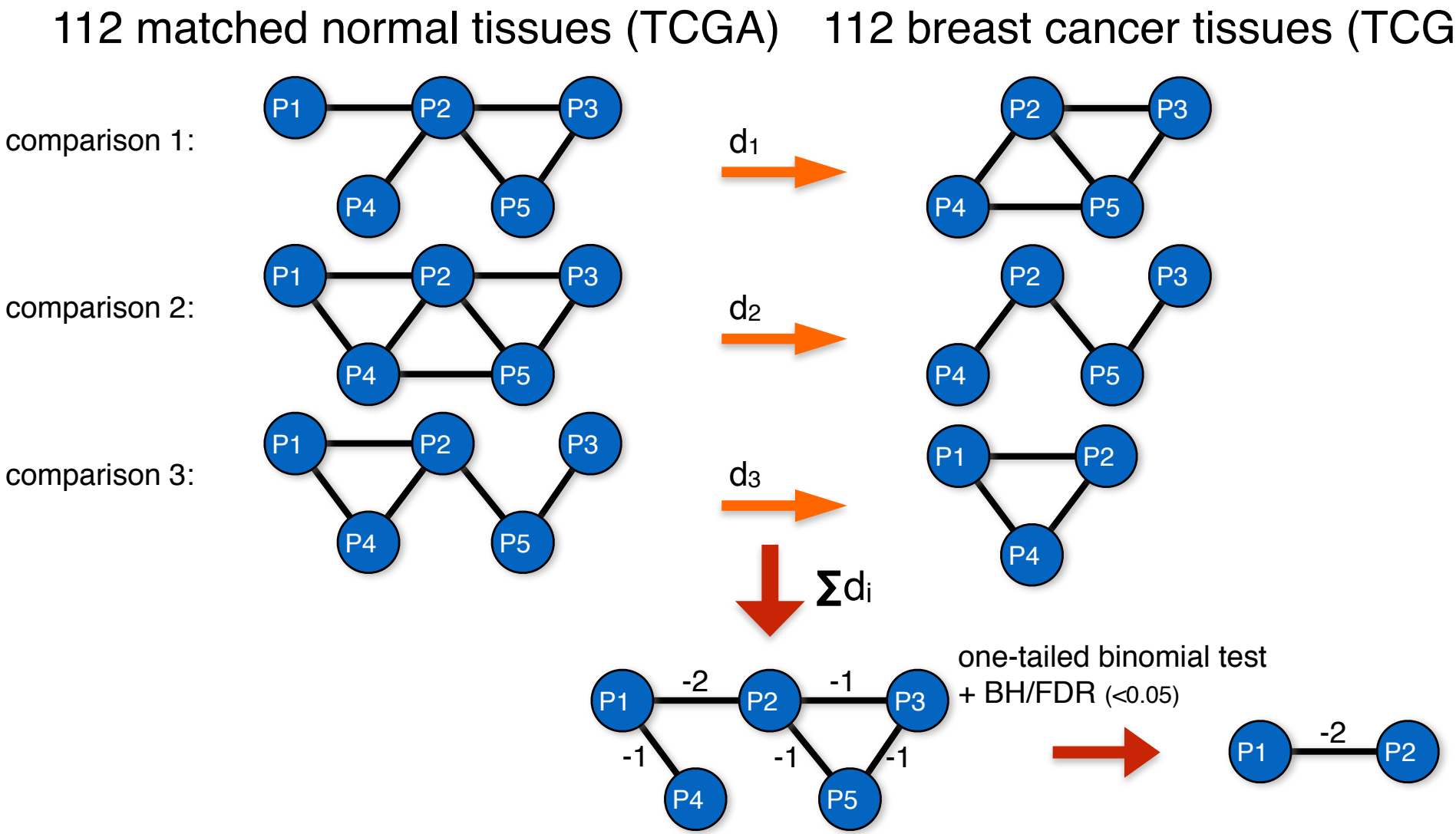
Wang et al. Nature (2008) 456: 470-6

Review from V3: Not considered yet: alternative splicing



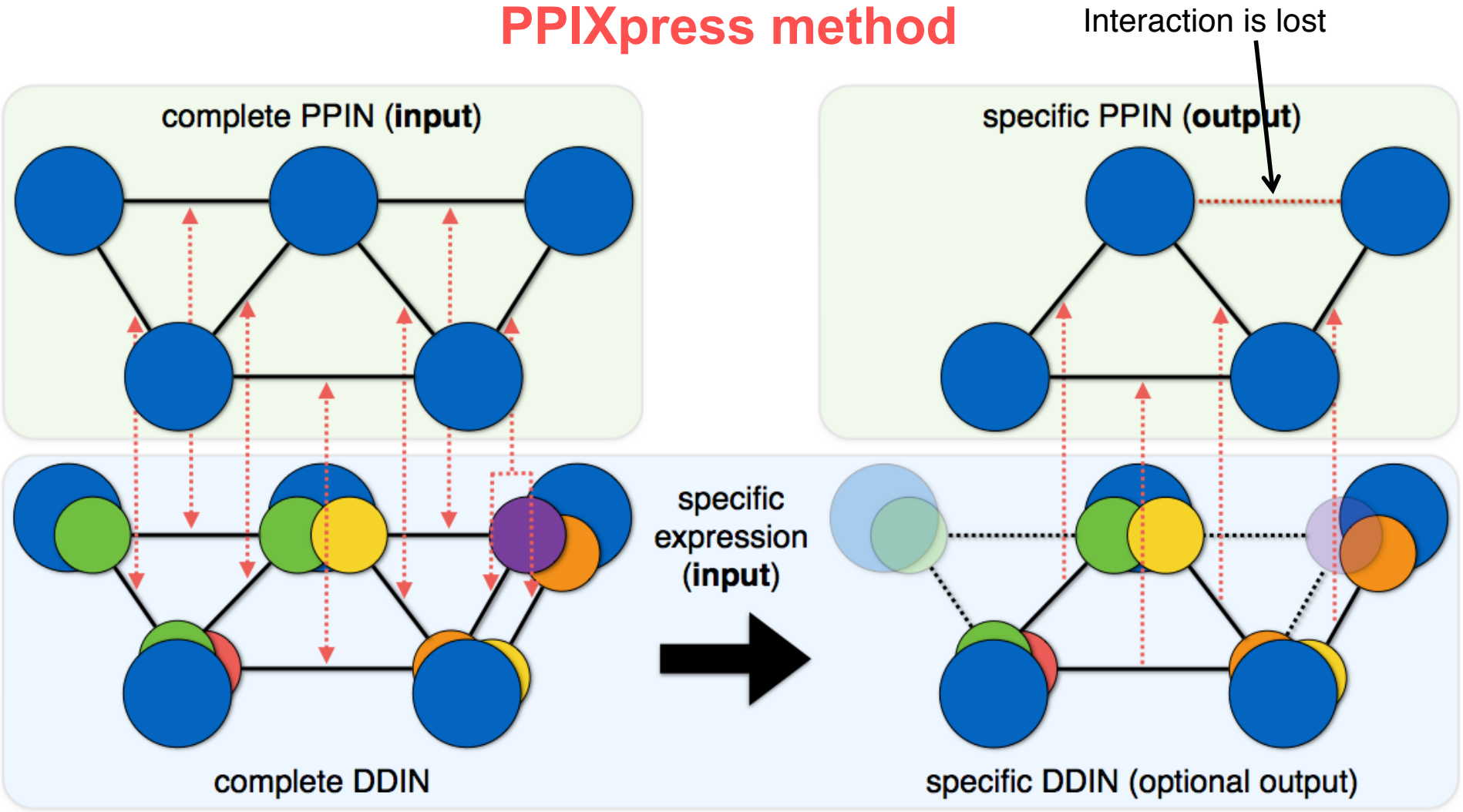
AS affects ability of proteins to interact with other proteins

Differential PPI wiring analysis



Check whether rewiring of a particular PP interaction occurs in a significantly large number of patients compared to what is expected by chance rewiring events.

PPIXpress method



reference: principal protein isoforms

built using most abundant protein isoforms

I. mapping

II. instantiation

Enriched KEGG and GO-BP terms in gene-level \ transcript-level set

	GENE		ALL-DDI	
	term	p	term	p
KEGG	hsa04012:ErbB signaling pathway	0.0013	hsa05200:Pathways in cancer	$1.5 * 10^{-17}$
	hsa05212:Pancreatic cancer	0.0491	hsa04110:Cell cycle	$1.8 * 10^{-15}$
			hsa05220:Chronic myeloid leukemia	$3.5 * 10^{-15}$
			hsa05212:Pancreatic cancer	$1.4 * 10^{-8}$
			hsa05223:Non-small cell lung cancer	$4.3 * 10^{-8}$
GO BP	GO:0007242 intracellular signaling cascade	$6.9 * 10^{-5}$	GO:0010604 positive regulation of macromolecule metabolic process	$4.3 * 10^{-16}$
	GO:0043065 positive regulation of apoptosis	0.0252	GO:0042981 regulation of apoptosis	$3.6 * 10^{-15}$
	GO:0043068 positive regulation of programmed cell death	0.0272	GO:0043067 regulation of programmed cell death	$6.1 * 10^{-15}$
	GO:0010942 positive regulation of cell death	0.0287	GO:0010941 regulation of cell death	$7.7 * 10^{-15}$
	GO:0051329 interphase of mitotic cell cycle	0.0409	GO:0007049 cell cycle	$1.7 * 10^{-14}$

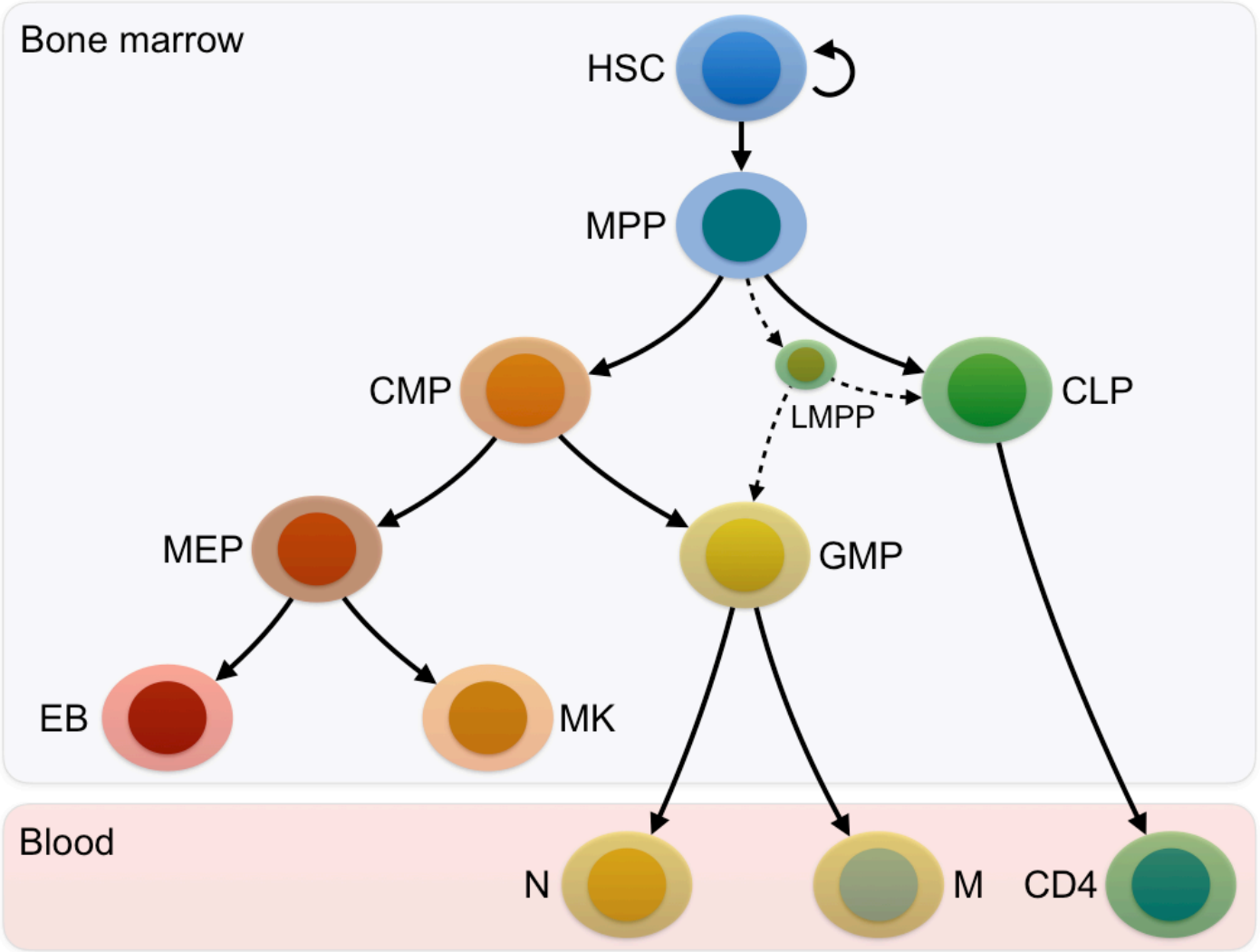
Table S16: *Comparison of rewiring results between the gene-based construction and a transcript-based construction method for the BioGRID network. Here, the top five enriched terms and their p-values are shown for the proteins affected by interactions exclusively found by the transcript-based method using the ALL-DDI dataset or the gene-based approach, respectively. Enrichment in KEGG pathways and GO biological processes was determined using DAVID [2] where we used the proteins included in the corresponding input network as the background. Enrichment was defined as $p < 0.05$ (Bonferroni-adjusted).*

The enriched terms that are exclusively found by the transcript-level method (right) are closely linked to carcinogenetic processes.

Hardly any significant terms are exclusively found at the gene level (left).

Will, Helms, Bioinformatics, 47, 219 (2015)
doi: 10.1093/bioinformatics/btv620

Hematopoiesis (development of blood cells)

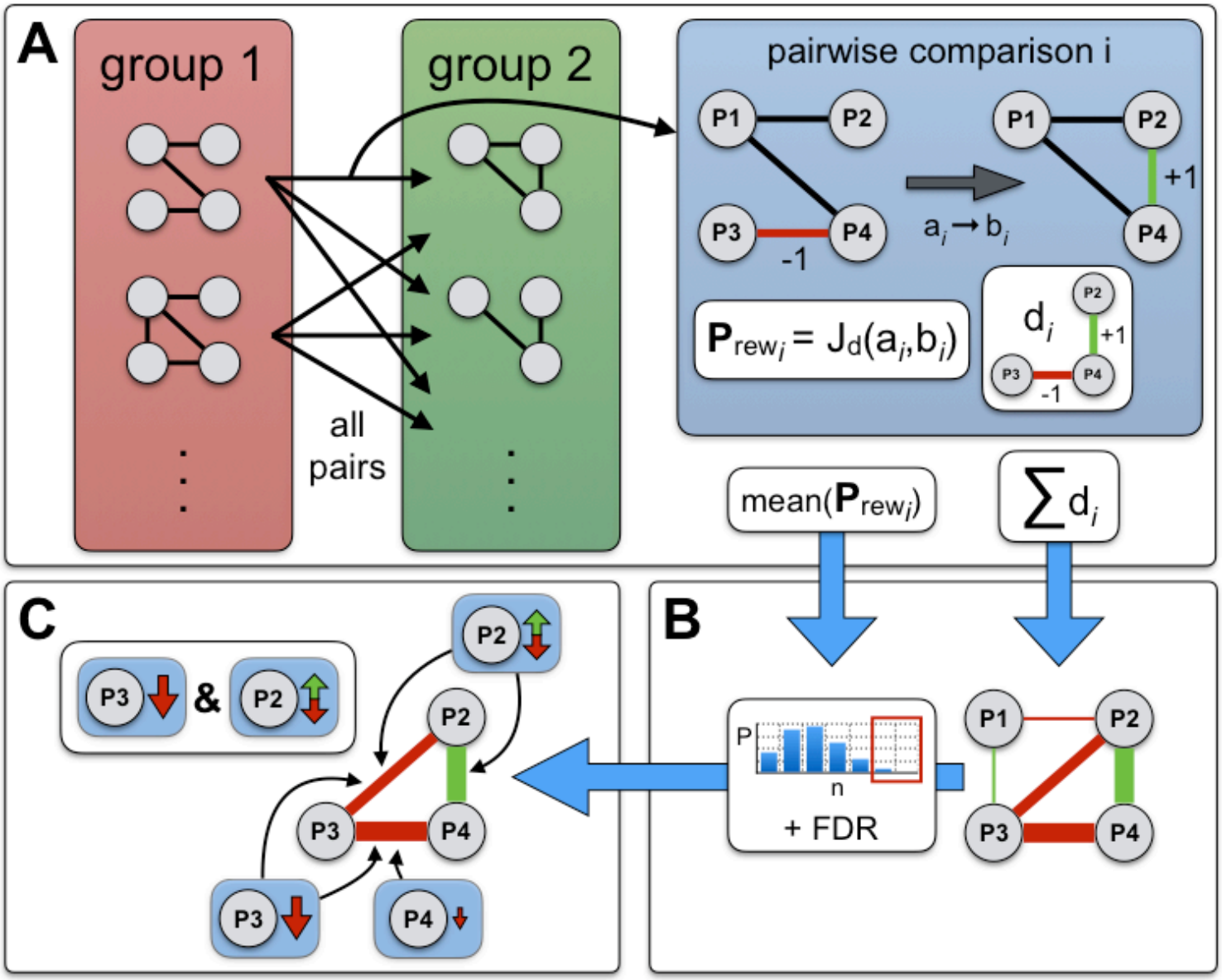


Hematopoietic **stem cells** (in bone marrow)

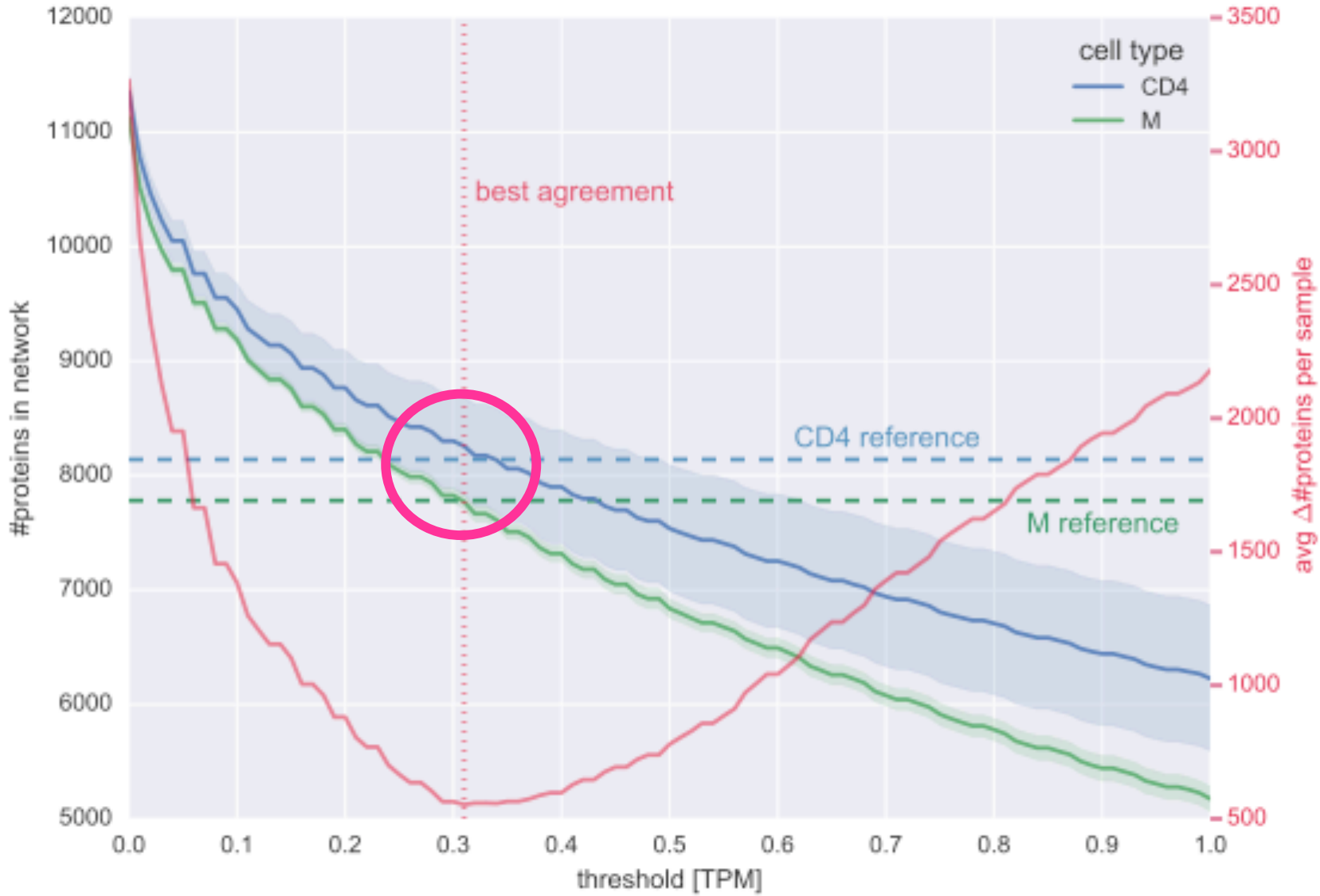
progenitor cells

terminally **differentiated cells**

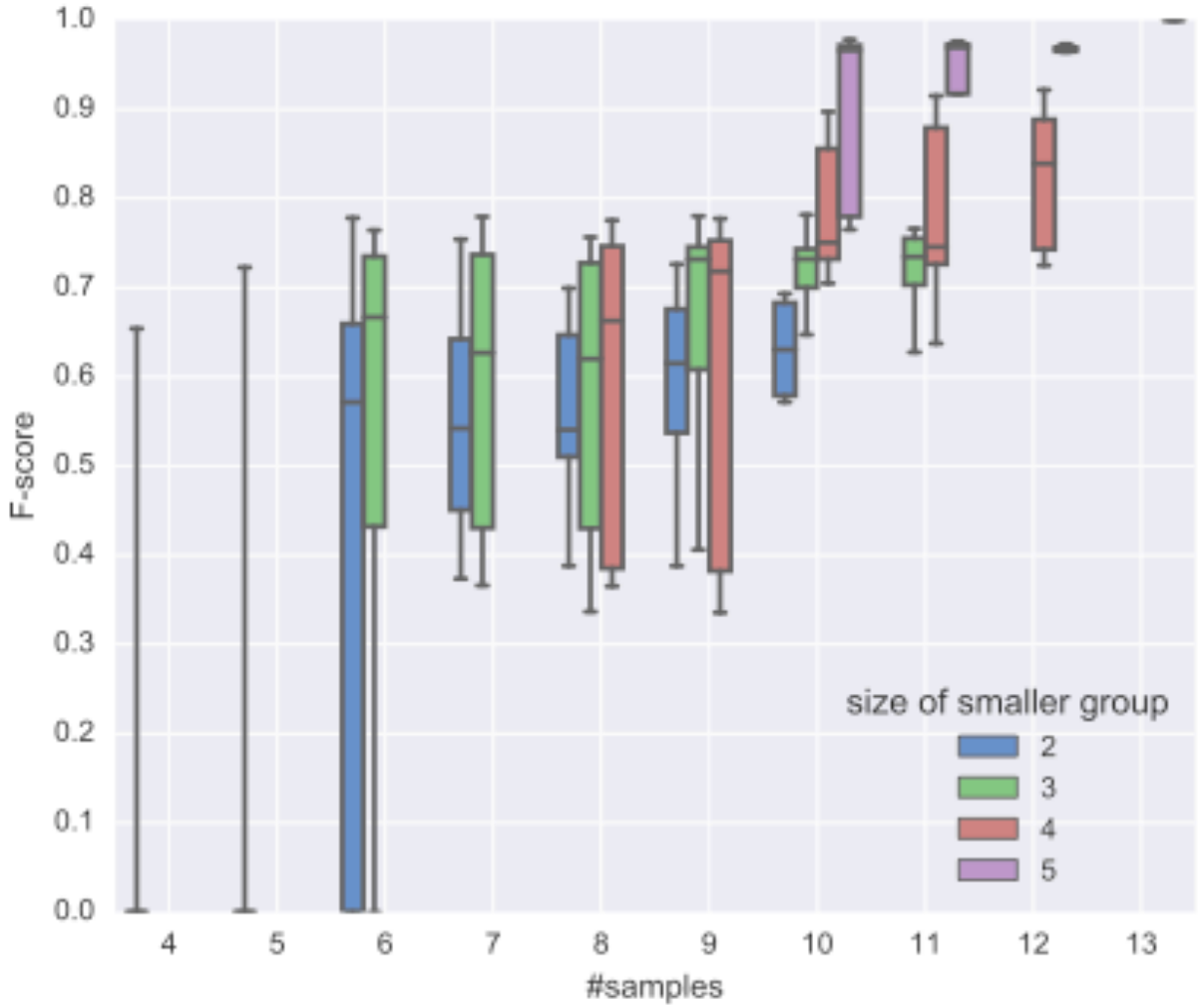
PPICompare workflow



Calibrating RNAseq on/off threshold against proteome data (MS)



How many RNAseq samples are needed?



X-axis:
Total number of samples in both groups

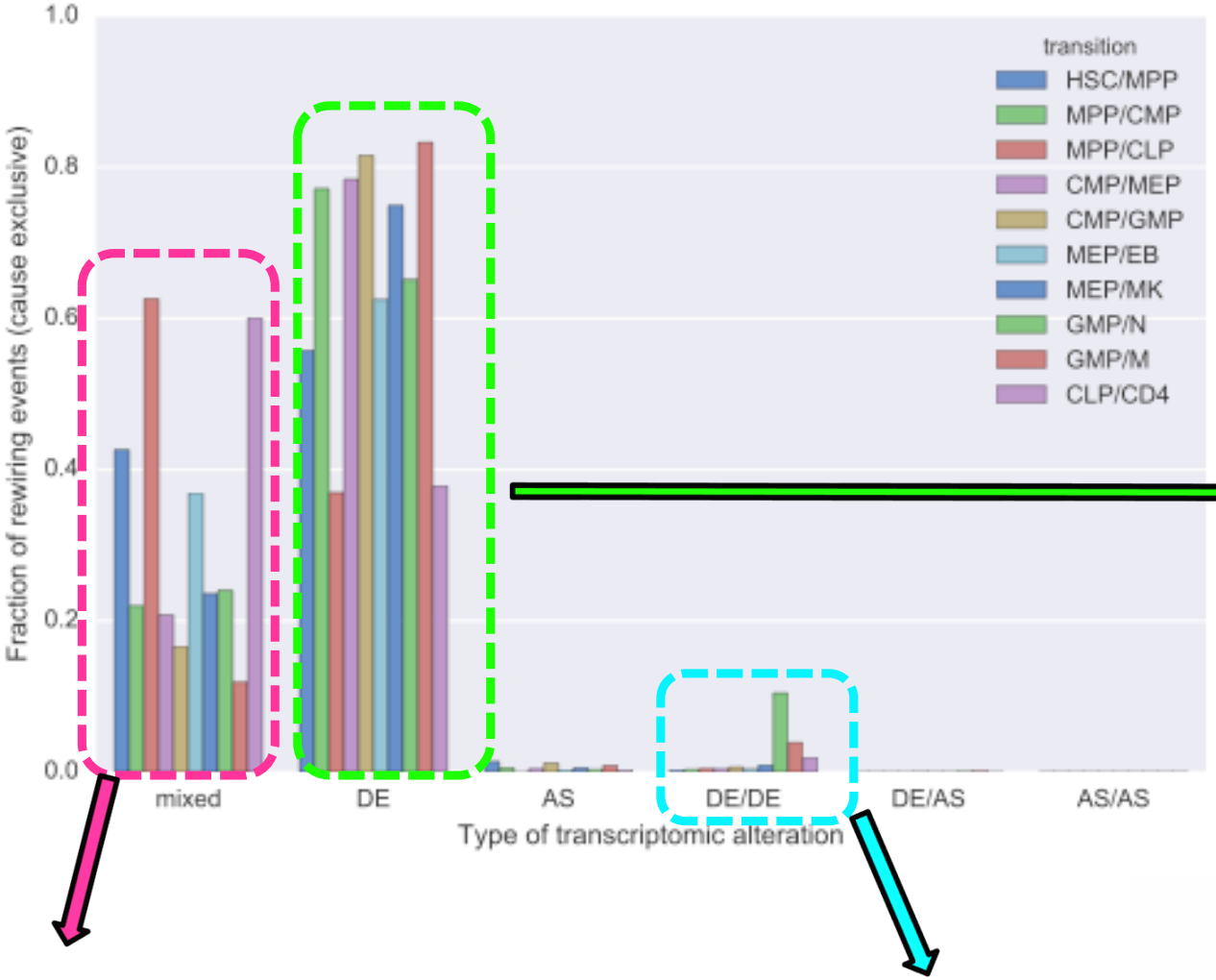
Blue -> violet:
Imbalance reduces

Subsampling shows that reasonable results are obtained for ≥ 3 samples

Rewiring is due to …

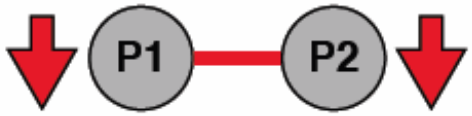
DE: differential expression
DE/DE: both proteins DE

AS: alternative splicing
DE/AS: one protein affected by DE, the other by AS



DE of a single protein is most frequent event for PPI rewiring

Simultaneous deregulation of interaction partners is often biologically meaningful.

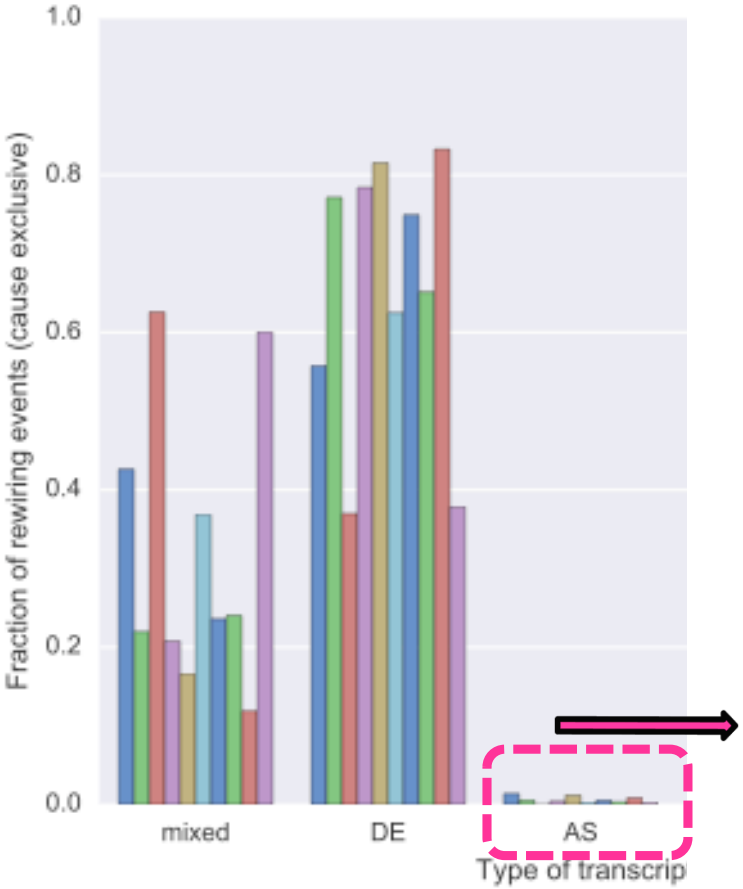


- more likely to participate in known protein complexes
- lower betweenness in interaction network
- more likely active in same biological process

→ **functional modules**

Different types of alterations can cause same rewiring event

contribution of AS seems minor (< 1%)



BUT 548 rewiring events in hematopoiesis are due to AS.

Rewiring events exclusively regulated by AS were enriched in GO terms related to:

- **post-elongation** processing of **mRNA**

- **cell cycle** (G2-M checkpoint and control of pre-replication complex)

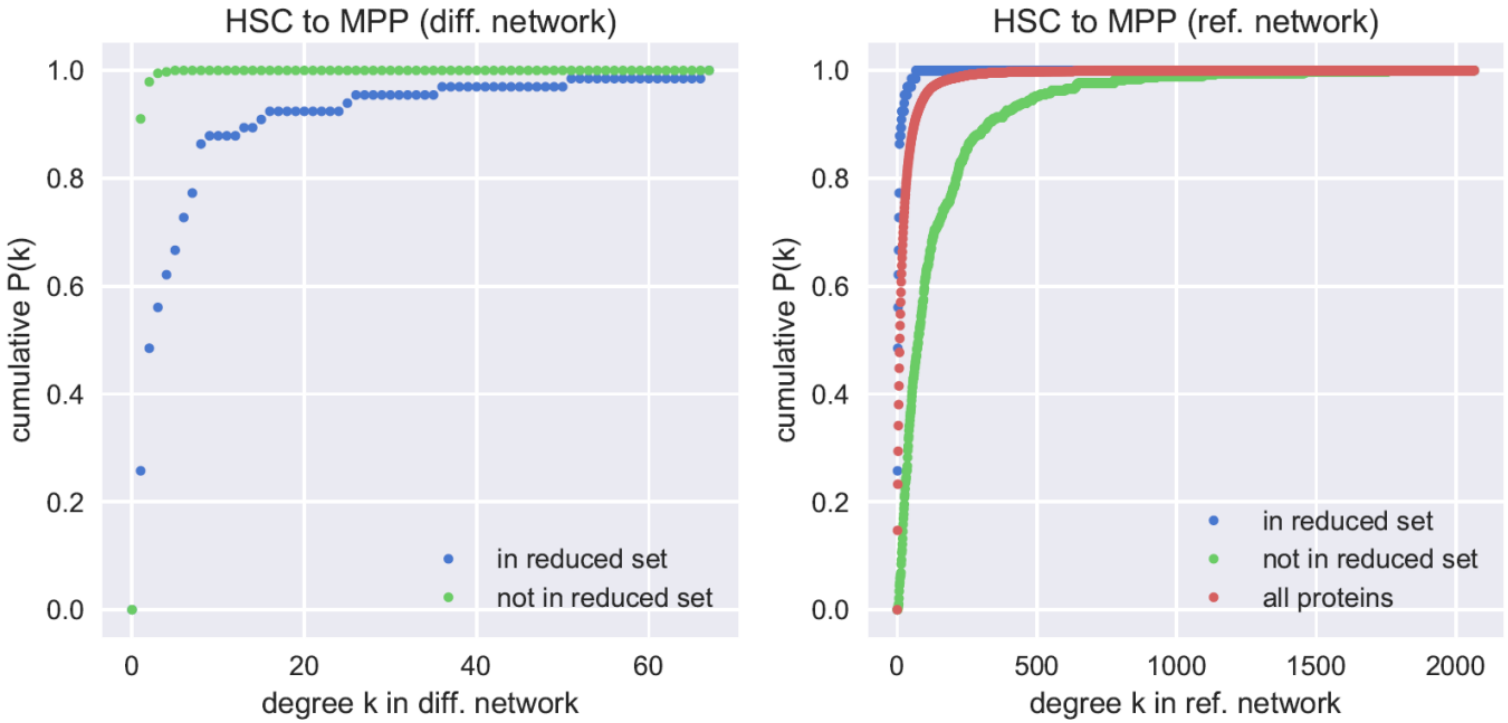
- **transport of mRNA from the nucleus to the cytoplasm**, Hippo signaling, as well as Interleukin receptor SHC signaling

reduced set

Identify **reduced set** of transcriptomic changes that

– explains all rewiring events (i.e. is very likely given the data) and

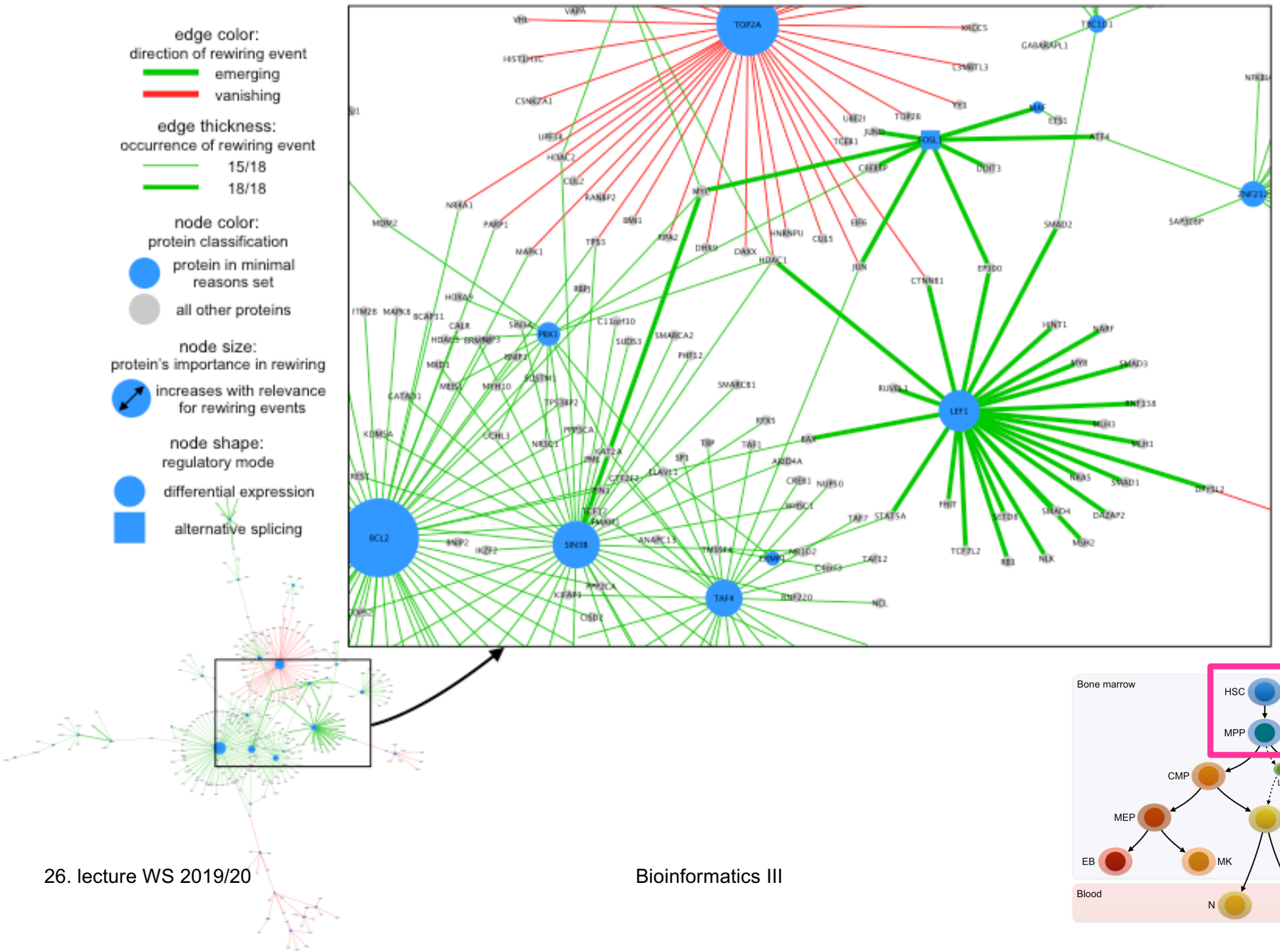
– is of small cardinality: **weighted set-cover problem**



→ (left) roteins in reduced set are **hub proteins** in the **differential network**, not in full reference network (right)

They tend to be **connectors** of **different functional modules**.

Rewiring HSC → MPP



involvement of epigenetics in alternative splicing?

V14 – V16 showed that chromatin state plays an essential role in regulating gene expression.

Although epigenetic signatures are mainly found to be enriched in **promoters**, it has become increasingly clear that they are also present in **exon** regions, indicating a potential link of epigenetic regulation to splicing.

Zhou *et al. BMC Genomics* 2012, **13**:123
http://www.biomedcentral.com/1471-2164/13/123

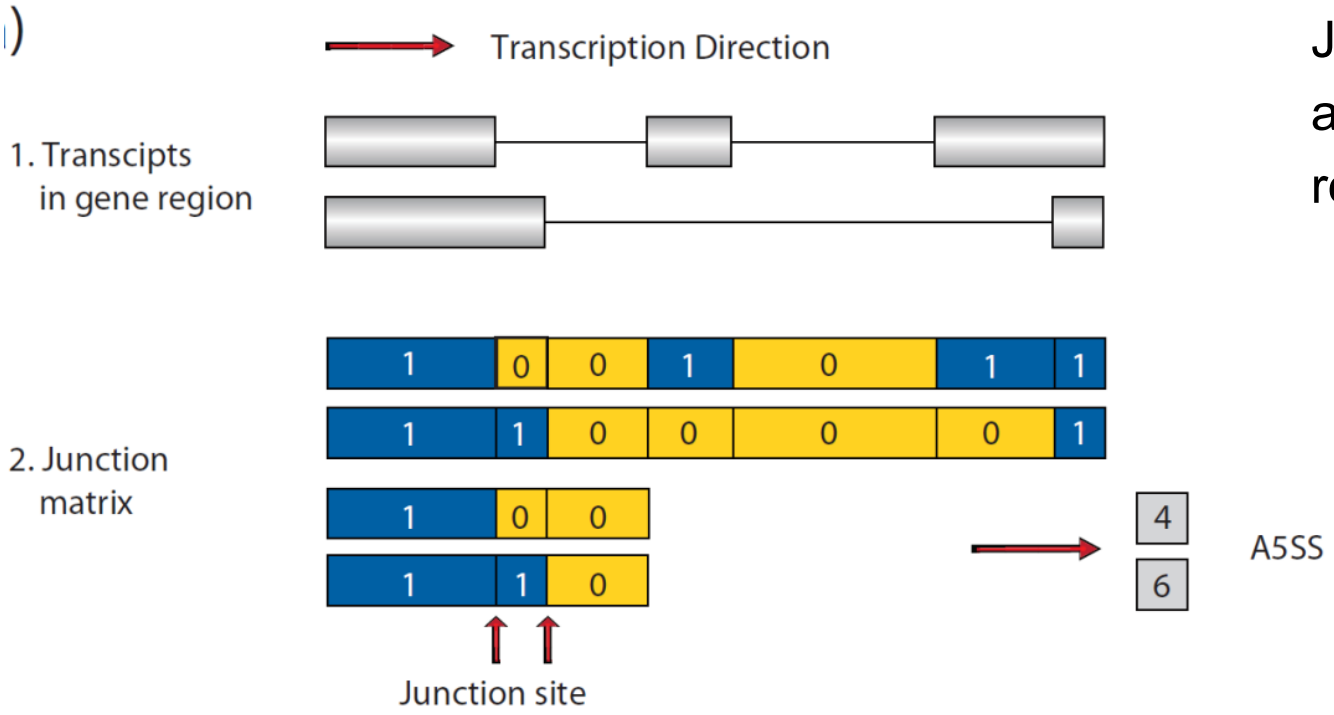
BMC Genomics

RESEARCH ARTICLE Open Access

Epigenetic features are significantly associated with alternative splicing

Yuanpeng Zhou, Yulan Lu and Weidong Tian*

Procedure to recognize AS events



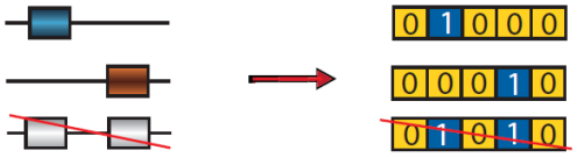
Junction site annotation and alternative splicing recognition process.

Data sources:

- Annotated AS types from Ensembl
- DNA methylation data from Salk institute
- Chip-seq data from ENCODE and elsewhere

Zhou et al. BMC Genomics (2012) 13:123

Procedure to recognize AS events

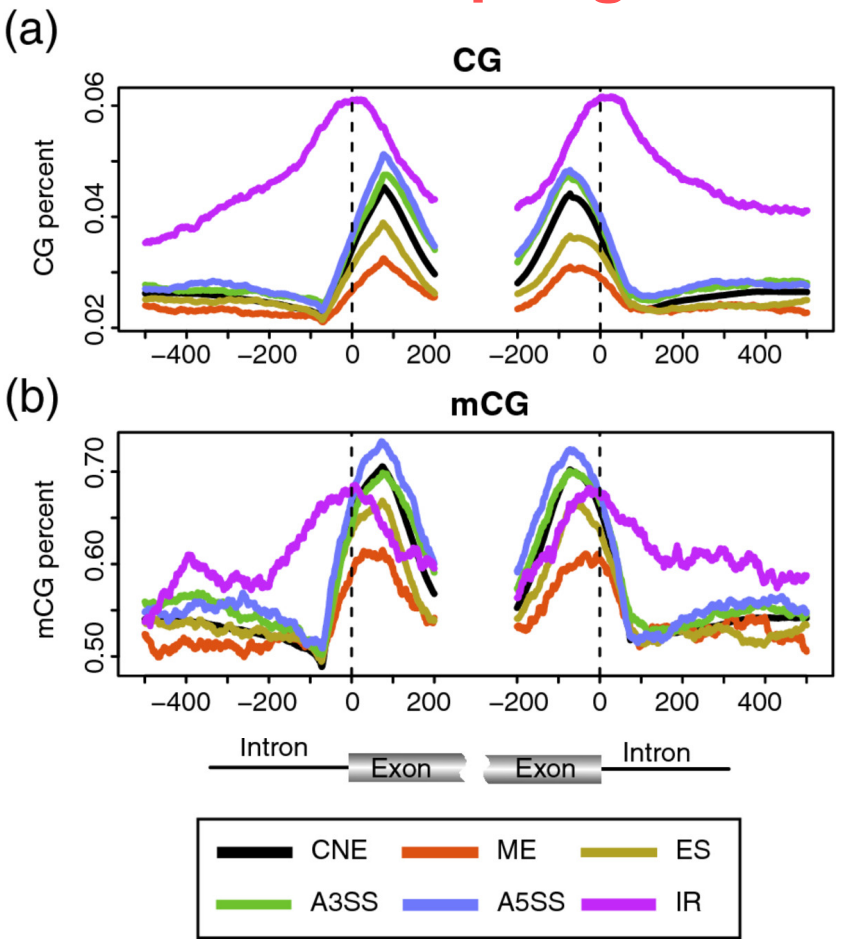
Sketch map	Recognition Code	AS Type	Number of Events
	2	CNE	103,806
	6 2	A3SS	2,521
	6 4	A5SS	1,884
	7 5	IR	563
	8 2 ~~10~~	ME	1,206
	42 34	ES	2,725

CNE: constitutively spliced exon (no AS)

The recognition code of AS events and the number of each type of splicing event

Zhou et al. BMC Genomics (2012) 13:123

Coupling AS ↔ epigenetic modifications



The association of DNA methylation and nucleosome occupancy with AS.

(a) Distribution of genomic CpG levels around the splice sites of different types of AS events. 6% is the expexted frequency of dinucleotides. However CpG levels are lower on average (V21).

(b) Distribution of DNA methylation level (mCG) around the splice sites of different types of AS events.

Both **a** and **b** use a sliding window of 147 bp

CNE: constitutively spliced exon (no AS)

ES: exon skipping

ME : mutually exclusive exon

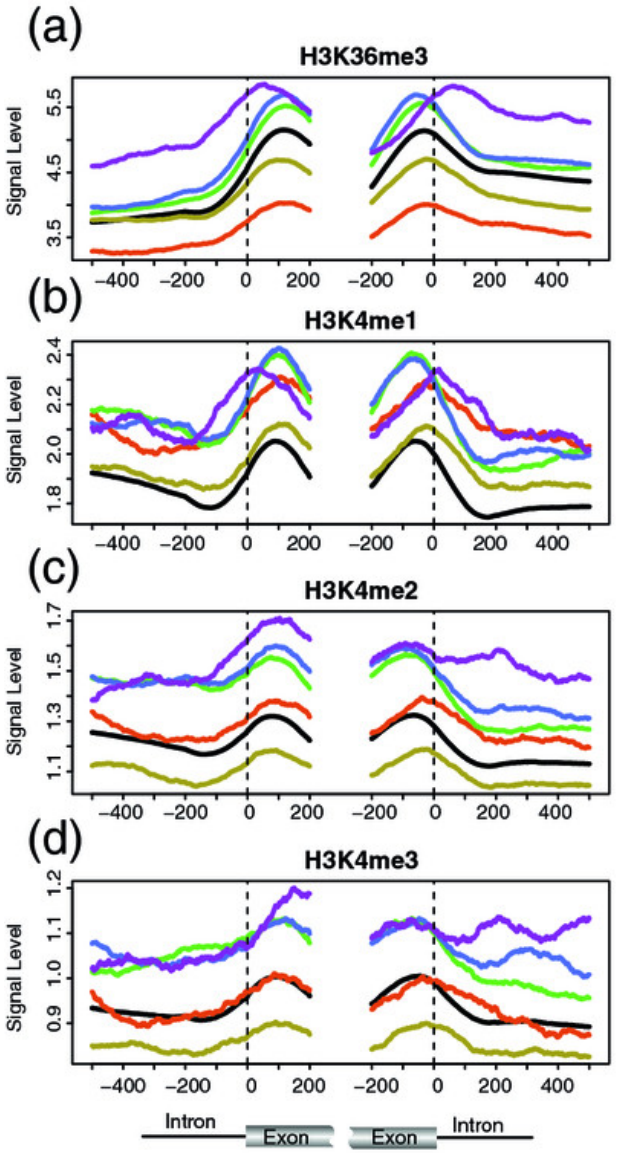
A5SS : alternative 5' splice site selection

A3SS : alternative 3' splice site selection

IR : intron retention.

Zhou et al. BMC Genomics (2012) 13:123

Association of histone modification with AS



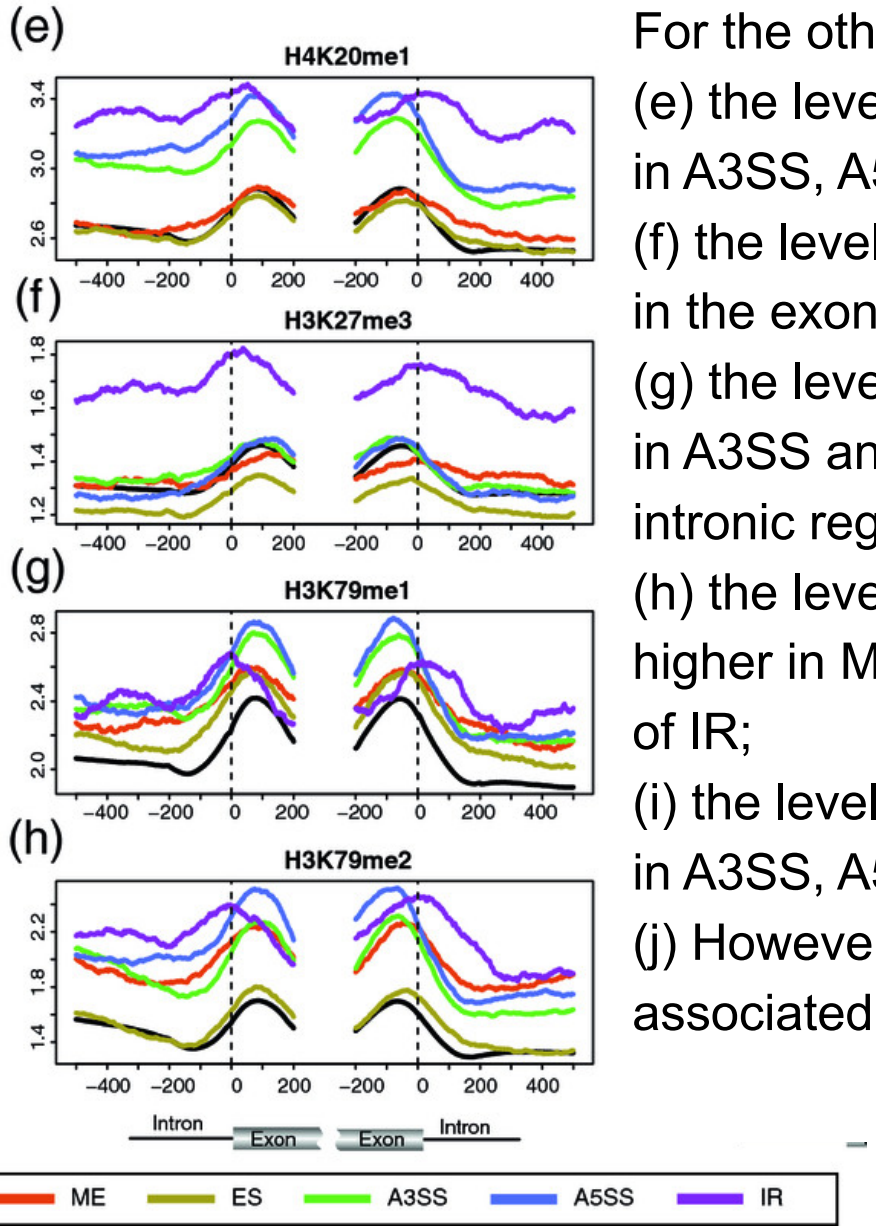
H3K36me3 (a) is the only histone PTM that is significantly associated with all types of AS events in all regions.

However the association patterns are different: H3K36me3 levels are significantly lower in **ME** and **ES** and significantly higher in **A3SS**, **A5SS** and **IR**.

The levels of H3K4 methylation, including H3K4me1, H3K4me2, and H3K4me3 (b - d), are almost all significantly higher in A3SS and A5SS.

Zhou et al. BMC Genomics (2012) 13:123

Association of histone modification with AS



For the other histone methylations,

(e) the level of H4K20me1 is significantly higher in A3SS, A5SS and IR;

(f) the level of H3K27me3 is significantly higher in the exonic region of ES;

(g) the level of H3K79me1 is significantly higher in A3SS and A5SS, and slightly higher in the intronic region of ES;

(h) the level of H3K79me2 (h) is significantly higher in ME, A3SS and A5SS, and most region of IR;

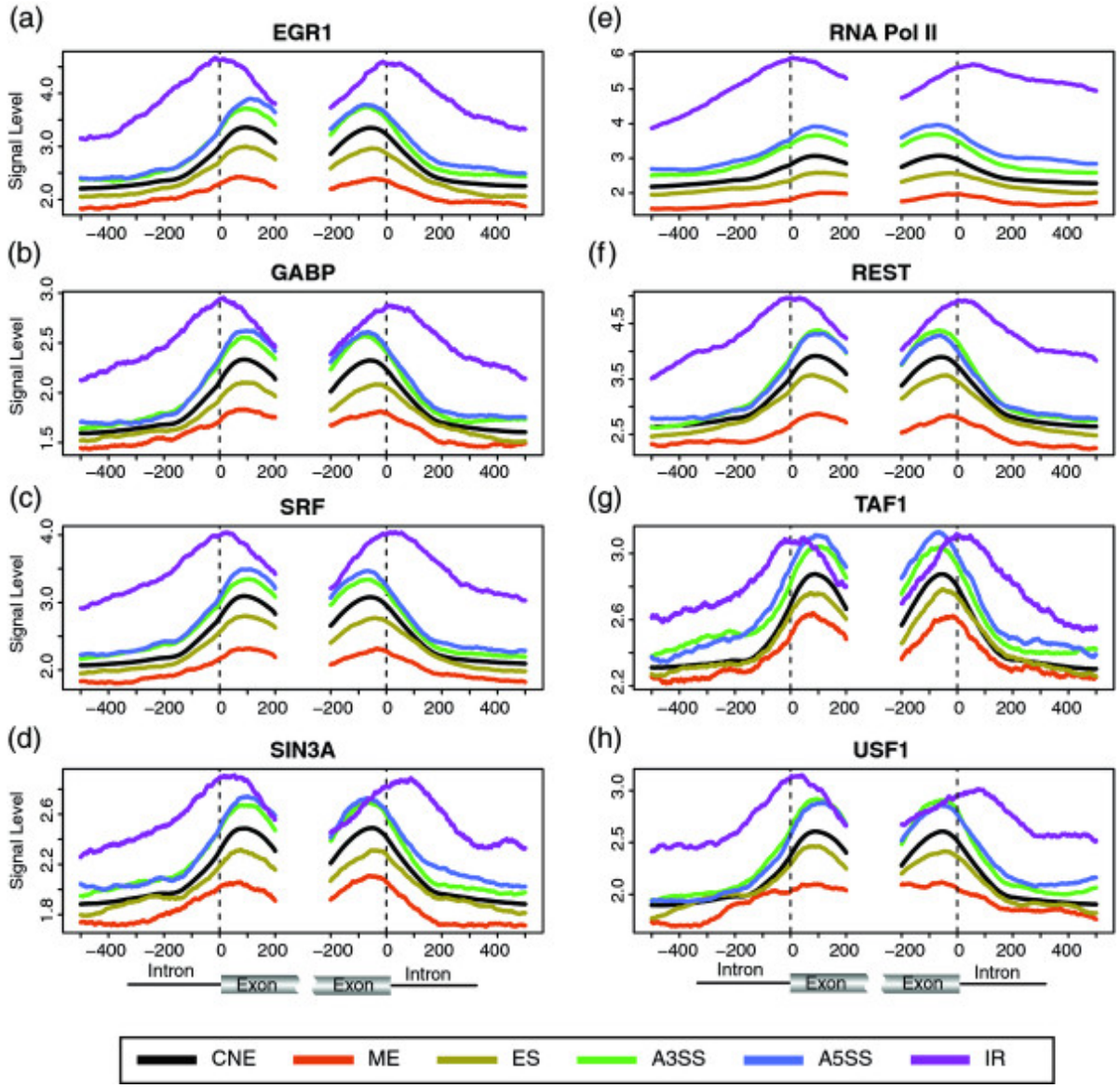
(i) the level of H3K9me1 is significantly higher in A3SS, A5SS and most regions of IR.

(j) However, H3K9me3 is not significantly associated with any type of ASE.

Zhou et al. BMC Genomics (2012) 13:123

Association of protein features with AS

ChIP-seq data for 9 TFs, CTCF and RNA Pol II

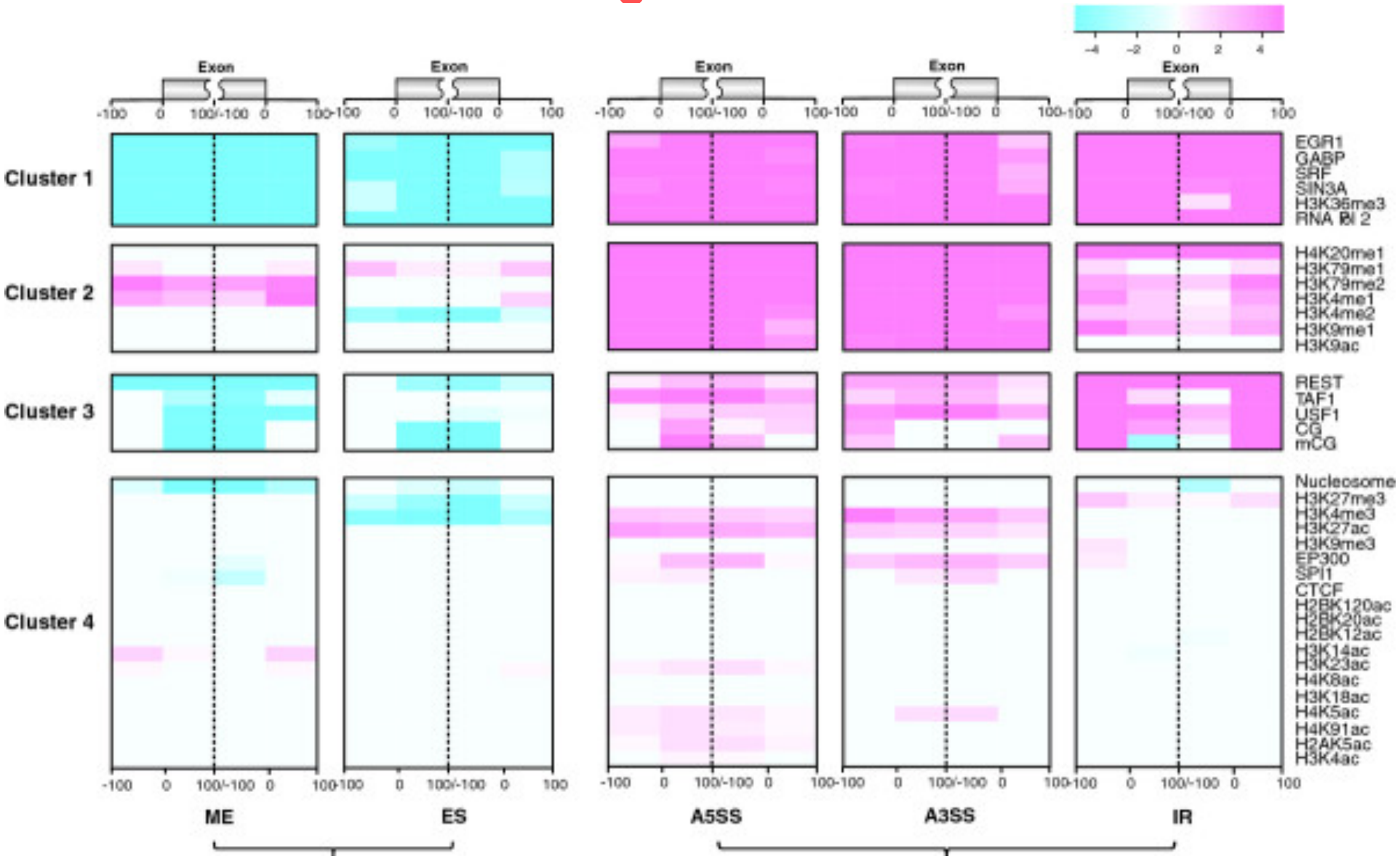


The binding levels of EGR1, GABP, SIN3A, SRF and RNA Pol II (a - e) are all signifycantly higher in A3SS, A5SS and IR, and significantly lower in ME and ES,

Their levels all steadily increase from ME, ES, CNE, A3SS, A5SS to IR.
This is similar to the results for H3K36me3.

Zhou et al. BMC Genomics (2012) 13:123

Clustering of associations



Zhou et al. BMC Genomics (2012) 13:123

epigenetic modifications that are associated with AS

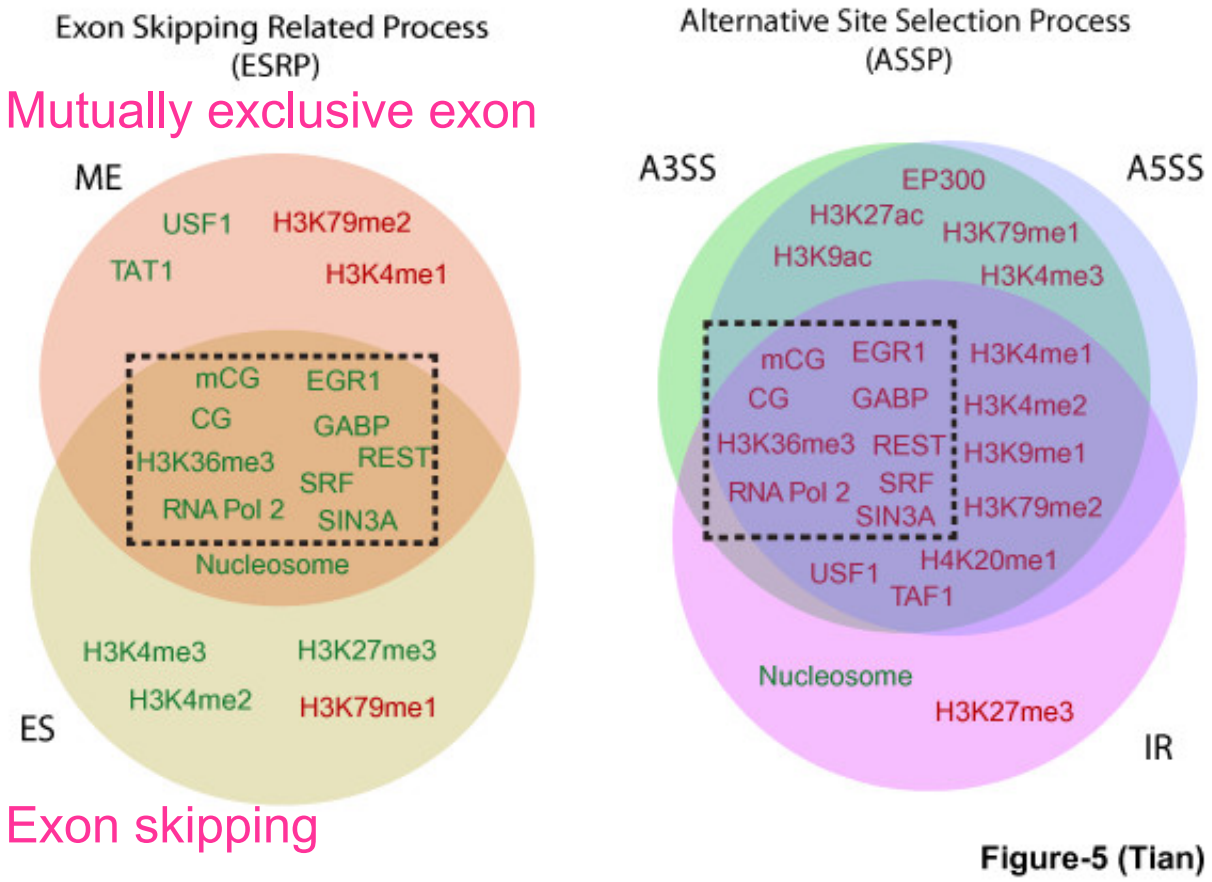


Figure-5 (Tian)

The Epigenetic features are strongly associated with different types of AS.

The features showing higher level and lower level in AS events than in CNE are colored in **red** and **green**, respectively. The features inside the dashed black box are those common in both ESRP and ASSP; note their association patterns are very different in between ESRP and ASSP.

Zhou et al. BMC Genomics (2012) 13:123

Coupling AS ↔ epigenetic modifications

Epigenetic features are strongly associated with AS.

This suggests that epigenetic regulation may be involved in AS.

Clustering yielded 4 tight clusters of epigenetic features that are associated with AS.

The AS events may be grouped into 2 classes on the basis of their association patterns with epigenetic features.
- the exon skipping related process (ESRP) (including ME and ES) and
- the alternative splice site selection process (ASSP) (including A3SS, A5SS and IR)

→ these 2 processes may involve different mechanisms of epigenetic regulation.

Zhou et al. BMC Genomics (2012) 13:123

Content of final exam

Lecture	Slides relevant for exam
1	15-21
2	1-11, 30-42
3	1-40
4	None
5	1-20,24-32,37-39,42
6	None
7	1-21
8	None (V1-V8 as announced in V8)
9	19-20,30-37
10	10-34, 42-54
11	9-16
12	17-30
13	3-33
14	11-30

Lecture	Slides relevant for exam
15	None
16	None
17	All slides (only proofs presented in lecture)
18	All slides (only proofs presented in lecture)
19	22-40
20	6-10, 24-37
21	2-6, 15-22, 26, 29-45 (main ideas)
22	1-34
23	1-8
24	1-13
25	None
26	None

Relevant are also the assignments !
(theoretical parts, not the programming parts)