

A WIDESPREAD ROLE FOR U-RICH MOTIFS AND TIA-1/TIAR PROTEINS IN ALTERNATIVE SPLICING REGULATION

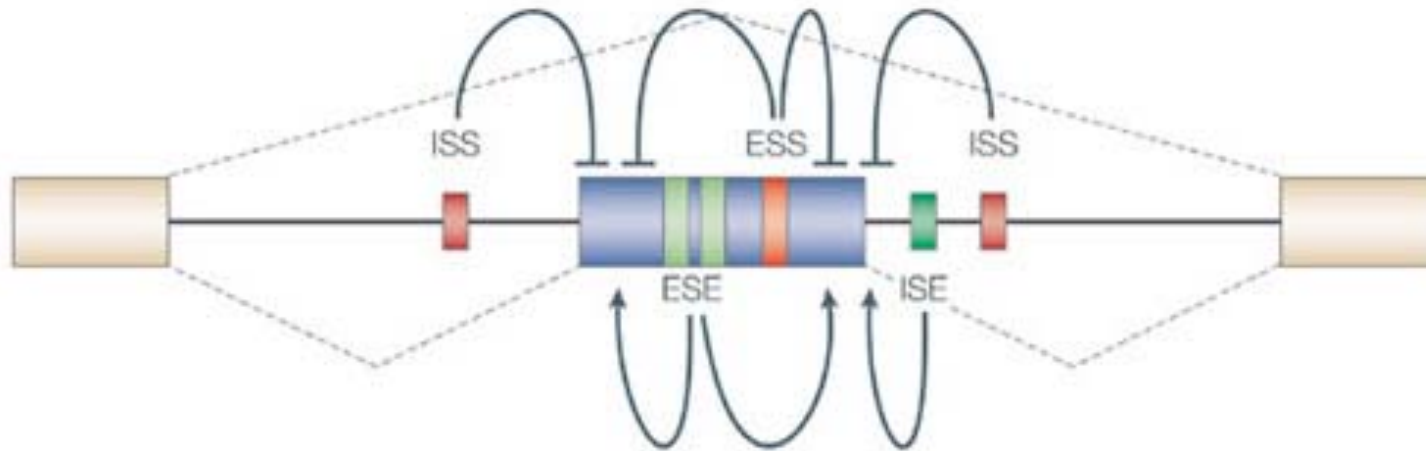
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The Hospital for Sick Children and the University of Toronto, Canada

SickKids



Splicing regulatory elements



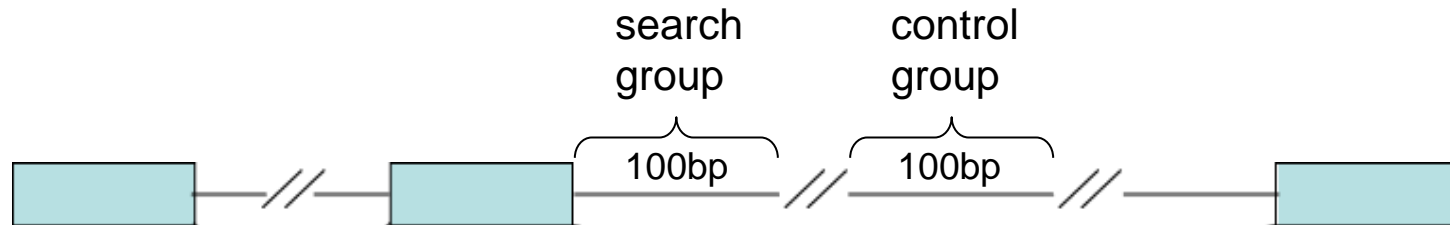
From Matlin et al, *Nat. Rev. Mol. Cell Biol.*, 2005

Goals

1. Systematic search for motifs in intronic sequences downstream of 5' splice sites
2. Determine the relationship between intronic and exonic splicing elements
3. Identify splicing factor(s) involved in 5' splice site selection
 - Enrichment of U-rich motifs downstream of 5' splice sites
 - Widespread role of TIA-1/TIAR in 5' splice site selection via U-rich motifs

Goal 1: Strategy

- Systematic search for motifs enriched in adjacent intronic regions



- Datasets:
 - Constitutive cassette exons (CS) $n = 109,225$
 - Alternative cassette exons (AS) $n = 3,872$
- Discriminative search - Seedsearcher algorithm (Barash et al, 2001) :
 - Search motifs of 5-12nt allowing “wild cards”
 - Estimate statistical significance, multiple hypothesis correction

U-rich motifs are prominent downstream of 5' splice sites

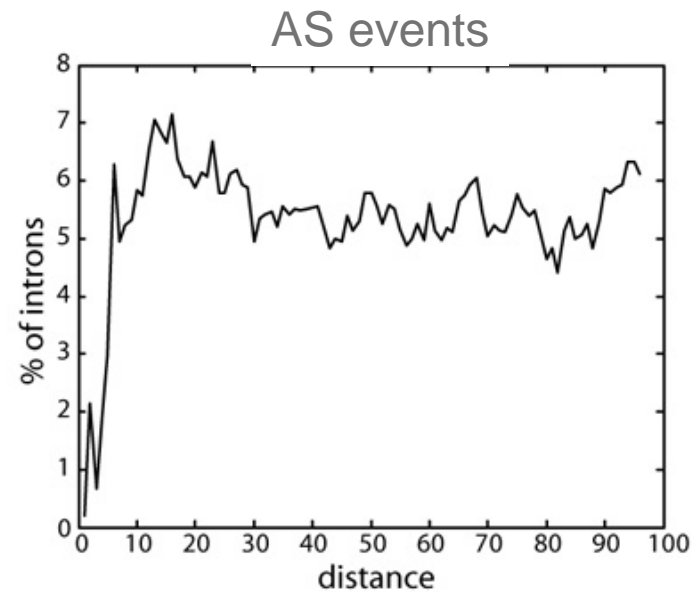
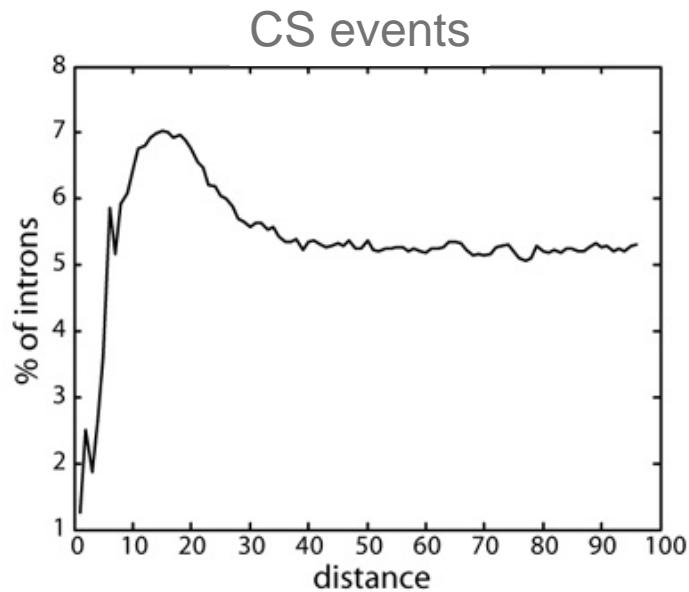
Constitutive (n=109,225)				Alternative (n=3872)	
Motif	Score*	Motif	Score*	Motif	Score*
GGG????GGG	inf	UUUC?UU	52	CCUUU	40
GGGGC	inf	UUG?UUU	50	UUUCU	40
GGG??GGG	inf	UUUCU	50	GGGGG	35
GGGGG	305	CCCCU	49	UUUUC	34
GGGCC	256	UUU?A?UU	48	UUCUU	34
UGGGG	222	UUUUC	48	UGGGG	33
GGGGA	208	UUU?AU?U	47	CUUUU	33
GAGGG	176	UUCUUU	46	GAGGG	32
GUGGG	165	UUGUU	46	GGGCC	29
AGGGG	150	U?U?CUUU	45	UCUUU	29
GGGCU	120	UUUCUU	44	GGGGC	28
GGCCC	119	U??U?U?CUU	42	AGGGC	27
GGGUG	97	GGAGC	42	GUUUU	27
GCCCC	97	U?U?AUUU	41	CUUUC	26
GGGGU	93	GCCCU	37	UUUCC	26
AGGGC	90	UUAUUU	35	UUCUU	26
UGUUU	72	CCCUC	35	GGGUG	26
UCUUU	63	UUUA?U?U	34	UCCUU	25
UUCUU	57	GCUGC	34	UGCUU	25
U?UGUUU	56	CUUUU	34	UGUUU	25
UUU?AUU	56	UU?UUUC	33		
CAGGG	53	U?UUCU?U	33		
UU?AUUU	53	UUUAUU	30		
UU?CUUU	53	UU?U?A?U?U	30		

Mutations introduced in U-rich sequences downstream of 5' splice sites of four genes (MYPT1, TIA-1, TIAR and CFTR) caused the skipping of the preceding exon *in vitro*

*corrected for multiple comparisons

Positional bias of U-rich motifs

Motifs: 4 Us out of 5 nucleotides

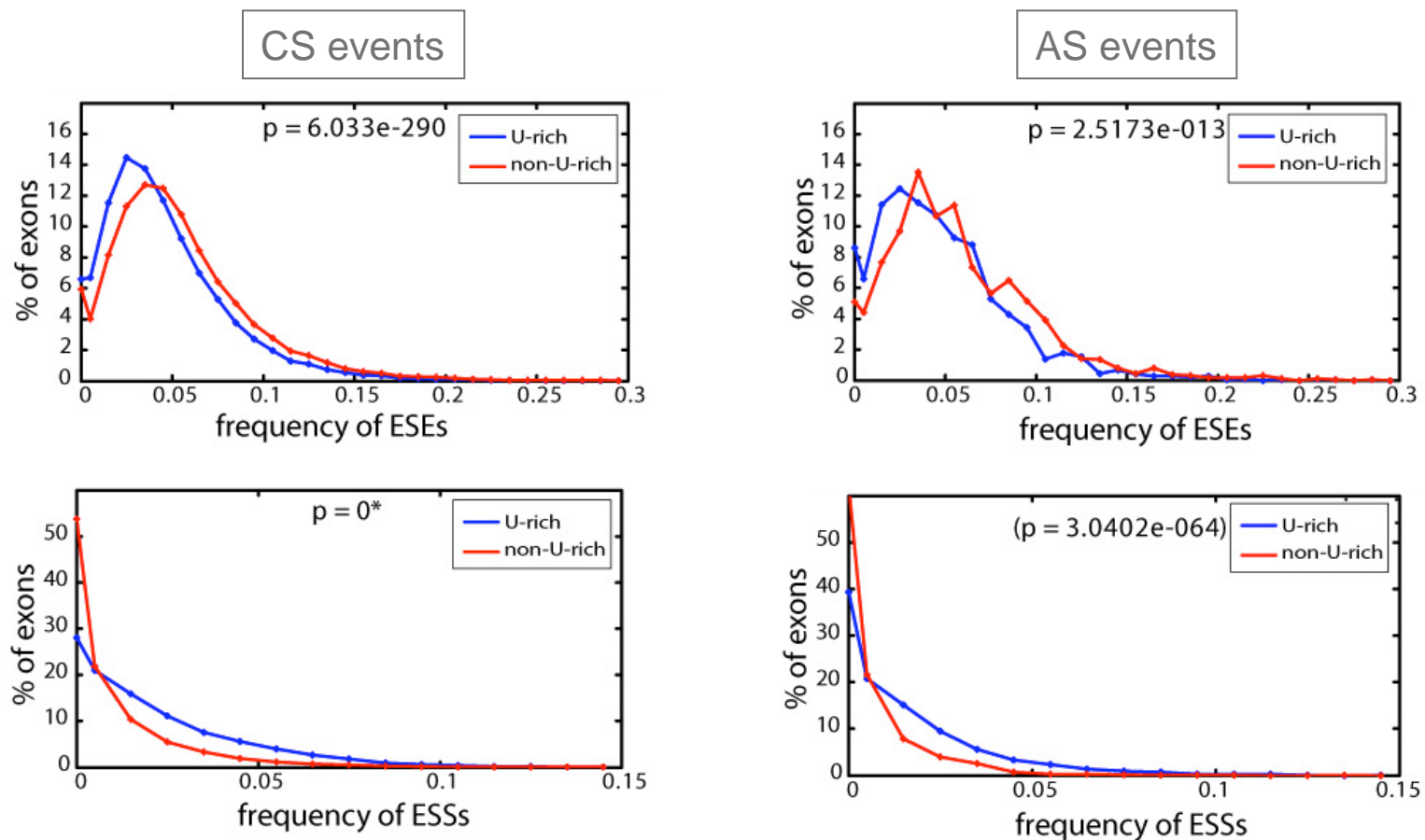


U-rich motifs are not enriched in pseudo introns at the same location

Goal 2: Relationship between U-rich intronic motifs and exonic splicing elements

U-richness defined as % of Us in the first 100nt of intron sequences ($\geq 30\%$ of Us = U-rich)

ESEs and ESSs consist of 8-mers defined by Zhang and Chasin, 2004

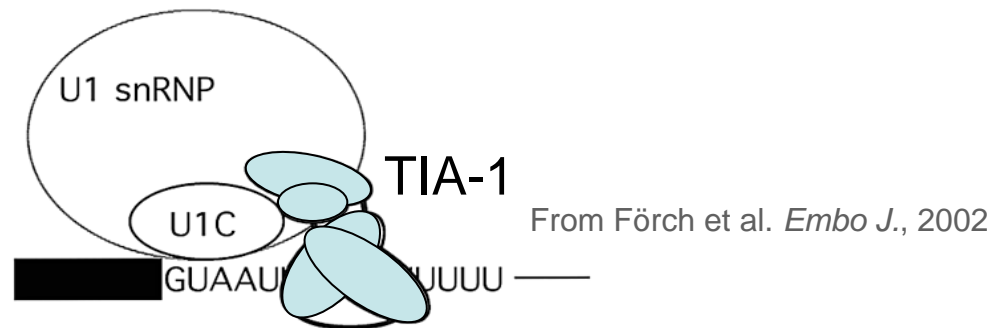


Goal 3: TIA-1 (T-cell intracellular antigen-1) and TIAR (TIA-1-related protein)

Evolutionary conserved splicing factors

Have preference for U-rich sequences shown by *in vitro* SELEX (Dember et al. *JBC*, 1996)

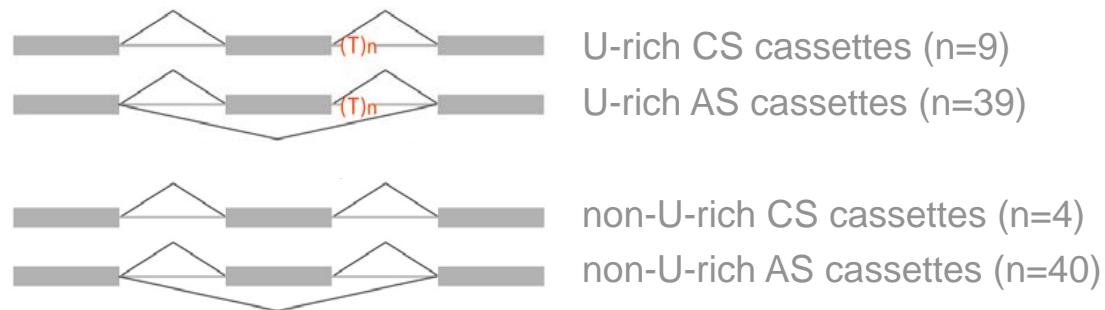
Aid the recognition of 5' splice sites through interaction between the Q-rich domain and U1C of the U1snRNP complex (Förch et al. *Embo J.*, 2002)



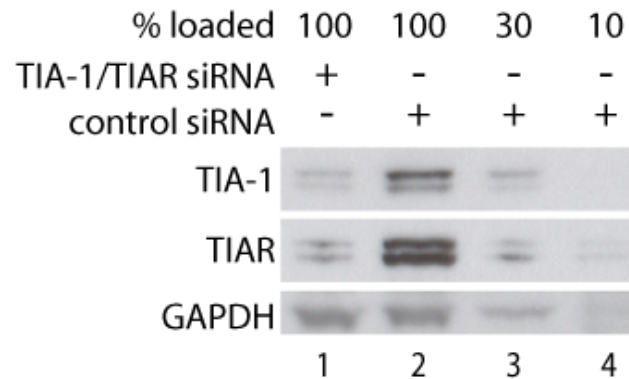
Knockdown of TIA-1/TIAR increased skipping of FAS and MYPT1 exons (Izquierdo et al. *Mol. Cell*, 2005; Shukla et al. *RNA*, 2005)

Assessment of the role of TIA-1/TIAR on splicing regulation via U-rich motifs

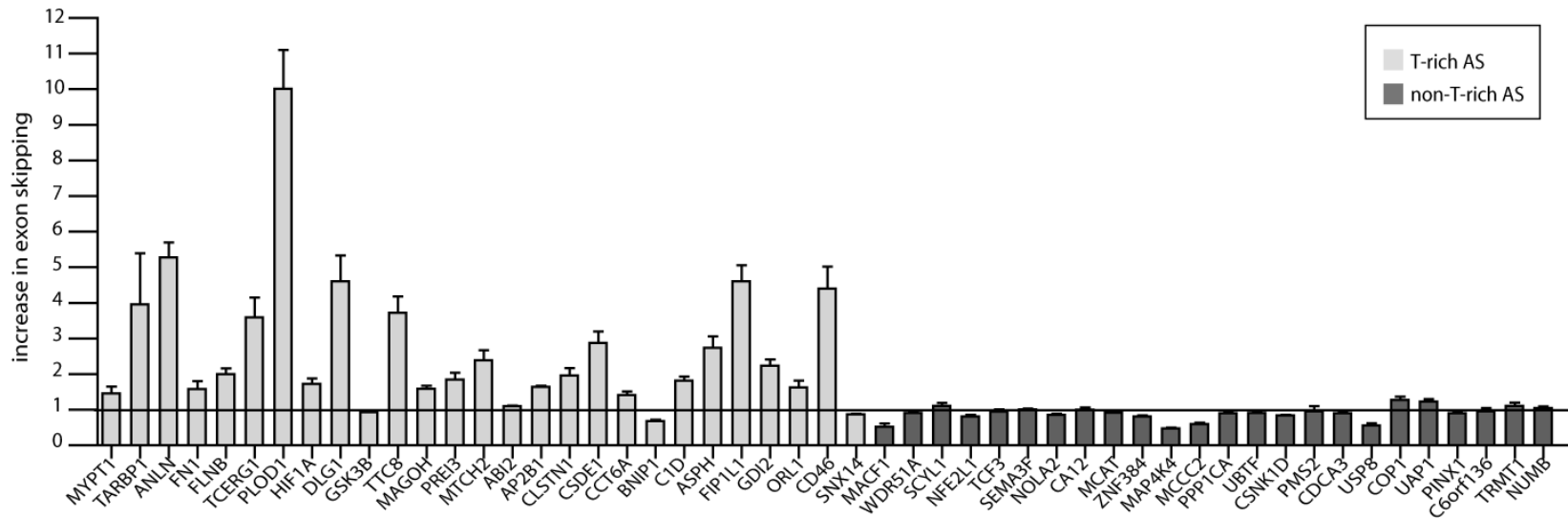
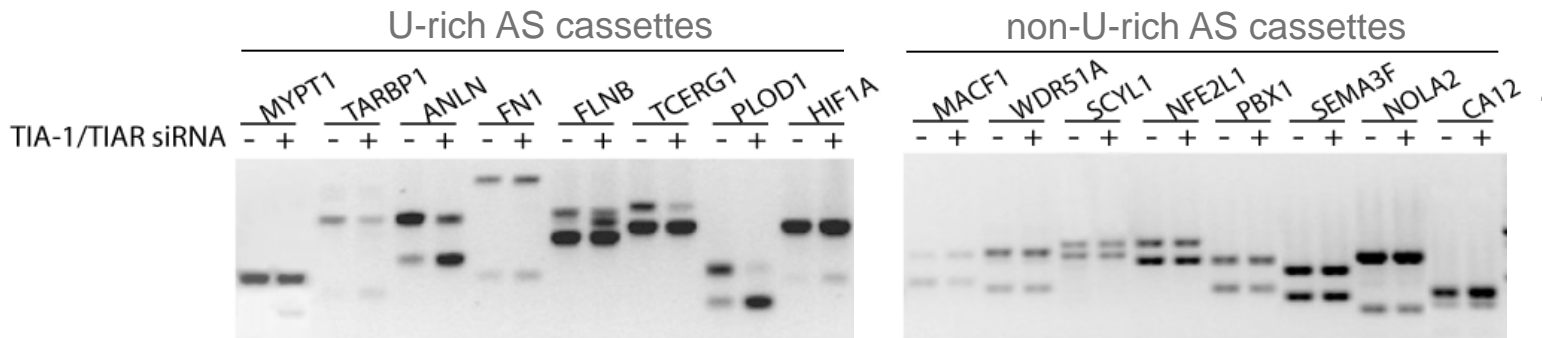
Selection of constitutive and alternative splicing cassettes of endogenous transcripts based on the U-richness of the second intron for RT-PCR analysis



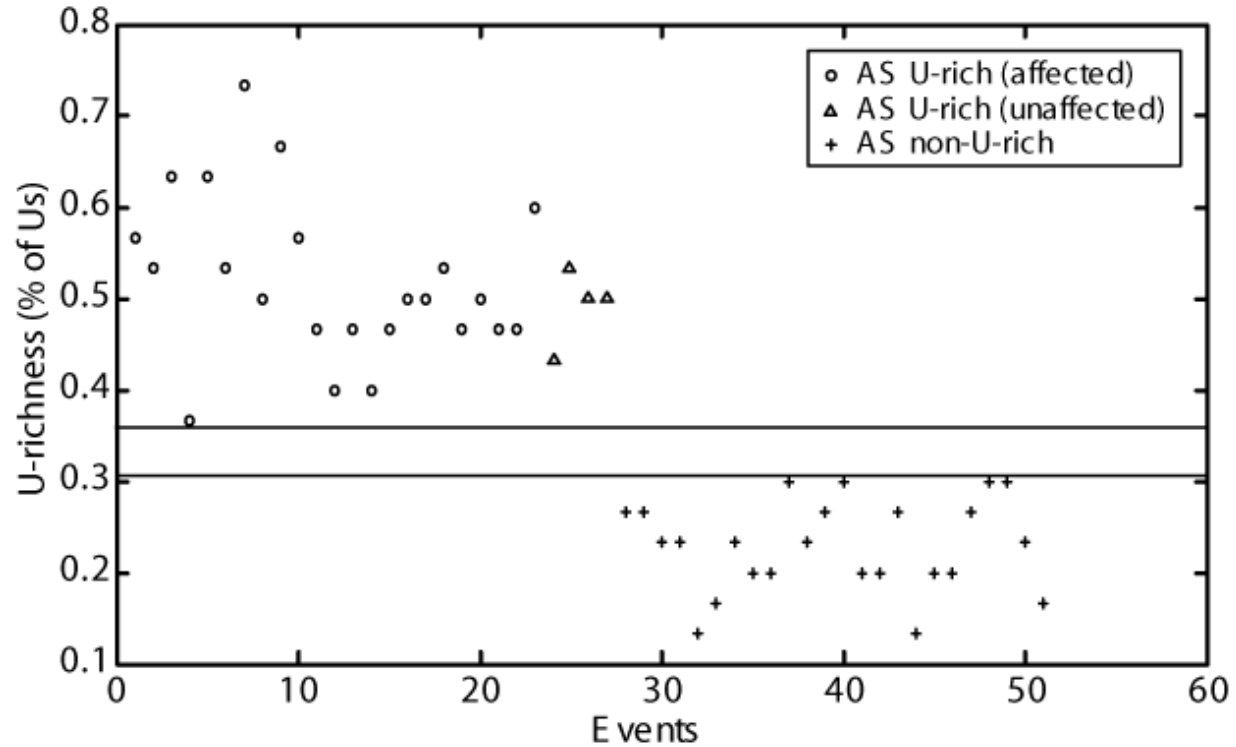
siRNA-mediated knockdown of TIA-1/TIAR in HeLa Cells



TIA-1/TIAR knockdown in HeLa cells increases the skipping of alternative exons followed by U-rich ISEs



Correlation between U-richness and effect of TIA-1/TIAR knockdown in alternative splicing



The inclusion of 85% of exons followed by introns that contain >35% of Us are regulated by TIA-1/TIAR

Diverse functional roles for TIA-1/TIAR-regulated AS genes

TIA-1/TIAR-regulated, U-rich AS events		
Gene	AS conserved	ORF preserved
→ MYPT1	no	yes
→ TARBP1*	no	no
→ ANLN	yes	yes
→ FN1	yes	yes
→ FLNB	yes	yes
→ TCERG1	yes	yes
→ PLOD2	yes	yes
→ HIF1A	yes	no
→ DLG1	yes	yes
→ TTC8*	no	yes
→ MAGOH	no	yes
→ PREI3	yes	yes
→ MTCH2*	yes	no
→ AP2B1	yes	yes
→ CLSTN1	yes	yes
→ CSDE1	yes	yes
→ CCT6A	no	yes
→ C1D*	n/a	no
→ ASPH	n/a	yes
→ FIP1L1	yes	yes
→ GDI2	no	yes
→ OLR1	no	no
→ CD46	n/a	yes

Higher than expected conservation (56%) of TIA-1/TIAR-regulated AS events between human and mouse

Summary

U-rich motifs are enriched downstream of 5' splice sites

U-rich motifs are present downstream of exons with low frequency of ESEs and high frequency of ESSs

The activity of the U-rich motifs is modulated by the splicing factors TIA-1 and TIAR

TIA-1/TIAR regulate the splicing of genes with a variety of functions

TIA-1/TIAR-regulated AS events are conserved between human and mouse more than expected

The estimated frequency of total events regulated by TIA-1/TIAR via U-rich intronic motifs is ~15%

Acknowledgements

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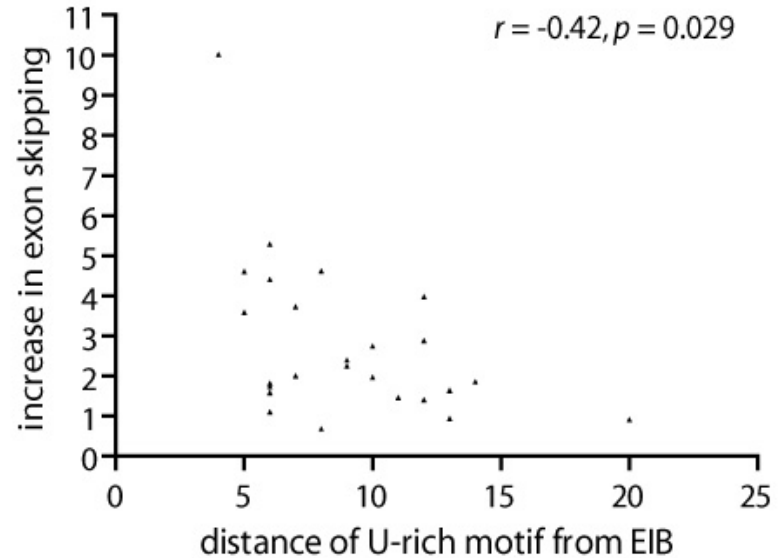
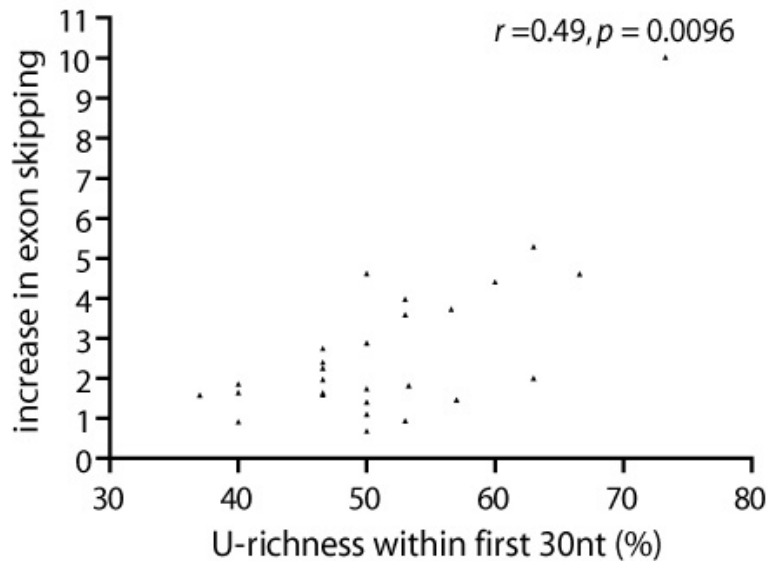
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Variables influencing the effect of TIA-1/TIAR knockdown on the increase in exclusion of alternative exons followed by U-rich ISEs

Variable tested using Spearman correlation test: 5' splice site strength, presence of ESEs, presence of ESSs, U-richness, distance of first motif to the intron-exon boundary (EIB)



Each dot corresponds to the mean increase in exon exclusion from two independent siRNA experiments performed in duplicate