Characterizing the regulation of splicing factors by alternative splicing and nonsense-mediated mRNA decay

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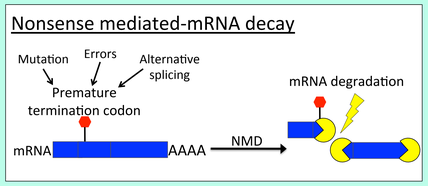
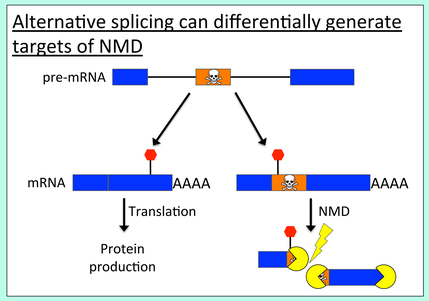
1 Nonsense-mediated mRNA decay is a quality control pathway that also regulates gene expression

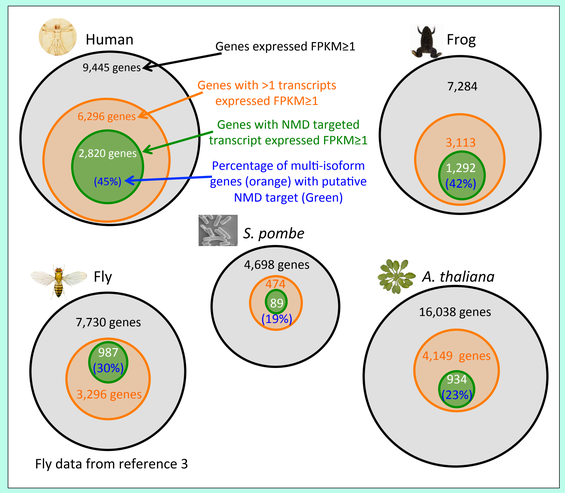


Nonsense-mediated mRNA decay (NMD) is a pathway conserved across eukaryotes that degrades transcripts with premature termination codons. Premature termination codons can arise from mutation or transcriptional/ processing errors.

Premature termination codons can also be introduced by regulated alternative splicing events. In this way, alternative splicing and NMD work together to regulate the expression of many endogenous genes in a process called regulated unproductive splicing and translation (RUST). Some alternative splicing events that introduce a premature termination codon into an transcript have been conserved for over a billion years[1-2].



2 Hundreds of genes produce NMD-targeted transcripts in diverse eukaryotes

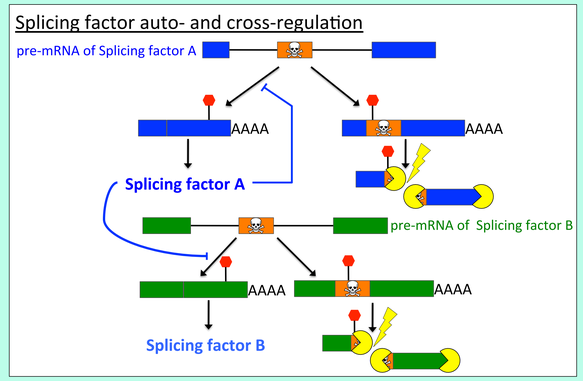


NMD was inhibited in a variety of eukaryotes. We identify putative NMD targeted genes as those with both an isoform that increases in abundance when NMD is inhibited and an isoform that does not change (to control for transcriptional upregulation). We found that 19-45% of alternatively spliced genes produce an isoform putatively targeted to NMD in examined organisms.

Therefore, alternative splicing coupled to NMD is potentially a major mechanism of gene regulation in a range of eukaryotes. Splicing factors are enriched among NMD targets in many species.

3 Splicing factors can regulate their own expression through splicing and NMD

Some splicing factors have been shown to auto-regulate their own expression by inhibiting the splicing of the productive isoform. Some also cross-regulate the expression of other splicing factors in a similar manner, allowing for the potential for a complex auto- and cross-regulatory network.

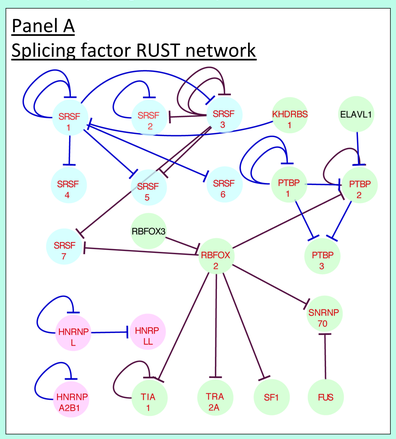


4 Highly connected splicing factor network suggests extensive auto- and cross-regulatory interactions via splicing and NMD

The prevalence of RUST as a mechanism for splicing factor regulation was assessed by searching the literature and building networks of known interactions [4-26] (Panels A and B).

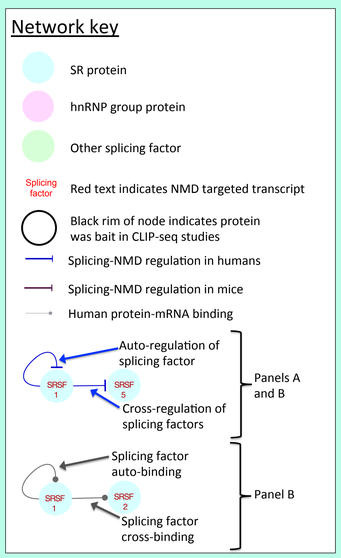
Panel A depicts the regulatory connections between splicing factors using RUST to inhibit the expression splicing factors and suggests RUST is an important mechanism of control for the examined splicing factors.

The protein-mRNA interactions denoted in panel B indicate that splicing factors could extensively regulate their own splicing and the splicing of other splicing factors making RUST of splicing factors a pervasive mechanism of gene control.



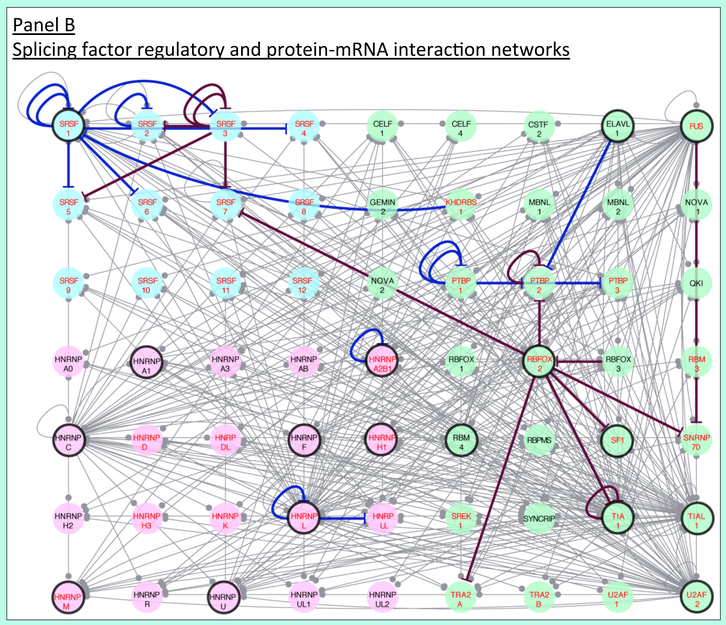
Network guide

In our networks, nodes represent both the protein and the mRNAs of a splicing factor. The start of edges (connections) represent the protein of a splicing factor and the end of edges represent the pre-mRNA of the splicing factor the protein is interacting with. See network key for more information. References used to create the networks are 4-26.



The high connectivity of the network is expected to lead to resilience to perturbation via overexpression and knockdown of splicing factors.

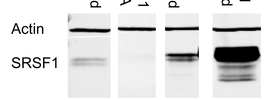
The uniform distribution of edges in our networks implies that there does not seem to be a hierarchy in which certain splicing factors are “master regulators”, as we see in transcription factor networks.



5 Manipulation of splicing factor expression will help us to identify new regulatory connections in the network

Our aim is to identify more instances of RUST of splicing factors, with the goal of producing a dynamic model that will allow us to predict the impact of splicing factor perturbation, as occurs in many disease states including cancer. By combining the overexpression or knockdown of a splicing factor with inhibition of NMD we can identify many splicing events normally hidden by degradation. We will start by studying SRSF1, SRSF6, hnRNP A1 and hnRNP A2. We have already been able to knockdown and overexpress SRSF1.

Knockdown and overexpression of splicing factor SRSF1 in HEK293 cells



Western blot showing knockdown and overexpression of SRSF1 in HEK293 cells

Planned experimental configuration

Splicing factor	+	-	+	-
NMD	+	+	-	-

Splicing factor	+	++	+	++
NMD	+	+	-	-

Combining splicing factor knockdown or overexpression with NMD inhibition will help us identify novel splicing factor-NMD regulatory interactions by qRT-PCR and RNA-seq

References and acknowledgments

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