# Pervasive unproductive splicing of SR proteins associated with ultraconserved elements

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SR proteins play critical roles in the regulation of pre-mRNA splicing. We show that most human SR protein genes are themselves alternatively spliced, and their alternative splice forms are often targets of a degradation pathway known as nonsense-mediated mRNA decay (NMD). By mining EST databases for sequences corresponding to the 11 human SR protein genes, we have identified premature stop (nonsense) codons in alternative mRNA forms of 10 of the genes. These isoforms are expected to be degraded by NMD rather than producing protein. The frequency with which these genes are alternatively spliced, the similar splice patterns, and the striking conservation imply that the alternative splice forms are functionally important.

We observe two classes of alternative splicing patterns among the SR proteins; in both cases the splicing is likely to decrease production of functional protein by targeting mRNA for decay. For some SR proteins, the minor splice form includes a cassette exon harboring a stop codon (figure 1). For others, the 3' untranslated region (UTR) is alternatively spliced to introduce a splice junction downstream of the normal stop codon, marking that stop codon as premature. Closely-related SR proteins tend to fall within the same class.

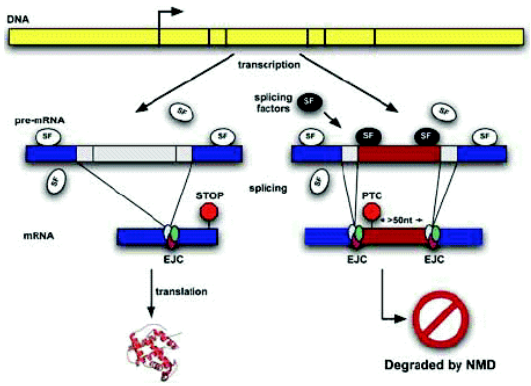


**Figure 1** Alternative splicing can include a cassette exon harboring a premature termination codon (PTC), and thus target the mRNA for

Alternative splice forms are not generally conserved between human and mouse. It is striking, then, that the mouse orthologs of human SR proteins exhibit the same unproductive splicing patterns. In at least two cases, the alternative splicing is also conserved between human and the basal chordate *Ciona intestinalis*. Further, the alternatively-spliced 3' UTRs or noncoding cassette exons and flanking introns show remarkable sequence conservation between human and mouse – some are more conserved than the protein-coding exons of the same genes. Five of the genes contain ultraconserved elements, long stretches of 100% nucleotide identity, in their alternatively spliced regions.

To demonstrate that our predicted SR isoforms are indeed NMD targets, we have established methods to detect specific transcript expression levels in the absence of NMD. After using RNAi to target UPF1, a key NMD effector, in HeLa cells, we measured the levels of transcripts containing premature stop codons using real-time PCR. Results from the first 5 SR genes display stabilization of predicted NMD target isoforms, in agreement with our predictions.

Some SR proteins have been shown to modulate splicing of their own transcripts. The SR protein SC35 is thought to autoregulate its splicing to produce unstable alternative forms that are likely to be degraded by NMD in order to attenuate the level of SC35 protein in the cell (1). Previously, we showed that a third of all human alternate splice forms may be targets of NMD, and that regulation of expression via degradation may be widespread (2,3). Our analyses suggest that the known cases of regulated alternative splicing of SR proteins may represent a highly conserved mode of gene regulation shared by almost all members of the SR protein family.

1. Sureau A, Gattoni R, Dooghe Y, Stevenin, J, Soret, J. 2001. SC35 autoregulates its expression by promoting splicing events that destabilize its mRNAs. *EMBO J.* 20:1785-1796.
2. Lewis BP, Green RE and Brenner SE. 2003. Evidence for widespread coupling of alternative splicing and nonsense-mediated mRNA decay in humans. *Proc Natl Acad Sci USA.* 100:189-192. doi:10.1073/pnas.0136770100
3. Hillman RT, Green RE and Brenner SE. 2004. An unappreciated role for RNA surveillance. *Genome Biol* 5:R8. doi:10.1186/gb-2004-5-2-r8