Stony Brook University
The Graduate School

Doctoral Defense Announcement

Abstract

Regulation and Target Specificity of Human Alternative Splicing Factors

SF2/ASF and Fox-1/2

By

Shuying Sun

Alternative splicing is a highly regulated process in eukaryotes. It greatly increases the diversity of proteins encoded by the genome, and its disruption can cause a number of genetic diseases. SF2/ASF is a prototypical serine/arginine-rich (SR) protein, with important roles in constitutive and alternative splicing and other aspects of mRNA metabolism. SFRS1 (SF2/ASF) is a potent proto-oncogene with abnormal expression in many tumors. We found that SF2/ASF negatively autoregulates its expression to maintain homeostatic levels of the protein. We characterized six SF2/ASF alternatively spliced mRNA isoforms: the major isoform encodes full-length protein, whereas the others are either retained in the nucleus or degraded by NMD. Unproductive splicing accounts for only part of the autoregulation, which occurs primarily at the translational level. The effect is specific to SF2/ASF and requires RRM2, the second of two RNA-recognition motifs. The ultraconserved 3'UTR (untranslated region) is necessary and sufficient for downregulation. SF2/ASF overexpression shifts the distribution of target mRNA towards mono-ribosomes, and translational repression is partly independent of Dicer and a 5' cap. Thus, multiple post-transcriptional and translational mechanisms are involved in fine-tuning the expression of SF2/ASF.

Fox-1 and Fox-2 are brain- and muscle-specific alternative splicing factors. Their single RRM is conserved from worm to human, and specifically binds the RNA element UGCAUG. We applied Solexa high-throughput mRNA sequencing to assess global changes of alternative splicing controlled by Fox-2. We generated ~110 million paired-end reads to compare target-isoform expression levels in cells expressing Fox-2 versus cells treated by RNAi to reduce Fox-2 expression. We identified about 150 high-confidence alternative exons with Fox-dependent splicing, of which 95% could be experimentally validated.

We also explored the mechanisms of splicing activation and repression by Fox-1. Fox-1/2 regulate alternative splicing positively or negatively in a position-dependent manner: they activate exon inclusion when binding to the downstream intron, and promote exon skipping when binding to the upstream intron. We found that Fox-1 can enhance exon inclusion of a heterologous gene when tethered to the downstream intron by a phage MS2 hairpin/coat-protein interaction, and its C-terminal domain is sufficient for this activity. However, both C-terminal domain and the central RRM are required for exon repression when tethered to the upstream intron. We used immunoprecipitation and mass spectrometry to identify proteins that interact with the C-terminal domain of Fox-1. Characterization of several interacting candidates to elucidate their potential roles in alternative splicing regulation by Fox-1 is in progress.

Date: April 28, 2010
Time: 1:00 PM
Place: James Building, CSHL

Program: Molecular and Cellular Biology
Dissertation Advisor: Prof. Adrian R. Krainer