

Genomewide RNA-Seq analysis in mutant zebrafish reveals role of U1C protein in alternative splicing

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Many disease-causing mutations in human genes have been mapped to splice sites or splice-regulatory sequences, resulting in alternative splicing defects. Surprisingly, mutations have also been found in several genes coding for general splicing factors that can cause human diseases, such as spinal muscular atrophy or Retinitis pigmentosa.

To address the question how mutated general splicing factors can yield such specific phenotypes and affect only specific cell types, we are using the zebrafish *Danio rerio* as a new model system to study the genomewide role of splicing factors.

The U1 snRNP specific protein U1C is known to be involved in 5' splice site recognition during early splicing complex formation and important for complex stabilization. A U1C knockout zebrafish mutant, which originates from a large-scale genetic screen (1), is embryonically lethal at day 5 and showed a strong reduction of U1C protein levels; surprisingly, however, U1 snRNA levels were not affected, and the U1 snRNP appeared to be overall stable; in addition, glycerol gradient centrifugation analysis provided evidence for a U1C-deficient U1 snRNP. We were able to rescue the wildtype phenotype in the knockout mutant embryos by injecting zebrafish U1C cRNA.

We next asked whether the U1C mutant zebrafish embryos may show splicing defects or aberrant alternative splicing patterns. To address this and to identify U1C-dependent target genes, we applied high-throughput sequencing (Solexa RNA-Seq; 76-bp reads), based on total RNA from three-day-old U1C mutant and wild-type zebrafish embryos. As a result, more than 100 mutant-specific exon-skipping events were detected, 30 alternative 5' splice site events, and 13 other splice pattern changes (intron retention, mutually exclusive exons), that could be RT-PCR-validated at a rate of over 90%. In sum, our analysis revealed a large group of target genes that exhibited in the absence of U1C altered splicing patterns, in particular increased exon skipping. Sequence motif analysis of the U1C-dependent 5' splice sites and their surrounding sequences suggests that U1C mediates the function of alternative splicing regulators bound to adjacent sequence elements. Thereby U1C-deficient U1 snRNPs may play a role in alternative splicing regulation. We currently work on testing these conclusions in mammalian cells.

1. Amsterdam, Nissen, Sun, Swindell, Farrington, Hopkins (2004) *Proc Natl Acad Sci USA* 101, 12792-12797.