In vivo Regulation of the Alternative Splicing of the Pro- and Anti-Apoptotic Gene *ced-4*

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The processing of pre-mRNAs by alternative splicing provides a cell with the ability to generate multiple mRNAs from a single gene. Despite many efforts to study this process, the regulation of alternative splicing *in vivo* and in a tissue- or developmental stage-specific manner as well as the functional implications of alternative splicing are not well understood.

The *C. elegans* CED-4 protein promotes the activation of the caspase CED-3 and is essential for canonical programmed cell death (PCD). However, the *ced-4* transcript is alternatively spliced, giving rise to two different isoforms with antagonistic functions generated by use of alternative 3' splice sites (ss) in exon 4. The main isoform, CED-4S, is pro-apoptotic, while CED-4L is anti-apoptotic. *ced-4* is the only apoptotic gene known to be alternatively spliced in *C. elegans*. How *ced-4* alternative splicing is regulated is largely unknown.

To study the regulation of *ced-4* alternative splicing *in vivo* we have generated fluorescent reporters so that expression of CED-4L will give rise to GFP, while expression of CED-4S will give rise to RFP. These reporters showed a higher levels of expression of the *ced-4S* isoform, consistent with the alternative splicing levels observed for the endogenous gene. A deletion analysis of the reporters indicated the presence of two sequences important for the regulation of *ced-4* splicing. One, located upstream of the exon 4L 3'ss, contains possible binding sites for the Fox-1 family members FOX-1 and ASD-1 and for the muscle-specific splicing factor SUP-12. These proteins might prevent the recognition of exon 4L 3'ss. Deletion of the FOX-1/ASD-1 but not of the SUP-12 binding site weakly increased CED-4L expression. However, single mutants of *fox-1*, *asd-1* or *sup-12* did not modify the *ced-4* isoform ratio and did not exhibit PCD defects in the anterior pharynx or ventral cord.

We are now testing the effects of double mutants of these genes as well as of other genes involved in alternative splicing, such those that encode the Serine/Arginine-rich proteins implicated in cell survival. We are also trying to identify factor/s that bind upstream of exon 4 using biochemistry. In addition, we are performing a genetic screen for mutants with an increased CED-4L signal.

We hope that by studying *ced-4* alternative splicing using our fluorescent reporter system we will identify not only factors involved in alternative splicing but also modulators of the apoptotic pathway itself.

C. elegans Development, Cell Biology, & Gene Expression Meeting
Poster
Primary session topic: Cell death
Secondary session topic: Gene regulation during development
No. characters (counting spaces): 2,500 (2,500 max)