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, thus enormously expanding proteome diversity. Despite 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. However, *ced-4* is alternatively spliced, giving rise to two different isoforms with antagonistic functions: 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 are generating reporters in which the alternatively spliced region of *ced-4* is followed by a fluorescent-protein cDNA specifically in-frame with one or the other isoform, so that expression of CED-4L will give rise to GFP while generation of CED-4S will give rise to RFP. This approach should allow us to determine the isoform ratio during development at a single-cell level and to correlate this ratio with a specific cell's fate. We then will characterize regulators of this alternative splicing event by performing a genetic screen for mutants with an altered fluorescent protein ratio (*e.g.*, decreasing the ratio of CED-4S to CED-4L). We will determine the expression patterns of new spicing regulatory factors and establish the mechanisms by which they modulate *ced-4* alternative splicing using biochemical and molecular approaches.

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

Poster
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