Reprogramming Alternative Splicing Using *Cis*-Acting Intronic Control Elements

Thesis by

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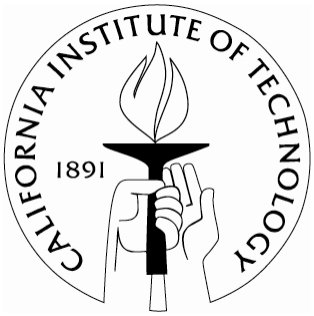

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Dedicated to Manigeh, Ron, and Bradley

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Abstract

Alternative splicing is a process by which multiple protein isoforms are generated from a single coding region by altering the ways in which exons are joined together. This pathway is used by cellular systems to both increase proteomic diversity from a limited number of genes and to precisely control gene expression. Bioinformatics and comparative genomics approaches have provided significant sequence and functional insight into the regulatory sequences that occur within exonic regions of a transcript. *In vitro* and *in vivo* strategies have also been developed to screen for exonic splicing enhancers and silencers (ESEs and ESSs) from small, randomized libraries. Much less is known about intronic splicing enhancers and silencers (ISEs and ISSs), although recent bioinformatics approaches are beginning to shed some light on these regulatory sequences. A thorough understanding of both exonic and intronic regulators is necessary to enable the programming of alternative splicing patterns, which will provide a powerful tool for interrogating and manipulating cellular function.

We developed a generalizable *in vivo* screening strategy for generating intronic splicing regulatory elements (ISREs). Our high-throughput approach employed a systematic screening strategy with extensive genome-wide bioinformatic analyses and experimental characterization which included a small-scale RNAi screen. Using this approach, we identified ISRE consensus motifs, characterized the splicing regulatory networks (SRNs) associated with these regulatory elements and generated a model for ISRE regulatory function. Highlighting the complexity of SRNs, we found that *cis*-acting intronic regulatory sequences function through combinatorial effects from multiple elements and trans-acting factors, and that the immediate transcript context has a

dominant effect on ISRE function. Overall, this screening strategy provides a general method for generating regulatory sequences of alternative splicing events, which provide powerful tools for gene expression control.

We next extended from our studies on cellular screening strategies for generating splicing regulatory elements, to build novel platforms that support the construction of protein-responsive alternative splicing control elements. Protein-binding RNA aptamers were inserted into key intronic locations of an alternatively spliced transcript to enable the detection of intracellular protein concentrations and to translate detection events to the regulation of alternative splicing patterns and thus gene expression. We demonstrate that these RNA elements can serve as autonomous control devices by linking endogenous nuclear protein levels to gene expression events and external stimuli to complex cellular phenotypes. These synthetic alternative splicing regulators can be implemented combinatorially to regulate alternative splicing patterns in response to multiple inputs. In addition, we applied these synthetic regulators to the rewiring of endogenous signal transduction pathways and building of novel regulatory networks for user-defined phenotypes. Our work provides an early example of a novel class of RNA-based "intelligent" therapeutics by directing increased signaling through pathways associated with disease to the triggering of apoptosis. These programmable sensing-actuation molecules will be broadly applied in health and medicine towards the early diagnosis and treatment of disease.

Table of Contents

LIST OF FIGURES

LIST OF TABLES