Abstract

In the field of functional genomics, significant attention has been paid to the effects of gene disruption on cellular homeostasis. In particular, reverse genetic screens have transformed our current understanding of gene function. Largely ignored in such screens are the functions of the diverse repertoires of transcript and protein isoforms derived from genes through a variety of co- and post-transcriptional processes, among which alternative splicing (AS) is widespread. Alternative isoforms are particularly abundant in the nervous system where they are coordinately regulated in networks of functionally related genes. Short, 3-27 nucleotide long, 'microexons' represent a significant proportion of neuronal-specific alternative exons. These exons exhibit high levels of conservation across vertebrates, are enriched in frame-preserving lengths, and often display decreased inclusion in the brains of individuals with autism spectrum disorder (ASD). While a growing number of studies have shown that microexons can have a profound impact on protein function and neuronal development, the function of the vast majority of these exons is unknown. To enable the systematic interrogation of isoform function, I developed CHyMErA-seq: a scalable CRISPR-based screening platform that leverages dual Cas-mediated genomic deletions paired to single-cell transcriptomics to generate interpretable readouts associated with individual exon loss. I applied this method to investigate the functions of microexons during neurogenesis and uncovered a convergent pathway in which these exons regulate neurodevelopmental timing. Microexon deletion-associated gene signatures overlap with those detected in the brains of individuals with ASD. Furthermore, downstream functional studies uncovered roles for *Gfra1* and *Clasp1* microexons in the regulation of neuritogenesis and neurogenic signalling pathways respectively. Collectively, this work establishes technology enabling the systematic profiling of

the functions of alternative isoforms, and provides a framework for understanding the roles of microexons during neurogenesis, and the impact of their disruption in the context of ASD.