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Technology Watch

COLOUR-CHANGING WORMS

Observing the development of a living worm in real time is now possible, according to a study by Ohno and colleagues. Using fluorescent proteins, they visualized developmentally regulated changes in live nematodes during alternative pre-mRNA splicing. Alternative splicing of the *Caenorhabditis elegans let-2* gene (encoding α2 (IV) collagen, which is expressed in the body-wall muscles) is strictly regulated and changes during the worm's lifetime: embryos express only exon 9, whereas adults express only exon 10. Using a green fluorescent protein (GFP) linked to exon 9 and a red fluorescent protein (RFP) linked to exon 10, the tissue-specific expression profiles of each *let-2* exon were revealed. Expression of the *let-2* reporters, which are controlled by the body-wall-specific gene promoter of *myo-3*, switched from GFP to RFP during the development of embryos to adults, in parallel with endogenous *let-2* mRNAs. This reporter system enabled the identification of splicing mutants and their partially spliced pre-mRNAs, with the advantage of analysing the abundance of two different mRNA isoforms with only a minimal effect on overall transcription levels. These reporters can assist expression-profiling efforts and the identification of auxiliary factors that regulate alternative splicing.

ORIGINAL RESEARCH PAPER Ohno, G., Hagiwara, M. & Kuroyanagi, H. STAR family RNA-binding protein ASD-2 regulates developmental switching of mutually exclusive alternative splicing *in vivo*. *Genes Dev.* **22**, 360–374 (2008)

EXONS EXPOSED

Spliceosomes are large macromolecular complexes that carry out mRNA splicing during gene expression. Although spliceosome structures have been elucidated by electron microscopy (EM) and single-particle reconstruction, they are difficult to interpret because individual protein or RNA elements are not visible. Now, Alcid and Jurica describe a protein-based label that can reveal the location of the two exons that are ligated together during splicing. The label is a fusion protein that comprises the sliding-clamp protein DnaN — which forms a ~9-nm doughnut-shaped structure that is easily identifiable by EM — fused to the RNA-binding protein PP7, which has a high binding affinity for a 24-nucleotide RNA hairpin sequence that can be engineered into the exons of interest. To label exons in spliceosomes, the authors inserted these hairpin sequences into a pre-mRNA substrate near the 5′ and 3′ splice sites at accessible regions in the assembled spliceosomes, and incubated spliceosomes with an excess of the DnaN–PP7 label. By comparing EM images of labelled and unlabelled spliceosomes, they confirmed that DnaN–PP7 labelled ~25% of spliceosomes. Compared with gold and antibody labelling techniques that are difficult and expensive to use, the ease of production and the higher labelling efficiency of this specific, readily identifiable RNA label makes it a useful tool for the analysis of RNA-containing macromolecular complexes.

ORIGINAL RESEARCH PAPER Alcid, E. A. & Jurica, M. S. A protein-based EM label for RNA identifies the location of exons in spliceosomes. *Nature Struct. Mol. Biol.* **15**, 213–215 (2008)