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GENE EXPRESSION

Controls and roles for *trans*-splicing

A few examples of *trans*-spliced RNAs — RNA sequences ligated from separate transcripts — have been experimentally validated and functionally characterized in various species. *Trans*-spliced RNAs are often inferred from transcriptome sequencing data sets, but dissecting the genuine *trans*-spliced RNAs from false positives resulting from technical artefacts remains a challenge. A new study reports a pipeline for prioritizing candidate *trans*-spliced RNAs from transcriptome sequencing data and uses this approach to identify a role for a *trans*-spliced non-coding RNA (ncRNA) in maintaining pluripotency in human cells.

Wu *et al.* carried out both long-read and short-read high-throughput transcriptome sequencing on the H9 human embryonic stem cell (hESC) line, and used equivalent publicly available sequencing data for the H1 hESC line. By aligning the long-read sequences to the reference human genome sequence, they identified 8,822

candidate *trans*-spliced RNAs. They then applied various bioinformatic filters to remove probable false positives, such as candidates that were not supported by short-read sequencing and by both cell lines, and those candidates with sequence features that were suggestive of artefactual generation. These filters eliminated ~99.9% of candidates; of the remaining nine high-priority candidates, four passed subsequent experimental validation.

All four validated *trans*-splicing events were intragenic; that is, they resulted in a mis-ordered arrangement of exons from the same gene. Sequencing and experimental data indicate that this splicing occurred through *trans*-splicing between two copies of the primary transcript rather than through, for example, intramolecular splicing to generate a circular mRNA.

Three of the *trans*-splicing events brought an alternative sequence upstream of a full-length coding region. These events were associated with a different expression pattern relative to

the regular, colinear isoforms across a panel of differentiated cell types.

Interestingly, the fourth *trans*-spliced RNA was a novel ncRNA, termed *tsRMST*. This ncRNA was found to be expressed at higher levels in pluripotent cells (both hESCs and human induced pluripotent stem cells (hiPSCs)) relative to differentiated cells. In support of a role for *tsRMST* in pluripotency, manipulation of *tsRMST* levels in hESCs showed that this ncRNA suppresses the expression of differentiation-associated genes. This function is mediated in part by recruiting the transcription factor NANOG and the repressive Polycomb protein SUZ12 to the promoters of these lineage-specific genes.

It will be interesting to use prioritization strategies in other cell types to determine how widespread *trans*-spliced RNAs are, and the scope of their biological functions.

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ORIGINAL RESEARCH PAPER Wu, C. S. *et al.* Integrative transcriptome sequencing identifies *trans*-splicing events with important roles in human embryonic stem cell pluripotency. *Genome Res.* <http://www.dx.doi.org/10.1101/gr.159483.113> (2013)