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SON sheds light on RNA splicing and pluripotency

Ilana Livyatan and Eran Meshorer

The role of RNA splicing in the regulation of stem cell properties has remained largely unexplored. The splicing-associated protein SON is now shown to be necessary for embryonic stem cell maintenance, by influencing the splicing of pluripotency regulators.

Embryonic stem cells (ESCs) are characterized by their ability to self-renew indefinitely and, when given the proper conditions, to differentiate into essentially every cell type or tissue, a quality known as pluripotency[1,2]. To maintain these features, ESCs express a core set of transcription factors that are important for maintaining the pluripotent state[3]. Once expressed, most genes are subject to RNA splicing, a process carried out by a macromolecular complex termed the spliceosome, whereby intronic portions of a nascent RNA are removed and the remaining exonic sequences are fused together to create a mature RNA transcript. Alternative splicing involves varying specifications of intronic and exonic regions within the gene, and gives rise to several different RNA products (or isoforms) from the same gene. This process enhances the transcriptional capacity of the cell beyond the number of genes it contains, and enlarges the repertoire of both protein-coding and non-coding RNA products[4,5]. Although RNA splicing and alternative splicing are well-characterized cellular processes, their role in stem cell biology has remained vague. In this issue, Lu *et al.*[6] shed light on this subject by identifying a connection between the splicing machinery and pluripotency that is mediated by a splicing-related protein named SON.

When ESCs are induced to differentiate, the alterations in their transcriptional program are accompanied by changes in their RNA splicing patterns[7]. These differences lay the groundwork for the very different biological functions of the cells. For example, an ESC-specific isoform of the *FOXP1* gene has different DNA-binding properties compared with the non-ESC-expressed FOXP1, and is able to stimulate the expression of pluripotency genes and to repress genes involved in lineage specification[8]. Gene expression profiling studies have already led to the identification of factors which maintain or confer the pluripotent nature of ESCs. This knowledge has been applied to completely reprogramming a terminally differentiated somatic cell into one that is almost identical to a pluripotent ESC, by the expression of several core pluripotency factors[9]. A comprehensive understanding of the spliced transcriptome and the factors that govern it is likely to translate into additional, or more fine-tuned, methodologies for achieving somatic cell reprogramming. Recently, two splicing regulators from the MBNL protein family were discovered to be significantly upregulated following ESC differentiation[10]. Overexpression of these factors in ESCs led to impaired splicing and, specifically, to the loss of the ESC-specific isoform of FOXP1, whereas depletion of the two factors increased reprogramming efficiency.

A previous genome-wide RNAi screen, designed to identify proteins that are essential for the maintenance of stem cells, reinforced the notion of a connection between splicing and pluripotency[11]. The SON protein, previously shown to be associated with splicing regulation, ranked in the top 10 potential regulators of pluripotency out of over 20,000 genes tested. In the present study, Lu *et al.*[6] analysed the effects of shRNA-mediated depletion of SON. As proposed by the original RNAi screen[11], the reduction of SON levels was deleterious to the maintenance of ESCs as they exhibited a spontaneous differentiation phenotype, followed by widespread cell death (Fig. 1). The authors used RNA sequencing to delve deeper into the molecular mechanism underlying this phenotype, and discovered a marked impairment in the splicing patterns of more than 1,000 genes. This gene group was significantly enriched with pluripotency-related and ESC survival genes, particularly *OCT4*, *PRDM14*, *MED24* and *E4F1*, all key players in stem cell biology. RNA crosslinking immunoprecipitation (RNA-CLIP) assays demonstrated that SON directly bound the RNA transcripts of these genes. Interestingly, intron retention, the phenomenon whereby an intronic sequence is retained in the mature RNA transcript, was a prevalent type of splicing defect under conditions of SON deficiency, with 1,994 events in 1,127 genes. The retained introns were shorter than average, with higher GC content, and contained weaker splicing signals. This phenomenon was specific to certain introns within particular genes, rather than a general preference of SON for this type of intron. The inclusion of an intron in the aberrant RNA isoform sometimes led to nonsense-mediated decay (NMD), a mechanism needed for the degradation of RNA transcripts that contain premature stop codons and which therefore terminate protein translation prematurely, uncovering a previously unrecognized link between SON-regulated splicing and RNA

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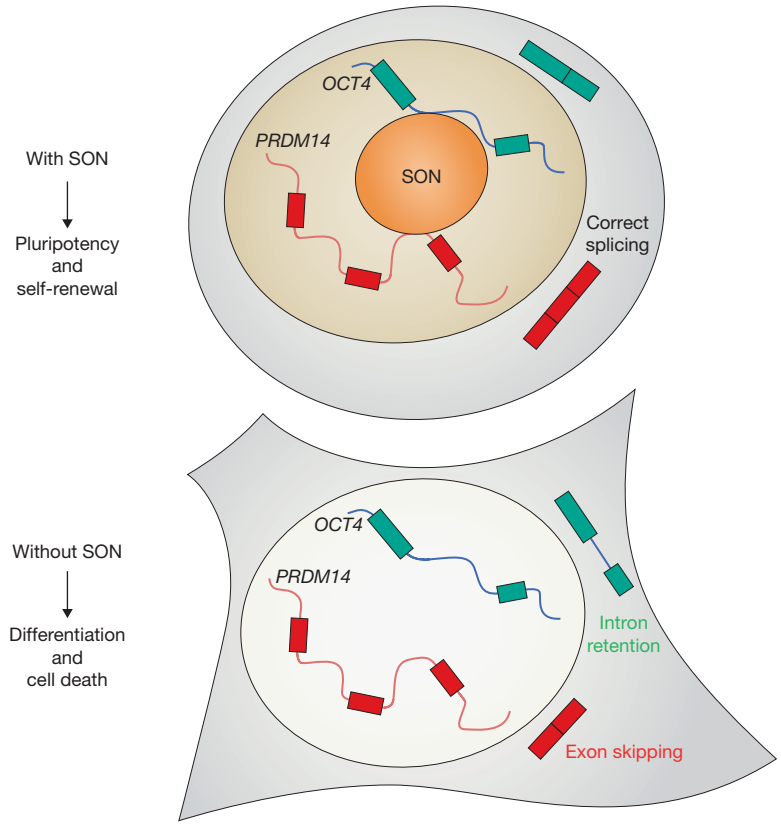

Figure 1 SON depletion results in aberrant splicing of pluripotency factors in ESCs. In wild-type ESCs, SON binds the RNA transcripts of several pluripotency-related genes, including *OCT4* and *PRDM14*, which are spliced correctly and function to maintain the pluripotent and self-renewing phenotype of the cells (top). Conversely, when SON is depleted, these genes and other pluripotent factors are aberrantly spliced, manifested by both intron retention and exon skipping. This results in the loss of stem cell qualities, the presence of differentiation characteristics, and cell death (bottom). The nucleus is shown in yellow (top) and light grey (bottom); cytoplasm is dark grey; and RNA is shown in red and green, with exons as rectangles and introns as lines.

decay. In addition to intron retention, 940 splicing defects of other types, including exon skipping, were detected in 694 genes following SON depletion. Among these genes, *PRDM14* exhibited both intron retention and skipping of exon 2. Intriguingly, the shorter protein resulting from the shorter isoform lacking exon 2 lost the ability to support pluripotency and to enhance reprogramming, when compared with the full-length version (Fig. 1).

These findings demonstrate the key role of RNA splicing in regulating the pluripotency phenotype of ESCs, in line with previous reports[10,12]. SON seems to regulate this phenotype in the opposite direction to the aforementioned MBNL proteins[10], and thus it will be very interesting to identify the upstream regulators of these two splicing regulators. The specificity of the MBNL and SON proteins to functional groups of genes, and particularly that of SON to certain types of introns, indicates a targeted mode of gene recognition. Very little is known about the binding specificity of splicing-related factors, including whether it stems from the RNA sequence or structure, or whether it is based on something else entirely. Employing assays such as RNA-CLIP to determine binding sites within RNAs bound by SON and other splicing factors will perhaps enhance our understanding in a manner comparable to the way widespread use of ChIP-seq data has elucidated the mechanisms of transcriptional regulation by transcription factors.

In the future it will be interesting to understand how SON interacts with the spliceosomal complex and other factors which may contribute to the specification of isoforms in pluripotent and differentiated cells, such as the MBNL proteins. Its co-localization with the splicing factor SRSF2 (ref. 13), for example, is potentially a first step in elucidating the exact molecular function of SON in the context of splicing. Moreover, the influence of SON downregulation on components of the NMD pathway infers a far more complex mechanism of co-regulation between splicing and other RNA post-transcriptional mechanisms. Elucidating how SON orchestrates this interplay may provide a more general perspective on RNA processing.

In addition to delving deeper into the regulation, mode of operation and downstream molecular and phenotypic effects of SON, our understanding of the relationship between splicing and stem cell biology can be expanded by further unbiased screens for factors that may be associated with splicing and also confer a molecular and/or phenotypic effect on stem cells. A genome-wide analysis of non-polyadenylated RNA expression in ESCs and differentiated cells has implicated components of the minor spliceosome in the regulation of *Eed*, which regulates lineage specification and differentiation[14]. Furthermore, a recent unbiased screen in ESCs for proteins that bind RNA (ref. 15) may provide new candidates of splicing-dependent regulation of pluripotency and differentiation.

From a more general perspective, given that phenotypic changes are so pronounced during development and differentiation in the embryo, ESCs are a good platform for discovering splicing regulators. These factors could represent potential drug targets for diseases caused by aberrant splicing such as spinal muscular atrophy, Hutchison-Gilford Progeria syndrome, some cases of cystic fibrosis, and an estimated 15% of all diseases stemming from genetic mutations. The characterization of SON is one of the first steps along a long road to understanding the impact of splicing on cellular biology.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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