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ALTERNATIVE SPLICING

The lord of the rings

Circular RNAs can regulate the alternative splicing profile of their parental genes by physically interacting with the DNA to form RNA:DNA hybrids.

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Eukaryotic genes are transcribed into primary RNAs that need to undergo several processes to generate a mature protein-coding messenger RNA (mRNA). One of these processes, known as splicing, removes fragments of the primary transcript (or introns) to yield an mRNA composed of consecutively joined exons. Alternative splicing of specific introns or skipping exons is the mechanism by which a single gene can be transcribed into various mRNA isoforms. Importantly, nearly all human intron-containing genes, as well as the majority of plant genes, can be alternatively spliced[1], greatly expanding the mRNA population in each cell type by combining different exons and boosting the variety of proteins encoded in the same DNA from a given organism. A range of factors modulating alternative splicing[2] have been identified, including specific protein complexes, associated epigenetic modifications, and even noncoding RNAs[3]. In this issue of *Nature Plants*, Conn *et al.*[4] demonstrate that circular RNA (circRNA) can modulate the alternative splicing of its own parental gene by directly interacting with its DNA, forming an RNA:DNA hybrid known as the R-loop[5].

Circular RNAs are covalently closed circular molecules of single-stranded RNA. They are transcribed from intergenic regions or can be formed from either exonic or intronic sequences by a particular splicing event, the so-called back-splicing. Although circRNAs have been identified in all eukaryotic kingdoms, their mechanisms of action remain unclear. We know that they can be developmentally regulated[6], some can act as microRNA sponges[6,7], and certain intron-containing circRNAs can modulate the expression of their parental genes[8,9]. Exonic circRNAs are back-spliced at canonical splice sites. These circular transcripts are abundant and highly stable, and they may efficiently compete with pre-mRNA splicing for the recognition of related protein complexes. Indeed, there is a correlation between the existence of exonic circRNAs and the exon-skipping event of the cognate linear alternatively spliced mRNA. However, it remains uncertain what mechanism is behind this process.

In their Article, Conn and co-workers identified loci across the genome that are precursors of circRNAs and also suffer exon-skipping. They did this by comparing publicly available information on *Arabidopsis* circRNAs[10,11] with alternatively spliced transcript variants that are compiled in the *The Arabidopsis Information Resource* database. As a result, the authors chose a few members from the MADS family of transcription factors (TFs) for further characterization. The alternative splicing of MADS TFs can affect the domains responsible for protein–protein interactions. Thus, the resulting splicing variants may determine their participation in various distinct multimeric protein complexes[12], modulating their transcriptional activity and impacting many developmental processes, including their floral homeotic functions. In particular, the authors found that overexpression of circRNA from exon 6 of the MADS gene *SEPALLATA3* (*SEP3*) enhances the accumulation of the naturally occurring *SEP3.3* isoform, which consists of the exon-6-skipped transcript. Furthermore, lines with higher levels of this circRNA produced flowers with altered floral organ number, for example, fewer stamen and additional petals. This developmental disorder was confirmed by the overexpression of the full *SEP3.3* isoform. This exciting result constitutes the first example of an organismal-level phenotype that is mediated by circRNA manipulation.

Strikingly, the authors found out that circRNA from exon 6 of the *SEP3* gene is capable of generating an R-loop by direct interaction with its own genomic locus (Fig. 1). R-loops occur when a transcript invades the DNA duplex and anneals to one of the strands to generate an RNA:DNA hybrid. R-loops have been identified in

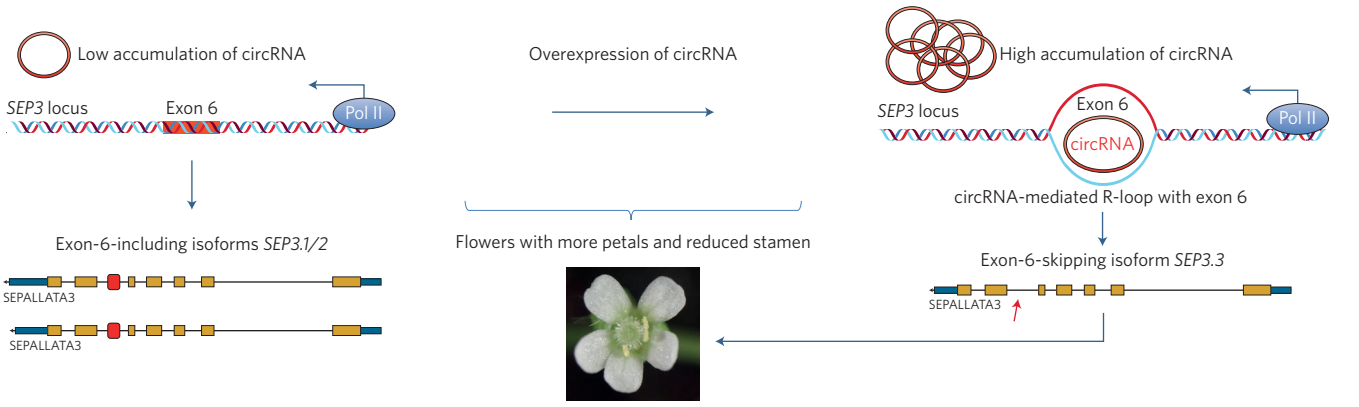

Figure 1 | The occurrence of circRNA promotes the formation of an R-loop between the circular transcript and the complementary strand of its parent locus. The RNA:DNA hybrid induces the skipping of exon 6 (red), thus favouring the processing of the pre-mRNA into isoform *SEP3.3*. The overexpression of circRNA causes an imbalance in the ratio among the isoforms, impacting flower development. Pol II, RNA polymerase II.

many eukaryotic organisms, with increasing occurrence at sites with high transcriptional activity[13], and R-loop interaction with nascent transcripts impacts chromatin patterning and gene regulation[14]. In this work, the formation of the R-loop mediated by circRNAs was linked to the determination of the splicing variants derived from the parental gene. These findings provide a new perspective on the field of RNA biology and further expand the role of noncoding RNAs in gene expression and splicing modulation. The development of high-quality antibodies that recognize RNA:DNA hybrids by DNA–RNA immunoprecipitation, together with the advent of powerful sequencing technologies, will allow us to decipher genome-wide formation of R-loops. The correlation of such genome-wide characterization with circRNA databases and known exon-skipped alternatively spliced variants will certainly shed light on this enigmatic mechanism, and let us assess how generally it occurs throughout eukaryotic genomes. ❐

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Competing interests

The authors declare no competing financial interests.