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Biogenesis of Circular RNAs

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Circular RNAs are generated during splicing through various mechanisms. Ashwal-Fluss et al. demonstrate that exon circularization and linear splicing compete with each other in a tissue-specific fashion, and Zhang et al. show that exon circularization depends on flanking intronic complementary sequences. Both papers show that several types of circular RNA transcripts can be produced from a single gene.

Circular RNAs are ubiquitous in molecular biology. Although observed for decades in eukaryotic cells but perceived as splicing errors at best, single-stranded circular RNAs have been coming back to the forefront with the discovery of their abundance thanks to the technological breakthroughs in high-throughput deep sequencing (Jeck and Sharpless, 2014). In this issue of *Cell* and in an upcoming issue of *Molecular Cell*, the papers by Zhang et al. (2014) and Ashwal-Fluss et al. (2014) add fundamental dimensions to our understanding of the molecular pervasiveness of circular RNAs in eukaryotic cells from flies to mammals and human.

Most eukaryotic circular RNA molecules are produced during splicing, a cellular process that is generally catalyzed by either the spliceosomal machinery or by groups I and II ribozymes (Figure 1). Typically, the split coding exonic sequences are reattached together in a continuous coding transcript. The spliced out noncoding intronic sequences are released as linear or lariat molecules and often play other important functions. Circular RNAs are distinct from their linear counterparts because they are devoid of the terminal structures (e.g., 5′ cap or a polyA tail) that frequently determine the fate of RNA transcripts. On the other hand, because of the lack of free ends, circular RNAs are resistant toward exonucleases, thereby escaping normal RNA turnover.

Why would circular RNAs get so much attention? Recent papers have described the presence of circular RNA species from back-spliced exons in mammals (circular exonic RNAs, circRNAs) and have established that they are very abundant and are differentially expressed (Jeck and Sharpless, 2014). Circular RNAs may, for example, serve as transcription regulators or as sponges for small RNA regulators (Hansen et al., 2013; Memczak et al., 2013). Evidence that circular intronic RNAs can get passed on to offspring in *Xenopus* oocytes hints at their role in RNA-mediated inheritance and epigenetics (Talhouarne and Gall, 2014).

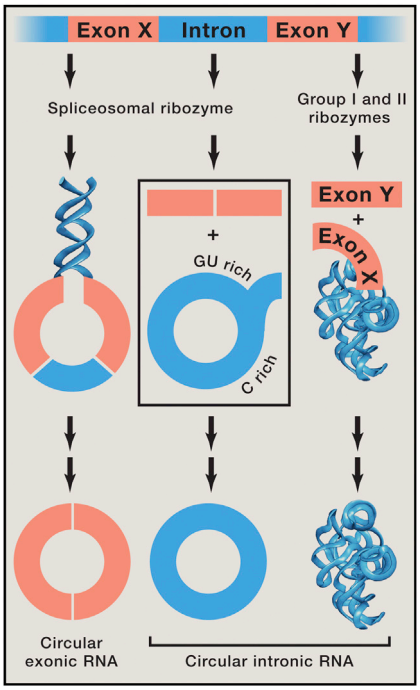

Figure 1. The Three-Ring Circus of the Posttranscriptional Fates of mRNA Transcripts
In addition to the standard pathway of collinear exon ligation with removal of a lariat intron (boxed), RNA architecture and chemistry make alternative pathways possible. (Left) circular exonic RNAs are produced when inverted repeats within the flanking introns close a circle, promoting regular splicing reactions to occur and leading to a circle of one or several exons or of the intron. For the circular exonic RNA, only one mechanism is depicted (called "direct back-splicing"); exon skipping can also produce circular exonic RNAs (Jeck and Sharpless, 2014). Both mechanisms require an action of the splicing machineries at either end, leading to lariats and other byproducts. (Middle) Circular intronic RNAs are produced when lariats that escape the debranching enzymes are processed by exonucleases (Talhouarne and Gall, 2014; Zhang et al., 2013). It is still not understood how the circular intronic RNAs escape debranching enzymes, but conserved RNA motifs near the 5′ splice site could contribute to the stabilization of the 2′-5′ linkage present in circular intronic RNAs or make it occluded by an RNA binding protein. (Right) The highly structured group I and II introns can also form circular intronic RNAs, following nucleophilic substitution at the 3′ splice site. In such introns, exons are brought close together by intronic guide sequences within the catalytic center of the ribozyme, thereby poising the intron for circularization.

By systematic deep RNA sequencing of fly and human cells, Ashwal-Fluss et al. (2014) demonstrate that exonic circular RNAs are generated cotranscriptionally at the expense of canonical linear mRNA splicing with a strong dependence on intronic sequences. They further show that the RNA-binding protein muscleblind—important for muscle and eye development and implicated in myotonic dystrophies—promotes exon circularization by binding to sequences in the flanking introns. Zhang et al. (2014) precisely pinpoint the sequence requirements in the flanking introns that promote exon circularization. They show that complementary sequences, which can be repetitive or not, are required. They also convincingly demonstrate that there is a competition between pairings of

complementary sequences within a single intron and pairings involving sequences from the flanking introns. Only the latter pairings would bring the exon-intron junctions in closer proximity and promote circularization. The nonrepetitive complementary sequences are not evolutionarily conserved, and thus, the detection of circular RNAs from a given locus is not always expected. As previously noted (Jeck et al., 2013), *Alu* sequences oriented in opposite directions stand out among the repetitive elements that promote circularization, as they are able to form inverted repeats (IR*Alus*).

This result is particularly significant, as it suggests a new critical role for *Alu* elements, which are specific to primate genomes, altogether representing 10.5% of the genome in humans. *Alu* elements are captivating molecular objects of roughly 0.3 kb in size that were originally derived from the RNA component of the signal recognition particle, a molecular complex that targets proteins to the endoplasmic reticulum. They are mobile elements that retrotranspose by hijacking a reverse transcriptase and an endonuclease from autonomous retrotransposons for reinsertion into the genome. Interestingly, about half of the IR*Alu* elements are found in the intronic regions (Zhang et al., 2014). Now, because they contain double-stranded RNA helices, *Alu* elements are attacked by double-stranded ribonucleases like Dicer and Drosha. In retina cells that do not contain the gene coding for Dicer, *Alu* elements are toxic and induce macular degeneration (Kaneko et al., 2011). Furthermore, they are subject to intense adenosine-to-inosine editing by the ADAR enzyme family, thereby causing nuclear retention of mRNAs (Chen and Carmichael, 2009).

What could the biological consequences of all those interrelated phenomena be? In particular, how is the network of multiple interactions maintained and controlled throughout circular RNA processing and beyond? How is the splicing machinery kept away from its regular splicing activities? Which geometrical constraints due to structural elements promote or restrict these alternative pathways? Ultimately, could circular RNAs contribute to epigenetic heritability? The papers by Ashwal-Fluss et al. (2014) and Zhang et al. (2014) put together several puzzle pieces and propose key factors for the biogenesis of circular RNAs. They also take our view of posttranscriptional regulation to a whole new level of fascinating complexity. Much still remains to be discovered about circular RNAs and their functions before coming full circle.

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