Forum
Are Circular RNAs New Kids on the Block?

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Circular RNAs (circ-RNAs), a novel class of noncoding RNAs, are a popular topic in animal research because they have potential as post-transcriptional regulators and diagnostic markers. Research in plants is only now emerging, but indicates that circ-RNAs could also be a crucial class of noncoding regulators.

In animals, many thousands of circ-RNAs have been identified, representing a relatively unexplored class of noncoding RNAs (see Glossary) compared to the better-studied microRNAs (miRNAs) and small interfering RNAs (siRNAs) [1]. The unique structure of circ-RNAs, a covalently closed continuous loop, means that they are more resistant to exonuclease attack than are linear RNAs [1]. circ-RNAs were initially thought to be the result of splicing errors, but more recently it has been shown that circ-RNAs can act as miRNA sponges or as sponges for RNA-binding proteins, and regulate post-transcriptional events [2].

A close association with human diseases, including cancers and neurologic diseases such as Alzheimer disease, has been demonstrated, and circ-RNAs are currently being explored as diagnostic biomarkers and therapeutic targets in such diseases [2,3]. Recently, transcriptome-wide sequencing studies have revealed that circ-RNAs are highly conserved and many circ-RNAs have also been identified in plants [4], but insight into their function is only now emerging.

We highlight here the distinct features of plant circ-RNAs and propose possible functions.

Genesis and Exodus from the Nucleus

The regulation and function of circ-RNAs in plants were largely unknown until recently, whereas circ-RNAs in human cells were identified as far back as 1994 [5]. In plants, viroids—pathogenic viruses without capsids that were identified in the 1980s—replicate using a rolling-circle mechanism and self-cleavage [6]. The circular structure and replication mechanism of viroids are features shared with intracellular circ-RNAs. However, in contrast to viroids, which enter cells from the outside, computational and molecular techniques initially developed in animals revealed that circ-RNAs are generated from plant genomes and are involved in cytosolic processes [4,7–9]. The genesis of circ-RNAs is a conserved feature in animal and plant cells, and depends on transcription by RNA polymerase II and back-splicing reactions of pre-messenger RNAs (pre-mRNAs). Regulation of the back-splicing reaction in the spliceosome requires a repeated sequence and RNA-binding proteins (Figure 1). Typically, spliceosome formation is initiated by the sequential assembly of small nuclear ribonucleoproteins (snRNPs) onto a specific region of the pre-mRNA downstream of the 5’ donor splice site (GT) and upstream of the 3’ acceptor site (AG). Depending on the initial snRNP binding site, exonic or intronic circ-RNAs are generated [8]. In addition, RNA-binding proteins such as serine/arginine-rich proteins (SR proteins) play important roles in the back-splicing reaction (Figure 1). In plants, circ-RNAs have a few repetitive elements and relatively long reverse complementary flanking introns for splicing knots [4,7]. Thus, different endogenous circRNA-silencing pathways may be involved in regulatory processes in plants.

What Do circ-RNAs Do?
Developmental Processes

Developmental regulation by circ-RNAs is poorly understood in plants, but has been intensely studied in humans and animals; for example, circ-RNAs are known to be involved in the epithelial–mesenchymal transition [2,7]. Interestingly, some plant circ-RNAs accumulate in a tissue- or organelle-dependent manner [8,9]. Recently, 30 putative circ-RNAs were found to be differentially expressed in rice (Oryza sativa) leaves and panicles [7]. In Arabidopsis (A. thaliana), 6% and 1% of putative circ-RNAs are present in chloroplasts and mitochondria, respectively [8]. In addition, some barley (Hordeum vulgare L.) circ-RNAs involved in micro-nutrient homeostasis are highly expressed in mitochondria [9]. These results indicate that plant circ-RNAs contribute to cellular development via tissue- or organelle-specific expression (Figure 1). However, there have been no reports
showing the involvement of circ-RNAs during plant development.

Stress Responses

Although functional studies are still lacking, some plant circ-RNAs have been identified under abiotic or biotic stress conditions (Figure 1). A total of 163 tomato circ-RNAs were predicted to be involved in chilling injury regulation, such as redox reactions and low temperature-induced transcription factors [10]. Another 62 wheat circ-RNAs are expressed under dehydration conditions and target drought-specific miRNAs involved in photosynthesis and oxidative phosphorylation [11]. Some circ-RNAs in rice and barley respond to imbalances in nutrients such as phosphate, iron, and zinc [4,9]. However, the detailed mechanism underlying circRNA-mediated regulation during abiotic stress has not been studied. Meanwhile, some Arabidopsis circ-RNAs that regulate immunity against Pseudomonas syringae pv. tomato influence biotic stress-related accumulation of miRNAs by acting as a miRNA sponge [8].
Collectively, these reports indicate that plant circ-RNAs can affect plant responses to abiotic and biotic stresses.

**miRNA Sponges**

Functional studies of animal circ-RNAs showed that they modulate gene expression via post-transcriptional regulation. For example, some circ-RNAs act as miRNA sponges, as shown by the competition between a circRNA cIRs-7 and miR-7 in the regulation of brain development in animals [2]. The first genome-wide study of plant circ-RNAs containing miRNA-binding sites identified 6.6% and 5.0% of putative circ-RNAs in rice and Arabidopsis, respectively, as miRNA sponges (Figure 1) [4]. Furthermore, 235 rice exonic circ-RNAs contain putative miRNA binding sites [7]. Recently, 24 of 102 tomato circ-RNAs and six of 62 wheat circ-RNAs were identified as miRNA sponges [10,11]. However, the functions of these miRNA sponges remain to be explored.

**Facing circ-RNAs: Identification Methods**

The emergence of high-throughput RNA sequencing technology and in silico analysis methods, such as ‘PcircRNA finder’, has facilitated the prediction of circ-RNAs in plants [12]. Approaches designed to determine circRNA abundance are limited, but include northern blot analysis, 2D gel electrophoresis, gel trap electrophoresis, and treatment with RNase R exonuclease [3].

**Where Do circ-RNAs Go from Here?**

Although circRNA research in plants is still in its infancy, insights gained in the circRNA field in animals could lead to new directions in plant circRNA research. Specifically four points stand out that need to be addressed in this context.

(i) Triggering signal: the triggering signals that originate outside the plant cell and stimulate the accumulation of circ-RNAs in specific organelles or nucleus remain elusive. (ii) Regulation: the mechanisms involved in the degradation and reassembly of circ-RNAs are unknown, but are likely to be novel given the unusual loop structure of these circ-RNAs. (iii) Mobile signal: recent findings of small RNAs moving via the phloem suggest that they may act as signaling molecules. Similarly, localization, lack of correlation between circ-RNAs, and their accumulation in response to specific stresses hint at their possible roles as mobile signals [3]. Nevertheless, the functional significance of organelle-specific localization or systemic movement of circ-RNAs orchestrated by highly regulated genetic circuits need to be investigated further. (iv) Biomarkers: similarly to the application of human circ-RNAs in the diagnosis of diseases such as cancer [3], circ-RNAs in plants may be used as biomarkers for the assessment of plant status under biotic and abiotic stress conditions.

**Concluding Remarks**

circ-RNAs constitute a new and exciting addition to the burgeoning field of plant RNAs. Although information on circ-RNAs in plants is still limited, considerable progress can be expected in characterizing their biogenesis and regulation, their modulation for the management of plant phenotypes, and the mechanisms involved in their fine-tuning of RNA processing. We propose that circ-RNAs could turn out to be a crucial class of noncoding regulators in plant.