EDITORIAL: REFLECTIONS ON PLANT CELL CLASSICS

MicroRNAs in Plants: Key Findings from the Early Years

Just two decades ago, the study of small RNAs (sRNAs) in plants had barely begun. Seminal papers published from 2000 to 2003 described a specific class of sRNAs, the microRNAs (miRNAs; typically 21 or 22 nt in length) found in both animals and plants. Several of these papers came out of the lab of James Carrington, then at Oregon State University; one of these, Llave et al. (2002), was published in this journal and was perhaps the first significant effort to characterize plant small RNAs by sequencing. This work was a forerunner of hundreds of later next-gen-based sequencing papers. In 2002, most of what we knew about sRNAs came from animals and involved forward genetics. In the Llave et al. (2002) work, the Carrington group developed and applied in plants a sequencing method for small RNAs from plants. They identified sRNAs from a GFP silencing system in tobacco, following up on earlier work from several other labs, including that of David Baulcombe, showing that small RNAs function in silencing. And, importantly, they started the first genomic analysis of plant small RNAs, sequencing just 125 Arabidopsis small RNAs and matching these back to the then-newly-sequenced Arabidopsis genome, demonstrating the utility to small RNA analyses of having a reference genome.

From these seemingly scant data, perhaps 0.0005% of what a typical illumina sRNA library might yield today, the outlines of the global sRNA population in plants were visible. The data displayed the now-characteristic size distribution of sRNAs in plants, with peaks at 21, 22, and 24-nt (Llave et al., 2002). Tissue-specific accumulation or enrichment of some sRNAs was observed on RNA gel blots used for validation. rRNA decay products, derived from structural RNAs in many sRNA libraries, and repeat-derived heterochromatic sRNAs (hc-sRNAs, a major pool) both feature prominently in the paper, and both are abundant components of sRNA populations in all modern-day data sets. Curiously, the sRNA-generating locus described in Figure 6, a long, non-coding RNA, is now known to yield copious sRNAs, but the function is still unknown. Several other sequenced small RNAs would later turn out to be important microRNAs (miRNAs), including miR167 and miR171 (named in subsequent papers); the latter (called sRNA39 in the paper) was mentioned as matching transcripts encoding a Scarecrow-like TF. Right around the same time, a collaboration between the siblings Bonnie Bartel and David Bartel (at Rice University and The Whitehead Institute, respectively) published their first sRNA sequencing effort from Arabidopsis (Reinhart et al., 2002). This analysis led to the first formal descriptions of plant miRNAs. Reinhart et al. (2002) also included the key observation that plant miRNA accumulation is dependent on the CARPEL FACTORY (CAF) locus. CAF, now referred to as DICER-LIKE 1 (DCL1), encodes an endonuclease that hydrolyzes miRNAs from stem-loop RNA hairpin precursors. The caf mutant allele (which causes a reduction of function, not a null allele) displays pleiotropic developmental defects (Jacobsen et al., 1999); its relationship to miRNA biogenesis immediately suggested a connection between plant miRNAs and development, a theme that was to be elaborated on in many subsequent studies.

This early sequencing work served as the basis for our subsequent efforts to codify the criteria for the annotation of plant miRNAs, also published in the pages of this journal (Meyers et al., 2008; Axtell and Meyers, 2018). Those efforts first defined the community standards and later refined the criteria based on a decade’s worth of massive, accumulated datasets – many millions-fold higher than the first efforts of the Carrington group in their 2002 paper – plus the many plant genomes that have been sequenced in the intervening years. Yet, earlier papers such as Llave et al. (2002) started the process for the field to think collectively about how to sort out the different classes and types of small RNAs that one obtains by size capture and sequencing.

Just a year later, Aukerman and Sakai (2003) published a seminal paper that, for the first time, demonstrated a direct role for one specific miRNA, miR172, in plant development. By then, the plant sRNA field was starting to explode with new results and insights – miRNAs were named and organized in miRBase (the central miRNA registry; http://www.mirbase.org), and their targets and possible functions, initially mostly in development, were beginning to be described and predicted. Aukerman and Sakai (2003) isolated a miR172 primary transcript from an activation-tagging screen in Arabidopsis. miR172 overexpression caused early flowering and floral development defects. Both the Aukerman and Sakai (2003) study and a contemporaneous study by Xuemei Chen (2004) showed that miR172 controls flower development and floral timing by control of the APETALA2 (AP2) and related TARGET OF EAT (TOE) miRNAs. An interesting observation, mentioned only in passing by Aukerman and Sakai (2003), was that the miR172 target sites were conserved broadly in AP2 family members in diverse flowering plants, including in several grasses, soybean, and tomato. This presaged many later efforts (including by both of our labs) to identify both conserved and lineage-specific miRNAs.

It was in this context that the Sunkar and Zhu (2004) paper was published, also exploring miRNAs in Arabidopsis by sequencing. Their efforts differed from previous studies in that RNA samples were taken from plants placed under various abiotic stresses. This resulted in the discovery of several novel miRNAs. The accumulation of several of these new miRNAs was found to be modulated by various stresses, and the predicted targets of several of them encoded proteins with known or predicted roles in stress responses. At about the same time, a partially overlapping set of miRNAs, some them stress-
related, was also reported by Jones-Rhoades and Bartel (2004) using a comparative genomics approach. These studies expanded the role of plant miRNAs beyond development and into stress responses. It's now known that plant miRNAs play key roles in mineral nutrition, as well as both biotic and abiotic stress responses.

As a result of this set of classic Plant Cell papers (Llave et al., 2002; Aukerman and Sakai, 2003; Sunkar and Zhu, 2004) and the many other seminal papers published in the early 2000s, we now know that miRNAs are important regulatory molecules in plants. Regulation of genes important to development, abiotic stress responses, and biotic interactions are among their primary roles. These three studies discovered and characterized miRNAs and other sRNAs by direct sequencing or by forward genetic analysis; two methods which the field has made great use of since. These are truly classic studies that continue to be influential and relevant to ongoing research.

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REFERENCES


