Expanding the plant non-coding RNA world

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Ribonucleic acid (RNA) is one of the biopolymers essential for organisms. In the “classical” view of molecular biology, functional RNA species have been categorized into three major groups, such as messenger RNAs (mRNAs), template molecules to biosynthesize proteins, ribosomal RNAs (rRNAs), components of huge molecular machinery ribosome for protein biosynthesis, and transfer RNAs (tRNAs), molecular couriers of amino acids to the ribosome. Function of these RNAs brought the perspective of so-called “central dogma”, in which RNA molecules are considered as the mediators of genomic information written within DNA molecules for protein biosynthesis to achieve required cell activity.

However, our view of RNA function has been greatly revised with the advent of the post genome era. One notable study was the discovery of small non-coding RNAs, called small interference RNAs (siRNAs), that function in post-transcriptional gene silencing (Fire et al. 1998). Later, the phenomenon known as co-suppression in plants and quelling in fungi, in which transgenes suppress the accumulation of endogenous transcripts based on the homology between transgenes and endogenous transcripts (Romano and Macino 1992; Napoli et al. 1990), can be mediated at least partly by the function of siRNAs. These earlier works vitalized the research to elucidate the mode of actions of small non-coding RNAs that regulate gene expression. In 2005, the FANTOM project for functional gene annotation

in mouse revealed “a new continent in the RNA world”, through the novel observation showing that ~73% of genomic regions are used for transcription of non-coding RNAs (Carninci et al. 2005; Katayama et al. 2005). The emergence of next-generation sequencing technologies encouraged this trend to identify a variety of non-coding RNAs. These big successes by non-coding RNA research have generated new RNA biology; now we know that RNA molecules are not only working as the intermediates between DNAs and proteins, but as key players of post-transcriptional and epigenetic regulation of gene expression. This is obviously true in plants, as many experimental reports have indicated the importance of non-coding RNA-based gene regulation in plant development, environmental responses, and biotic and/or abiotic stress responses.

In this special issue of JPR Symposium “Expanding the plant non-coding RNA world”, our current understanding of biogenesis, metabolisms, and function of plant non-coding RNA is reviewed and discussed with updated review articles, an original paper, and a technical note. First, we tried to provide detailed molecular views of small RNA biogenesis in plants. The siRNA and microRNA (miRNA), classes of small RNA with 18–25 nucleotides (nt) in length, pursue their function as the guides to target specific mRNAs for gene silencing, after incorporation into an RNA-induced silencing complex (RISC). These small RNAs are generated through multiple steps of RNA metabolic regulation; (1) formation of inter- or intramolecular double-stranded RNA (dsRNA) precursors, (2) biogenesis of small RNA duplexes through catalyzing dsRNA by dsRNA-specific endoribonucleases, Dicer-like (DCL) proteins, (3) loading of small RNA duplex onto ARGONAUTE (AGO) proteins, and (4) elimination of miRNA* or passenger siRNA strand, which are complementary strands for guide strand RNAs in small RNA duplex, by unwinding of

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duplex during RISC formation. Iki (2017) reviewed these molecular processes through the viewpoint of small RNA duplexes, to describe how molecular characters of small RNA duplexes regulate the RISC formation. Fukudome and Fukuhara (2017) focused on the biochemical properties of plant DCL proteins for small RNA biogenesis pathways. Fukuhara's group also reported the functional analysis of *Arabidopsis thaliana* (Arabidopsis) dsRNA-binding protein DRB3, a member of dsRNA-binding (DRB) proteins that are co-factors of DCL proteins, and suggested negative regulation of anthocyanin biosynthesis by modulating *PAP1* expression by DRB3 (Sawano et al. 2017). In addition, Komiya (2017) reviewed the phased small interfering RNAs (phasiRNAs) recently identified in plants, small RNAs that are generated from a long RNA precursor at intervals of 21–26-nt (Komiya et al. 2014). phasiRNAs are also loaded on AGO proteins, and the phasiRNA biogenesis was initiated by miRNA-dependent cleavage of precursor in plants. These articles describe plant-specific aspects of small RNA biogenesis and subsequent gene silencing by RISC, possibly leading to plant-specific physiological phenomena and environmental responses. The novel in vitro system to detect pre-miRNA processing activity using nuclear extract of Arabidopsis suspension cells reported by Yoshikawa (2017) would provide additional information on molecular basis of small RNA biogenesis, along with the known in vitro system using tobacco BY-2 nuclear extracts (Iki et al. 2010).

Ohtani (2017) reviewed the biogenesis of small nuclear RNA (snRNA), a distinct type of small RNAs from siRNAs and miRNAs, with special attention to evolutionary conservation among organisms. snRNAs are non-coding RNA accumulated in the nucleus with 60–220 nt length, and function in pre-mRNA splicing and rRNA processing. Based on the analysis of a temperature-sensitive Arabidopsis mutant defective in snRNA transcription (Ohtani and Sugiyama 2005), the developmental roles for snRNA transcriptional regulation in plants were discussed. While molecular mechanisms of snRNA transcription have been well studied in animal cells (Hernandez 2001), the physiological significance of snRNA transcriptional regulation is still unclear in animals, because of the difficulties generating animal knock-out lines of such essential genes. Thus, the plant snRNA biogenesis research using temperature-sensitive Arabidopsis mutants would be a good example to show the potential to work on plant cells to investigate importance of non-coding RNA function.

The regulatory system for not only RNA biogenesis but also RNA degradation is critical for RNA metabolism. Kurihara (2017) and Tsuzuki et al. (2017) reviewed and discussed the RNA degradation pathways in plants, and showed many plant-specific regulatory aspects of RNA turnover. Recent Arabidopsis mutant work suggested that

the defects in function of 5'–3' and/or 3'–5' exoribonucleases can change what has happened on RNA molecules, e.g. mRNA molecules can be recognized by RNA-dependent RNA polymerases to produce siRNA when exoribonuclease-based RNA degradation was impaired (Martínez de Alba et al. 2015). So far, many proteins involved in RNA degradation were reported (Tsuzuki et al. 2017). To understand further how plant cells manage to regulate RNA metabolism, a detailed functional analysis on these proteins is expected.

Moreover, Yamada (2017) introduced the current knowledge of long intergenic non-coding (linc) RNAs in plants. Although examples of functional analysis on lincRNAs are still limited in plants, the importance of lincRNAs was already shown for (1) epigenetic regulation of gene expression, (2) inhibition of physical interaction between miRNAs and target mRNAs, and (3) regulation of alternative pre-mRNA splicing events (Yamada 2017). Very recently, a group of cell-type specific lincRNAs was newly identified in Arabidopsis (Li et al. 2016). A part of them were co-expressed with specific protein-coding genes, suggesting functional importance of these lincRNAs (Li et al. 2016). Further experimental analysis of these lincRNAs may reveal novel functions of lincRNA in plants.

As mentioned above, the information on plant non-coding RNAs is continuously increasing. Taken together with the fact that plant cells can generate secondary RNA molecules by the RNA-dependent RNA polymerases (Willmann et al. 2011), it can be speculated that plant cells can team with RNA molecules. The RNA metabolic regulation based on non-coding RNAs would be critical to trim and shape transcriptomic information, as well as to epigenetically regulate gene expressions, for proper cell activities in plants. The view of “expanding the plant non-coding RNA world” described in this special issue will bring you new perspectives for the understanding of gene expression regulatory system in plants.

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