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HIGHLIGHT

MicroRNAs and their targets from *Arabidopsis* to rice: half conserved and half diverged

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MicroRNAs are 21- to 24-nucleotide long, endogenous non-coding RNAs in eukaryotes (Hannon, 2002). Mature microRNAs generated by Dicer are incorporated into an RNA-induced silencing complex (RISC), resulting in gene silencing via the cleavage of a target mRNA or the repression of target mRNA translation (Qi et al, 2005). Thus, microRNAs play a key role in post-transcriptional gene silencing, and microRNA-based gene silencing pathways are critical in developmental regulation, biotic and abiotic adaptations, and hormone responses in plants (Baulcombe, 2004; Jones-Rhoades et al., 2006).

In microRNA-based gene silencing, the microRNA-target mRNA pair determines the specificity of the biological effect. Hundreds of microRNAs have been identified in plants by cloning and deep genomic sequencing (Griffiths-Jones et al., 2008). Thus, a key step in determining the biological function of a microRNA is to identify its target. However, our knowledge of microRNA targets is incomplete due to limitations imposed by the methods used to identify microRNA targets. Traditionally, microRNA targets have been identified from computational predictions based on sequence matching between a microRNA and its target mRNA (Rhoades et al., 2002). Thus, a new approach is needed to facilitate the identification of microRNA targets on a global scale.

Degradome sequencing is a new approach that integrates high-throughput sequencing, experimental approaches, and bioinformatic analyses (German et al., 2009). More than 100 microRNA targets in *Arabidopsis* have been isolated using this method (Addo-Quaye et al., 2008; German et al., 2008), suggesting that degradome sequencing is a powerful tool for microRNA-target pair identification.

The isolation and experimental validation of microRNA-target pairs from the monocot model plant rice using a degradome sequencing approach was reported in this issue by Dr. Xiaofeng Cao's group (Zhou et al., 2010). They identified 177 mRNAs that were targeted by 87 unique microRNAs (Zhou et al., 2010). Among them, microRNAs known to be conserved in the plant kingdom targeted about half of the identified target mRNAs, and, interestingly, about 70% of the target mRNAs encoded transcription factors (Zhou et al., 2010). One example is the mi156-SBP-box gene mRNA pair (Fig. 1A). The *Arabidopsis* genome encodes thirteen SBP-box genes (*SPLs*), ten of which are mi156 targets (Schwab et al., 2005; Wu et al., 2009; Wang et al., 2009). The mi156-SPL pathway regulates the vegetative-phase transition, flowering, leaf initiation, and apical dominance in *Arabidopsis* (Schwab et al., 2005; Wu et al., 2009; Wang et al., 2009). Consistent with the microRNA-target of the mi156-*SPL* mRNA pairs in *Arabidopsis*, eleven *SPL* mRNAs are mi156 targets in rice (Zhou et al., 2010). These conserved microRNA-target pairs may represent common regulatory nodes for the regulation of fundamental developmental processes and responses to environmental signals in the plant kingdom.

The second half of the identified target genes were found to be targeted by non-conserved microRNAs (Zhou et al., 2010). These non-conserved microRNA-target pairs may be involved in rice- or grass-specific processes or responses, such as osa-miR528/Os0738290 interaction (Fig. 1B). Interestingly, a large proportion of the non-conserved microRNAs targeted the 5'- or 3'-untranslated region of their target mRNAs (Zhou et al., 2010). Thus, these non-conserved microRNAs may regulate gene expression via manners other than mRNA cleavage, and translational repression by microRNAs is more common in rice than in *Arabidopsis*. Therefore, rice is a better model than *Arabidopsis* to use in determining the mechanism of microRNA-mediated translational repression in plants.

What will be the next step after this useful and much needed atlas of microRNA-target interactions is completed? First, it will be possible to address the biological functions of microRNA-target transcript pairs in rice. The genetic manipulation of microRNA-mRNA pairs *in planta*

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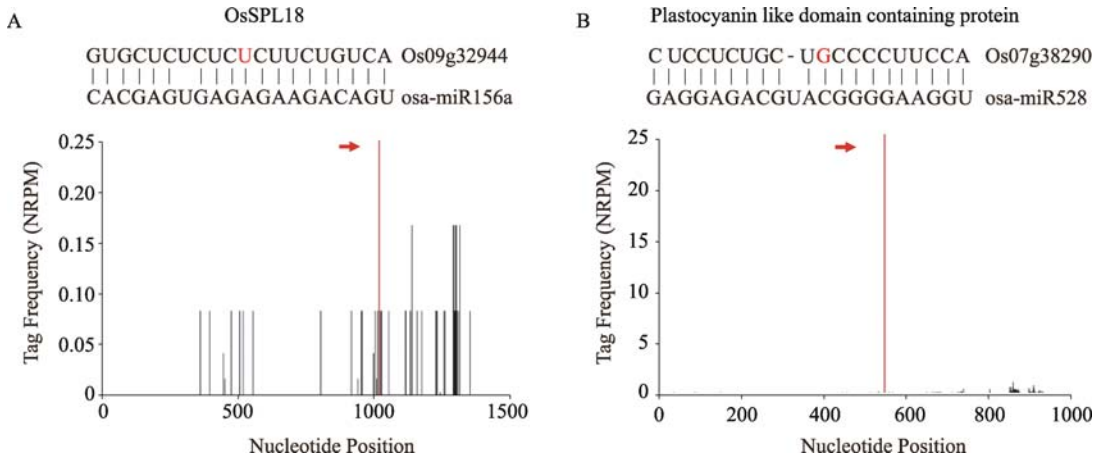

Fig. 1 Degradome sequencing reveals miRNA targets in rice

(A) Alignment of the miRNA with a portion of its target sequence (top) and distribution of 5' end of the degradome tags within the full-length target mRNA sequence (bottom) corresponding to Os09g32944 which encodes a SBP-box protein. The cleavage site is shown as a red letter. Tags aligned with the ninth through eleventh nucleotides of the osa-miR156a complementary sites are combined and shown as a red line indicated by a red arrow. (B) As in (A) for Os07g38290 which encodes a plastocyanin like domain containing protein, the target for osa-miR528.

will enable us to determine the function of microRNA-mRNA pairs in the control of rice development or the response to environmental signals. Second, it will be possible to assess whether the repression of translation by microRNAs is a widespread mechanism in rice. The regulation of gene expression at the translational level by microRNAs is a common feature in many animal RNAi pathways (Hannon, 2002). The microRNA-target mRNA pairs identified by Zhou et al. (2010) will enable us to investigate the microRNA-mediated inhibition of gene expression at the translational level in plants. Third, it will be possible to determine whether reciprocal regulation between a microRNA and its target is common in plants. In *Arabidopsis*, mi156 silences *SPL* expression by mediating the cleavage of *SPL* mRNA by an RISC, while SPL activates *mi156* precursor expression, thereby providing an additional level of control to make the regulation of expression more precise (Wu et al., 2009). It would be interesting to know how common this type of reciprocal regulation between a microRNA and its target is in plants. Fourth, additional microRNA-target mRNA pairs need to be identified. Almost certainly, the total number of microRNA-target mRNA pairs in plants greatly exceeds the number identified thus far. Additional microRNA-target mRNA pairs may be identified by this new approach using specific tissues or plants collected following growth under various conditions.

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