Post-Transcriptional Regulation of Nutrient Transporters

Most protein-coding genes in eukaryotes contain introns, and splicing of precursor mRNA (premRNA) is an essential step in gene expression. This posttranscriptional step of gene regulation takes place in the nucleus, is highly conserved among eukaryotes, and involves the coordinated and specific activity of splicing factor proteins. Some splicing factors directly bind RNA, while others are involved in overcoming the spatial and structural difficulties inherent to splicing events. For example, the animal Smu1 and its homolog RED are splicing factors that aid in splicing of short introns. In addition to this general role in splicing, Smu1 and RED are involved in regulating alternative splicing (Spartz et al., 2004; Keiper et al., 2019). Similarly, in plants, the two Smu1/RED homologs, SMU1 and SMU2, are crucial for the correct splicing of genes. SMU2 was first identified in maize (*Zea mays*), where loss of function leads to a variety of developmental defects in the endosperm, the embryo, and meristematic tissues (Chung et al., 2007). A yeast two-hybrid screen using Arabidopsis (*Arabidopsis thaliana*) SMU2 (At2g26460) as bait then revealed SMU1 (At1g73720) to be a protein interacting partner, and *smu1* knockdown showed similar defects compared to *smu2* (Chung et al., 2009). It was therefore proposed that SMU1 and SMU2 function as a complex to regulate splicing of premRNA and are mainly active in developmental tissues.

In this issue of *Plant Physiology*, Feng et al. (2020) show that complex formation of SMU1 and SMU2 is not obligatory for the function of the proteins in splicing; and that SMU1/SMU2-mediated regulation of gene expression is important for magnesium (Mg^{2+}) homeostasis. In a forward genetic screen, they identified plants hypersensitive to low Mg^{2+} that harbor a mutation in *SMU1*. They then show that the SMU1/SMU2 complex is crucial for the correct processing of premRNA of the Mg^{2+} transporter *MRS2-7* (At5g09690).

Previously, *MRS2-7* was shown to have several alternatively spliced variants. Therefore, Feng et al. (2020) investigated the *MRS2-7* mRNA in their *smu1* mutant. They additionally included SMU2 in their studies and found that alterations in the SMU1/SMU2 complex lead to mis-spliced *MRS2-7* variants that encode proteins lacking transporter activity, which ultimately renders the plants hypersensitive to low environmental Mg^{2+}. In addition, Feng et al. (2020) further demonstrated that, contrary to a previous suggestion (Chung et al., 2009), complex formation of SMU1 and SMU2 is not obligatory for their splicing activity. Overexpression of SMU1 in a *smu2* knockout, and vice versa, restores the splicing of some introns in *MRS2-7*. Interestingly, the respective splicing activities of SMU1 and SMU2 are specific, and loss of either homolog results in a different erroneous splicing pattern of *MRS2-7* mRNA. Splicing within the nucleus occurs in nuclear speckles, which are defined subdomains with clear boundaries separating them from other nuclear regions. Split-yellow fluorescent protein (YFP) assays using SMU1 and SMU2 each tagged with a YFP-half showed reconstitution of fluorescence in nuclear speckles. This suggests that stabilized SMU1 and SMU2 dimers localize in a spliceosomal complex and that dimerization ensures correct splicing of all introns (Feng et al., 2020).

This study shows insights into both the interaction of two important splicing factors and the control of Mg^{2+} transport. It is one of the few studies showing a direct connection between specific splicing factors and a nutrient transporter. We know from previous work looking at whole transcriptome changes that alternative splicing plays a critical role in nutrient homeostasis (Li et al., 2013; Dong et al., 2018), and further research might reveal more specific interactions. Other aspects of posttranscriptional regulation also play crucial roles. For example, mutations in the RNA processing protein complex THO/TREX have been shown to result in defective transcription termination at the often-utilized NOS (nopaline synthase) terminator (Khan et al., 2020).

These examples show that posttranscriptional regulation is complex, and its accuracy depends very often on nucleotide sequences outside of the coding sequence. DNA sequences outside of the coding sequence are often not present in transgenic plants expressing, for example, constructs of proteins of interest tagged with fluorescent proteins. The rapid advances being made in the use of CRISPR/Cas9 will hopefully soon allow us to easily integrate protein modifications, such as tags, into the original genome position. This will vastly decrease possible secondary effects due to loss of posttranscriptional regulation of our genes of interest.

Stefanie Wege[1,2]
ORCID ID: 0000-0002-7232-5889
Australian Research Council Centre of Excellence in Plant Energy Biology, Plant Research Centre, School of Agriculture, Food and Wine, Waite Research Institute, University of Adelaide, Waite Campus, Glen, Osmond, South Australia 5064, Australia

[1]Author for contact: stefanie.wege@adelaide.edu.au.
[2]Senior author.
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