

# Post-Transcriptional Regulation of Nutrient Transporters

Most protein-coding genes in eukaryotes contain introns, and splicing of precursor mRNA (pre-mRNA) 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 pre-mRNA 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 pre-mRNA 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<sup>1,2</sup>

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

## LITERATURE CITED

Chung T, Kim CS, Nguyen HN, Meeley RB, Larkins BA (2007) The maize *zmsmu2* gene encodes a putative RNA-splicing factor that affects protein

<sup>1</sup>Author for contact: stefanie.wege@adelaide.edu.au.

<sup>2</sup>Senior author.

www.plantphysiol.org/cgi/doi/10.1104/pp.20.01017

- synthesis and RNA processing during endosperm development. *Plant Physiol* **144**: 821–835
- Chung T, Wang D, Kim C-S, Yadegari R, Larkins BA** (2009) Plant SMU-1 and SMU-2 homologues regulate pre-mRNA splicing and multiple aspects of development. *Plant Physiol* **151**: 1498–1512
- Dong C, He F, Berkowitz O, Liu J, Cao P, Tang M, Shi H, Wang W, Li Q, Shen Z, et al** (2018) Alternative splicing plays a critical role in maintaining mineral nutrient homeostasis in rice (*Oryza sativa*). *Plant Cell* **30**: 2267–2285
- Feng Z, Nagao H, Li B, Sotta N, Shikanai Y, Yamaguchi K, Shigenobu S, Kamiya T, Fujiwara T** (2020) An SMU splicing factor complex within nuclear speckles contributes to magnesium homeostasis in Arabidopsis. *Plant Physiol* **184**: 428–442
- Keiper S, Papasaikas P, Will CL, Valcárcel J, Girard C, Lüthmann R** (2019) Smu1 and RED are required for activation of spliceosomal B complexes assembled on short introns. *Nat Commun* **10**: 3639
- Khan GA, Deforges J, Reis RS, Hsieh Y-F, Montpetit J, Antosz W, Santuari L, Hardtke CS, Grasser KD, Poirier Y** (2020) The transcription and export complex THO/TREX contributes to transcription termination in plants. *PLoS Genet* **16**: e1008732
- Li W, Lin W-D, Ray P, Lan P, Schmidt W** (2013) Genome-wide detection of condition-sensitive alternative splicing in Arabidopsis roots. *Plant Physiol* **162**: 1750–1763
- Spartz AK, Herman RK, Shaw JE** (2004) SMU-2 and SMU-1, *Caenorhabditis elegans* homologs of mammalian spliceosome-associated proteins RED and fSAP57, work together to affect splice site choice. *Mol Cell Biol* **24**: 6811–6823