From Leaf to Kernel: Trehalose-6-Phosphate Signaling Moves Carbon in the Field

Nearly 20 years ago, company scientists attempted to induce trehalose biosynthesis in plants via the expression of microbial genes encoding trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP; Goddijn et al., 1997). The trehalose biosynthetic pathway condenses Glc-6-P and UDP-Glc into a molecule of UDP and trehalose 6-phosphate (T6P), which is subsequently dephosphorylated to form trehalose and inorganic phosphate (Fig. 1A). At the time, the aim was to overproduce the protein structure-stabilizing agent trehalose in plants and to study the protective effects of trehalose accumulation on plant performance under stress. At about the same time, it was realized that the trehalose biosynthetic pathway is present in all plants, not only in resurrection plants such as *Selaginella lepidophylla*, where it was first noticed in high abundance. ESTs (copy DNAs) and genome-sequencing projects uncovered the presence of extensive TPS and TPP gene families in plants, the function of which was (and still is) unclear (Vandesteene et al., 2012). Unexpectedly, overexpression of trehalose biosynthetic genes in plants produced detrimental phenotypes that could be linked to the accumulation of the biosynthetic intermediate T6P (Schluepmann et al., 2004). Further studies showed T6P to be a powerful signaling metabolite that controls carbohydrate metabolism and that is essential for plant growth and reproduction (van Dijken et al., 2004; Wahl et al., 2013).

Suc specifically induces T6P accumulation, which seems to function as a signaling intermediate for reporting the cellular Suc status (Lunn et al., 2006; Yadav et al., 2014). T6P somehow operates as a licensing factor that allows sugar utilization for growth. Already early on, it was evident that the powerful regulatory effects of T6P on metabolism held potential for manipulating carbon partitioning and plant yield. Now, research by scientists from Syngenta and Rothamsted Research, reported by Nuccio et al. (2015) in *Nature Biotechnology*, shows that maize (*Zea mays*) yield stability can be significantly increased by the expression of the rice (*Oryza sativa*) *OsTPP1* gene in developing ears (female reproductive organs). In multiyear and multilocation field trials and greenhouse experiments, it was found that the *OsTPP1* transgene substantially improved grain yield under normal growth conditions but significantly more so when plants were exposed to mild drought stress, particularly at the highly susceptible flowering stage. Selection of the rice *OsMADS6* promoter that targets the expression of *OsTPP1* to specific cells of the developing female floret was key to the success the project. In rice, the *OsMADS6* promoter regulates floral organ and meristem identities and endosperm nutrient accumulation. By contrast, the related *OsMADS13* promoter, which in maize ears shows a more restricted expression pattern with lower expression in the vasculature, had detrimental effects on yield. This provides an excellent demonstration that spatiotemporal regulation of a transgene can significantly impact phenotypic outcome.

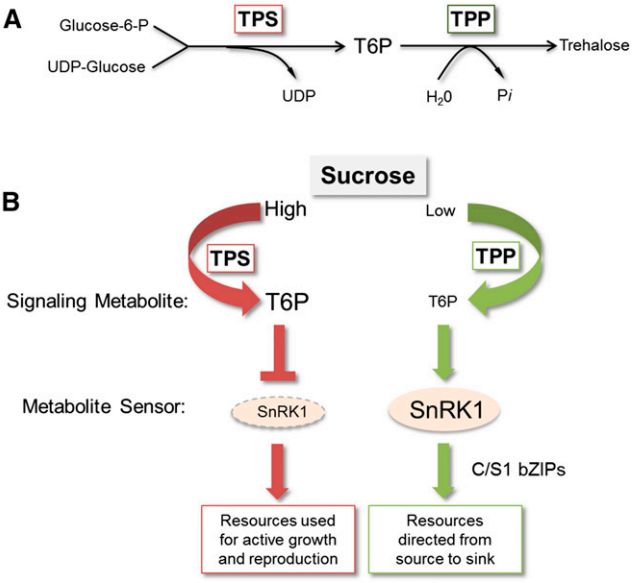

Figure 1. A, The trehalose biosynthetic pathway in plants. TPS converts Glc-6-P and UDP-Glc to UDP and T6P, which can be dephosphorylated to trehalose by TPP. Pi, Inorganic phosphate. B, Model explaining the key function of T6P in balancing growth and reproduction with resource availability. Suc stimulates T6P accumulation, thereby inhibiting SnRK1 and allowing growth. Low sugar promotes T6P conversion to trehalose by TPP, which signals resource sequestration in sinks, probably mediated by SnRK1. SnRK1 phosphorylates and activates C/S1 bZIP transcription factors that induce *TPP* gene expression, potentially creating a feed-forward loop for SnRK1 activation. C/S1 bZIPs are likely to have a function in promoting resource flow to sinks. T6P levels and SnRK1 activities are indicated by font sizes.

In ear spikelets of *OsTPP1*-expressing maize transgenics (*OsMads6-Tpp1*), lower T6P and increased Suc levels were observed compared with nontransgenic plants, suggesting an improved sink function of the reproductive tissues. The *OsMads6-Tpp1* phenotypes include increased kernel number and weight as well as an increased harvest index. These qualities were observed with multiple independent transgenic events, at several field sites in different years, and with high penetrance and expressivity. Likely, in the developing ears,

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reduced T6P levels somehow signal increased sink activity, allowing the emergence of more and bigger kernels.

Previous research findings suggest that T6P inhibits Sucrose nonfermenting1-Related Kinase1 (SnRK1; Zhang et al., 2009; Nunes et al., 2013), a protein kinase that responds to the cellular metabolic status and that is a central regulator of metabolism in all eukaryotic organisms. In this model (Fig. 1B), increased SnRK1 activity and its downstream effectors then deliver the sink signal. The C/S1 subgroup of plant basic region-leucine zipper (bZIP) motif transcription factor proteins are among the SnRK1 targets, and interestingly, overexpression of the Arabidopsis C/S1 group member bZIP11 in Arabidopsis was found to induce TPP gene expression and to increase the sugar content of such overexpressing plants (Ma et al., 2011).

The molecular and genetic underpinnings of this T6P-C/S1 bZIP-SnRK1 regulatory module have been presented (O'Hara et al., 2013; Lunn et al., 2014), but the molecular details and mode of action need further investigation. Currently, no solid molecular explanation is available for the enhanced sink function of *OsMads6-Tpp1*-expressing maize ears. As mentioned above, T6P somehow licenses sugar utilization and is essential for growth. Therefore, T6P appears to be a balancing signal that connects cellular sink activity (low T6P) with cellular growth (high T6P).

Irrespective of the precise mechanism involved, the impressive TPP gene-to-field study presented by Nuccio et al. (2015) provides the first example of effective spatial and temporal regulation of a nutrient-sensing system that provides enhanced yield stability under drought in a major crop species.

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