eXtra Botany

Insight

Fuel for the road – sugar transport and pollen tube growth

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Pollen tubes require the activity of carbohydrate uptake transporters to sustain their high growth rate. The work of Rottmann and colleagues in this issue (pages 2387–2399) provides evidence that expression of a hexose transporter in pollen tubes may be regulated via the hexokinase signaling pathway.

Pollen, the male gametophyte (haploid generation) of flowering plants, is responsible for delivering sperm cells to the egg sac, a process essential for sexual reproduction. Interaction between pollen and stigma (pollination) initiates secretion in the stigma and pollen germination. The pollen tube emerges and grows through the style to the ovaries where it delivers the sperm cells to the ovule to accomplish fertilization (Box 1) (Edlund *et al.*, 2004). In Arabidopsis, the male gametophyte consists of three cells – one vegetative cell that encloses two sperm cells. Despite this seemingly simple system, about half of all Arabidopsis genes are expressed in pollen (Bock *et al.*, 2006). There has been much recent progress in the field of pollen biology, especially in the area of pollen tube guidance and reception by the female gametophyte (reviewed by Kanaoka and Higashiyama, 2015).

Pollen tubes have been studied intensively as a model system for tip growth, a process vital in diverse cell types from fungal hyphae to cancer cells (Sanati and Geitmann, 2013). Basic research on pollen biology also has many direct practical applications. The vast majority of the world's harvest depends upon successful plant reproduction. In many crop plants the male gametophyte is sensitive to environmental stress, such as heat and drought, which can lead to serious crop losses (Zinn *et al.*, 2010). Understanding pollen function is therefore also of important practical interest.

Carbohydrate uptake into pollen tubes

Pollen tubes can sustain very rapid growth rates (5.3 µm min^{-1} in Arabidopsis; Wilhelmi and Preuss, 1996) and achieve lengths of 25 cm or more in maize. Carbohydrates and other storage compounds stored in mature pollen are sufficient to support pollen survival and germination but pollen tubes use carbohydrate secretions from the stylar canal to support growth (Labarca and Loewus, 1973). Since the pollen tube is symplastically isolated from the surrounding tissue of the style and transmitting tract, the activity of membrane transporters is required in order to import nutrients from the sporophyte tissue to contribute to cell wall synthesis and respiration of the growing pollen tube. It is therefore interesting to note that genes encoding membrane transporters are overrepresented in the pollen transcriptome (Bock *et al.*, 2006). Pollen can germinate *in vitro* in relatively simple media, which made the first pollen transcriptome studies possible. More recent work using a semi-*in vivo* method where pollen is applied to a stigma, after which the style is cut – allowing the pollen tubes to grow out onto the medium – showed that certain genes are specifically induced after growth through the pistil tissue (Qin *et al.*, 2009). Interestingly, there are not many examples of sugar transporters for which a function in pollen has been demonstrated. One example is the Arabidopsis sucrose transporter AtSUC1, which can be localized to the plasma membrane in pollen tubes. Loss of function affects pollen germination *in vitro* and causes segregation distortion *in vivo* (Sivitz *et al.*, 2008).

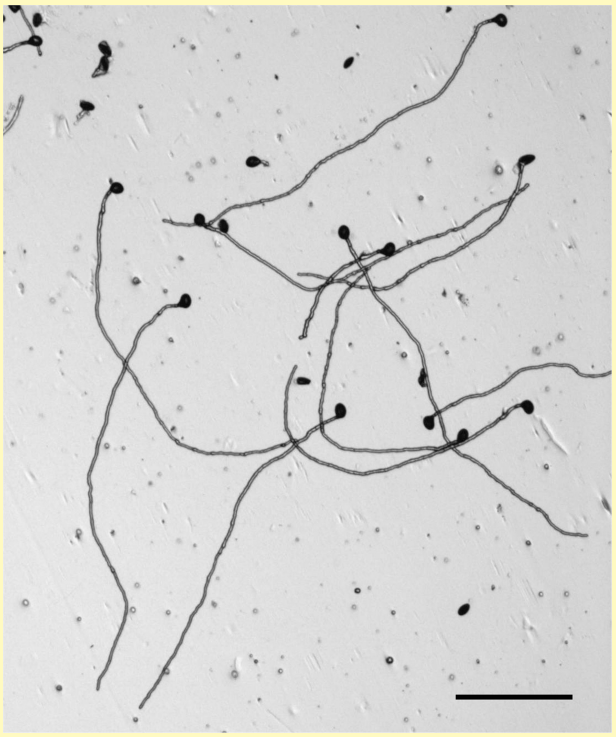
However, pollen tubes also express a cell wall-localized invertase (Singh and Knox, 1984) and are capable of hydrolysing all of the sucrose in germination media to fructose and glucose (Ylstra *et al.*, 1998). It is not known to what extent pollen tubes take up sucrose compared to its hydrolysis products fructose and glucose. There are a large number of hexose transporters expressed in pollen and direct evidence for their involvement in pollen function has probably been hampered by redundancy. In Arabidopsis there are 14 members of the SUGAR TRANSPORT PROTEIN (STP) family alone, and six of them are expressed in pollen (reviewed in Büttner, 2010; Rottmann *et al.*, 2016). *STP2*, *STP4*, *STP6*, *STP9* and *STP11* are expressed at different stages of pollen development. *STP4*, *STP10* and *STP11* are expressed in pollen tubes and may be necessary for the uptake of hexoses from the surrounding tissue. Even though all evidence points to a role for STPs in pollen, no pollen-related loss-of-function phenotype has been described for any of the STPs.

Glucose repression of *STP10* via hexokinase 1

The paper by Rottmann *et al.* (2016) describes the characterization of Arabidopsis STP10. Two aspects are especially interesting for understanding the role of STPs in pollen: evidence for possible hexokinase-dependent repression of expression and for expression pattern regulation by intragenic domains. Expression of *STP10* in yeast was used to show that it encodes

Box 1. Pollen tube growth

The images show pollen tubes growing through the ovary to reach the egg cells in wild-type Arabidopsis (above, scale bar = 50 μm; aniline blue staining) and *stp10-1* pollen tubes *in vitro* (below, scale bar = 100 μm). Courtesy of Dr Ruth Stadler.



a hexose transporter with high affinity for glucose. STP10 also transports galactose and mannose, but not fructose. While no impairment of pollen function was found in an *stp10* loss-of-function mutant, possibly due to redundancy, Rottmann *et al.* provide intriguing insight into the regulation of *STP* expression in pollen. *STP10* is highly induced during pollen germination, both *in vitro* and even more in semi-*in vivo* pollen tubes (Qin *et al.*, 2009; Rottmann *et al.*, 2016). The authors show that expression is only detectable after pollen germination and that the protein is localized to the pollen tube plasma membrane. Induction was lost during *in vitro* germination of pollen when 50 mM glucose was added to the medium; fructose and mannose did not have this effect, nor did adding 50 mM glucose and 50 mM fructose at the same time. Expression of *STP4*, another pollen tube-expressed hexose transporter, was affected in the same way. The downregulation of expression of *STP10* and *STP4* no longer occurred in two independent hexokinase 1 mutants. Hexokinase 1 has a dual role, both as a glucose sensor and as an enzyme that converts glucose to glucose-6-phosphate (Sheen, 2014). The findings by Rottmann *et al.* suggest that the glucose signal that leads to the decrease in *STP10* and *STP4* expression may be transmitted via the hexokinase pathway.

More information is available concerning transcriptional control of *STP10*. In a triple *myb97 myb101 myb120* mutant, the pollen tubes fail to burst upon reaching the synergid and therefore do not deliver the sperm to the ovule. Interestingly, in this mutant a number of genes are no longer induced during pollen germination, among them *STP10* (Leydon *et al.*, 2013). *STP10* may therefore be a direct or indirect target of MYB regulation. The three *MYBs* themselves are induced during growth through the pistil tissue.

An additional interesting finding by Rottmann *et al.* (2016) is that intragenic regions of *STP10* seem to be involved in providing specificity to the expression of *STP10*. A promoter–GUS fusion showed expression in many more tissues than a whole-gene–GUS fusion, indicating that a transcriptional repressor may be present in intron, exon or UTR sequences.

Next steps for understanding sugar uptake by pollen

Future work will undoubtedly have to address the issue of redundancy, and the creation and analysis of double or higher-order multiple mutants in several hexose/sugar transporter genes. We also need to know what sugars are available in the transmitting tract, especially in Arabidopsis. Such data would provide a realistic picture of the conditions encountered by growing pollen tubes and help us understand how they get the nutrition necessary for rapid growth.

Key words: Arabidopsis, glucose signaling, hexokinase 1, hexose transport, major facilitator superfamily, pollen tube growth.

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Insight

Lights, P450, action! Metabolite formation in chloroplasts

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The capacity of plants to convert light into sugar and, consequently, to build specialized compounds of vast complexity is a well-established phenomenon. In their study in this issue of *Journal of Experimental Botany*, Gnanasekaran *et al.* (pages 2495–2506) show that the most versatile catalysts of specialized metabolism can be fueled directly by photosynthetic electron flow. This finding might have great implications for future plant metabolic engineering endeavors.

Plants, being sessile organisms, rely on myriad chemicals as the means of interaction with their environment. Collectively referred to as 'specialized compounds', they comprise structurally variable metabolites from diverse classes, such as terpenoids, phenylpropanes and alkaloids, to name only a few major groups. Besides helping plants to attract pollinators or deter enemies, many of these compounds have become directly important to us. Applied as fragrances, flavors, fine chemicals or medicines, they are being extracted in large amounts, traded and widely utilized – they have been rightly dubbed 'plant natural products' (PNPs). Understanding the biosynthetic capacity behind specialized compound accumulation and exploiting this natural 'warehouse' has, therefore, become a major scientific goal. Ultimately, elucidation of metabolite formation will facilitate targeted engineering of pathways – modifying plants to spur production of compounds of interest in defined amounts and at desired time points and, further, generate novel structures (Staniek *et al.*, 2013).

Dhurrin, the metabolite in question in the study of Gnanasekaran *et al.* (2016) is a cyanogenic glucoside originating from *Sorghum* spp. Although probably of no direct use to people, it confers enormous advantage to its host plants by deterring feeding insects (Tattersall *et al.*, 2001). For delineation of plant metabolism the pathway leading to dhurrin seems to provide an ideal playground. First, it takes only three enzymatic steps (from tyrosine, the primary metabolism-derived amino acid) to yield the final product, making it a relatively short and straightforward metabolic route. Second, the pathway enzymes have to act in a tightly regulated and choreographed manner. Since intermediates on the way from tyrosine to dhurrin are toxic to the cell, the catalysts need to operate in a kind of 'bucket chain', handing over the product of one reaction to the following actor, thereby minimizing leakage and preventing toxicity. While unequivocally demonstrated for dhurrin biosynthesis, the so-called *metabolon* principle is postulated to be relevant to many more pathways (Moller, 2010). Third, the three actors in this short pathway are members of large enzyme families – cytochrome P450 monooxygenases ('P450s') and UDP-glucosyl transferases – whose relatives can be found in almost all pathways of plant metabolism. Therefore, the findings of Gnanasekaran *et al.* could possibly be extrapolated to numerous PNP pathways and might help engineer synthetic metabolic pathways for enhanced product formation.