A perspective on photoperiodic phloem-mobile signals that control development

David J. Hannapel*

Edited by: Jannasen Tom, University of Sheffield, UK
Reviewed by: Jannasen Tom, University of Sheffield, UK
Friedrich Kragler, Max Planck Institute for Molecular Plant Physiology, Germany

*Correspondence: David J. Hannapel, Plant Biology Major, Iowa State University, 253 Horticulture Hall, Ames, IA 50011-7002, USA
e-mail: djh@iastate.edu

INTRODUCTION

Day length is critical in plants as an environmental cue for regulating numerous developmental processes. Recent reviews have addressed the remarkable similarities between photoperiodic signaling in both flowering and tuberization (Suárez-López, 2005; Tamaki et al., 2007; Yo et al., 2013). FT is a member of a family of proteins that contain a phosphatidylethanolamine-binding domain (PEBP) and is not itself a TF (Kardailsky et al., 1999). FT acts as a co-regulator of FD to facilitate binding to floral identity genes like FLOWERING LOCUS T (FT; reviewed by Turck et al., 2008). Under inductive conditions, the B-box zinc finger protein CONSTANS (CO) induces transcription of FT in the phloem. The FT protein then moves through the sieve element system into the shoot apex where it interacts with the bZIP transcription factor (TF), FLOWERING LOCUS D (FD), to activate the floral pathway. Several studies have identified FT in the shoot apex or phloem exudate of plants induced for flowering (Corbesier et al., 2007; Lin et al., 2007; Tamaki et al., 2007; Yoo et al., 2013). FT is a member of a family of proteins that contain a phosphatidylethanolamine-binding domain (PEBP) and is not itself a TF (Kardailsky et al., 1999; Kobayashi et al., 1999). FT acts as a co-regulator of FD to facilitate binding to floral identity genes like APETALA1. In this way, FD provides spatial control of flowering and FT provides temporal control. Recent studies suggest that an anti-florigenic signal may also be trafficking long distance. This CEN/TFL1 homolog, designated ATC, is expressed in the phloem and not the shoot apex. Genetic analysis showed that ATC suppresses flowering and that both its mRNA and protein can move through a graft junction (Huang et al., 2012). After entering the shoot apex, ATC may then compete with FT for binding to FD. So what can we learn from flowering that will help us better understand the phloem-mobile signal that regulates tuberization?

Phloem-mobile signals that are regulated by day length activate both flowering and tuber formation. Both signaling processes have numerous elements in common. In this review, FLOWERING LOCUS T and the three signals currently implicated in controlling tuberization, SPL9, mR172, and the SiBEL5 complex, are discussed with a focus on their functional roles, their mechanisms of long-distance transport, and their possible interactions.

Keywords: FLOWERING LOCUS T, mobile RNAs, potato, SiBEL5, SPL9

The advent of genome sequence and extremely sensitive molecular detection methods have made it possible to identify several potential signals involved in the process. As potential standards, let us consider some experimental criteria for assessing the functional role of a putative phloem-mobile tuberization signal. (1) If it is phloem-mobile then it should be detected in phloem cells or sap and it should transverse a heterograft. Of course, this presents detection issues for specific agents and is a limiting factor with some prime candidates. (2) Because movement may be technically difficult to confirm, then at least, accumulation of the signal should be associated in someway with tuber initiation or development. Over-expression or suppression should affect tuber development or morphology. Over-expression should be able to overcome the negative effects of long days (LDs). Certainly a knock-out mutant would be advantageous but redundancy is very likely built into this important biological process. (3) And finally, some component of the complex should be photoperiod regulated, verifying that the leaf is the origin of the activator or repressor signal. The three best candidates for phloem-mobility signals that regulate tuber formation and will be discussed in this perspective are SPL9/SPL6A, mR172, and the SiBEL5 RNA complex. Other candidate signals will likely emerge in the near future, which would add to the growing notion that there is redundancy in phloem-mobile tuberization signals. Some of these signals may also function as components in flowering pathways. This review discusses the current evidence for phloem-mobile signals controlling tuberization and/or flowering.

Phloem-mobile signals that are regulated by day length activate both flowering and tuber formation. Both signaling processes have numerous elements in common. In this review, FLOWERING LOCUS T and the three signals currently implicated in controlling tuberization, SPL9, mR172, and the SiBEL5 complex, are discussed with a focus on their functional roles, their mechanisms of long-distance transport, and their possible interactions.
StSP6A

It is now readily apparent that FT-like genes function in a wide range of developmental events beyond flowering (Pin and Nils- son, 2012). Consistent with a role for StFT/SP6A as the tuber signal, transgenic over-expression lines tuberized under non-inductive LDs, whereas transgenic suppression lines exhibited a strong reduction in tuber production under SDs (Navarro et al., 2011). Local induction of StSP6A transcripts in stolons activated several tuber-identity genes including SGA2ox1 (Navarro et al., 2011). Potato SCO which has a negative effect on tuberization (Gonzalez-Scham et al., 2012) also represses StSP6A gene expression under LDs. Whereas StSP6Axox icons grafted onto wild-type stocks induced the stocks to tuberize, there was no detection of StSP6A protein moving through the graft unions. This could be due to technical limitations. More support for activity by phloem-mobile FT proteins came from the demonstration that the rice FT orthologue Hd3a fused to GFP can move through a graft into a stolon (Navarro et al., 2011). Supporting the common theme with flowering, this Hd3a construct was able to increase tuber production in over-expression lines under LDs as well as through heterografts with wild type (WT) stocks (Navarro et al., 2011). Hd3a functions in a hexameric floral activation complex composed of three homodimers of OsFT, OsFD and a 14-3-3 protein.

The data available on StSP6A strongly implies it is a very likely candidate for a mobile tuber signal. Despite the evidence for StSP6A as a tuber-inducing signal, several questions remain to be answered. How is StSP6A gene expression induced in leaves under SDs? If the protein moves from leaf to stolon, why does StSP6A RNA accumulate in stolons in response to SDs? What is the mechanism for StSP6A LD repression in leaves? Is it by SCO activity as was previously assumed or by StSP6G competition in a mechanism similar to the antagonistic interplay between FT-orthologs as was previously assumed or by StSP6A LD repression in leaves? Is it by StCO activity that functions as a scaffold (T aoka et al., 2011).

The data available on StSP6A strongly implies it is a very likely candidate for a mobile tuber signal. Despite the evidence for StSP6A as a tuber-inducing signal, several questions remain to be answered. How is StSP6A gene expression induced in leaves under SDs? If the protein moves from leaf to stolon, why does StSP6A RNA accumulate in stolons in response to SDs? What is the mechanism for StSP6A LD repression in leaves? Is it by SCO activity as was previously assumed or by StSP6G competition in a mechanism similar to the antagonistic interplay between FT-orthologs as was previously assumed or by StSP6A LD repression in leaves? Is it by StCO activity that functions as a scaffold (T aoka et al., 2011).

miR172

Movement of miR172 represents a unique and interesting aspect of regulation in the tuberization system. The processing of miR172 is known to be mediated by GIGANTEA and it is involved in the photoperiodic control of flowering (Jung et al., 2007). Over-expression of this microRNA in potato promotes flowering and activates tuber formation under LDs (Martin et al., 2009). Although no movement of miR172 was detected, the presence of this microRNA could be detected in the vascular bundles and its effect on tuberization was graft transmissible. A model was proposed wherein miR172 acts downstream of the tuberization repressor phytochrome B and upstream of the tuberization activator StBEL5. As a hint to function, a miR172 binding site was identified in an APETALA2-like mRNA, RAP1, which was down-regulated in a phytochrome B antisense line. In this model, miR172 induces the degradation of RAP1 which may then influence StBEL5 expression. Because of its role in suppressing translation and enhancing degradation of target RNAs, it is difficult to separate direct movement of miR172 and a localized function in stolons from repression (via transcript degradation) of the movement of one of its targets that may influence tuberization.

Overall, these results suggest, however, that miR172 plays important roles in regulating both flowering and tuber induction in potato.

THE StBEL5 RNA COMPLEX

FT-like transcription factors function by binding to KNOTTED1-types (Hamant and Pasquet, 2010). These ubiquitouss families regulate a number of pathways controlling hormone synthesis and signaling in plants (Bolduc et al., 2012). There is considerable information available on the transcriptional role of StBEL5 and one of its KNOTTED1 partners, POTH1, and their putative role as mobile signals (Chen et al., 2003, 2004; Banerjee et al., 2006, 2009, Mahajan et al., 2012). Movement and accumulation of StBEL5 RNA have been consistently associated with enhanced tuberization even under LDs. But StBEL5 also increases earliness (initiation) in tissue culture plants under both LD and SD conditions (Chen et al., 2003). StBEL5 RNA has been detected in phloem cells using three different approaches: in situ hybridization and RT-PCR of phloem sap and RNA extracted from phloem cells harvested by using laser capture microdissection (Banerjee et al., 2006; Vu et al., 2007; Campbell et al., 2008).

Movement into stolons was confirmed in heterografts and in two transgenic whole plant systems with two different promoters (Banerjee et al., 2006; Figure 1). The use of transgenic over-expression lines with non-plant sequence tags has been critical in establishing movement assays and clarifying the role of untranslated regions (UTRs) in this process. Without such an approach, it would be impossible to detect mobility or to distinguish endogenous StBEL5 RNA from transgenic. The source of StBEL5 RNA has also been clearly established providing further insight on the mechanism of its mobility. For example, despite the observation that there are copious amounts of StBEL5 transcripts in the stem of WT plants, promoter activity is essentially absent in this organ (Banerjee et al., 2006). Both POTH1 and StBEL5 RNAs move freely throughout the plant with a concentration of StBEL5 transcripts in SD stolons (Figure 1, Hannapel, 2013).

Movement into stolons is regulated by photoperiod and enhanced by the UTRs of StBEL5. StBEL5 UTRs were fused to another RNA, StREL14, to make it more mobile (Banerjee et al., 2009). This directed accumulation of this non-mobile StREL RNA was correlated with enhanced yields. SD induction of StBEL5 promoter activity in dark-grown stolons has been observed (Chatterjee et al., 2007) and explained by a mechanism of auto-regulation (Lin et al., 2013). Such auto-regulation by a RNA induced to move by short-day conditions is a classic example of light transduction to an underground organ. Several potential targets of the StBEL5 protein have been identified and specific tandem TTGAC target elements have been confirmed by gel-shift analysis (Hannapel et al., 2013; Lin et al., 2013). These include GAZ1, ISOPENTENYL TRANSFERASE, YUCCA1 and several other genes involved in hormone metabolism. Putative protein
chaperones that may facilitate StBEL5 movement and stability have been identified (Cho et al., 2012; Mahajan et al., 2012). Despite the fact that neither protein has been detected in phloem cells, one cannot rule out the possibility that StBEL5 and/or POTH1 proteins act as long-distance signals in this developmental process. In addition, StBEL5 and POTH1 are not specific and/or POTH1 proteins act as long-distance signals in this developmental process. In addition, StBEL5 RNA is ubiquitous and even present in stolons from plants cultivated under LDs. Although lower in abundance, RNA of POTH1 is also ubiquitous, which contradicts the need for StBEL5 and POTH1 RNAs to function as phloem-mobile tuberization signals. However, from animal systems it is known that RNA-binding proteins not only facilitate intracellular localization, but they can also contribute to repression of translation (St. Johnston et al., 2005). Hence, specificity of phloem-mobile RNAs might be incurred by translational repression of RNA-binding proteins in non-target tissues. As examples, the UTRs of both POTH1 and StBEL5 suppress translation and these UTRs both bind to specific RNA-binding proteins (Banerjee et al., 2009; Mahajan et al., 2012). It must be made clear, however, that there is no direct evidence that mobile StBEL5 and POTH1 RNAs are required for tuberization. In this regard, however, one must consider the possibility of redundancy. There are several lines of information that suggest this possibility. Early antisense lines of both BEL5 and POTH1 showed no phenotype. Early antisense lines of both BEL5 and POTH1 showed no phenotype. There are three other StKN1-type transcription factors that exhibit greater levels of RNA in phloem cells than POTH1 (unpublished RNA-Seq data). Any of these are potentially mobile and could act in direct interaction with StBEL5. In addition to POTH1, there are two other reports of KN1-type mRNAs that are phloem mobile (Kim et al., 2001; Ham et al., 2009). It is conceivable that there might be more than one pathway leading to tuber formation. There are five major pathways controlling flowering time in Arabidopsis (Yamaguchi and Abe, 2012) and each is adapted to respond to different environmental conditions. To ensure efficiency, genetic control of these pathways is mediated by regulatory “hubs” like FLOWERING LOCUS C, FT, SUPPRESSION OF OVEREXPRESSION OF CO1 and LEAFY. Formation of a tuber represents a similar substantial investment in photosynthesis and is a very costly bioenergetic process that may also be

**CONCLUSION: DO PHLOEM-MOBILE SIGNALS HAVE OVERLAPPING FUNCTIONS?**

It is conceivable that there might be more than one pathway leading to tuber formation. There are five major pathways controlling flowering time in Arabidopsis (Yamaguchi and Abe, 2012) and each is adapted to respond to different environmental conditions. To ensure efficiency, genetic control of these pathways is mediated by regulatory “hubs” like FLOWERING LOCUS C, FT, SUPPRESSION OF OVEREXPRESSION OF CO1 and LEAFY. Formation of a tuber represents a similar substantial investment in photosynthesis and is a very costly bioenergetic process that may also be

![FIGURE 1](image-url)
regulated by such “hub” genes. It is feasible that overlap in function may occur among STS6pA, ST5, miR172, and STBEL5 and that back-ups are in effect to ensure normal vegetative and reproductive development. Studies suggest that STBEL5 plays a crucial role during tuber formation by reducing GA levels in the stolon tips and transcription factors increase more than 70-fold at the onset of tuberization (Kloosterman et al., 2007). What elements are present in upstream sequence of STGA2ox1 that may provide insights as to the regulator that controls its expression? Of the eight tuber identity genes that are induced by STS6pA (Nacenta et al., 2011), all contain tandem TTGAC elements in their upstream sequences and are likely target candidates of a STBEL5 complex. STGA2ox1 contains five tandem TTGAC elements present in the first intron and upstream sequence of its gene including two tandem motifs 85 nucleotides apart, both containing TGAC elements on opposite strands two nucleotides apart that form a palindrome (Lin et al., 2013). The maize ortholog of STGA2ox also contains a tandem TTGAC element in its first intron (Bolude and Hake, 2009), suggesting conservation of this transcriptional complex among species. The fact that STGA2ox1 and other tuber genes may be regulated by both STBEL5/KNOX and STS6pA complexes implies cross-talk or direct interaction between these regulatory pathways. In planning future research, let us consider that we need to know more about miR172 targets, that STS6pA requires a transcription partner to make it a factor in expression, and that tuber-specific activity of STBEL5 may require an additional co-factor. And lastly, post-transcriptional control mechanisms to allow for targeted movement, enhanced stability, and translational repression.

ACKNOWLEDGMENTS

Thanks to Anjan Banerjee and Hao Chen for their contributions to our understanding of STBEL5 biology. Thanks also to Pooja Sharma and Sung KI Cho for their critical reviews of this perspective. Recent work on STBEL5 was funded by National Science Foundation-Plant Genome Research Program award no. 0820653.

REFERENCES

Abekeda, J. A., Navarro, C., and Prat, S. (2011). From the model to the crop: genes controlling tuber formation in potato. Crop J. Open Biotechniol. 22, 287–292. doi: 10.1016/j.copbio.2010.11.013
Banerjee, A. K., Chatterjee, M., Yu, C., Sub, S. G., Miller, W. A., and Hamangui, D. J. (2008). Dynamics of a mobile RNA of potato involved in a long-distance signaling pathway. Plant Cell 18, 3443–3457. doi: 10.1105/tpc.108.064273
Banerjee, A. K., Colegrove-Otero, L. J., Devaux, A., Stankiewicz, N., Sur, S. K., and Hannapel, D. J. (2009). Untranslated regions of a mobile transcript mediate long-distance RNA metabolism. Plant Physiol. 151, 1851–1863. doi: 10.1104/pp.109.144428
Bernard, N. (1902). Studies of tuberization in stolons.

Front. Plant Sci. 4:257. doi: 10.3389/fpls.2013.00257
Huang, N. C., Jane, W. N., Chen, L., and Yu, T. S. (2012). Arabidopsis Skn7-like CENTRORADIALIS homologue (LACS) acts dynamically to inhibit floral initiation in Arabidop- si. Plant J. 72, 177–185. doi: 10.1111/j.1365-313X.2012.05076.x
Li, J. H., Yu, Y. H., Song, F. Y., Repen, L. J., Yan, J., Chua, N. H., et al. (2007). The EIGANTEA-regulated microRNA172 mediates photoperiodic flowering in Arabidopsis. Science 318, 1151–1155. doi: 10.1126/science.1151115
Gregory, L. E. (1956). Some factors influencing the flowering of potato. Am. J. Bot. 43, 281–288. doi: 10.2307/2438945
Kardailsky, I., Shukla, V. K., Aln, J. S., Nguyen, J. T., et al. (1995). Activation tagging of the floral inducer FLC in Arabidopsis. Plant Cell 7, 195–210. doi: 10.1105/tpc.7.2.195
Kim, M., Cattis, W., Keshet, S., and Sinha, N. (2005). Developmental changes due to long-distance movement of a homeobox fusion transcript in tomato. Science 305, 297–299. doi: 10.1126/science.1100963
King, M. L., Mauz, T. J., and Mover, K. L. (2005). Putting RNAs in the right place at the right time: RNA localiza- tion in the frog oocyte. Biol. Cell 97, 19–35. doi: 10.1042/BC20040987
Kloosterman, B., Navarro, C., Bijsterbosch, G., Lang, T., Prat, S., Vinier, R. G., et al. (2007). STGA2ox1 is induced prior to tuber formation and controls GA levels during potato tuber development. Plant J. 52, 355–357. doi: 10.1111/j.1365-313X.2007.03245.x
A pair of related genes with antagonistic roles in mediating flowering signals. Science 286, 1960–1962. doi: 10.1126/science.286.5456.1960

Kumar, D., and Waring, P. F. (1973). Studies on tuberization in Solanum andigena. J. Evidences for the existence and movement of a specific tuberization stimulus. New Phytol. 72, 285–287. doi: 10.1111/j.1469-8137.1973.tb02344.x

Liu, M. K., Bringer, H., Lee, Y. J., Varkonyi-Gasic, E., Taska, K., Miura, E., et al. (2007). FLOWER-ERING LOCUS T protein may act as the multifaceted roles of FLOWERING LOCUS T in plant development. Plant Cell Environ. 30, 1742–1755. doi: 10.1111/j.1365-3090.2007.015560.x

Rodríguez-Falcón, M., Bou, J., and Pin, P. A. (2012). The mRNA of a Knotted1-like transcription factor of potato is involved in the vegetative development. Front. Plant Sci. 35, 131–140. doi: 10.1146/annurev.arplant.59.032607.092755

Shen, Z., Paquin, N., Forget, A., and Chartrand, P. (2009). Nuclear shuttling of She2p couples ASH1 mRNA localization to its translation repression by recruiting Loc1p and Puf6p. Molec. Mol. Biol. Cell 20, 2265–2275. doi: 10.1091/mbc.E08-11-1131

Prat, S. (2006). Seasonal control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Annu. Rev. Plant Biol. 57, 595–608. doi: 10.1146/annurev.arplant.57.032005.105224

Rice, F. M., Hart, J. K., Horner, H. T., Davies, P. J., and Hannapel, D. J. (2003). Tissue integrity and analysis of the tuber crop potato. Planta 216, 745–750. doi: 10.1007/s00425-003-0931-0

Martin, A., Adam, H., Diaz-Schum, N. D., and Suárez-López, P. (2009). Graft-transmissible induction of potato tuberization by the RNAi silencing of class I microRNA miR172. Development 136, 2873–2881. doi: 10.1242/dev.025589

Nasrallah, M. E., Ham, B. K., Araki, T., et al. (2013). Phloem long-distance delivery of FLOWERING LOCUS T (FT) to the apex. Plant J. 74, 1033–1036. doi: 10.1111/tpj.12213

Shen, Z., Paquin, N., Forget, A., and Chartrand, P. (2009). Nuclear shuttling of She2p couples ASH1 mRNA localization to its translation repression by recruiting Loc1p and Puf6p. Molec. Mol. Biol. Cell 20, 2265–2275. doi: 10.1091/mbc.E08-11-1131

Prat, S. (2006). Seasonal control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Annu. Rev. Plant Biol. 57, 131–140. doi: 10.1146/annurev.arplant.57.032005.105224

Rodríguez-Falcón, M., Bou, J., and Pin, P. A. (2012). The mRNA of a Knotted1-like transcription factor of potato is involved in the vegetative development. Front. Plant Sci. 35, 131–140. doi: 10.1146/annurev.arplant.57.032607.092755

Shen, Z., Paquin, N., Forget, A., and Chartrand, P. (2009). Nuclear shuttling of She2p couples ASH1 mRNA localization to its translation repression by recruiting Loc1p and Puf6p. Molec. Mol. Biol. Cell 20, 2265–2275. doi: 10.1091/mbc.E08-11-1131

Prat, S. (2006). Seasonal control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Annu. Rev. Plant Biol. 57, 131–140. doi: 10.1146/annurev.arplant.57.032607.092755

Rodríguez-Falcón, M., Bou, J., and Pin, P. A. (2012). The mRNA of a Knotted1-like transcription factor of potato is involved in the vegetative development. Front. Plant Sci. 35, 131–140. doi: 10.1146/annurev.arplant.57.032607.092755

Shen, Z., Paquin, N., Forget, A., and Chartrand, P. (2009). Nuclear shuttling of She2p couples ASH1 mRNA localization to its translation repression by recruiting Loc1p and Puf6p. Molec. Mol. Biol. Cell 20, 2265–2275. doi: 10.1091/mbc.E08-11-1131

Prat, S. (2006). Seasonal control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Annu. Rev. Plant Biol. 57, 131–140. doi: 10.1146/annurev.arplant.57.032607.092755

Rodríguez-Falcón, M., Bou, J., and Pin, P. A. (2012). The mRNA of a Knotted1-like transcription factor of potato is involved in the vegetative development. Front. Plant Sci. 35, 131–140. doi: 10.1146/annurev.arplant.57.032607.092755

Shen, Z., Paquin, N., Forget, A., and Chartrand, P. (2009). Nuclear shuttling of She2p couples ASH1 mRNA localization to its translation repression by recruiting Loc1p and Puf6p. Molec. Mol. Biol. Cell 20, 2265–2275. doi: 10.1091/mbc.E08-11-1131

Prat, S. (2006). Seasonal control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Annu. Rev. Plant Biol. 57, 131–140. doi: 10.1146/annurev.arplant.57.032607.092755

Rodríguez-Falcón, M., Bou, J., and Pin, P. A. (2012). The mRNA of a Knotted1-like transcription factor of potato is involved in the vegetative development. Front. Plant Sci. 35, 131–140. doi: 10.1146/annurev.arplant.57.032607.092755