A Model System of Development Regulated by the Long-distance Transport of mRNA

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Abstract

BEL1-like transcription factors are ubiquitous in plants and interact with KNOPTED1-types to regulate numerous developmental processes. In potato, the RNA of several BEL1-like transcription factors has been identified in phloem cells. One of these, StBEL5, and its Knox protein partner regulate tuber formation by targeting genes that control growth. RNA detection methods and grafting experiments demonstrated that StBEL5 transcripts move across a graft union to localize in stolon tips, the site of tuber induction. This movement of RNA originates in source leaf veins and petioles and is induced by a short-day photoperiod, regulated by the untranslated regions, and correlated with enhanced tuber production. Addition of the StBEL5 untranslated regions to another BEL1-like mRNA resulted in its preferential transport to stolon tips leading to increased tuber production. Upon fusion of the untranslated regions of StBEL5 to a β-glucuronidase marker, translation in tobacco protoplasts was repressed by those constructs containing the 3’ untranslated sequence. The untranslated regions of the StBEL5 mRNA are involved in mediating its long-distance transport and in controlling translation. The 3’ untranslated sequence contains an abundance of conserved motifs that may serve as binding motifs for RNA-binding proteins. Because of their presence in the phloem sieve tube system, their unique untranslated region sequences and their diverse RNA accumulation patterns, the family of BEL1-like RNAs from potato represents a valuable model for studying the long-distance transport of full-length mRNAs and their role in development.


Introduction

In animals, mRNAs are transported within the cell in a tightly regulated process to facilitate their function. With the extensively studied Staufen protein of Drosophila (St Johnston et al. 1991; Ferrandon et al. 1994), binding to the bicoa and oskar mRNAs occurs to determine their localization in the egg cell, either anterior or posterior (Figure 1). This directed localization involves RNA–protein, RNA–RNA (Ferrandon et al. 1997) and protein–protein interactions (Elvira et al. 2006) during transport and repression of translation of the mRNAs. Repression of translation ensures that the mobile mRNAs are functional only at the target site (King et al. 2005). Commonly, it is the untranslated regions (UTRs) that function in binding to proteins that facilitate movement of a transcript (Jansen 2001), in mediating RNA stability (Demigo et al. 2000; Lee and Jeong 2006), or in regulating the efficiency of translation (Gualerzi et al. 2003; Barreau et al. 2006). Without the protection of these RNA-binding proteins, naked, cellular RNA molecules quickly fall prey to degradative processes (Shyu et al. 2008). Intracellular localization of mRNAs in plants is an important process for targeting expression to specific cellular regions...
system, predominantly the phloem. This information superhighway transports sugars, metabolites, proteins, and RNAs via a combination of plasmodesmata and the sieve tube system. The delivery of these information molecules has been implicated in regulating developmental processes, responses to biotic stresses, delivery of nutrients, and as a vehicle used by viruses for systemic infection (Lough and Lucas 2006).

Numerous full-length mRNAs have been identified in the sieve tube system of several plant species (Asano et al. 2002; Vilaine et al. 2003; Omid et al. 2007; Deeken et al. 2008; Gaupels et al. 2008), but only a few have been confirmed to be transported to distantly-located sink organs (Kehr and Buhtz 2008). Of these, three are from Cucurbita maxima, namely CmGAIP, CmNACP, and CmPP16 (Ruiz-Medrano et al. 1999; Xoconostle-Cazares et al. 1999; Haywood et al. 2005); DELLA-GAI from Arabidopsis (Haywood et al. 2005); PFP-LeT6 from tomato (Kim et al. 2001); and StBEL5, a BEL1-like transcription factor, is expressed in potato (Banerjee et al. 2006). Recently, an RNA-protein complex that transports GAI RNA of pumpkin has been identified that contains six mRNAs and up to 16 proteins (Ham et al. 2009). The key RNA-binding protein of this complex was identified as a polypyrimidine tract binding protein, designated CmRBP50.

Our understanding of the long-distance movement of full-length RNAs in plants and the sequences that mediate this process continues to expand (Kehr and Buhtz 2008; Ham et al. 2009; Huang and Yu 2009; Li et al. 2009). Based on animal models, these sequences likely form recognition stem/loop structures, designated zip code elements that function in recognizing RNA-binding proteins (reviewed in Jansen 2001). Whereas these zip codes may be located anywhere in the transcript, they are most predominant in the 3′ UTR (Macdonald and Kerr 1997; Chartrand et al. 1999; Thio et al. 2000). Viroid RNA motifs have also been identified that facilitate selective RNA movement through plant cells (Qi et al. 2004; Zhong et al. 2007, 2008). These short sequences most likely mimic endogenous plant RNA motifs that are recognized by cellular factors for transport. Viroid movement in plants and the elements that mediate this movement are reviewed by Wang and Ding (2010).

One of the mobile mRNAs of potato that is transported through the phloem, designated StBEL5, is a member of the BEL1-like family of transcription factors (Müller et al. 2001; Chen et al. 2003; Smith and Hake 2003). RNA detection methods and grafting experiments have been used to demonstrate that StBEL5 transcripts are present in phloem cells and move across a graft union to localize in stolon tips, the site of tuber induction (Banerjee et al. 2006; Yu et al. 2007). This movement originates in the veins of source leaves and petioles and is induced by a short-day photoperiod, regulated by the UTRs, and correlates with enhanced tuber production. Whereas photoperiod mediates the movement of StBEL5 RNA,

Long-distance Transport of Full-length Transcripts in Plants

Plants have developed a unique long-distance signaling pathway that takes advantage of connections in the vascular
transcription of the StBEL5 gene in leaves is activated by either red or blue light, regardless of photoperiod or light intensity (Chatterjee et al. 2007).

In this review the mechanisms that regulate the metabolism of a mobile RNA of potato that acts as a long-distance signal during development are discussed. Because of their diversity in RNA accumulation patterns, the uniqueness of their untranslated RNA sequences, and location in phloem cells, the RNA family of BEL1-like transcription factors of potato represents a valuable model for studying the processes that control the long-distance transport of mRNA in plants.

The BEL1 Family of Potato: A Model for RNA Transport

Tuber formation in potatoes (Solanum tuberosum L.) is a complex developmental process involving a number of important biological systems. It has long been known that under short-day photoperiodic conditions receptors in the source leaves perceive the light cue and activate a transmissible signal that moves from the leaf through the phloem down to the stolon to induce growth in the apical stem of the stolon (Figure 2). This graft-transmissible signal initiates processes that lead to cell division and expansion as well as a change in the orientation of cell growth in the subapical region of the stolon tip, leading to starch accumulation and formation of the tuber.

As one approach to identifying this mobile signal, research efforts have focused on the role of transcription factors in regulating tuber formation. One important group of transcription factors includes the TALE superfamily. In plants, these include members from the BEL1- and the KNOTTED1-like families: both are involved in pattern formation and maintenance of the shoot apical meristem (Reiser et al. 2000; Kanrar et al. 2008). In potato, seven BEL1-like transcription factors have been identified (Chen et al. 2003). This family of transcription factors is ubiquitous among plant species and these proteins interact with KNOTTED1-like transcription factors (Figure 3) to regulate a range of developmental processes (Müller et al. 2001; Chen et al. 2003; Smith and Hake 2003; Kumar et al. 2007; Pagnussat et al. 2007; Kanrar et al. 2008; Ragni et al. 2008).

In potato, the BEL1-like transcription factor, StBEL5 and its Knox protein partner, POTH1, regulate tuber formation by mediating hormone levels in the stolon tip (Rosin et al. 2003; Chen et al. 2004). This change in hormone level leads to the establishment of a strong sink organ, the tuber, by activating cell division and enlargement in specific cells of the stolon tip (Xu et al. 1998; Hannapel et al. 2004). Upon this activation, abundant amounts of sucrose are transported to the stolon tip for the synthesis of starch in the newly formed tuber.

Transgenic approaches have confirmed a positive correlation between the accumulation of StBEL5 RNA and tuber formation (Figure 4). Overexpression of StBEL5 and its partner POTH1.

Figure 2. Photoperiod-mediated response that activates a long-distance signal (white arrows) that moves from source leaves, via the phloem, to induce tuber formation in potato.

In photoperiod-sensitive lines of potato, short days induce tuber formation, whereas long-day growth conditions inhibit tuberization.
Figure 3. Model for POTH1/BEL5 interaction.

(1) BEL1- and KNOTTED1-like transcription factors (TFs) work in tandem. Protein interaction is mediated by BELL and KNOX domains (Chen et al. 2003). (2) DNA interaction is mediated by the respective homeodomains that interact with one of the TTGAC motifs in the tandem repeat (Chen et al. 2004). POTH1 is a potato KNOTTED1-type transcription factor (Rosin et al. 2003). All seven of the BEL1-like transcription factors of potato interact with all known KNOTTED1-type TFs of potato and tobacco.

have consistently produced plants that exhibit enhanced tuber production, both earliness and overall yield. Because of the aberrant phenotype produced by POTH1, increased tuberization is observed only under tissue culture conditions. POTH1 overexpression lines display a dwarf phenotype and malformed leaves (Rosin et al. 2003). With StBEL5, overexpressing RNA constructs with and without the UTRs produced plants with enhanced tuber formation. Full-length transgenic RNA (including both UTRs) tuberized earlier, with greater yields and with the capacity to tuberize under non-inductive (long-day) conditions (Banerjee et al. 2006).

Movement of BEL5 RNA through the Phloem

As previously mentioned, the promoter of StBEL5 is activated by light in the veins of leaves but is not active in stems. Despite this activity profile, an abundant amount of StBEL5 RNA could be detected in stems (Banerjee et al. 2006), suggesting the possibility that the RNA was being transported from leaves to stems through the phloem translocation stream. If this were the case, then BEL5 RNA would be present in stem phloem cells of the plant. This hypothesis was tested by using two different approaches, namely in situ hybridization of stem and stolon sections with probes specific for StBEL5 (Figure 5A–D) and laser capture microdissection (Figure 5E–F). Both techniques verified that BEL5 RNA plus several more from the StBEL family and POTH1, the KNOTTED1-type protein partner of BEL5, are detectable in RNA extracted from phloem tissues. Using in situ hybridization, RNA of POTH1 was also detected in phloem cells of stolons (Rosin et al. 2003).

Movement of StBEL5 transcripts was first confirmed by grafting an overexpression line for a full-length StBEL5 construct to a wild-type plant (Figure 6A). In three separate plants, the StBEL5 RNA moved from the transgenic scion into the wild-type stock accumulating specifically in the stolon tip (Figure 6B). As with the previous positive correlations with tuber enhancement, transport of transgenic StBEL5 RNA was correlated to an increase in tuber production on the grafted plants (Figure 6C).

Whereas there are several reports available on non-cell-autonomous movement of RNAs in plants (Ruiz-Medrano et al. 1999; Kim et al. 2001; Nakajima et al. 2001; Haywood et al. 2005), the rationale for movement of some of these transcripts is not readily apparent. However, in the StBEL5 case, it is reasonable to assume that the movement of StBEL5 RNA acts as a signal that mediates tuber development. Overexpression lines of StBEL5 exhibited enhanced tuber production (Chen et al. 2003), but other than this phenotype, these transgenic lines appeared to be normal. Overexpression lines of POTH1 and other Knox genes produced plants with abnormal leaf architecture (Parnis et al. 1997; Nishimura et al. 2000; Rosin et al. 2003). No such foliar aberrations have been observed in StBEL5 overexpression lines. The StBEL5 protein works with one of its Knox partners, POTH1, to target specific genes. Using the ga20ox1 promoter as a model target, no effect on transcription was observed without the involvement of both partners (Chen et al. 2004). RNA levels for POTH1 are constitutive in stems and stolons, whereas RNA accumulation of StBEL5 increases in stems and stolons in response to short days, the environmental signal for tuber formation. In this context, the rationale for the movement of StBEL5 RNA as a signal for tuber formation, in conjunction with its Knox partner, is compelling.

The Role of Untranslated Regions in RNA Transport

That addition of the UTRs of StBEL5 could affect a phenotype in transgenic plants related to tuberization was surprising
Figure 4. An increase in tuber number occurs in transgenic potato (*Solanum tuberosum* ssp. *andigena*) lines overexpressing *StBEL5* and *StPOTH1*.

(A) Tuber production in wild-type and three independent transgenic lines that overexpress *StPOTH1*. Plants were grown in culture. (B) Tuber production in wild-type and three independent transgenic lines that overexpress *StBEL5*. Plants were grown in soil. All constructs were driven by the CaMV 35S promoter and the plants were grown under short-day conditions. Bars = 0.6 cm and 2.0 cm for (A) and (B), respectively. Panel (A) is reprinted from Rosin et al. (2003; Copyright American Society of Plant Biologists, www.plantphysiol.org).

(Banerjee et al. 2006). *BEL5* RNA has a rather large 3′ UTR of 505 nt with several conserved motifs. These regions include clusters of CUUC and UAGUA motifs (Banerjee et al. 2009) and are conserved in putative *BEL5* orthologs from tomato and tobacco as well as in *StBEL11* and *StBEL29*. Interestingly, a genome-wide analysis of RNA protein binding motifs of yeast identified GUAR, RUUC, and CUUC as conserved elements in several transcripts (Hogan et al. 2008). Overall, a considerable amount of diversity exists within the 3′ UTR sequences of the RNAs from the *StBEL* family, ranging from 60 and 90 nt for *BEL30* and *BEL14*, respectively, to 424 and 505 nt for *BEL29* and *BEL5*, respectively. These same RNAs exhibit diverse patterns of transcript accumulation in organs of the potato plant (Chen et al. 2003). Because most of the RNAs of the *StBEL* family are detected in phloem cells, could it be possible that their respective UTRs are directing their transport to functional sites throughout the plant?

The mRNA UTRs are critical in numerous aspects of RNA metabolism. In particular, there are several examples confirming the role of the 3′ UTR in recognizing RNA-binding proteins...
that regulate metabolism and movement (Ferrandon et al. 1994; Corral-Debrinski et al. 2000; Padmanabhan and Richter 2006; Irion and St Johnston 2007). In the Staufen/bicoid complex, the localization of bicoid RNA requires specific binding to proteins in an endosomal sorting complex to an element in the 3′ UTR (Irion and St Johnston 2007). In Saccharomyces cerevisiae, ASH1 mRNA is localized to the bud tip of daughter cells, where it plays key roles in development (Olivier et al. 2005). The localization of these transcripts depends on interactions between localization elements in ASH1, some of which are located within the 3′ UTR; these are recognized by the RNA-binding protein, She2p. The 3′ UTR of the RNA for Pmp1, a short plasma membrane protein of yeast, mediates its cellular localization to membrane compartments (Loya et al. 2008). For the GAI RNA of Arabidopsis, motifs in the coding sequence and the 3′ UTR display functional roles in regulating RNA movement (Huang and Yu 2009).

In the oocyte of Xenopus laevis, Vg1 RNA is transported to the vegetal cytoplasm, where localized expression of the encoded protein is critical for embryonic polarity (King et al. 2005). Interaction between specific repeated motifs within the 3′ UTR and RNA-binding proteins mediates the targeted localization of Vg1 RNA to the vegetal pole of the developing embryo (Lewis et al. 2004).

Do the UTRs of StBEL5 have any effect on RNA metabolism or location? Three experiments were carried out to explore this question. First, to determine if the StBEL5 UTRs play a functional role in regulating movement, the relative
accumulation of \textit{StBEL5} RNAs in whole plants of transgenic lines that expressed only the coding sequence (\textit{StBEL5-cds}) or the coding sequence plus both UTRs (\textit{StBEL5-FL}) was compared. Here, both constructs were driven by the \textit{Cauliflower mosaic virus 35S} promoter. Transgenic plants with each of the two constructs were grown under short-day (SD) conditions and RNA extracted from new leaves (\textbf{Figure 7A}) and 5.0 mm stolon tips (\textbf{Figure 7B}) and RNA for the \textit{BEL5} construct was quantified. In samples from SD plants, more polymerase chain reaction (PCR) product for the full-length transcript was detected in stolons compared with leaves (\textbf{Figure 7C}), whereas the yield of product for the \textit{BEL5} cds RNA from leaf and stolon exhibited a ratio of 1:1 (\textbf{Figure 7C}). When transgenic lines expressing the full-length \textit{BEL5} transcript were grown under long-day (LD) conditions, preferential accumulation of the transgenic RNA in stolons did not occur and the ratio of leaf : stolon RNA was 1:1 (\textbf{Figure 7C}).

To confirm that the preferential accumulation of full-length \textit{BEL5} RNA, driven by the \textit{35S} promoter, was due to photoperiod-mediated movement, the leaf-abundant galactinol synthase (GAS) promoter from \textit{Cucumis melo} (\textit{Ayre et al. 2003}) was used to drive \textit{StBEL5} expression. With this promoter, in the samples from plants grown under SD conditions, the ratio of quantified \textit{StBEL5} RNA that moved to stolons in relation to the source leaf was sevenfold greater in SD compared with LD plants (\textbf{Figure 7D}), indicating enhanced mobility of the \textit{StBEL5} RNA under SD conditions. This increased RNA mobility was correlated with earliness (more tubers after 10 d of SD inductive conditions) and enhanced tuber yields (\textit{Banerjee et al. 2006}). Collectively, these studies demonstrated that the addition of the UTRs facilitated the transport of \textit{BEL5} RNA into stolon tips and that a short-day photoperiod enhances this process.

\textbf{How Does Photoperiod Affect RNA Movement?}

If photoperiod mediates the onset of \textit{StBEL5} RNA movement from leaf veins through the phloem to stolon tips, what
Figure 7. Effect of untranslated regions (UTRs) and photoperiod on long-distance transcript movement into stolon tips.

(A) Movement of transcripts with and without the UTRs (FL and cds, respectively) was assayed in three transgenic plants (for each construct) grown under short day (SD) conditions for 14 d. RNA was extracted from recently matured source leaves.

(B) Stolon tips (0.5 cm in length) on plants described in (A) were used to extract RNA.

(C) RNA levels in leaf and stolon tissues obtained from transgenic potato lines described in (A); constructs driven by the CaMV 35S promoter. One-step reverse transcription-polymerase chain reaction (RT-PCR) was carried out using 20 ng of total RNA.

(D) As in (C), except that full-length StBEL5 transcripts were driven by the leaf-abundant galactinol synthase (GAS) promoter (Ayre et al. 2003), which in potato is active only in the minor veins and 500 ng total RNA was used in RT-PCR assays. Note that, as for experiments described in Figure 6, a non-plant sequence tag was fused to all constructs to allow discrimination from native transcripts. All PCR reactions were standardized and optimized to yield product in the linear range, normalized using rRNA primers, and quantified by using ImageJ software (Abramoff et al. 2004). Values represent Mean ± SEM for three biological replicates. (Reprinted from Banerjee et al. 2006; Copyright American Society of Plant Biologists, www.plantcell.org).

pathway is regulating this process? A clue to this answer was provided by examining the role of phytochrome B (phyB) in this system. Phytochrome B is a photoreceptor that acts as a negative regulator of tuber formation in potato (Jackson et al. 1996). Consequently, transgenic lines of photoperiod-responsive potato plants with reduced levels of phyB RNA will tuberize even under long-day conditions (Jackson et al. 1998). In these phyB antisense lines, even under LD conditions, transcripts of SIBEL5 move freely from leaves to stolon tips in correlation with the onset of tuber formation (Figure 8, Martin et al. 2009). This pattern of movement is similar to wild-type plants grown under SD conditions. As expected, wild-type plants grown under long days do not produce tubers and most of the SIBEL5 RNA remains in leaves and stems (Figure 8). Based on our understanding of the location of BEL5 promoter activity, the differences in the patterns of accumulation for SIBEL5 RNA very likely reflect the capacity of the full-length transcript for transport. Phytochrome B in response to long days may function to modify or close down protein gateways that facilitate the movement of SIBEL5 RNA. Under short days, these same putative gateways are opened to allow selective RNA transport through the phloem.

Enhancing RNA Mobility through the Addition of the StBEL5 UTRs

If the BEL5 UTRs mediate long-distance movement, then it should be possible to fuse both UTRs to a less mobile RNA and enhance its overall mobility. This hypothesis was tested by developing a chimeric construct containing both SIBEL5 UTRs fused to the coding sequence of a less mobile BEL1-like RNA, SIBEL14 (Figure 9A, BEL14 + BEL5 UTRs). A construct consisting of the full-length SIBEL14 sequence without the SIBEL5 UTRs was used as a base-line control (Figure 9A, FL-BEL14). As a positive control, movement was assayed for a transgenic line previously characterized (Banerjee et al. 2006) expressing the full-length SIBEL5 transcript (Figure 9A, FL-BEL5). To ensure that the source of most of these transgenic RNAs was the source leaves (similar to the SIBEL5 promoter), the leaf-abundant GAS promoter was used for all three constructs.

The most efficient movement of transgenic RNA was observed with the full-length SIBEL5 RNA (Figure 9B). The difference in RNA accumulation in leaves relative to stolon tips between transgenic lines with BEL14 plus the SIBEL5 UTRs...
et al. 2004; Colegrove-Otero et al. 2005; Paquin et al. 2007), the process of transporting several mobile non-plant mRNAs (Gu 2003). Because translational repression is a key component in the sequence of translation, the patterns of accumulation for BEL5 RNA reflect its capacity for transport. Under long-day conditions (♦), RNA movement to stolon tips is enhanced. Under short-day conditions (▲), RNA movement to stolon tips is reduced. Under LD conditions, WT plants do not form tubers. The phytochrome B antisense lines (♦, phyB AS) tuberize even under long-day conditions. The data for the long-day plants (LD), graciously provided by P. Suarez-Lopez, was derived from quantitative reverse transcription-polymerase chain reaction (qRT-PCR) (Figure 7F from Martin et al. 2009). The data for the short-day plants (WT SD) was derived from RNA blot hybridization (Banerjee et al. 2006). Based on analyses of BEL5 promoter activity and transcript movement, the patterns of accumulation for SIBEL5 RNA reflect its capacity for transport. Under long-day conditions (■), very little BEL5 RNA moves down to stolons, whereas under short-day conditions (▲), RNA movement to stolon tips is enhanced. Under LD conditions, WT plants do not form tubers.

or BEL14 without these UTRs was 2.7-fold. Hence, almost threefold more BEL14 transcripts were translocated into stolon tips in the presence of the StBEL5 UTRs. Such enhanced movement was correlated with a fourfold increase in tuber yield for the BEL14 + BEL5 UTR lines compared with the FL-BEL14 lines (Figure 9C). Clearly, the addition of the UTRs of BEL5 to another, less mobile, BEL RNA can enhance its phloem mobility and targets the chimeric RNA to a specific organ.

The Effect of the StBEL5 Untranslated Sequences on Translation

Because translational repression is a key component in the process of transporting several mobile non-plant mRNAs (Gu et al. 2004; Colegrove-Otero et al. 2005; Paquin et al. 2007), the effect of BEL5 UTRs on translation was assessed. Here, the coding sequence of β-glucuronidase (GUS) with and without the BEL5 UTRs was cloned and assayed in a transient translation system using tobacco protoplasts. Four constructs were analyzed: GUS plus both UTRs attached, and the GUS coding-sequence alone (Figure 10A). Translation of GUS was reduced by 12% in the construct containing the 5’ UTR of SIBEL5 relative to GUS alone (Figure 10B). With the GUS + 3’ UTR and the 5’ UTR + GUS + 3’ UTR constructs, translation was repressed by 32% and 50%, respectively (Figure 10B). As a comparison, using in vitro systems with marker genes, regulatory sequence from the transcript, and co-expression of established RNA-binding proteins, levels of translational repression for four mRNAs, GluR2, Vg1, Ringo/SPY, and ASH1, were 30 to 40%, 40 to 60%, 70 to 90%, and 80 to 90%, respectively (Colegrove-Otero et al. 2005; Huang et al. 2006; Padmanabhan and Richter 2006; Deng et al. 2008). Repression of translation ensures that the mobile mRNAs mentioned above, most of which encode developmentally important transcription factors, are functional only at their target sites. Taken together, studies with fusion of the BEL5 UTRs to the GUS coding sequence indicated a clear reduction in the level of GUS translation in a protoplast expression system.

Future Perspectives on this Signaling Network

The mRNA of SIBEL5 is one of the few, full-length mRNAs in plants that has been confirmed to move a long distance through the phloem from leaf veins to underground stolon tips (Banerjee et al. 2006; Kehr and Buhtz 2008). The other SIBEL RNAs have also been confirmed to be present in phloem cells. All seven BEL1 proteins of potato interact with the six known KNOTTED1-types of potato (D. Hannapel, unpubl. data, year). These observations set up the intriguing possibility that the BEL1 RNAs of potato participate in a complex network of phloem-mediated transport to regulate development and to respond to environmental cues. The existence of such a complex is a potentially new paradigm in plant biology. Couple this discovery with the fact that post-transcriptional regulation mediated by protein/mRNA complexes occurs widely among eukaryotes (Sanchez-Diaz and Penalva 2006; Keene 2007; Halbeisen et al. 2008) and it is clear that RNA metabolism and protection are important mechanisms for regulating gene expression. Without protection, phloem mobile RNAs may well be rapidly degraded and recycled, as in animal systems (Shyu et al. 2008).

The long-distance transport of SIBEL5 RNA from leaves to stolon tips, to activate tuber formation in potato, represents a dynamic signaling system. Through light-activation of the BEL5 promoter in foliar veins and SD-induced movement of the transcript, light signals from the leaf are communicated to underground stolon tips growing in the dark. Phytochrome B appears to play a role in regulating this movement (Figure 8). In this model system for...
non-cell-autonomous movement of full-length mRNAs, it is readily apparent that escort factors that facilitate transport are not the only interactions putatively mediated by the UTRs of the mobile RNA. RNA-binding proteins function in numerous aspects of post-transcriptional biology (Bailey-Serres et al. 2009; Lorkovic 2009; Martin and Ephrussi 2009) and in this capacity, the 5′ and 3′ UTRs of most RNAs are critical for protein recognition (Curry et al. 2009). In this light, it makes sense that so many proteins and transcripts (up to six) have been identified in the ribonucleoprotein complex of phloem-mobile RNAs of pumpkin (Ham et al. 2009).

In an analysis of RNA-binding proteins in yeast, the bait proteins were associated with specific sets of a few to several hundred RNAs (Hogan et al. 2008). On average, each distinct yeast mRNA interacted with three of the RNA-binding proteins, suggesting the potential for a dynamic complex network of regulation. These results strongly suggest that combinatorial interaction of RNA-binding proteins with specific recognition elements in mRNAs is a pervasive mechanism for multi-dimensional regulation of their post-transcriptional fate. Plants add another layer of complexity to this process as they implement the means by which RNA is transported over long distances through the vascular system to specific target organs. The major challenges that lie ahead for plant biologists are to identify the numerous RNA/protein complexes that mediate this long-distance transport network and to understand how they function in this unique special-delivery system.

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Figure 10. Effect of the SIBEL5 untranslated regions (UTRs) on translational efficiency of β-glucuronidase (GUS) in tobacco protoplasts.

(A) Four constructs driven by the CaMV 35S promoter in pBI221 were analyzed; GUS + the 3' UTR of SIBEL5, the 5' UTR of SIBEL5 + GUS, GUS + both UTRs, and the GUS coding sequence alone. The luciferase gene in pBI221 under the control of the CaMV 35S promoter was included as an internal control.

(B) Relative values for GUS expression were obtained by dividing the GUS activity by the specific luciferase activity. Each transfection was carried out three times. Values represent mean ± SEM. Levels of GUS translation have been adjusted to reflect differences in RNA accumulation for each of the constructs. cds, coding sequence; NOST, nopaline synthase terminator. (Reprinted from Banerjee et al. 2009; Copyright American Society of Plant Biologists, www.plantphysiol.org).

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