**AtTCTP2 mRNA and protein movement correlates with formation of adventitious roots in tobacco**

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The Translationally Controlled Tumor Proteins, or TCTP, is a superfamily of exclusively eukaryotic proteins essential in the regulation of proliferation and general growth. However, it is clear that these are multifunctional proteins given (1) the pleiotropic effects of its mutations, and (2), the multiple processes in which this protein is involved. TCTP function in general is conserved, since Arabidopsis *AtTCTP1* can rescue a Drosophila mutant, and vice versa. It has become clear, however, that these proteins may have “taxon-specific” functions. In the case of plants, mRNA and/or proteins have been found in the phloem translocation stream of different species, suggesting a role in long-distance signaling. We have found that a second Arabidopsis *TCTP* gene, *AtTCTP2*, codes for a protein that moves long-distance through a graft union in tobacco. Interestingly, the mRNA is also transported long-distance. Both mRNA and protein move long-distance; interestingly, the movement, while more efficient from source to sink tissues, also occurs in the opposite direction. The protein reaches the nuclei of parenchyma cells and adventitious roots. Furthermore, it is clear that the long-distance delivery of *AtTCTP2* protein and mRNA is required for the induction of adventitious roots. A model is presented that accounts for these observations.

**TCTP is essential for growth and development in eukaryotes**

The translationally Controlled Tumor Proteins (TCTP) are found in most eukaryotes in which they have an essential role in regulating general growth and proliferation. Knock out of this gene in certain species, such as mouse, Drosophila and Arabidopsis leads to lethality during early stages of development; this underscores its central role in development. On the other hand its function appears to be rather conserved, since the expression of an Arabidopsis *TCTP* gene can complement a Drosophila TCTP mutant, and vice versa, but some members may have functions specific for each taxon. For example, TPT1 mRNA, the human isoform of TCTP, activates Protein Kinase R (which is part of the Interferon pathway and is also induced by viral double-stranded RNA); this protein in turn down-regulates translation. Furthermore, TPT1 induces histamine release from mast cells [hence its other name, Histamine Release Factor (HRF)] and regulates B and T cell proliferation, demonstrating an important role in the modulation of the immune response, at least in mammals. TPT1 also activates transcription of genes involved in pluripotency, such as *oct4* and *nanog*. Other members of this family engage in molecular mimicry, such as the *Plasmodium falciparum* TCTP; this may be a strategy to suppress the host’s immune response. The fact that this protein is secreted into serum and acts as a chemokine illustrates in some cases its non-cell autonomous function.

In plants, the induction of TCTP by different types of stress and signaling molecules (such as heavy metals, pathogen attack, salt, and methyl jasmonate, among several others) suggests a role in maintenance of homeostasis in response to environmental stimuli. The over-expression of the Arabidopsis thaliana *TCTP* gene, *AtTCTP1*, induces drought tolerance via stomatal closure, while a *Nicotiana benthamiana* TCTP negatively

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bacterium rhizogenes. We have also tested tobacco explants when harbored by Agrobacterium rhizogenes.24,25 We have recently found that some plant TCTPs enhance in vitro plant regeneration in tobacco explants when harbored by Agrobacterium rhizogenes.24,25 We have also proposed that, while the phylogenetic relationships of plant TCTPs are difficult to establish perhaps due to unequal evolutionary rates, extensive horizontal gene transfer, or both, the predicted structures of plant TCTPs fall within 2 groups. Interestingly, those that are capable of enhancing regeneration fall within one of these groups, the pumpkin TCTP (CmTCTP) and the Arabidopsis thaliana TCTP2 (AtTCTP2).27 This regeneration capacity may be related to a role in differentiation, rather than proliferation, and, on a speculative note, could be important for vegetative reproduction in certain species. The fact that some of these species in which vegetative reproduction occurs, such as potato and strawberry, harbor TCTP isoforms that are structurally related to CmTCTP and AtTCTP2, supports this notion.27

Arabidopsis AtTCTP2 induces adventitious roots upon its long-distance transport

AtTCTP2 is one of 2 TCTP genes in Arabidopsis, AtTCTP1 having been more thoroughly studied.4,27 Evidence has been found that the latter is a central mitotic regulator in plants, and probably also in animals; it is also required for gametophyte development, and, more generally, knock-out of this gene results in early embryonic lethality.4,27 AtTCTP2, on the other hand, has been considered a pseudogene; however, some evidence from our group indicates otherwise.25 Indeed, AtTCTP2-GFP fusions are expressed; the resulting protein accumulates in stomata, trichomes and root cortex nuclei in Arabidopsis and tobacco (Fig. 1). Importantly, AtTCTP2 also enhances in vitro plant regeneration of tobacco explants when harbored by A. rhizogenes.25

It was observed that the accumulation pattern of AtTCTP2-GFP in regenerated tobacco plants was similar to that in Arabidopsis, i.e. in root cortex nuclei.25 Since the upstream region of this gene drove the expression of a GUS reporter gene in the vasculature,25 it was proposed that the products of this gene, too, are transported long-distance through the vasculature. To determine whether this was the case, regenerated tobacco expressing an AtTCTP2-GFP fusion was grafted onto WT tobacco, and vice versa.28 Given that the stock plant included mature leaves, while the grafted scion included mostly sink tissues, phloem movement would be expected to occur in direction of the scion. In this case, RNA and protein corresponding to the AtTCTP2-GFP fusion were detected moving from transgenic stock to non-transgenic stock. Importantly, the tissue selected for analysis in the scion was at a considerable distance from the graft union; RNA was detected by qRT-PCR, but also the fusion protein (through Western blot and by analysis of GFP-associated fluorescence by confocal microscopy). The fraction of AtTCTP2 RNA found in the scion (i.e., the mobile form) relative to the transgenic stock AtTCTP2 RNA (measured as the ratio of AtTCTP2 mRNA per 100 mg of scion sink leaf to AtTCTP2 mRNA in 100 mg of source leaf tissue) was close to 9%. This approach may be useful to determine the rate of movement for phloem mobile transcripts. Interestingly, AtTCTP2-GFP RNA movement was detected from sink to source tissue; i.e., when the scion expressed this fusion, although the long-distance movement ratio was much lower (ca. Three%). This raises several questions, the main one as to the route through which this RNA, and several others, move long distance from sink to source tissues. Similar results were observed at the protein level. An additional conundrum is evident from the analysis of the AtTCTP2-GFP protein transport across the graft union. Indeed, GFP-associated fluorescence is observed in non-transgenic scion or stock in stomata grafted with AtTCTP2-GFP-expressing plants. These cell types are symplastically isolated upon reaching maturity; thus, it is not clear how AtTCTP-GFP RNA or protein gain access to them. An obvious possibility is that this occurs before plasmodesmata are sealed by deposition of β-glucans; or that such movement occurs through the extracellular space. Thus, AtTCTP2 would be exported and afterwards imported into the guard cells, presumably via a putative PTD in this protein (although this has not been described yet in plant TCTPs). Likewise, this protein accumulates in nuclei of cortex cells after its movement across the graft union. In any case, the accumulation pattern of AtTCTP2 after its long-distance delivery is the same as in the tissue that...
expresses it. Thus, once the protein is transported across a graft union, it must interact with factors that direct AtTCTP2 to its proper localization, i.e., in the cortex, where it possibly induces adventitious roots.

Another important point mentioned above is that AtTCTP2 RNA and protein movement occurs from sink to source tissue. It is not clear the pathway followed, but it is unlikely that the phloem is the conduit involved in its delivery. A cell-to-cell pathway would be the alternative pathway (Fig. 2). This possibility is indirectly supported by the analysis of RNA movement between a parasitic plant (Cuscuta reflexa) and its host (Tomato and Arabidopsis); the former functions as a strong sink for the plant, to which it is symplasmically connected. Indeed, several different C. reflexa transcripts are transported into Arabidopsis or tomato. Similar results have been obtained during the analysis of RNA movement across a graft union between different Arabidopsis accessions and when parasitized by C. reflexa. Interestingly, in both cases, AtTCTP1 was a prominent mobile mRNA. Thus, it is becoming clear that cell-to-cell and long-distance movement pathways for proteins and RNAs operate in plants.

It is also clear that massive long-distance mRNA transport occurs in plants, the role of which in whole plant physiology is poorly understood. To date, there are no examples of mutants lacking in such movement, and given the massive nature of such transport, it is unlikely that such mutants would be viable. However, analysis of gain-of-function could be accomplished by grafting experiments in which either stock or scion express one of such mobile RNAs, such as the one described here. An interesting observation was that, when the stock expressed AtTCTP2, but also the scion (albeit less prominently), adventitious roots were formed in close proximity to the graft union. Of note, AtTCTP2-GFP (as GFP-associated fluorescence) was detected in these roots, and more precisely, in the nuclei of root cortex cells, which is the site of AtTCTP2 accumulation in Arabidopsis and in transgenic tobacco. These roots were not observed in WT tobacco or WT tobacco homografts, but neither in ungrafted AtTCTP2-expressing tobacco plants; thus, the transport of AtTCTP2 (protein or mRNA) through a graft union is required for the formation of these roots. Adventitious roots are those that are formed in tissues that normally do not give rise to them, most notably stems, and are a response to stress such as drought or wounding. It is likely that the the long-distance transport of AtTCTP2, is necessary, but not sufficient to induce emergence of aerial roots; the grafting procedure (as a form of stress) and the A. rhizogenes rol genes, may be the other triggering factors. It is evident that the endogenous tobacco TCTP mRNA and/or protein do not induce aerial roots in homografts; it is possible that these are not phloem-mobile or capable of intercellular movement. Indeed, it is also possible that cell-to-cell or long-distance transport of TCTP mRNA or protein occurs only in certain vascular plants. Another possibility is that AtTCTP2 mRNA and/or protein is not subject to the same regulatory constraints (at the postranscriptional, translational and/or post-translational levels) as the endogenous tobacco TCTP, the misexpression of which could also result in the induction of aerial roots. Regardless, this illustrates that the long-distance transport of an RNA and its encoded protein results in a discernible phenotype.

It has been pointed out that plant regeneration and lateral root formation share common pathways. It has also been demonstrated that regenerating tissue does not arise from undifferentiated callus, but rather from founder cells that resemble those from root pericycle. Given the expression pattern of AtTCTP2 in Arabidopsis and in transgenic tobacco, it is tempting to speculate that the
capacity of certain TCTP isoforms, such as CmTCTP and AtTCTP2, to enhance plant regeneration reflects its involvement in lateral root formation, although this awaits experimental confirmation, for example, by complementation of mutants unable to form lateral or secondary roots with these genes. It could also be speculated that some TCTP isoforms are involved in vegetative reproduction that occurs via roots, stolons or tubers in certain plants; presumably, these isoforms originally had a role only in lateral root formation in their ancestors. Finally, plant regeneration and lateral root formation would appear to require long-distance transport of TCTP protein and/or mRNA. An obvious question that is raised by these assumptions is where do these mRNA and proteins originate. Since the AtTCTP2 gene promoter is active in certain root tissues, it is not clear why or how its mRNA and protein are transported to its target tissue, which is in root pericycle. It must be mentioned that GUS activity is detected in a region from which lateral roots emerge; no other root cell type shows such activity. Thus, it is possible that a certain “input” from distantly transported AtTCTP2 (protein and/or mRNA), presumably from leaves, is required for the emergence of lateral roots in the correct position. However, the endogenous transport of AtTCTP2 mRNA and protein remains to be demonstrated in Arabidopsis. A model for AtTCTP2 function is shown in Figure 2. According to this model, the mRNA and protein are synthesized constitutively, albeit at low levels in certain cell types in different plant tissues, for example in mesophyll. Upon a certain stimulus (for example, root wounding), the mRNA and/or protein are transported to root pericycle, where lateral root formation takes place; the mRNA must be translated, and once this occurs, the newly synthesized protein (or the distantly transported protein) enters the nucleus, where it induces adventitious roots. The lines that form an angle represent the graft interface.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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