Systemic signalling through translationally controlled tumour protein controls lateral root formation in Arabidopsis

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Abstract

The plant body plan and primary organs are established during embryogenesis. However, in contrast to animals, plants have the ability to generate new organs throughout their whole life. These give them an extraordinary developmental plasticity to modulate their size and architecture according to environmental constraints and opportunities. How this plasticity is regulated at the whole-organism level is elusive. Here we provide evidence for a role for translationally controlled tumour protein (TCTP) in regulating the iterative formation of lateral roots in Arabidopsis. AtTCTP1 modulates root system architecture through a dual function: as a general constitutive growth promoter enhancing root elongation and as a systemic signalling agent via mobility in the vasculature. AtTCTP1 encodes mRNAs with long-distance mobility between the shoot and roots. Mobile shoot-derived TCTP1 gene products act specifically to enhance the frequency of lateral root initiation and emergence sites along the primary root pericycle, while root elongation is controlled by local constitutive TCTP1 expression and scion size. These findings uncover a novel type for an integrative signal in the control of lateral root initiation and the compromise for roots between branching more profusely or elongating further. They also provide the first evidence in plants of an extracellular function of the vital, highly expressed ubiquitous TCTP1.

Keywords: Grafts, lateral root initiation, long-distance mobility, TCTP, translationally controlled tumour protein, root development, root system architecture, scion control of rootstock development.

Introduction

Plant development is highly plastic. This is essential to survival and adaptation to a wide range of environments from which, being sessile, plants cannot escape. That plasticity manifests itself as an extraordinary capacity of the plant to modify the number, size, shape, patterning, and spatial deployment of its organs, above and below ground, to adapt efficiently to environmental constraints.

As is typical of dicotyledonous species, the Arabidopsis root system arises from a primary root, initiated in the embryo, and de novo organogenesis of secondary and higher order lateral roots (LRs), post-embryonically (Peret et al., 2009; Bellini et al., 2014). LRs constitute the major part of the root system, and are major determinants of water and nutrient uptake and colonization of new soil pockets. Despite their high agronomic and ecological relevance, the molecular mechanisms that determine the placement of LRs, in space and time, and their number are still little known. LRs originate from inner root pericycle founder cells, through a pre-patterning (priming) activation and cell fate redefinition process that gives them competence to divide and differentiate in an orderly fashion to generate a highly organized root primordium (Malamy and Benfey, 1997; De Smet et al., 2007; Peret et al., 2009; Moreno-Risueno et al., 2010). The
establishment of LR initiation sites and the subsequent actual initiation process are not well understood. They are thought to involve an oscillating transcriptional network in interaction with auxin and other as yet unidentified mobile signals and to depend critically on intercellular connectivity (De Smet et al., 2007; Moreno-Risueno et al., 2010; Benitez-Alfonso et al., 2013).

There is intense trafficking of a vast array of molecules between shoot and roots—photoassimilates and a range of growth–enabling metabolites, numerous hormones, and also a vast number of proteins and RNAs (Turgeon and Wolf, 2009; Batailler et al., 2012; Turnbull and Lopez-Cobollo, 2013; Kim et al., 2014; Thieme et al., 2015). Besides specialized mRNAs and siRNAs, translocated RNAs include a huge cohort of protein-encoding mRNAs of various kinds. It is now well recognized that hundreds to thousands of mRNAs transit in the phloem and have long-distance mobility between aerial organs and roots (Notaguchi et al., 2015; Thieme et al., 2015; Yang et al., 2015; W. Zhang et al., 2016; Xia et al., 2018). Long-distance movement of proteins has also been demonstrated, many of which can be unloaded in the root tip (Atkins et al., 2011; Paultre et al., 2016). An emerging consensus is that this movement does not simply reflect passive diffussion and mass flow, but is in part actively controlled movement, probably serving for the delivery of systemic signalling agents (Jorgensen et al., 1998; Lough and Lucas, 2006; Calderwood et al., 2016; Paultre et al., 2016; Kehr and Kragler, 2018; Morris, 2018). There are a few established cases of genes regulating plant development through mobility of their mRNA and/or encoded protein, such as FT in the regulation of flowering time (Corbesier et al., 2007; Jaeger and Wigge, 2007), GIBBERELLIC ACID-INSENSITIVE (GAI) or Mouse ear (me) in the regulation of leaf development (Kim et al., 2001; Haywood et al., 2005), the SIBEL5 transcription factor gene in the control of tuber formation (Banerjee et al., 2009), or shoot-derived Auxin/Indole-3-Acetic Acid IAA18 and IAA28 in the regulation of LR formation (Notaguchi et al., 2012). However, nothing is known of the physiological significance of the vast majority of mobile mRNAs or proteins transiting through the phloem and roles in receiving cells, nor of the mechanisms controlling their excretion, transport, and delivery.

Among the large number of mRNAs with demonstrated long-distance mobility between above- and below-ground organs are transcripts encoding transcriptionally controlled tumour proteins (TCTPs). These include Arabidopsis AtTCTP1 (Notaguchi et al., 2015; Thieme et al., 2015) and AtTCTP2 (Toscano-Morales et al., 2014), a Vitis vinifera TCTP (GSVIVG01017723001; Yang et al., 2015), or Csa3M154390, a Cucumis TCTP (Z. Zhang et al., 2016). TCTP is a highly conserved ubiquitous protein found in almost all eukaryotes. Its molecular function is still a matter of debate, but clearly relates to the regulation of GTPase activity (Hsu et al., 2007; Dong et al., 2009). Fitting with this, TCTP is involved in a number of fundamental biological processes. It is known as an essential mitotic factor and a promoter of cellular growth interacting with the protein synthesis machinery, and also as a cytoprotective and anti-apoptotic protein (reviewed by Bommer and Thiele, 2004; Bommer, 2017). Although best characterized in animals given their high relevance to malignancy and cancer progression, these core functions appear largely conserved in other eukaryotes, including plants, and make TCTPs essential proteins for embryogenesis and early development, organ patterning, regulation of organ size, and cellular homeostasis (Bommer, 2017). In Arabidopsis, knock-out mutations of either AtTCTP1 or its homologue, AtTCTP2, are lethal (Berkowitz et al., 2008; Brioudes et al., 2010), as is the case of TCTP loss of function in mice (Chen et al., 2007) or Drosophila (Hsu et al., 2007). Reduced AtTCTP1 expression through RNAi causes general cell proliferation and growth inhibition, in both vegetative and reproductive organs (Berkowitz et al., 2008; Brioudes et al., 2010; Betsch et al., 2019), and plant TCTPs have been linked to resistance to various abiotic stresses, including salinity, drought, flooding, and suboptimal temperatures (Cao et al., 2010; Kim et al., 2012; Li et al., 2013; Chen et al., 2014; Deng et al., 2016; de Carvalho et al., 2017), and also to biotic stresses (Malter and Wolf, 2011; Du et al., 2015; Gawehns et al., 2015).

In addition to its core functions at the cellular level, mammalian TCTP has long been known to act as an extracellular protein in the immune system, and was in fact first characterized as a histamine-releasing factor (HRF) (MacDonald et al., 1995). Human TCTP has since been shown to modulate the release of cytokines and other signalling molecules (MacDonald, 2012) and to have a broad role in immunity (reviewed in MacDonald, 2017). Whether plant TCTPs also assume non-cell-autonomous functions is unknown. The demonstrated long-distance translocation of TCTP transcripts and proteins between scion and rootstock in various species would support that possibility, but in itself does not prove it. Another most interesting indication in that direction is the earlier finding by Aoki et al. (2005) that pumpkin TCTP (CmaCh11G012000) moved rootward in a selectively destination-controlled manner when introduced in rice sieve tubes, and furthermore in complex with RNA-binding proteins and the conserved eukaryotic translation initiation factor eIF5A. Moreover, this association was found to be necessary for the selective movement of the protein complex.

Together, these observations raise the prospect that plant TCTPs might have physiologically important systemic signalling functions, through mobility. This is what we sought to examine, focusing on Arabidopsis AtTCTP1 (Berkowitz et al., 2008). We asked whether long-distance movement of AtTCTP1 gene products occurs under physiological conditions and plays a role in shaping root architecture.

### Materials and methods

#### Plant material and growth conditions

The transgenic pACTCTP1::gTCTP1-GFP::NOS (TCTP1-GFP) and the TCTP1-RN3 lines are as in Berkowitz et al. (2008), and the 35S::YFP::cDNAATCTP1 is as in Thieme et al. (2015). All lines are in the Arabidopsis thaliana Columbia (Col-0) background (wild type, WT).

The hypocotyl micro-grafting method is as in Marsch-Martinez et al. (2013) with minor adaptations. Seeds were surface-sterilized for 5 min with a solution of sodium 0.125% (v/v) hypochlorite and 90% (v/v) ethanol, rinsed in 100% ethanol, and dried before being reseeded in sterile water and sown on the surface of a nitrocellulose membrane strip (membrane filter; cellulose nitrate 0.45 μm diameter, Whatman code NC45ST) laid on top of a nutrient agar gel [2.15 g l⁻¹ Murashige and Skoog (MS)] salts, 0.5% sucrose, 1% agar type-M, pH 5.7] in Petri plates.
The plates were sealed with porous micropore tape (3M), stratified for 2 d at 4 °C in the dark, and incubated in a vertical position at 21 °C, under a 12 h photoperiod with 120 μmol quanta m⁻² s⁻¹ light intensity. Four to six days later, under sterile conditions, the two cotyledons were severed and the seedlings positioned with the hypocotyl perpendicular to the nitrocellulose strip, and the shoot (scion) overhanging on the agar. A sharp cut was then made through the hypocotyl. The upper part (scion) and bottom part (rootstock) were grafted onto the relevant rootstock and scion, respectively, as indicated, by simple hydrophilic contact maintained by dint of water surface tension, ensuring cotyledon petioles were slightly above the agar surface. This generated reciprocal heterografts (WT scion/ transgenic rootstock and transgenic scion/WT rootstock) and homografts (scion and rootstock of the same genotype). The plates were rescaled and returned to the growth chamber. Five days later (5 DAG, 5 days after grafting), the seedlings were transferred to larger plates filled with similar medium but without sucrose. For the auxin experiment, 20 μM NPA (N-1-naphthylphthalamic acid in DMSO) or an appropriate volume of DSMO solvent was supplied to the medium, and the thickness of agar gel underneath the scion was removed to prevent any contact with the scion. All scion–rootstock combinations compared within an experiment were raised alongside each other within each replicated plate.

For soil-grown plants, on 5 DAG, grafted seedlings were transferred to pots filled with seed raising mix and Osmocote (5g l⁻¹), and then raised alongside seedlings in plates, in the same growth chamber.

**Microscopy**

For in vivo confocal microscopy, the root, scion, or part thereof were mounted on slides in half-strength MS (0% sucrose, pH 5.7, 21 °C) and imaged using a TCS-SP8 microscope (Leica, Germany) equipped with a ×10/0.3 NA or ×63/1.2 NA water immersion objective. Autofluorescence spectra were acquired on the same samples for spectral unmixing. Autofluorescence was excited using a 488 nm argon laser and acquired in λ-mode (from 495 nm to 600 nm, 5 nm acquisition window) with the pinhole opened at 2.8 Airy units (AB). The same parameters were used to acquire green fluorescent protein (GFP) or yellow fluorescent protein (YFP) fluorescence, and spectra were subsequently unmixed using LAS-X (Leica) software. For monitoring the patterns of dye localization, the root, rootstock, and shootstock were imaged using in vivo confocal microscopy (Leica DM5000B, ×10/0.3 NA objective and a 510–525 nm acquisition window, keeping all settings constant within an experiment, and each positive signal was confirmed by spectral unmixing.

For light microscopy, samples were mounted in water, and imaged in DIC (differential interferential contrast) mode with a Leica DMI5000B microscope fitted with a ×40/0.85 NA dry objective and a Leica DFCL 310FX camera Leica Instruments). Individual cell lengths were measured along epidermal files from the quiescent centre to the root differentiation zone, using a custom macro in Fiji (code available upon request). Mature cell length was estimated by means of 10 consecutive cells in the root zone, using a custom macro in Fiji (code available upon request). Mature cell length was estimated by means of 10 consecutive cells in the root zone, using a custom macro in Fiji (code available upon request). Mature cell length was estimated by means of 10 consecutive cells in the root zone, using a custom macro in Fiji (code available upon request).

**Phenotyping**

For determination of the kinetics of root elongation, plates were scanned daily from seed germination at a resolution of 600 dpi, 8-bits per channel, and saved in JPEG. The raw images were automatically pre-processed with a custom ‘auto align’ macro in Fiji, using RootTrace as described in French et al. (2009). At least six plants per condition with a complete trace were available for each of the compared genotypes and conditions, and thus were used for subsequent analysis. Traces of the primary root were manually curated, and erroneous data were manually corrected using the ‘segment line’ tool in Fiji software. The relative root elongation rate (RER; mm mm⁻¹ h⁻¹) was computed for each root as:

\[ \text{RER(\text{mm mm}^{-1} \text{h}^{-1})} = \frac{\text{d}x}{\text{d}t} \]

For normalization of average scion size between WT/RNAi and RNAi/WT grafts, at 12 DAG (i.e. a week after the grafted seedlings had been transferred to a new plate as described above), the first leaf of WT scions was severed from half of the WT/RNAi heterografts, generating WT_Amp scions, and left intact in the other half. To minimize potential artefacts due to confounding wounding effects when comparing amputated WT_Amp scions and scions left intact (WT or RNAi), one of the cotyledon stumps in these grafts was gently squeezed with a pair of very fine tweezers. All plates were scanned daily. Primary and secondary LR lengths were determined using Fiji software.

Scion sizes were measured in all grafts at the end of each experiment through destructive sampling, laying leaves flat on a film of 0.8% phytagel without overlaps, and scanning them at 2400 dpi resolution for leaf area measurement using Fiji. Roots were fixed in a fixing solution [phosphate-buffered saline, 10% (v/v) formaldehyde, 0.1% (v/v) Triton X-100], and imaged by light microscopy in DIC mode. Epidermal cell length profiles along the primary root were determined using the macro ‘Cell length profile’. The positions of all non-emerged lateral root primordia (LRPs) and emerged LRs between the root–hypocotyl junction and root tip were recorded. The distance between the most basal (oldest) LR and the most acropetal LRP was defined as the ‘zone of lateral root formation and emergence’, enabling determination of LRP and LR densities along that zone (number mm⁻¹).

**Molecular analysis**

For quantitative reverse transcription PCR (qRT–PCR), fresh tissues were snap-frozen in liquid nitrogen and ground using a Tissue Lyzer (Qiagen). Total RNA was obtained using a chloroform/TRIzol (Thermofisher) extraction protocol, following the manufacturer’s instructions. mRNAs were purified from 40 μg of total RNA using TYGR Dynabeads® oligo(dT)₃₂₅ and subsequently used for cDNA synthesis using 200 U of M-MLV (Promega). The qRT–PCR was performed with 2.5 μl of the diluted reverse transcription mix in a final volume of 10 μl of FastStart Universal SYBR® Green Master (Roche) and using a V917 real-time PCR system (Thermofisher). Primer efficiencies were calculated using LinRegPCR (Ramakers et al., 2003), and the relative expression of target genes was normalized to four reference genes (TIP41, APT1, UBC9, and PFD2) using the delta-Ct method (Livak and Schmittgen, 2001). Primers used for quantification of AtTCTP1 and reference gene mRNAs are as in Berkowitz et al. (2008). GFP-specific primers were either GFP₁₅′ 5'GATCCTGTTGACGAGGGTGT3' and GFP₁₃₅ rev 5'GGATACGTGCAGGAGAGGAC3', or GFP2 for 5'GATCCGGCTTCTTTTGGCTTGC3', or GFP₂₅′ 5'GATCCTGTTGACGAGGGTGT3' and GFP₂₅₃ rev 5'GGATACGTGCAGGAGAGGAC3'. The two sets of primers produced almost identical delta-Ct values, which were thus averaged to calculate expression levels. Primers for quantification of CybB₁₁ expression were CybB₁₅′ 5'TCAGCTCTAGGAGGCTGCAC3' and CybB₁₅₃ rev 5'GATCCTGTTGACGAGGGTGT3'. In all experiments, measurements were replicated on 3–6 independent pools, each consisting of scion or rootstock from at least five plants, unless specified otherwise.

**Results**

AtTCTP1 mRNA moves through the shoot–root graft junction, in both directions

To investigate the long-distance mobility of endogenous AtTCTP1 mRNA and its encoded protein in a physiologically
relevant context, and to be able to differentiate between locally expressed *ArTCTP1* and *ArTCTP1* originating from distant sources, we performed reciprocal grafts between WT (Col-0) and a TCTP1–GFP line expressing a AtTCTP1–GFP fusion protein under the control of the *AtTCTP1* native promoter (pArTCTP1::gArTCTP1-GFP (Berkowitz et al., 2008; Fig. 1A). To avoid potential confounding effects from the uncontrolled formation of adventitious roots post-grafting, we developed a modified micro-grafting technique where these roots are not formed (see the Materials and methods). At 14 DAG, the scions had developed 5–6 leaves of normal size (Fig. 1B), indicating that the scion–rootstock junction was fully functional. Microscopic observation confirmed a clean junction, with continuous vasculature and absence of adventitious root primordia (Fig. 1C–F).

*TCTP1*-GFP transcripts were detected in both the scion of WT/TCTP1–GFP grafts and the rootstock of TCTP1–GFP/WT reciprocal grafts. This was observed in young seedlings grown *in vitro* (14 DAG; Fig. 1G) and also at a much later stage (early flowering) in soil-grown grafts (80 DAG; Fig. 1H). Mobile *TCTP1*-GFP transcripts were more abundant in the latter, both in absolute terms and relative to the amount

**Fig. 1.** *AtTCTP1* mRNA moves over hypocotylar graft junctions in young and adult plants, in variable proportions. (A–D) Representative images of: scion–rootstock junction (j) immediately after grafting (A); whole seedling (B) and graft junction (C and D) 14 DAG. DIC image (C) and autofluorescence image (D). Scale bars, 250 µm (A), 10 mm (B), 200 µm (C and D). (E and F) *TCTP1*-GFP fluorescence at the graft junction imaged by confocal microscopy. WT root grafted to a pTCTP1::gTCTP1-GFP scion (E) and a WT scion grafted to a pTCTP1::gTCTP1-GFP root (F). Scale bar, 200 µm. (G and H) *AtTCTP1*-GFP and endogenous *AtTCTP1* transcripts were quantified by qRT–PCR in the scion (values above the x-axis) and rootstock (values below the x-axis) of reciprocal grafts between WT and transgenic pTCTP1::gTCTP1-GFP seedlings grown *in vitro* and sampled at 14 DAG (G) or transferred to soil 10 DAG and sampled at 80 DAG (H). Scion–rootstock combinations are indicated below each panel, and schematized beside each bar. Values are means ±SE of fold change in target gene expression relative to expression of four reference genes (*in vitro* seedlings, *n*=5 biological replicates each consisting of pooled rosettes or rootstocks from five plants; soil-grown plants, *n*≥6 biological replicates, each consisting of pooled rosettes or rootstocks from two plants). Asterisks denote statistically significant expression differences compared with the WT/WT control homograft by one-tailed Student’s *t*-test (*P*<0.05; ***P*<0.001). Similar results were obtained with two sets of GFP-specific primers as listed in the Materials and methods. The percentages above or below bars denote the abundance of mobile *AtTCTP1*-GFP transcripts measured in WT/TCTP1–GFP and TCTP1–GFP/WT grafts relative to the total amount of *AtTCTP1*-GFP transcripts produced by the *TCTP1*-GFP scion or rootstock, respectively, in the two sets of heterografts.
of *AtTCTP1*-GFP transcripts produced (0.4% and 2.3% of shootward and rootward mobile *AtTCTP1*-GFP transcripts in WT/*AtTCTP1*-GFP and *TCTP1*-GFP/WT, respectively, in soil-grown plants; 0.001% and 0.00009%, respectively, in seedlings raised on agar media). These data demonstrate sustained bi-directional mobility of *AtTCTP1* mRNA, of variable magnitude, in both the shootward and rootward direction.

**AtTCTP1 protein of scion origin is detected in WT rootstock, with preferential accumulation in pericycle cells at sites of lateral root formation**

We next examined the presence of the encoded TCTP1–GFP protein in these grafts by confocal laser microscopy. A weak TCTP1–GFP fluorescence signal was consistently detected in the primary root of *pAtTCTP1:gAtTCTP1-GFP/WT* heterografts (Fig. 2A–E), whether in the agar-grown seedlings (Fig. 2A–C, 8–13 DAG) or the older soil-grown plants (Fig. 2E; 80 DAG). Imaging the scion with the same microscope settings completely saturated the confocal photomultiplier (images therefore not shown). GFP fluorescence localized along the vascular strands, with acropetally increasing intensity towards the root tip, down to ~250 µm from the quiescent centre (251±11.8 µm at 13 DAG, n=5); that is, within the transition zone from the root elongation zone to the root meristem, where the GFP signal completely disappeared (Fig. 2A, B, E; Supplementary Fig. S1 at JXB online), coinciding with the end of the protophloem. Closer inspection at higher resolution along the root elongation and differentiation zones showed patchy GFP signal intensity, reflecting preferential protein accumulation in pericycle cells at the sites of LR initiation (Fig. 2C, F, G). WT roots grafted to scions expressing a *p35S::YFP:*TCTP1 construct providing a much stronger fluorescence signal, also clearly showed pericycle-specific localization of YFP fluorescence (Fig. 2D). TCTP1–GFP fluorescence was also detected in LRPBs, but was much weaker, sometimes barely detectable, until the root had emerged and started to elongate rapidly (Fig. 2H). The pattern of GFP fluorescence in control roots grafted on a scion expressing GFP alone under the same promoter was very different, with a very high ubiquitous signal in the whole stele and throughout the root meristem (Supplementary Fig. S2), as previously reported (Stadler et al., 2005; Ross-Elliott et al., 2017). Taken together, these results suggest that rootward TCTP1 mobility is actively controlled and may have a specific signalling function in root development, targeted to cells involved in the spatial patterning of LRPBs along the primary root.

To examine this in more detail, we monitored the appearance of TCTP1–GFP fluorescence in the rootstock of *AtTCTP1–GFP/WT* grafts over a 10 d period following grafting. The earliest evidence of GFP fluorescence was on 7 DAG (23 out of 28 roots; Supplementary Fig. S3), consistent with reports of establishment of a fully functional graft junction in Arabidopsis (Mehnyk et al., 2015). Strikingly, the preferential TCTP1–GFP accumulation in the primary root elongation zone observed in older roots (Fig. 2) was already obvious (Fig. 3A). Moreover, when imaging the entire root from base to tip, TCTP1–GFP fluorescence was first encountered in two patches localized in pericycle cell at the base of the two youngest LRPBs, at both initiation stages I and II (Malamy and Benfey, 1997) (Fig. 3H, I). This result further supports the notion of a destination-selective signalling function of mobile TCTP1 gene products originating from the shoot, in the initiation of LRs. Given the bi-directional mobility of TCTP1 mRNA (Fig. 1G, H), we examined the presence of GFP fluorescence in WT scions grafted onto *pAtTCTP1:gAtTCTP1-GFP* roots. GFP fluorescence was undetectable, whether in young or mature leaves, or in the shoot apical meristem (Fig. 2I, J).

**Constitutive expression of AtTCTP1 in scion promotes scion growth which in turn stimulates root growth**

In our earlier characterization of *AtTCTP1*, we showed that *AtTCTP1* gene products, mRNA and protein, are constitutively highly expressed in the primary root meristem and LRPBs, and that *AtTCTP1* silencing inhibits root elongation and LR branching (Berkowitz et al., 2008). The above results in the present study suggested a role for *AtTCTP1* also in root development through mobility. To investigate that, due to lack of roots devoid of constitutive *AtTCTP1* expression given the embryo lethality of total *AtTCTP1* knock-out and the dwarfism of *ctp1* seedlings rescued through embryo culture (Berkowitz et al., 2008; Brioudes et al., 2010), we severed TCTP1-RNAi roots from 5–day-old seedlings expressing a constitutive *AtTCTP1* silencing construct (Berkowitz et al., 2008) (hereafter referred to as TCTP1-RNAi or RNAi), and grafted them to either a WT scion of the same age, or to a homologous TCTP1-RNAi scion. Root development in these grafts was then monitored over the next 3–4 weeks. We reasoned that the 100-fold higher *AtTCTP1* constitutive expression in WT scions than TCTP1–RNAi scions (Berkowitz et al., 2008) should translate into a significantly increased amount of scion to root mobile *AtTCTP1* mRNA and protein in WT/RNAi compared with RNAi/RNAi grafts, and thus enable us to determine whether the distinctive short root and reduced branching of the rootstock is root autonomous or involves signalling by *AtTCTP1* from the scion. WT/RNAi reciprocal heterografts were grown alongside each other and control WT/WT and RNAi/RNAi homografts, in replicated plates. WT primary roots grew faster than TCTP1–RNAi roots irrespective of scion genotype (Fig. 4A, B). Both WT and TCTP1–RNAi roots elongated faster when grafted onto a WT rather than a TCTP1–RNAi scion (26% and 21% increase in maximum relative elongation rate at 13 DAG, respectively; Fig. 4C). It is known, however, that siRNA can move long distances through the plant (Mohar et al., 2010; Bai et al., 2011; Mehnyk et al., 2011; Liang et al., 2012). To test whether this explained the root growth inhibition associated with TCTP1–RNAi scions, we measured *AtTCTP1* transcript abundance in homo- and heterograft scions and rootstocks by qRT–PCR. *AtTCTP1* mRNA levels in WT roots showed a nearly 6-fold reduction in RNAi/WT seedlings compared with WT/WT seedlings (*P*=0.005; Fig. 4D). This is much higher than could be expected from simply a reduction of mobile rootward *AtTCTP1* mRNAs (see Fig. 1G, H) and hence suggested some down-regulation of *AtTCTP1* expression in these roots by mobile siRNA from the RNAi
In contrast, *AtTCTP1* transcript abundance in RNAi roots was similar regardless of scion genotype (0.68±0.118 and 0.77±0.013 relative transcript abundance in RNAi/RNAi and WT/RNAi grafts, respectively, *P*=0.55; Fig. 4D), ruling out that simple explanation for their slower elongation rate. To verify that mobile *AtTCTP1* transcripts from the scion were not silenced by siRNA upon delivery to the root, we grafted TCTP1–GFP scions on TCTP1–RNAi roots and quantified transgenic TCTP1–GFP mRNAs in the rootstock (Fig. 4E). TCTP1–GFP mRNAs were present in roots of TCTP1–GFP/RNAi grafts, in low but significant abundance, representing ~0.006% of scion total TCTP1–GFP mRNAs, that is in fact a similar or even higher proportion than found in WT rootstocks of TCTP1–GFP/WT grafts in our earlier experiments, under similar conditions (e.g. Fig. 1G). This result shows that a significant amount of *AtTCTP1–GFP* transcripts translocated from the scion to RNAi rootstock escaped silencing. GFP fluorescence from the encoded TCTP1–GFP protein was also consistently detected, with the same distinctive spatial expression pattern (Fig. 4F compared with Fig. 2B). These results suggest the presence of a larger amount of intact TCTP1 gene products of scion origin in TCTP1–RNAi roots grafted to a WT scion instead of a homologous TCTP1–RNAi scion. The low abundance of translocated TCTP1–GFP transcripts compared with the high abundance of native root endogenous TCTP1 transcripts is consistent with the only small change in total TCTP1 transcript abundance measured earlier in rootstocks of WT/RNAi grafts compared with RNAi/RNAi grafts (Fig. 4D).

Despite all being trimmed to a similar size at the time of grafting (see the Materials and methods), WT scions quickly became larger than TCTP1–RNAi scions (Supplementary Fig. S4), consistent with the high expression of *AtTCTP1* in the shoot apical meristem and its growth-promoting effect in leaves (Berkowitz et al., 2008). This suggested that the faster elongation rates of TCTP1–RNAi roots in WT/RNAi than in RNAi/RNAi grafts might then at least partly reflect an increased photoassimilate supply from larger scions, rather than higher abundance of *AtTCTP1* mRNA translocated from the scion.

To address this, we grafted TCTP1–RNAi roots onto WT scions or onto homologous TCTP1–RNAi scions of the same age as earlier. However, at 7 DAG, when the graft junction was fully established and TCTP1–RNAi root lengths were still similar regardless of scion genotype (12.8±1.25 mm and 11.4±0.91 mm Fig. 2.

**Fig. 2.** Distinctive pattern of TCTP1–GFP fluorescence in WT rootstock grafted to TCTP1–GFP scions, with preferential accumulation in pericycle cells at sites of lateral root formation. (A–H) Confocal laser microscopy images of WT roots grafted onto scions expressing *pTCTP1::gTCTP1-GFP* (A–C and E–H) or *p35S::cYFP-TCTP1* (D). Roots were imaged in seedlings grown in agar plates, 8 DAG (A) or 13 DAG (B–D) (*n=*30 primary roots), and in flowering soil-grown plants, 80 DAG (E–H), *n*≥10; primary root and LRP (E–G); elongating lateral root (H). Note the localization of the GFP fluorescence in parallel strands, the increasing signal closest to the root tip, and the preferential accumulation of the GFP-tagged TCTP1 protein in pericycle cells (C, D and F, G) at the sites of lateral root initiation (labelled LRP in F and G). GFP fluorescence (green) and autofluorescence (magenta/red) were separated by spectral unmixing. Arrowheads in (B–D) denote the boundary between root meristem, transition zone, and elongation zone only of the root. Inserts in (C) and (D) show TCTP1–GFP (C) and YFP–TCTP1 (D) localization, respectively, in the pericycle (p); GFP fluorescence was undetectable in the epidermis (e), the endodermis (en), or the cortex (c). (I and J) Absence of detectable GFP fluorescence signal in WT scions grafted to *pTCTP1::gTCTP1-GFP* roots, 8 DAG in agar-grown plants (I) or 80 DAG in soil-grown plants (J). Scale bars=200 μm except in (J) 100 μm. Given the low-intensity GFP signal when TCTP1–GFP expression in the scion is driven by the native *AtTCTP1* promoter, imaging had to be done at low magnification and larger pinhole aperture (×10 and 4.5 AU, respectively, A–C and I–J); limiting resolution, in contrast to roots grafted onto *p35S::cTCTP1-GFP* (×63, 1 AU, D).
Signalling through TCTP1 controls LR formation in Arabidopsis

in WT/RNAi and RNAi/RNAi grafts, respectively; \( P = 0.13 \); Fig. 4B), we normalized WT scion sizes to RNAi scion sizes, via tip amputation of leaf 1 (scions hereafter denoted \( \text{WT}_{\text{AMP}} \)). To minimize potential confounding wounding effects in subsequent phenotypic analyses, one of the tiny remaining stumps of the two cotyledons cut out 7 d earlier at the time of grafting was manually pinched in control grafts, at its very tip, causing a similar very brief and spatially restricted micro-wounding event in all scions. Over the next 1 and 2 weeks, scion sizes were still similar in the two sets of grafts (\( \text{WT}_{\text{AMP}}/\text{RNAi} \) and RNAi/RNAi; Fig. 4G; Supplementary Fig. S5A), and much smaller than non-amputated WT scions in WT/RNAi grafts (~2-fold difference at 22 DAG; Fig. 4G). Remarkably, associated TCTP1-RNAi rootstocks showed similar primary root lengths (Fig. 4H, I; Supplementary Fig. S5B), significantly shorter than roots of WT/RNAi grafts. When individually plotted against scion sizes, root lengths described a unique relationship for the three sets of grafts, and data points for TCTP1-RNAi roots associated with \( \text{WT}_{\text{AMP}} \) or TCTP1-RNAi scions overlapped (Fig. 4H). Consistently, relative root elongation rates over the monitoring period (7~22 DAG) were similar (4.17±0.15 h\(^{-1} \) and 4.07±0.14 h\(^{-1} \) in \( \text{WT}_{\text{AMP}}/\text{RNAi} \) and RNAi/RNAi grafts, respectively, \( P = 0.09 \)). Moreover, the cell length profiles in the root elongation zone also showed complete overlap, and final cell lengths were similar (Fig. 4J). These results indicate that, at the same scion size, differences in constitutive \( \text{AtTCTP1} \) expression levels in the scion and rootward mobile \( \text{AtTCTP1} \) gene products have little impact on primary root elongation during early post-embryonic development.

Graft-mobile \( \text{AtTCTP1} \) transcripts and encoded protein promote lateral root initiation and emergence

As scion-derived TCTP1–GFP showed preferential accumulation at sites of LR initiation (Fig. 2), we next closely examined root branching patterns. The number of LRs varied between plants. That variation was closely correlated to variation in scion size (Fig. 5A). Data points for \( \text{WT}_{\text{AMP}}/\text{RNAi} \) and WT/RNAi grafts fell on the same line (slopes 0.38±0.02 and 0.36±0.04, respectively), indicating that partial amputation of \( \text{WT}_{\text{AMP}} \) scions per se had no unwanted confounding effects on root development. Remarkably, LR numbers in RNAi/RNAi grafts fell significantly below those counted in \( \text{WT}_{\text{AMP}}/\text{RNAi} \) heterografts. The density of LR formation sites along the primary root was decreased by 41% on average (Fig. 5B), while being as high in roots grafted to \( \text{WT}_{\text{AMP}} \) rather than much larger WT scions (\( P = 0.36 \); Fig. 5B).

We next used DIC microscopy to examine the entire length of the primary root in \( \text{WT}_{\text{AMP}}/\text{RNAi} \) and RNAi/RNAi grafts, for non-emerged LRP stages I–VII (Malamy and Benfey, 1997) in addition to emerged LRs, also separately scoring those found on the primary root segment formed pre- or post-grafting and scion size normalization (Fig. 5C). The former were confined to the basal 8–9 mm of the primary root, and in total amounted to 2.9 on average in the two sets of grafts (\( P = 0.89 \)). The density of both non-emerged LRP stages and emerged LRs on the younger, proximal primary root segment formed post-scion size normalization was significantly greater in \( \text{WT}_{\text{AMP}}/\text{RNAi} \) than RNAi/RNAi grafts (\( P < 0.05 \); Fig. 5C, D). This was already clear at 12 DAG and even more obvious 6 d later. Consistent with the increased LRP density, the expression of \( \text{CYCB1;1} \), a marker of the early divisions of pericycle cells that initiate LR formation (Beeckman et al., 2001), was also increased in roots from \( \text{WT}_{\text{AMP}}/\text{RNAi} \) grafts compared with those of RNAi/RNAi grafts (\( P < 0.004 \); Fig. 5E). In the course of these experiments, we noticed that the youngest, most acropetal LRP stages seemed to be located closer to the root tip in those grafts. Systematic measurements of its coordinate along the primary root showed that the zone of LR formation indeed
Fig. 4. Constitutive expression of AtTCTP1 in the scion promotes scion growth which in turn stimulates primary root elongation. (A) Representative images of reciprocal grafts between WT and TCTP1-RNAi lines, and control WT/WT and TCTP1–RNAi/TCTP1-RNAi homografts, 22 DAG. Scale bar, 10 mm. (B and C) Primary root lengths (B) and relative root elongation rates (C) versus time. Means ±SE, n = 6–9. (D) AtTCTP1 relative gene expression levels in rootstocks and scions, 22 DAG (means ±SE, n = 3 pools of at least five plants each). (B and C) Asterisks denote statistically significant differences by two-tailed Student’s t-test; *P < 0.05; **P < 0.01; ***P < 0.001. (E) AtTCTP1-GFP relative expression in the rootstocks of reciprocal grafts between TCTP1-RNAi and TCTP1-GFP lines (denoted for brevity ‘RNAi’ and ‘GFP’, respectively, in the figure), and control homografts. Different letters indicate statistically significant differences from noise levels in roots from RNAi/RNAi grafts by one-way ANOVA followed by Tukey’s post-hoc test, n = 4 pools of
extended significantly closer to the root meristem in WT Amp/RNAi than RNAi/RNAi grafts ($P=4\times10^{-4}$; Fig. 5F). Root patterning was completely normal, however (Supplementary Fig. S6). Taken together, these data indicate a stimulation of LR initiation and emergence in WT Amp/RNAi grafts, at the same scion size and primary root length and anatomy, thus probably being related to enhanced delivery of mobile AtTCTP1 gene product(s) from WT scions compared with RNAi scions. The identical cell length profiles along the whole transition, elongation, and differentiation zones of the two sets of roots imply an increased frequency of LR priming events along the primary root pericycle.

While initiated and emerging in greater number in WT Amp/RNAi than RNAi/RNAi grafts, LRs were on average shorter ($P=4\times10^{-4}$; Fig. 5G). Strikingly, the overall result was that their cumulated length over the whole primary root was unaffected, similar to that measured for RNAi/RNAi grafts ($P=0.32$; Fig. 5H; Supplementary Fig. S7). Plant to plant variation of cumulated LR root length was correlated to individual variation in scion size (Fig. 5H), as found for primary root length, and data points fell on the same relationship for the two sets of grafts (Fig. 5H). Taken as a whole, these results indicate a signalling role for AtTCTP1 rootward mobility in root development, specifically targeted at the regulation of early events of LR formation and of LRP emergence from covering layers.

Discussion

TCTP function and physiological roles in plants are only beginning to be unravelled. The possibility of a dual role, as a protein acting not only cell autonomously, but also extracellularly as found in mammals and other eukaryotes, remains unknown. This study identified a targeted, selective function of scion-encoded mobile endogenous Arabidopsis AtTCTP1 mRNA and translated protein in shaping the deployment of the root system.

AtTCTP1 mRNA movement was reported to occur in a strictly rootward direction (Thieme et al., 2015). Here, we consistently detected a bi-directional movement, whether in plate assays with young seedlings raised under similar conditions to those in the earlier study, or after graft transfer to soil. This discrepancy may be related to the different reporter constructs used: full genomic TCTP1 here, including the 5′-untranslated region (UTR) and native upstream promoter region; TCTP1 cDNA was fused to the constitutive Cauliflower mosaic virus promoter in the previous study. The 5′-UTR of AtTCTP1 contains a conserved 5′-TOP (terminal oligopyrimidine tract) motif, and two AUUUA motifs are present in the 3′-UTR. TOP mRNAs are highly sensitive to translational control, and AUUUA motifs are also important in mRNA stability and translation (Barreau et al., 2005). In animals, these motifs are thought to be important in the control of TCTP translation (Bommer and Thiele, 2004), and in Arabidopsis itself have been suggested to influence the expression pattern of AtTCTP1 (Brioude et al., 2010). It may be that the 5′- and 3′-UTRs also play a role in the mobility and/or transport of AtTCTP1 transcripts, as shown for SIBEL5 mRNA movement from leaf and petioles to stolon tips in the regulation of tuber formation in Solanum tuberosum (Banerjee et al., 2009). Supporting our results, a bi-directional endogenous movement of the native Vitis vinifera TCTP1 mRNA homologous to AtTCTP1 has also been documented in heterografts of two polymorphic Vitis genotypes, through genome-wide sequencing of scions and rootstock mRNAs (Yang et al., 2015).

The consistency of our observations in seedlings grown in vitro and older soil-grown plants provides evidence that long-distance movement of endogenous AtTCTP1 mRNA between the shoot and root is not a transient occurrence, nor an experimental artefact, but a sustained phenomenon, which takes place under physiological conditions.

If that phenomenon has a physiological function, one would expect it to be actively controlled, in a growth condition- or development stage-dependent manner. Fitting with this, while always representing a small fraction of the transcripts produced in the source organ as seems the norm (Notaguchi et al., 2015; Yang et al., 2015; Morris, 2018), the proportion of AtTCTP1 mRNAs transmitted across root–shoot graft junctions showed significant variation from plant to plant and between in vitro and soil experiments (Fig. 1). Consistently, for rootward movement, the fluorescence signal associated with TCTP1–GFP protein derived from mobile scion TCTP1 mRNAs was also of variable intensity, especially in the population of elongating LRs of soil-grown plants, even being undetectable in some. No conclusion is possible about TCTP1–GFP protein in the scion of reciprocal WT/TCTP1–GFP grafts. The fact that its presence was not detected in our experiments could indicate absence of translation under our experimental conditions—TCTP proteins are notoriously sensitive to translational control, as reflected in their name—or very low abundance, below the limits of detection, but nevertheless could have functionality as found for other regulatory proteins of root development, such as BREVIS RADIX (Mouchel et al., 2004).

Another expectation of mobile gene products serving a physiological function is that they give rise to distinct, quantifiable phenotypes. To examine this, we focused on AtTCTP1 rootward mobility. Our results provide convergent evidence towards a systemic signalling role targeted at cells involved in the initiation and emergence of LRs. First, the scion-derived TCTP–GFP protein detected in the WT primary root of

±6 roots. (F) Representative image of GFP fluorescence in TCTP1-RNAi roots grafted to TCTP1–GFP scions. Scale bar, 200 μm. (G–J) Comparison of grafted seedlings sharing the same rootstock (TCTP1-RNAi) but differing in scion genotype or size (‘AMP’ subscript denotes WT scions trimmed 7 DAG; see the Materials and methods): (G) scion sizes 22 DAG; (H) individual primary root lengths versus scion projected area, 22 DAG; (I) primary root lengths, ≥6 roots). (J) Epidermal cell lengths along the primary root meristem (MZ) and elongation zone (EZ), n=5 roots per graft type. Shown are moving averages of cellular lengths over 20 μm windows. The inset depicts mature cell lengths (means ±SE). Different letters in (G), (I), and (J) denote statistically significant differences by one-way ANOVA (G) or Student’s t-test (I, J) ($P<0.05$ unless indicated).
TCTP1–GFP/WT grafts consistently showed a distinctive localization pattern, being (i) absent from the root meristem; (ii) confined to the vasculature, with recurrent peaks of higher intensity, systematically coinciding with sites of LRP initiation in the pericycle; and (iii) highly abundant in the root elongation and transition zones upstream of the root meristem proper, which encompass a region of ‘oscillatory gene expression’ where priming of LR initiation takes place (De Smet et al., 2007; Moreno-Risueno et al., 2010). This pattern is in stark contrast to the ubiquitous expression, in all root layers and also the root meristem, of the constitutive root AtTCTP1 protein, translated from locally transcribed *AtTCTP1* transcripts.
It is also distinct from the pattern observed for mobile GFP or YFP alone, and passive mass flow transport through the phloem and leakage from companion cells to pericycle cells. These results indicate the presence of active targeting, capture, or unloading mechanisms in the root of *AtTCTP1* gene products encoded in the shoot. Secondly, TCTP1-RNAi roots of similar structure, anatomy, size, and elongation rate, but associated with WT*AMP* scions rather than TCTP1-RNAi scions of similar size, exhibited an extended zone of LR formation starting closer to the root tip. Thirdly, the densities of root branching sites (emerged LRs) and of LR initiation sites (non-emerged LRPs at more acropetal positions) along that zone were both increased. The specificity of these changes and the shift towards more numerous and on average shorter LRs, in an extended zone, while the overall length of root over the whole root system was unaffected, contrasts with the general promotion of both primary root and LR elongation associated with increased local constitutive *AtTCTP1* expression (Berkowitz et al., 2008), with increased scion size (Figs 4, 5), or with independently induced increases of photoassimilate supply via stimulation of carbon metabolism (Freixes et al., 2002; Lee-Ho et al., 2007). Differential sucrose uptake by TCTP1-RNAi and WT*AMP* scions directly from the medium—which is known to influence LR emergence (MacGregor et al., 2008)—can also be ruled out as an explanatory factor for the effects of scion genotype observed here on root system architecture, as our grafts were raised in the absence of exogenous sucrose supply.

The phloem is the long-distance delivery path to the roots of auxin synthesized in leaves and cotyledons. Auxin has a pivotal role in orchestrating root architecture (Laskowski et al., 2008; Lewis et al., 2011; Raya-González et al., 2012), being required for the activation of LR potential initiation sites in the pericycle, the subsequent process of primordium initiation and formation, and the breaking of overlying tissues for its function of plant TCTPs in protein synthesis, as established in animals (Cans and Passer, 2003; Wu et al., 2015). Under either scenario—mobility of the messenger only, or also of the protein—it will be intriguing to elucidate how destination cells recognize the mobile *AtTCTP1* gene products generated in distant cells from those they autonomously produce, and what their mechanisms of action are.

The plasticity of root system architecture is pivotal to plant adaptation to changes in environmental conditions, and strategic mining of spatially and temporally variable soil resources. *AtTCTP1* appears as a central controller of that plasticity. Combining a dual function, namely as a general constitutive growth promoter and a mobile signalling agent between shoot and roots, in a vital, highly expressed gene, furthermore encoding a protein highly sensitive to translational and post-translational modifications, appears to be a clever strategy for a sensitive and dynamic tuning of root system architecture to optimize the compromise for roots between reaching deeper or branching more profusely depending on growth conditions. Interestingly, the much lower and more specifically expressed Arabidopsis *AtTCTP1* orthologue, *AtTCTP2*, was reported to encode an mRNA and protein with bi-directional long-distance mobility when overexpressed in *Nicotiana benthamiana* (Toscano-Morales et al., 2014). Movement across
the graft junction appeared to correlate with the formation of aerial roots at the interface of the grafted stem segments (Toscano-Morales et al., 2014). This raises the possibility of a broad role for TCTP mobile mRNAs/proteins in de novo root organogenesis, whether LRs or shoot-born roots, with specificity between gene isoforms in cell types targeted for developmental reprogramming and formation of root primordia from a variety of tissues (Bellini et al., 2014), whichever is most appropriate.

Plant TCTP genes show high similarity among species. TCTP mRNAs and proteins have been detected in the vasculature of diverse species, including the monocot rice where the TCTP family is reduced to one member (Supplementary Table S1). This suggests that the mobility and extracellular signalling function of AtTCTP1 to control root organogenesis might be widely conserved within the plant kingdom, and highly relevant to a better understanding of post-embryonic formation of lateral organs in plants, and the elusive coordination of shoot and root morphogenesis.

**Supplementary data**

Supplementary data are available at JXB online.

- Fig. S1. TCTP1–GFP fluorescence is restricted to specific strands along the vasculature.
- Fig. S2. Distinctive GFP fluorescence pattern in roots grafted to a pTCTP1::gTCTP1-GFP scion compared with a p35S::GFP scion.
- Fig. S3. Time-course of appearance of TCTP1–GFP fluorescence in pTCTP1::gTCTP1-GFP/WT heterografts.
- Fig. S4. Kinetics of scion expansion in homografts and heterografts between WT and TCTP-RNAi seedlings.
- Fig. S5. Similar scion and primary root sizes in WTAMP and TCTP1-RNAi scions following scion size normalization.
Fig. S6. Root patterning in TCTP1-RNAi seedlings shows no deviation from the stereotypical structure of WT Arabidopsis roots.

Fig. S7. At the same scion size, primary and overall lateral root lengths are independent of TCTP1 expression in the scion and mobility to the rootstock.

Fig. S8. The auxin transport inhibitor NPA does not modify GFP fluorescence of scion-derived TCTP1–GFP protein in the root.

Table S1. Plant TCTP/TCTP-like mRNAs or proteins detected as present in the vasculature or found to be moving through graft junctions, in published studies and publicly available databases.

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References

Aoki K, Suzuki N, Fujimaki S, Dohmne N, Yonekura-Sakakibara K, Fujiwara T, Hayashi H, Yamaya T, Sakakibara H. 2005. Destinational-selective long-distance movement of phloem proteins. The Plant Cell 17, 1901–1914.

Atkins CA, Smith PM, Rodriguez-Medina C. 2011. Macromolecules in phloem exudates—a review. Protoplasma 248, 165–172.

Bai S, Kasai A, Yamada K, Li T, Harada T. 2011. A mobile signal transported over a long distance induces systemic transcriptional gene silencing in a grafted partner. Journal of Experimental Botany 62, 4561–4570.

Banerjee AK, Lin T, Hnappel D. 2000. Untranslated regions of a mobile transcript mediate RNA metabolism. Plant Physiology 151, 1831–1843.

Barreau G, Paillard L, Osborne HB. 2005. AU-rich elements and associated factors: are there unifying principles? Nucleic Acids Research 33, 7138–7150.

Batailler B, Lemaitre T, Vilaine F, Sanchez C, Renard D, Cayla T, Benetauce J, Dinant S. 2012. Soluble and filamentous proteins in Arabidopsis sieve elements. Plant, Cell & Environment 35, 2171–2184.

Beneteau J, Dinant S. 2012. Soluble and filamentous proteins in Arabidopsis roots. The Plant Journal 71, 136–147.

Bensch S, Babich T, Pelletier G. 2019. 2019. TCTP and CSN4 control selective long-distance movement of phloem proteins. The Plant Cell 31, 1831–1843.

Betsch L, Boltz V, Brioudes F, Pontier G. 2019. TCTP and CN4 control cell cycle progression and development by regulating CULLIN1 neddylation in plants and animals. PLOS Genetics 15, e1007890.

Bommer U-.A. 2017. The translational controlled tumour protein TCTP: biological functions and regulation. In: Telerman A, Amson R, eds. TCTP/tpt1—remodeling signaling from stem cell to disease. Cham: Springer International Publishing, 69–126.

Bommer UA, Thiele BJ. 2004. The translationaly controlled tumour protein (TCTP). International Journal of Biochemistry & Cell Biology 36, 379–385.

Brioudes F, Thierry A-M, Chambrier P, Mollerue B, Bendahmane M. 2010. Translationaly controlled tumour protein is a conserved mitotic growth integrator in animals and plants. Proceedings of the National Academy of Sciences, USA 107, 16384–9.

Calderwood A, Kopriva S, Morris RJ. 2016. Transcript abundance explains mRNA mobility data in Arabidopsis thaliana. The Plant Cell 28, 610–615.

Cans C, Passer B. 2003. Translationaly controlled tumor protein acts as a guanine nucleotide dissociation inhibitor on the translation elongation factor eEF1A. Proceedings of the National Academy of Sciences, USA 100, 13892–13897.

Cao B, Lu Y, Chen G, Lei J. 2019. Functional characterization of the translationaly controlled tumour protein (TCTP) gene associated with growth and defense response in cabbage. Plant Cell, Tissue and Organ Culture 103, 217–226.

Casimiro I. 2001. Auxin transport promotes arabidopsis lateral root initiation. The Plant Cell 13, 843–852.

Chen SH, Wu P-S, Chou C-H, Yan Y-T, Liu H, Weng S-Y, Yang-Yen H-F. 2007. A knockout mouse approach reveals that TCTP functions as an essential factor for cell proliferation and survival in a tissue- or cell type-specific manner. Molecular Biology of the Cell 18, 2525–2532.

Chen Y, Chen K, Wang H, Bao Y, Zhang W. 2014. Examination of the leaf proteome during flooding stress and the induction of programmed cell death in maize. Proteome Science 12, 1–18.

Corbesier L, Vincent C, Jang S, et al. 2007. FT protein movements contribute to long-distance signaling in floral induction of Arabidopsis. Science 316, 1030–1033.

de Carvalho M, Acencio ML, Laitz AVN, de Araujo LM, de Lara Campos Arcuri M, de Nascimento LC, Maia IG. 2017. Impacts of the overexpression of a tomato translationaly controlled tumour protein (TCTP) in tobacco revealed by phenotypic and transcriptomic analysis. Plant Cell Reports 36, 887–900.

De Rybel B, Vassileva V, Parizot B, et al. 2010. A novel aux/IAA28 signaling cascade activates GATA23-dependent specification of lateral root founder cell identity. Current Biology 20, 1697–1706.

Deng Z, Chen J, Leclercq J, Zhou Z, Liu C, Liu H, Yang H, Montoro P, Xia Z, Li D. 2016. Expression profiles, characterization and function of HtTCTP in rubber tree (Hevea brasiliensis). Frontiers in Plant Science 7, 789.

De Rybel B, Vassileva V, Parizot B, et al. 2010. A novel aux/IAA28 signaling cascade activates GATA23-dependent specification of lateral root founder cell identity. Current Biology 20, 1697–1706.

Dong X, Yang B, Li Y, Zhong C, Ding J. 2009. Molecular basis of the acceleration of the GDP–GTP exchange of human ras homolog enriched in brain by human translationaly controlled tumor protein. Journal of Biological Chemistry 284, 23754–23764.

Du B, Wei Z, Wang Z, Wang X, Peng X, Du B, Chen R, Zhu L, He G. 2015. Phloem-exudate proteome analysis of response to insect brown plant-hopper in rice. Journal of Plant Physiology 183, 13–22.

Dubrovsky JG, Sauer M, Napsucialy-Mendivil S, Ivanchenko MG, Frim J, Shishkova S, Celenza J, Benkova E. 2008. Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. Proceedings of the National Academy of Sciences, USA 105, 8790–8794.

Freixes S, Thibaud M-C, Tardieu F, Muller B. 2002. Root elongation and branching is related to local hexose concentration in Arabidopsis thaliana seedlings. Plant, Cell & Environment 25, 1357–1366.

French A, Ubeda-Tomas S, Holman TJ, Bennett MJ, Pridmore T. 2009. High-throughput quantification of root growth using a novel image-analysis tool. Plant Physiology 150, 1784–1795.

Gommes F, Ma L, Boudoire J, Houterman PM, Boeren S, Cornelissen BJ, Rep M, Takken FL. 2015. The effector repertoire of Fusarium oxysporum determines the tomato xylem proteome composition following infection. Frontiers in Plant Science 6, 967.

Haywood V, Yu TS, Huang NC, Lucas WJ. 2005. Phloem-long-distance trafficking of GIBBERELLIC ACID-INSENSITIVE RNA regulates leaf development. The Plant Journal 42, 49–68.

Hsu YC, Chern JJ, Cai Y, Liu M, Choi KW. 2007. Drosophila TCTP is a conserved mitotic growth integrator in animals and plants. Proceedings of the National Academy of Sciences, USA 104, 13982–13987.

Jaeger KE, Wigge PA. 2007. FT protein acts as a long-range signal in Arabidopsis. Current Biology 17, 1050–1054.

Jorgensen RA, Atkinson RG, Forster RL, Lucas WJ. 1998. An RNA-based information superhighway in plants. Science 279, 1486–1487.
Kehr J, Kragler F. 2018. Long distance RNA movement. New Phytologist 218, 29–40.
Kim G, LeBlanc ML, Wafula EK, dePamphilis CW, Westwood JH. 2014. Genomic-scale exchange of mRNA between a parasitic plant and its hosts. Science 343, 808–811.
Kim M, Canio W, Kessler S, Sinha N. 2001. Developmental changes due to long-distance movement of a homeobox fusion transcript in tomato. Science 293, 287–289.
Kim YM, Han YJ, Hwang OJ, Lee SS, Shin AY, Kim SY, Kim JL. 2012. Overexpression of Arabidopsis translationally controlled tumor protein gene ATCTP enhances drought tolerance with rapid ABA-induced stomatal closure. Molecules and Cells 33, 617–626.
Laskowski M, Grienisei VA, Hofhuis H, Hove CA, Hogeweg P, Marée AF, Scheres B. 2008. Root system architecture from coupling cell closure. Molecules and Cells 33, 617–626.
Overexpression of Arabidopsis translationally controlled tumor protein gene 2012.
Lewis DR, Negi S, Sukumar P, Mudyai GK. 2011. Ethylene inhibits lateral root development, increases IAA transport and expression of PIN3 and PIN7 auxin efflux carriers. Development 138, 3485–3495.
Li D, Deng Z, Liu X, Qin B. 2013. Molecular cloning, expression profiles and characterization of a novel translationally controlled tumor protein in rubber tree (Hevea brasiliensis). Journal of Plant Physiology 170, 497–504.
Liang D, White RG, Waterhouse PM. 2012. Gene silencing in Arabidopsis spreads from the root to the shoot, through a gating barrier, by template-dependent, nonvascular, cell-to-cell movement. Plant Physiology 159, 984–1000.
Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25, 402–408.
Lough TJ, Lucas WJ. 2006. Integrative plant biology: role of phloem long-distance macromolecular trafficking. Annual Review of Plant Biology 57, 203–232.
Macdonald SM. 2012. Potential role of histamine releasing factor (HRF) as a therapeutic target for treating asthma and allergy. Journal of Asthma and Allergy 5, 51–59.
MacDonald SM. 2017. History of histamine-releasing factor (HRF)/translationally controlled tumor protein (TCTP) including a potential therapeutic target in asthma and allergy. In: Telemacher A, Amson R, eds. TCTP/tpt1—a therapeutic target for treating asthma and allergy. Journal of Biological Chemistry 281, 51–59.
Morris RJ. 2018. On the selectivity, specificity and signalling potential of the long-distance movement of messenger RNA. Current Opinion in Plant Biology 43, 1–7.
Souza M, Briggs GC, Hardtke CS. 2004. Natural genetic variation in Arabidopsis identifies BREVIS RADIX, a novel regulator of cell proliferation and elongation in the root. Genes & Development 18, 700–714.
Muraro D, Byrne H, King J, Bennett M. 2013. The role of auxin and cytokinin signalling in specifying the root architecture of Arabidopsis thaliana, Journal of Theoretical Biology 317, 71–86.
Notaguchi M, Higashiyama T, Suzuki T. 2015. Identification of mRNAs that move over long distances using an RNA-Seq analysis of Arabidopsis/Nicotiana benthamiana heterografts. Plant & Cell Physiology 56, 311–321.
Notaguchi M, Wolf S, Lucas WJ. 2012. Phloem-mobile Aux/IAA transcripts target to the root tip and modify root architecture. Journal of Integrative Plant Biology 54, 760–772.
Pautire DSG, Gustin MP, Molnar A, Oparka KJ. 2016. Lost in transit: long-distance trafficking and phloem unloading of protein signals in Arabidopsis homografts. The Plant Cell 28, 2016–2025.
Péret B, Larrieu A, Bennett MJ. 2009. Lateral root emergence: a difficult birth. Journal of Experimental Botany 60, 3637–3643.
Péret B, Swarup K, Ferguson A, et al. 2012. AUX/LAX genes encode a family of auxin influx transporters that perform distinct functions during Arabidopsis development. The Plant Cell 24, 2874–2885.
Ramakers C, Ruijter JM, Deprez RH, Moorman AF. 2003. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neuroscience Letters 339, 62–66.
Raya-González J, Pelagio-Flores R, López-Bucio J. 2012. The jasmonate receptor COI1 plays a role in jasmonate-induced lateral root formation and lateral root position in Arabidopsis thaliana. Journal of Plant Physiology 169, 1349–1358.
Ross-Elliot TJ, Jensen KH, Haaning KS, et al. 2017. Phloem unloading in Arabidopsis roots is convective and regulated by the phloem-pole pericycle. eLife 6, 1–31.
Stadler R, Wright KM, Lauterbach C, Amos G, Gahtzit M, Feuerstein A, Oparka KJ, Sauer N. 2005. Expression of GFP-fusions in Arabidopsis companion cells reveals non-specific protein trafficking into sieve elements and identifies a novel post-phloem domain in roots. The Plant Journal 41, 319–331.
Thieme CJ, Rojas-Triana M, Stecyk E, et al. 2015. Endogenous Arabidopsis messenger RNAs transported to distant tissues. Nature Plants 1, 15025.
Toscano-Moraes R, Xocconoste-Cázares B, Martínez-Navarro AC, Ruiz-Medrano R. 2014. Long distance movement of an Arabidopsis Translationally Controlled Tumor Protein (ATCTP) mRNA and protein in tobacco. Frontiers in Plant Science 5, 705.
Turgeon R, Wolf S. 2009. Phloem transport: cellular pathways and molecular trafficking. Annual Review of Plant Biology 60, 207–221.
Turnbull CG, Lopez-Cobollo RM. 2013. Heavy traffic in the fast lane: long-distance signalling by macromolecules. New Phytologist 198, 33–51.
Wu H, Gong W, Yao X, Wang J, Perrett S, Feng Y. 2015. Evolutionarily conserved binding of translationally controlled tumor protein to eukaryotic elongation factor 1B. Journal of Biological Chemistry 290, 8694–8710.
Xia C, Zheng Y, Huang J, et al. 2018. Elucidation of the mechanisms of long-distance mRNA movement in a Nicotiana benthamiana/tomato heterograft system. Plant Physiology 177, 745–758.
Yang Y, Mao L, Jittayasothorn Y, Kang Y, Jiao C, Fei Z, ZhongGY. 2015. Messenger RNA exchange between scions and rootstocks in grafted grapevines. BMC Plant Biology 15, 251.
Zhang W, Thieme CJ, Kollwig G, Apelt F, Yang Y, Mao L, Jittayasothorn Y, Kang Y, Jiao C, Fei Z, Lucas WJ. 2016. Vascular-mediated signalling involved in early phosphate stress response in plants. Nature Plants 2, 16033.