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# Endogenous *Arabidopsis* messenger RNAs transported to distant tissues

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**The concept that proteins and small RNAs can move to and function in distant body parts is well established. However, non-cell-autonomy of small RNA molecules raises the question: To what extent are protein-coding messenger RNAs (mRNAs) exchanged between tissues in plants? Here we report the comprehensive identification of 2,006 genes producing mobile RNAs in *Arabidopsis thaliana*. The analysis of variant ecotype transcripts that were present in heterografted plants allowed the identification of mRNAs moving between various organs under normal or nutrient-limiting conditions. Most of these mobile transcripts seem to follow the phloem-dependent allocation pathway transporting sugars from photosynthetic tissues to roots via the vasculature. Notably, a high number of transcripts also move in the opposite, root-to-shoot direction and are transported to specific tissues including flowers. Proteomic data on grafted plants indicate the presence of proteins from mobile RNAs, allowing the possibility that they may be translated at their destination site. The mobility of a high number of mRNAs suggests that a postulated tissue-specific gene expression profile might not be predictive for the actual plant body part in which a transcript exerts its function.**

The phloem is a plant-specific conductive system that consists of columns of living cells connected to the surrounding tissues by intercellular pores, called plasmodesmata, stretching through the cell wall. The resulting cytoplasmic continuum extends from one end of the plant to the other and facilitates transport of sugars, amino acids and hormones as well as macromolecules in the form of small silencing RNAs and proteins from source (exporting) to sink (importing) tissues[1]. *Arabidopsis* hypocotyl grafting experiments established that small interfering RNA (siRNA) is able to move through *Arabidopsis* graft junctions to distant plant parts including flower tissues[2–5]. It is thought that mobile siRNA and micro-RNA (miRNA) signals regulate many aspects of plant growth, including cell differentiation, adaptation to abiotic and biotic stresses, and metabolism. In addition, phloem content analyses on *Cucurbita* spp. suggested that many long RNA molecules including messenger RNAs (mRNA)[6–10] are transported via the phloem system. Furthermore, heritable changes may occur after grafting induced by mobile siRNA signals resulting in epigenetic modifications of genomic DNA (refs 2,3,11). However, only a small number of mRNAs were analysed further and found to have a signalling function after transport. Also, the extent of mRNA transport to distant plant tissues is largely unknown.

By applying grafting techniques to mutant or transgenic plants it has been shown that specifically allocated mRNAs induce phenotypic alterations in targeted tissues. These include decreased plant size in *A. thaliana* and tomato[12], and changes in tuber size in potato[13] or leaf shape in tomato[14]. Notably, the observation that protein-encoding RNA molecules can move to distant tissues seems to spatially unlink mRNA synthesis from protein production, with the implication that simple promoter studies might not reveal the actual location of transcript or protein function in a plant body. Hence, determining mobile mRNA species is an important step towards understanding spatial gene function in general and how higher plants might coordinate cellular differentiation, growth and metabolic activity between tissues. An agronomically important aspect is that the exchange of macromolecules between stock and scion plant parts might be linked to graft-induced alterations of phenotypes, altered resistance or graft incompatibilities.

## Results

***Arabidopsis* ecotype grafting reveals a high number of mobile transcripts.** To identify mobile transcripts and reveal the extent of their mobility, we devised a strategy based on grafting of two different *Arabidopsis thaliana* ecotypes displaying a high frequency of genomic sequence single nucleotide polymorphisms (SNPs). The ecotype-specific SNPs present in long RNA molecules allowed us to identify their origin and, thus, mobility in chimaeric root–shoot grafted plants (Fig. 1a and Supplementary Fig. 1). Examination of available sequences of ecotypes from the Arabidopsis 1,001 Genomes Project resource[15–17] revealed a number of ecotypes such as Ped-0, Don-0 and Bik-1 that have diverged substantially from the Col-0 reference with one polymorphism every ~130 nucleotides (Supplementary Fig. 1). For the grafting experiments we chose the Ped-0 (PED)/Col-0 (COL) combination, because stable hypocotyl graft connections could be easily established between these two ecotypes. COL and PED seedlings were autografted (control) and heterografted at the hypocotyl 10 days after germination. They were grown in full nutrient conditions, and additionally, in phosphorus limitation (–P) and nitrogen limitation (–N) for 2 weeks. Root and shoot materials from rosette stage plantlets (Fig. 1a) were harvested and evaluated by the polymerase chain reaction (PCR) and quantitative

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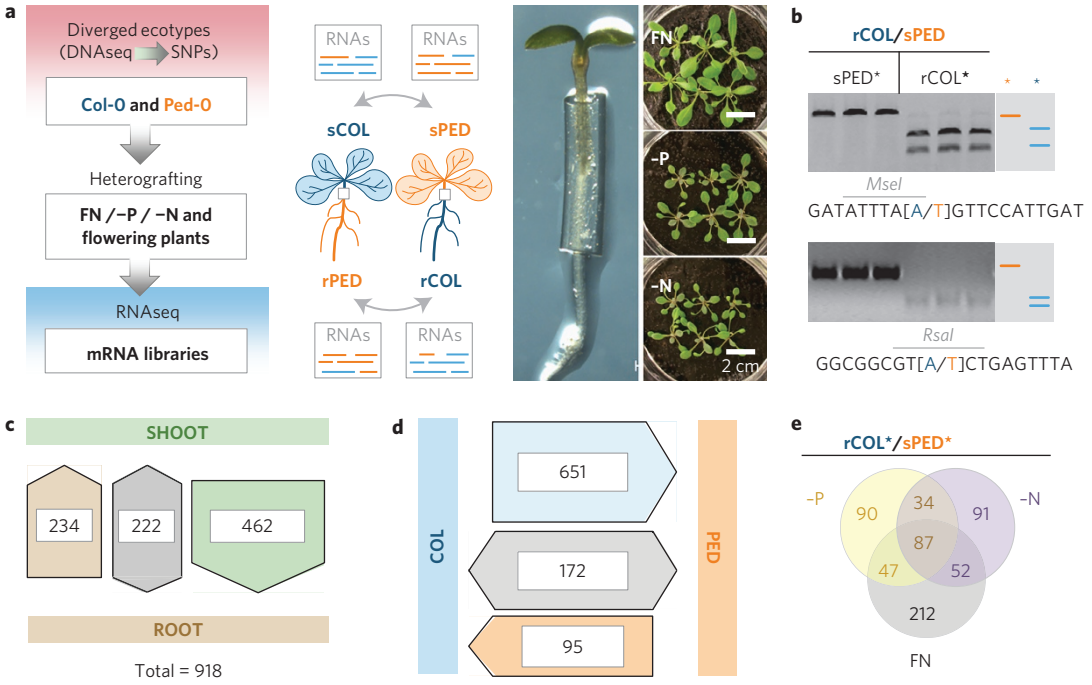


**Figure 1 | Hypocotyl-grafting of the distantly related *Arabidopsis thaliana* ecotypes Col-0 and Ped-0 exposed to nutritional stresses and number of identified mobile transcripts. a**, Outline of the experimental strategy and schematic representation of the performed reciprocal micro-grafts. Chimaeric seedlings were generated by micro-grafting of the highly divergent ecotypes Col-0 (COL) and Ped-0 (PED) and grown under three different nutritional conditions. **b**, Genotype verification of harvested material using SNP-derived molecular cleaved amplified polymorphic sequences markers. **c**, Number of transcripts moving bidirectionally or unidirectionally into root (stock) or shoot (scion) tissues, or **d**, between COL and PED ecotypes grown in full nutrient conditions. **e,** Venn diagram showing the numbers and overlap of the identified mobile transcripts found during phosphorus (–P) and nitrogen (–N) starvation and full nutrition (FN). Asterisks in **b** and **e** indicate the analysed grafted tissue.

reverse transcription (qRT)–PCR for tissue contamination (Fig. 1b) and the expected changes in marker gene expression due to nutrient limitations (Supplementary Fig. 1), prior to submitting the samples to next-generation sequencing (NGS). The obtained genomic DNA and RNA sequencing (DNA-/RNA-seq) data were used to identify heterologous transcripts moving into grafted tissues. In addition, samples from flowering PED/COL heterografted plants grown in nutrient-sufficient full nutrient conditions were used to identify the heterologous RNA present in stock, rosette leaves, lower and upper stem, and flower tissues (Supplementary Fig. 2).

In the first round of sequence analysis, we inspected the overall presence of substitution SNPs in the RNA-seq data set. Here, we discovered a relatively high number of polymorphic loci that had not been previously annotated (16%)[16,17]. Thus, we generated a custom allelic SNP reference map based on the resequenced genomic DNA data of the used COL and PED ecotypes. This reference map allowed proper identification of heterologous SNPs in grafted tissues. Next, we established a processing pipeline including a set of filtering steps (Supplementary Fig. 3). We excluded (1) SNPs found to be present in RNA-seq data but not in genomic data, (2) SNPs that showed more than two alleles across the data sets and (3) all organelle-encoded sequence information to avoid false predictions due to potential RNA-editing events[18]. To be indicative of a mobile transcript and to avoid false prediction due to sequencing errors, an SNP site had to be covered by at least three RNA-seq reads, confirmed in all four ecotype autograft data sets obtained from COL root/COL shoot (rCOL/sCOL) and PED root/PED shoot (rPED/sPED) samples (Supplementary Information), and all supporting reads had to map uniquely to this transcript position. In addition, if for a given SNP, based on homograft RNA-seq data, the associated genotype-specific alleles were not reliably confirmed (>5% deviating calls across all reads mapping to this position), this particular SNP position was not considered in the mobile annotation analysis. Furthermore, to ensure consistent information across all SNP sites in a given transcript a binomial score was calculated for each SNP site, indicating the likelihood that the observed read distribution (heterologous versus reference) at a particular SNP site is consistent with the background probability calculated from the total read set for a given transcript. By application of these filtering and scoring steps we were able to unambiguously assign 9,345 transcripts (~28% of the 33,602 known *A. thaliana* genes) to one of the two ecotypes (Supplementary Fig. 3). On the basis of this ecotype-specific assignment we identified 2,128 transcripts produced by 2,006 genes in the analysed RNA-seq data obtained from the heterologous samples. As these 2,006 gene-specific RNA sequences showed the allele of the corresponding other ecotype, they were designated mobile RNA (Supplementary Information). Of these 2,006 mobile RNA-producing genes, 1,992 are predicted to be protein-encoding mRNAs. The average number of informative SNPs per transcript is 6.03 for all transcripts, 10.05 for the mobile and 5.16 for the non-mobile-assigned transcripts. Here, the number of transcripts with informative SNPs is higher in the mobile data set. This was expected, as a lack of informative SNPs is penalized during assignment. In general, we found a balanced distribution of the predicted mobile RNA-producing genes on all five chromosomes, indicating that the used annotation pipeline is free from a positional bias (Supplementary Fig. 3). Finally, these mobile transcripts were ranked by their penetrance, that is the number of heterologous transcripts detected in the grafted ecotype reference tissue, and their presence in different samples (see Supplementary Methods for details).

**Transcript mobility is directional and depends on growth conditions.** The mobile RNAs identified in root and shoot samples from the reciprocally grafted plantlets grown in full

nutrient conditions ($n = 918$) allowed us to trace the direction of movement into heterologous tissues and an ecotype dependency (Fig. 1c,d and Supplementary Fig. 4). We classified the directionality of the identified mobile mRNAs into three categories by comparing PED mRNAs found in COL shoots (sCOL) grafted on PED roots (rPED) with COL mRNAs found in PED shoots (sPED) grafted on COL roots (rCOL) (Fig. 1c–e). Approximately half of the identified transcripts ($n = 462$) were found to migrate exclusively from shoot to root (Fig. 1c), matching the major flow of sucrose in the phloem from source leaves to the root, which is a major sink. However, ~24% ($n = 222$) of the 918 transcripts appeared in both heterologous root and shoot tissues pointing to bidirectional transport. Notably, ~25% ($n = 234$) of the transcripts exclusively moved from root to shoot, against the phloem source-to-sink flow. Furthermore, the majority of transcripts ($n = 651$) seem to preferably move from COL to PED tissues (Fig. 1d). This might be due to either decreased export activity in PED shoot tissues induced by the heterologous COL root system or an ecotype bias in the bioinformatic analysis. The latter appears unlikely as the RNA-seq reads mapped independent of SNPs to a similar number of transcripts (~82% of the annotated genes) in PED and COL as well as root and shoot samples (Supplementary Information). This implies that no bias existed due to the number of reads and supports the notion that there are interecotypic differences in mobile RNA export/import ability, which could be due to delayed flowering observed in PED plants or other, yet unknown, factors. However, the preference of PED to uptake COL transcripts reveals that natural variation-based approaches could be instrumental in a genetic dissection of RNA movement factors.

The analysis of mobile RNAs detected in sPED/rCOL chimaeric seedlings grown in parallel in full nutrient –P or –N conditions (Fig. 1e and Supplementary Figs 4a,b and 5) uncovered a significant number of conditionally mobile RNAs. For example, 90 and 91 transcripts were only found to be mobile in P- or N-limited plantlets, respectively, and were not found mobile under full nutrition conditions. Similarly, 212 mobile transcripts were not detected to be mobile in P- or N-limited plantlets. Further inspection of the 90 mobile transcripts exclusively detected in P-limited plantlets revealed that ~50% of these did not change transcript abundance, whereas 37% displayed a more than twofold increase and only 9% showed a >twofold decrease during P limitation (Supplementary Fig. 5c). This strongly suggests that the mobility of a given RNA is not exclusively determined by the intrinsic properties (*cis* dependent) of the RNA in question, but also by factors other than the RNA itself (that is *trans* dependent). Consequently, macronutrient stresses in plants do not only lead to major changes in transcript abundances[19,20] but also seem to change transcript mobility and, hence, the spatial transcript range by penetrating other tissues. In line with a possible role of the mobile mRNAs in adjusting tissue responses, transcripts belonging to 'signalling' Gene Ontology pathways[21] were statistically enriched in the set of mobile RNAs exclusively found under P-limited conditions (Supplementary Fig. 5c).

**Distinct subsets of mobile root transcripts move into different shoot organs.** Inspection of the heterologous transcripts present in various shoot organs (flower, upper stem, lower stem, and rosette leaves) of adult rPED/sCOL grafted plants revealed 1,606 mobile RNAs (Fig. 2). Taken together this resulted in an additional set of distinct 953 mobile RNAs that were not present in the set of 1,053 mobile transcripts of non-flowering and nutrition-depleted plants (Supplementary Information). In adult plants, most of the root-produced mobile transcripts ($n = 577$) were transported to rosette leaves. Remarkably, according to the calculated expression levels, 23 of the 577 PED root-produced mobile transcripts showed a higher presence in the COL rosette

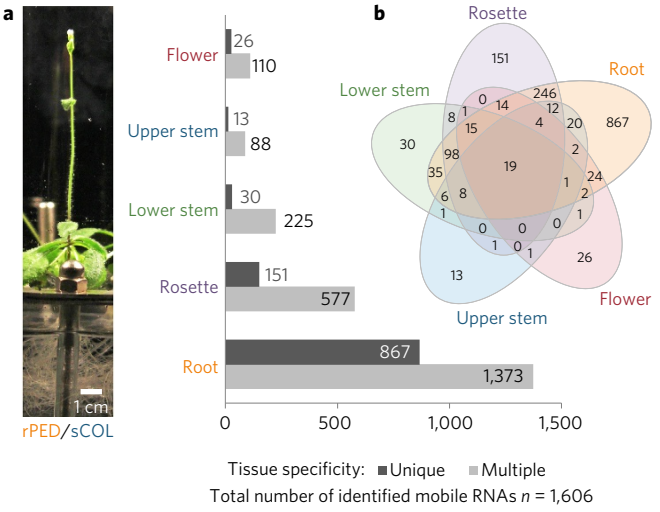


**Figure 2 | Mobile transcripts transported from PED roots into flowering COL shoots after grafting. a**, Image of a grafted plant representative of the flowering stage when the samples were harvested. The columns show the number of PED root (rPED)-specific transcripts that were detected in COL shoots (sCOL) samples harvested from rosette leaves, lower stem, upper stem and flower tissues, and the sCOL-produced transcripts present in rPED samples (root). Light grey columns give numbers of all detected mobile transcripts (multiple), dark grey columns give numbers of mobile transcripts exclusively detected in the indicated tissues (unique). **b**, Venn diagram of the mobile RNA distribution in the indicated tissues.

than the locally produced orthologous transcripts (Supplementary Information). A decreasing number of mobile root-produced transcripts were observed in the lower ($n = 225$) and upper stem ($n = 88$) tissues, indicating a gradient for RNA movement. Notably, 110 root-produced transcripts were detected in flowers (Fig. 2b and Supplementary Information). This latter number was higher than in upper stem tissue and higher than expected from an axial RNA transport based on a diffusion gradient or an exponential regression, and points to enrichment at the apex. Furthermore, a significant number of predicted mobile mRNAs appear to move into distinct tissues only. For example, 26 of all 739 root-exported transcripts were exclusively detected in the inflorescence (Fig. 2b). This directionality and tissue-specific accumulation of subsets of mobile transcripts support the notion of an active and targeted mRNA transport.

**Parasitic dodder plants take up a high number of mobile *Arabidopsis* transcripts.** To further estimate the extent of mRNA mobility, we used the parasitic dodder plant *Cuscuta reflexa*. Members of this phloem-feeding plant family form a symplasmic connection through haustoria to the host vasculature and, besides nutrients and water, take up a wide range of RNA molecules from host plants[22]. We harvested *C. reflexa* feeding on *Arabidopsis* host plants (Fig. 3a) and identified host mRNA present in the samples. To annotate these mRNAs as mobile, we compared the available *Arabidopsis* transcriptome information to the RNA-seq information obtained from *C. reflexa* samples feeding on *A. thaliana* C24. To establish a control data set, we assembled the *C. reflexa* transcriptome from autofeeding plants growing on tomato (*S. esculentum*) and filtered ambiguous reads (Supplementary Fig. 6; see Supplementary Methods). In total, this led to the identification of 2,110 transcripts that move from *Arabidopsis* into *C. reflexa* (Fig. 3b and Supplementary Information). This number is lower than the number of RNA molecules found in a recent study of a *C. pentagona* species parasitizing *Arabidopsis*[22], which possibly reflects a species dependency, a higher number of sequenced

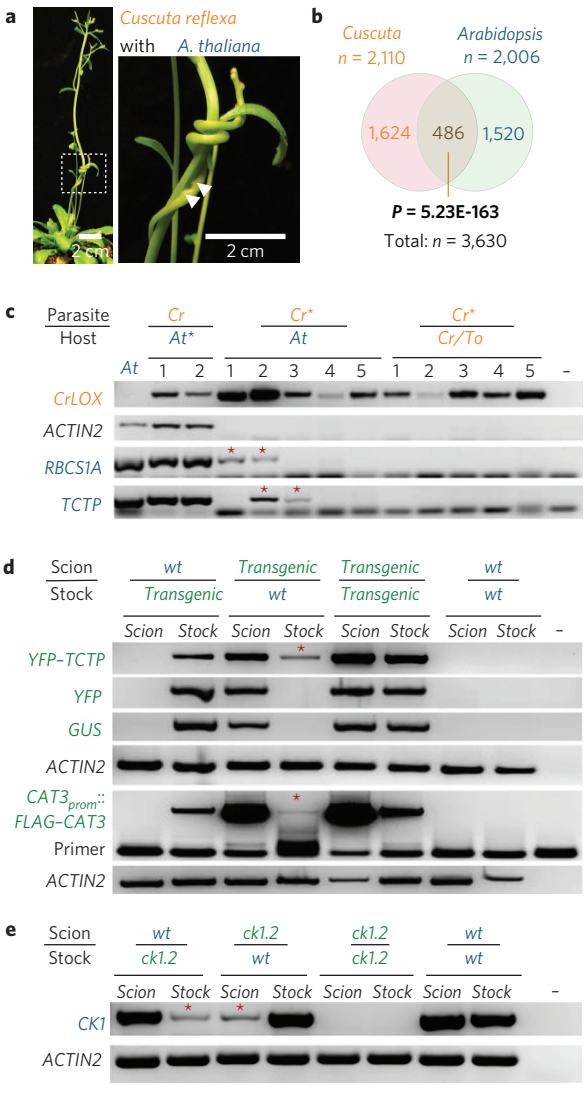


**Figure 3 | Analysis of mobile transcripts found in *Cuscuta reflexa* and in *A. thaliana* grafts. a**, Image of *C. reflexa* feeding on *A. thaliana* C24 plants. Arrows indicate haustorial connection to the vasculature of the stem. **b**, A significant number (hypergeometric test, $N = 33{,}602$) of $n = 486$ mobile-annotated *A. thaliana* transcripts move into Cuscuta. **c**, RT-PCR assays confirm *RBCS1A* and *TCTP* presence in *C. reflexa (Cr*)* feeding on *A. thaliana (At)* but not in *C. reflexa* feeding on other *C. reflexa (Cr*)* individuals supported by tomato plants (*Cr/To*). *ACTIN2* was not detected in *C. reflexa* samples but Cuscuta-produced *CrLOX* was detected in *A. thaliana* host (*At**). **d**, RT-PCR on hypocotyl-grafted *A. thaliana*. *YFP–TCTP* and *FLAG-tagged CAT3,* but not *YFP* or *GUS* transcripts, were detected in wild-type (*wt*) root (stock). **e**, *CK1* mRNA was detected in stem-grafted *ck1.2* mutant stock and scion samples. **c**–**e**, Red asterisks indicate mobile *Arabidopsis* mRNA presence.

samples or lower stringency used for the classification of mobile RNAs. In any case, of the identified *A. thaliana* long RNAs present in *C. reflexa* a larger than randomly expected number ($n = 486$) was also found mobile in PED/COL grafted plants ($P = 5.23\text{E-}163$, hypergeometric test). Thus, in total we identified 3,630 *Arabidopsis* genes (Fig. 3b) producing mobile transcripts, representing ~10% of all *A. thaliana* nuclear genes.

**Cell-autonomous transcripts fused to mobile transcripts move across graft junctions.** To confirm our findings, we performed RT–PCR assays on RNA samples from *C. reflexa* feeding on *A. thaliana* stems or on itself (=*Cuscuta* feeding on *Cuscuta* control) and on grafted transgenic and mutant *A. thaliana* plants (Fig. 3c–e). By this means we established that the predicted translationally controlled tumor protein (*TCTP*) and ribulose bisphosphate carboxylase small chain 1A (*RBCS1A*) mobile mRNAs (Supplementary Information) were indeed transferred from *A. thaliana* to *C. reflexa*, whereas the highly expressed *ACTIN2* mRNA, predicted as non-mobile, was not detected in these samples. To further evaluate the specificity of transport of endogenous plant transcripts, we used transgenic plants producing non-plant mRNAs such as *GUS* (β-glucuronidase) or *YFP* (yellow fluorescent protein) via a strong *35S* promoter. *YFP* and *GUS* mRNAs were not detected in wild-type scions or stocks in contrast to a *35S*-driven *YFP–TCTP* mRNA fusion construct. Thus, the *TCTP* sequence was sufficient to trigger *YFP* RNA transport. Next, we tested a transcript found to be mobile in *A. thaliana* grafts and in the *A. thaliana–C. reflexa* system. A *FLAG*-tagged endogenous *CATALASE 3* (*CAT3*) mRNA expressed from its own promoter[23] was detected in low amounts in stock (that is root) RNA samples. This suggested a directional *CAT3* transport with the phloem flow for *CAT3* transcripts in hypocotyl-grafted seedlings. Another predicted mobile transcript, *CHOLINE KINASE 1 (CK1),* exclusively found in *A. thaliana* grafts, was tested for mobility in stem-grafted wild-type–mutant *ck1.2* chimaeras. Owing to an insertion after the stop codon in the untranslated region (UTR), a mutant *ck1.2* plant does not produce a full length *CK1* transcript and, thus, can be discriminated by RT–PCR from wild-type *CK1* transcripts (Fig. 3e). Here, the appearance of the wild-type mRNA RT–PCR signal in both mutant stock and scion samples indicated that the wild-type *CK1* transcript moved bidirectionally from stock to scion and vice versa.

**Classification of mobile transcripts suggests multiple functions of allocated mRNAs.** To further evaluate the 3,630 mobile-annotated transcripts we analysed their expression patterns and searched for potential functional biases (Fig. 4). The expression profile in relation to their mobility revealed that, in general, the mobile transcripts correspond to highly expressed genes (Fig. 4a). In particular, root-to-shoot transported RNAs seem to be expressed at higher levels in root than in shoot. Otherwise, no apparent difference could be detected in the expression profile of the subsets (Fig. 4b, Supplementary Fig. 7a,b). Comparison of expression differences between shoot and root of the mobile transcripts revealed that some could move against an RNA gradient and the phloem flow by showing higher expression in the targeted tissue. In contrast, some transcripts move from their site of high expression to heterologous tissue with no or very low expression (Supplementary Fig. 8), suggesting a potential signalling function. To further evaluate the significance of the findings, we next identified the orthologous sequences in databases containing predicted or identified phloem-specific mRNA (refs 24–28) (Supplementary Fig. 7c and Supplementary Information). A significant number ranging from 22% to 49% of the mobile *A. thaliana* mRNAs could be identified in these phloem databases, indicating enrichment of phloem-related transcripts in our mobile mRNA data set.

Our results imply that not all highly expressed mRNAs including transgenes are systemically delivered to distant tissues (Figs 3c,d and 4b, Supplementary Fig. 8). Thus, we scrutinized the enrichment for a particular gene function in our data sets relative to all annotated transcripts and to a set of non-mobile transcripts matched for expression level distribution (Supplementary Fig. 7d). On the basis of this unbiased, expression level-adjusted analysis, we conclude that a complex over-/under-representation of terms exists. These include enriched cellular process terms, such as response to cadmium, glycolysis, ribosome and hypersensitive response, and enriched component terms, such as thylakoid, cytosolic ribosome, apoplast, and plasmodesmata (Supplementary

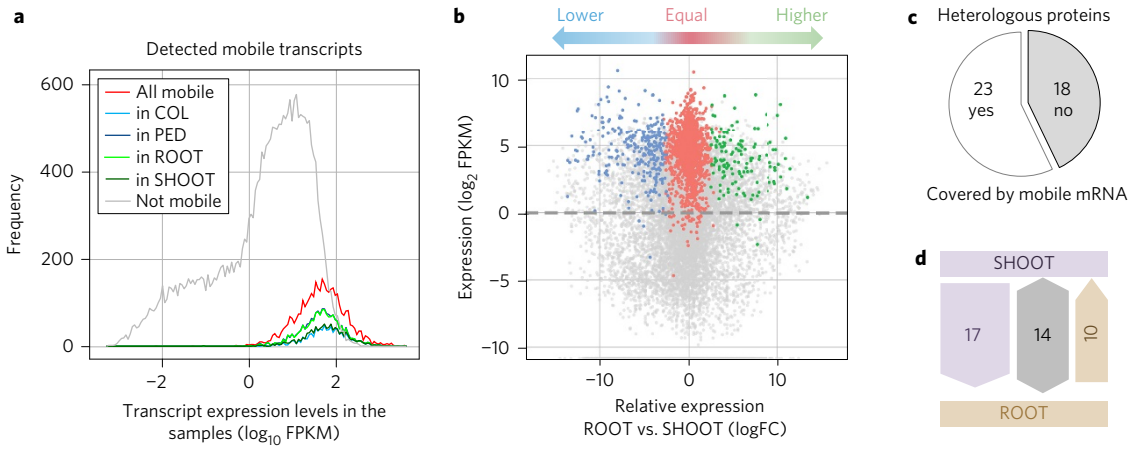


**Figure 4 | Tissue expression biases analysis of transcripts and heterologous proteins identified in grafted plants. a**, FPKM (fragments per kilobase transcript per million mapped reads)-estimated expression levels of mobile transcripts versus not mobile transcripts suggested that mobile transcripts are expressed at higher levels. **b**, Diagram showing the logarithmic fold change (logFC) of transcript abundance (mean of FPKM counts) according to its presence in root or in shoot. Mobile-annotated transcripts with more than twofold significant difference (false discovery rate < 0.05) between root and shoot are coloured as follows: green, higher expression in root; blue, higher expression in shoot; red, no significant difference; grey, transcripts not identified as mobile (see Supplementary Fig. 7a,b,e). **c**, Pie chart showing the fraction of the identified 41 proteins with SNP-based altered amino acid composition found in heterologous protein samples (Supplementary Information). **d**, Transport direction of detected heterologous proteins according to their root or shoot origin.

Information), pointing to a broad range of diverse functions of the mobile transcripts.

**Heterologous proteins related to mobile mRNA can be identified in grafted tissues.** Finally, the SNP-annotated transcript data set allowed us to perform peptide identification of mobile proteins or proteins produced by mobile mRNA present in grafted plant material. For this purpose, protein extracts from the grafted plant samples used for RNA-seq were submitted to a peptide liquid chromatography tandem mass spectrometry analysis for unambiguous identification of amino acid differences predicted to be present in 6,348 proteins by a non-synonymous SNP in a codon (Supplementary Information). This analysis revealed that 41 proteins were present in heterologous tissues (Fig. 4c). The peptide analysis was expected to identify a small number of proteins due to the low abundance of mobile proteins present in targeted tissues and limited technical sensitivity. Nonetheless, 23 (56%) out of the 41 proteins found in heterologous tissues corresponded to mobile RNAs (Fig. 4c), and, thus, could have originated from translation in the final destination tissue or been transported in parallel to their mRNA. By contrast, the 18 proteins associated with the transcripts that were not predicted to be mobile could represent cases in which the protein itself rather than its mRNA is mobile. Thus, it appears that mobile mRNAs may provide a means for protein localization distant from the site of gene expression. Indeed, the fraction of mobile proteins corresponding to SNP-tagged mobile mRNAs (that is 23 of 1,395 proteins produced by mobile mRNA versus 18 of 4,953 proteins encoded by identified non-mobile mRNAs) implies that a relatively high number of mRNAs may be translated after transport.

## Discussion

Initially, the presence of mRNA in phloem exudate was explained as potential contamination by surrounding tissue or a remnant of differentiating phloem cells[29]. This explanation implies that phloem mRNA signals move against the source-to-sink phloem flow as phloem vessels mainly form in young sink tissues. However, the observed distribution pattern and transfer of mRNA supports the notion of the existence of both transfer with and against phloem flow. The latter is supported by the presence of heterologous root-produced transcripts in leaves, stem and flower tissues (Fig. 2). In addition, we detected more heterologous transcripts in flowers than expected from a passive gradient. This distribution of allocated transcripts along the root-to-shoot axis of *A. thaliana* correlates with the siRNA signal spread in grafted *A. thaliana* plants. Here, siRNA signals were observed to be delivered into above-ground tissues after hypocotyl grafting[4] and to silence most flower tissues including meiotic precursor cells[5].

PED and COL ecotype grafting yielded data for ~28% (9,345 transcripts) of all annotated *A. thaliana* genes, and of these ~21% were detected in heterologous plant parts. Thus, extrapolated to the entire complement of the *Arabidopsis* transcriptome, as many as ~7,000 of the 33,602 known *A. thaliana* genes might be exchanged between tissues or cells. The systemic transfer of such a high number of small non-coding RNAs and full-length transcripts over graft junctions raises numerous questions regarding the specific role of mobile mRNAs in distant body parts, the regulatory mechanisms that establish tissue identity and the mechanism that facilitates directional transport. Conceivably, the mobile mRNAs uncovered in this study might function widely as specific signalling molecules coordinating growth, cell differentiation and stress adaptation of distant body parts. For example, the phosphate homeostasis regulatory *At4* (*AT5G03545*) transcript, which might sequester the shoot-to-root mobile miRNA *miR-399* by target mimicry[30], was found to be bidirectionally mobile. Mobile *miR-399* regulates $P_i$ throughput from root to shoot by targeting *PHO2* (AT2G33770) mRNA (ref. 31). Thus, mobile *At4* may build a depletion gradient against mobile *miR-399*, and provide another layer of control of $P_i$ transport to the shoot and $P_i$ homeostasis. Intriguingly, we also found *PHO2* in the mobile mRNA data set (Supplementary Information), which suggests that *PHO2* together with *At4* could limit the range of *miR-399* signalling activity.

In general, mobile transcripts could act as regulatory RNA molecules or might produce functional proteins in the targeted tissues. The presence of heterologous proteins related to mobile mRNAs in distant tissues (Fig. 4c), however, stresses the difficulty of distinguishing between mRNA mobility and protein mobility. Mobile mRNA moving from leaves to stolon tips in potato plants indicate the signalling potential of mobile mRNAs. Here it seems that the transported mRNA acts as a signal by producing a homeobox transcription factor inducing potato tuber formation in the targeted tissue[13]. That mobile proteins play a substantial role was already

shown with leaf-produced florigenic proteins initiating the apical flower programme[32]. In any case, a function of mobile transcripts could be to alert distant body parts, facilitating the systemic adaptation to stresses and defence against pathogens. In addition, mobile transcripts producing key metabolic enzymes or regulators thereof might alter the energy status and, consequently, growth of distant tissue in response to nutritional changes detected in roots or shoots. Furthermore, it is well known that some heterografts of anatomically similar plant species may have either beneficial or detrimental effects on plant growth; the identification of a large set of interorgan mobile mRNAs raises the possibility of their contribution to heterograft performance. Grafting between different plant genotypes has long been a common agricultural practice, particularly with fruit trees and in viticulture to increase productivity; however, the genetic determinants of beneficial graft combinations are not well known. Here, mobile mRNAs could play a pivotal role facilitating or limiting growth of a particular scion–stock combination used in agriculture. In addition, the existence of mRNA movement through graft junctions provides a potential basis for postulated horizontal gene transfer events[33]. Finally, the mobility of a significant number of protein-encoding transcripts suggests that gene expression and transcript function can be spatially unlinked. Thus, gene action can extend beyond or even become uncoupled from gene expression.

## Methods

**Plant material and growth conditions.** *A. thaliana* wild-type Columbia-0 (COL) seeds were obtained from the in-house collection of the Max-Planck Institute of Molecular Plant Physiology (MPI-MP). Seeds for the Pedriza-0 (PED) accession were provided by Dr Carlos Alonso Blanco (Centro Nacional de Biotecnología – CSIC, Madrid, Spain). For *A. thaliana* growth, wild-type COL and PED seeds were surface sterilized in 70% (v/v) ethanol for 1 min and afterwards incubated for 10–12 min in sodium hypochlorite solution containing 0.1% (v/v) Triton X-100. After repeated (fourfold), thorough washing with sterile water, seeds were suspended in 0.15% (w/v) agar and kept in the dark at 4 °C for 2 days. After this stratification period, sterile seeds were laid onto sterile half-strength (1/2) Murashige and Skoog (MS) medium supplemented with 1% (w/v) sucrose and solidified with 0.7% (w/v) agar. Seeds were germinated by keeping the plates vertically in a growth chamber under a controlled 9 h light photoperiod at 22 °C for 3 days and then at 26 °C for 3 more days. Tomato (*Solanum lycopersicum* cv. *Moneymaker*) plants were used as host for *C. reflexa* propagated under greenhouse conditions (relative humidity, day 50%; day/night temperature, 21 °C/19 °C; diurnal cycle, 16 h light/8 h darkness; seasonal-dependent light intensity range was between 190 and 600 μE $m^{-2}$ $s^{-1}$). To propagate *C. reflexa* feeding on tomato the parasite stems were cut ~30 cm below the apex, transferred onto adult tomato stems, sprayed with water every 2 days and covered with a plastic bag for 5–7 days until a haustorial connection was established. To establish *C. reflexa* feeding on *A. thaliana* C24, the parasite stem was cut ~30 cm below the apex and transferred onto shooting *Arabidopsis* plants grown in the greenhouse. *C. reflexa* stems were supported by a rod, sprayed with water, and covered with a plastic bag until haustorial connections were visible.

***Arabidopsis thaliana* grafting and nutrient limitation experiment.** To graft *A. thaliana* seedlings (COL and PED) the plants were grown on plates and cut 6 days after germination with a sterile razor blade. Transverse cuts were performed in the upper half of the hypocotyl. Silicon tubing with 0.3 mm internal diameter was used to support the graft junction, transferred onto new sterile plates with 1/2 MS 1.5% (w/v) agar medium supplemented with 1% (w/v) sucrose, and transferred to growth chambers (9 h/15 h light/dark cycle) set at 26 °C. After 3 days the temperature was lowered to 22 °C for 11 days. Adventitious roots that formed on the scion after grafting were regularly removed with surgical forceps. The grafted plants were then transferred to a hydroponic system and exposed to three different nutrient regimes: full nutrition, 1 mM $KNO_3$, 0.5 mM $NH_4NO_3$, 1.5 mM $KH_2PO_4/K_2HPO_4$ pH = 5.7–5.8, 2 mM $CaCl_2$, 0.5 mM $MgSO_4$, 1 mM $K_2SO_4$, 1.5 mM MES, 2 μM $Na_2FeEDTA$, 30 μM $H_3BO_3$, 7 μM $MnSO_4$, 0.5 μM $ZnSO_4$, 0.3 μM $CuSO_4$, 0.2 μM $NiCl_2$, 0.15 μM $HMoO_4$, 10 nM $CoCl_2$; phosphorus limitation (–P), full nutrition media but with limiting phosphate (0.1 mM $KH_2PO_4/K_2HPO_4$) and 1.25 mM KCl supplement, and completely omitting $KH_2PO_4/K_2HPO_4$ after 1 week; nitrogen limitation (–N), full nutrition media but with low $KNO_3$ (50 μM), $NH_4NO_3$ (25 μM) and KCl (0.75 mM). Note that the low P medium was applied immediately while low N medium was applied only 1 week after transferring the plantlets to hydroponic culture. Hydroponic growth was under 12 h light conditions at 22 °C. After 2 weeks in the different nutrient conditions, the stock and scion material were harvested, carefully omitting the areas close to the graft junctions. Plantlets with adventitious roots at the time of harvest were discarded outright.

**RNA and DNA sequencing and bioinformatic analysis.** PCR-verified total RNA and genomic samples from at least 12 plants were pooled in three biological replicates for each condition. The *Arabidopsis* pooled RNA (quantity ≥ 10 μg, concentration 20 ng $\mu l^{-1}$) and genomic DNA were subjected to next-generation genome and transcriptome sequencing (DNA- and RNA-seq, respectively). Total RNA (quantity ≥ 10 μg, concentration 20 ng $\mu l^{-1}$) from *C. reflexa* was subjected to RNA-seq. NGS libraries were generated and sequenced to obtain paired-end reads with a length of 99 bp or 150 bp on the Illumina HiSeq platform (LGC or MPI, Cologne), and paired-end 90 bp reads were obtained from BGI (http://www.genomics.cn/). For details of the sample IDs, read numbers and statistics see Supplementary Information. For details of the samples and NGS analysis, data processing, protein identification and PCR primers used see the Supplementary Methods. All sequencing data sets are available at the NCBI Small Read Archive (SRA), ID PRJNA271927.

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## Author contributions

C.J.T., C.S. and D.W. devised and implemented the bioinformatic methodology and analysis; E.S. and M.R-T. performed ecotype-grafting experiments and analysed data; W.Z. and L.Y. performed *A. thaliana* grafting and *C. reflexa* experiments and analysed data; M.M. provided constructs; W.X.S. performed protein identification and annotation analysis; J.P-A. supervised M.M.; F.K. supervised W.Z., L.Y. and E.S.; W-R.S. supervised E.S. and M.R-T.; D.W. supervised C.J.T. and C.S.; F.K. wrote, supported by all co-authors, the manuscript; W-R.S. and F.K. analysed results and implemented ideas; J.P-A.,W-R.S. and F.K. are co-principal investigators who conceived the study.

## Additional information

Supplementary information is available online. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to F.K.

## Competing interests

The authors declare no competing financial interests.

CORRIGENDUM

# Endogenous *Arabidopsis* messenger RNAs transported to distant tissues

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In Figure 3e of the version of this Article originally published, the wt/wt control graft and *ck1.2*/*ck1.2* mutant control graft were mislabelled. This error has been corrected.

# Corrigendum: Endogenous *Arabidopsis* messenger RNAs transported to distant tissues

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In the version of this Article originally published, the *x*-axis scale in Fig. 4b was incorrectly labelled and should have ranged from -10 to 10. This has been corrected in all versions of the Article.