Hitchhikers, highway tolls and roadworks: the interactions of plant viruses with the phloem

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The phloem is of central importance to plant viruses, providing the route by which they spread throughout their host. Compared with virus movement in non-vascular tissue, phloem entry, exit, and long-distance translocation usually involve additional viral factors and complex virus–host interactions, probably, because the phloem has evolved additional protection against these molecular 'hitchhikers'. Recent progress in understanding phloem trafficking of endogenous mRNAs along with observations of membranous viral replication 'factories' in sieve elements challenge existing conceptions of virus long-distance transport. At the same time, the central role of the phloem in plant defences against viruses and the sophisticated viral manipulation of this host tissue are beginning to emerge.

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Introduction

For plant-infecting viruses, the phloem is of particular importance, as it provides the fastest way to spread throughout the host in a race against systemic defence responses, in order to optimize viral load and reach tissues favouring host-to-host transmission [1,2]. Perhaps because it is a gatekeeper to systemic infection, the phloem appears to be specially protected against viruses, as its successful invasion often requires additional viral proteins compared with non-vascular movement. Recent studies on the unexpectedly widespread phloem trafficking of endogenous plant mRNAs suggest that host transport systems suitable for viral exploitation may exist and viral long-distance movement (LDM) requirements may provide insights into them. A few recent studies have also raised the possibility that the xylem might function as an additional/alternative systemic transport route [3•,4]. Due to space limitations, we do not discuss these findings, but we highlight the increasing evidence that viruses actively manipulate phloem cells to their advantage. Although viruses might be safe from many plant defences within dead xylem vessels, the predominance of the phloem as the route for viral LDM could be related to its susceptibility to being manipulated, as well as the amplification along the LDM pathway possible in living companion cell (CC)–sieve element (SE) complexes, and contact with vectors.

In what form do viral RNAs move within SEs?

Many plant viruses require their capsid protein (CP) for systemic movement. However, this does not necessarily mean that virions are the entities moving in the sieve elements, as the CP can also be involved in entry to and exit from the phloem, or in suppression of host defences (e.g. [5]). On the other hand, viruses capable of systemic spread in the absence of CP are generally assumed to be transported as ribonucleoprotein (RNP) complexes involving viral movement proteins or host RNA binding proteins.

Now, two studies have addressed the general phloem mobility of mRNAs. Whilst LDM of proteins and small RNAs has long been established [6,7], observations of phloem-mobile mRNAs have been more sporadic. An elegant approach avoiding invasive phloem sampling [8•,9•] combined grafting of different Arabidopsis eco-types or grape varieties, respectively, with whole tissue RNA-seq, identifying ∼2000 (Arabidopsis) and >3000 (grape) mRNAs that were systemically mobile, indicating that phloem mobility of mRNAs has so far been vastly underestimated. Modelling based on mRNA abundance alone successfully explained the data of [8•], suggesting that mobility of most of the mRNAs could be due to unregulated, non-sequence-specific 'leaking' into the phloem stream [10]. If this was the case, one might wonder why viruses, whose RNA genomes can be highly abundant after replicating in the CCs and repeatedly amplifying along the phloem pathway, would need a phloem transport mechanism at all, especially, since the mobile mRNA dataset from [8•] included transcripts of similar size to viral genomes. Indeed, replication-

incompetent RNA3 of Brome mosaic virus can move systemically independent of viral factors [11].

However, the model [10] did not take into consideration that the size exclusion limit of plasmodesmata between CCs and SEs is ~40–60 kDa [6], which is significantly smaller than the size of most mRNAs (~340 kDa/kb). Furthermore, all three studies [8•,9•,10] found indications of selective movement of certain mRNAs, such as mobility greater than expected from abundance and size, transport against the shoot-to-root phloem direction, selective trafficking into specific aboveground organs, and mobilization of immobile mRNAs by transcriptional fusion to mobile species. Generic mobility of mRNAs is also contradicted by studies which detected systemic movement of proteins, but not their corresponding mRNAs (e.g. [12,13]). It, therefore, seems more likely that a transport system for specific endogenous mRNAs exists that may permit some abundance-dependent unspecific 'leakage'. This transport system may rely on RNA secondary structures conferring mobility, as [14••] demonstrated that stem–loop secondary structures in tRNAs can mobilize transcriptionally fused mRNAs (Figure 1), and phloem-mobile transcriptomes [8•,9•] contained significant numbers of mRNAs either containing tRNA-like structures or transcribed as dicistronic fusions with proximal tRNA genes. Importantly, some RNA viruses have tRNA-like hairpins in their 3′UTRs, and the majority of viral RNAs contain extensive secondary structures involved in regulating various infection steps, which might interact with such a system for LDM [15]. Segmented multipartite viruses may traffic as a net of inter-segmentally base-paired RNAs stabilized by proteins including CP and host proteins, in order to ensure that recipient cells receive all genome components [16]. Viroids, non-coding pathogenic RNAs, also rely on their complex secondary structures for systemic transport [17], as well as on host proteins, some of which are graft-mobile [18]. Thus, intra-molecular or intermolecular RNA stem–loop-type structures are probably available in all RNA viruses that do not move systemically as encapsidated virions.

Viral interactions with cellular RNA-binding proteins are, therefore, of general interest for phloem RNA transport. Among these, the nucleolar RNA-binding protein and methyltransferase fibrillarin (FIB) that functions in maturation of ribosomal RNAs is particularly noteworthy. FIB is involved in the LDM of at least four different viruses [19–21] and interacts with the movement proteins of another two [22,23]. In the best-studied case of FIB–umbravirus interaction, FIB is recruited to the cytoplasm, where it forms ring-like oligomers together with a viral protein, which then encapsidate viral RNA, likely the systemic transport entity [19,24,25]. Recently, it was shown that FIB also aids LDM of satBaMV, a satellite RNA of Bamboo mosaic virus, but not BaMV itself [21].

FIB co-purified with the movement complex and was required within the phloem, as satBaMV was expressed from a 35S promoter and thus, in CC.

Given its prominence for systemic movement of viruses, it will be interesting to see if FIB also plays a role in endogenous mRNA trafficking. Both FIB and its mRNA were detected in Arabidopsis phloem exudate [26,27], and the latter was identified as mobile [8•]. It remains to be tested if silencing [19] or knock out of FIB also affects the transport of graft-mobile mRNAs or tRNA-fused reporter constructs [8•,9•,14••]. Such findings would strengthen the case for an endogenous RNA systemic transport system exploited by viruses and open the door for its characterisation.

At the other extreme of potential transport forms of viral LDM, [3•] have observed up to ~10 μm aggregations of vesicles associated with viral replicase, dsRNA, and CP in mature SEs of *Nicotiana benthamiana* stem internodes above leaves inoculated with Turnip mosaic virus (TuMV), proposing that entire virus replication complexes (VRCs) move systemically. SE occlusion-related (SEOR) protein, which forms aggregates up to several μm in size, is systemically mobile [28]. Thus, it seems possible that VRCs could also pass through sieve plates, particularly, as agglomerations of smaller membrane structures. Whether entire VRCs in SEs are just a fortuitous outcome of phloem infection, or actually required for systemic transport, or for virion assembly of aphid-transmitted viruses like TuMV in SEs, remains to be shown.

Viral re-programming of the phloem

The phloem is known as the conduit for systemic RNA silencing and systemic acquired resistance (SAR) [7,29], and the requirement of viral suppressors of RNA silencing for LDM [2] highlights that viruses have to overcome plant defences in the phloem itself. Now, a study [30••] analysing the phloem translatome in naïve and Tobacco mosaic virus (TMV)-infected Arabidopsis and *N. benthamiana* plants, found phloem responses to be several-fold higher than in non-phloem tissues, and most biological processes were only altered in the phloem. These results impressively demonstrate the importance of the phloem during virus infection. It is, therefore, not unexpected that viruses have evolved mechanisms to subvert phloem responses.

These authors also found that TMV reprograms CC transcription by disrupting nuclear localization of auxin/indole acetic acid (AUX/IAA) responsive transcription factors [31••]. TMV-interacting AUX/IAA isoforms were shown to be CC-expressed, and their recruitment to the cytoplasm by the TMV replicase had a positive effect on virus phloem loading and systemic transport. Conversely, CC over-accumulation of a stabilized AUX/IAA