Systemic transport of RNA in plants

Plant vasculature was once thought to function as a mere conduit for nutrients and hormones. However, recent evidence indicates that this transport system can participate in the dissemination of various signal molecules throughout the plant. Interestingly, these signals include not only traditional signaling factors, such as proteins and growth-regulating small molecules, but also RNA.

Once RNA was thought to be an information carrier, functioning as a passive template for protein synthesis. It is now also thought to act as an active signal molecule, regulating gene expression and development in plants. Various types of RNA molecules travel long distances from their site of synthesis to different parts of the plant. This systemic movement occurs through plant vasculature by as yet unknown mechanisms. Here, three major types of RNA systemic transport are summarized (Fig. 1):

- Long distance movement of plant virus genomic RNA.
- Systemic transport of RNA elicitors of post-transcriptional gene silencing (PTGS).
- Long distance transport of specific endogenous RNA molecules.

Systemic transport of plant RNA viruses

Following initial infection, usually by mechanical inoculation, plant RNA viruses spread from cell to cell through plasmodesmata until they reach the vascular system; the virus is then transported systemically through the vasculature (reviewed in Ref. 1; Fig. 1). Virus-encoded nonstructural movement proteins (reviewed in Refs 2,3) mediate the cell-to-cell spread of infection. The best studied movement protein is the 30 kDa protein of the tobacco mosaic virus (TMV). A TMV movement protein binds viral RNA, forming an extended TMV movement protein–RNA transport complex[4], which is targeted to plasmodesmata, potentially, through its interaction with cytoskeletal elements of the host cell[5,6]. The movement protein then acts to increase the size exclusion limit of plasmodesmata[7,8], allowing intercellular movement of TMV movement protein–RNA complexes. In addition to the TMV movement protein, these protein activities have also been demonstrated for movement proteins of many other plant viruses (reviewed in Refs 2,3).

Although movement proteins alone are sufficient to transport viral RNA genomes cell to cell to the vicinity of the host plant vascular system, virus entry into the vasculature and its subsequent systemic spread require an additional function generally encoded by viral coat proteins (reviewed in Refs 2,3). The exact nature of coat protein activity in viral systemic movement is unknown. Potentially, a coat protein, in concert with a movement protein, might act to dilate plasmodesmata at the boundary between vascular and non-vascular tissues. Indeed, although the TMV movement protein specifically accumulates in these plasmodesmata, it does not increase their permeability, suggesting that another factor, such as a coat protein, is required for this function[9].

The molecular pathway by which RNA viruses spread systemically is obscure. It is likely that entrance into the host plant vasculature differs from egress back into non-vascular tissues. For example, exposure of tobacco plants to non-toxic concentrations of the heavy metal cadmium prevents tobamoviral disease[10] by specifically blocking viral exit from the vascular tissue into the non-inoculated systemic organs, whereas viral entry into the vasculature is unaffected[11]. Potentially, non-toxic levels of cadmium trigger the synthesis of cellular factors that interfere with systemic viral movement, and might represent one of the regulatory mechanisms for RNA transportation throughout the plant.

Systemic transport of RNA signals for post-transcriptional gene silencing

Because viruses often adapt existing cellular machinery for their own needs, they probably employ an endogenous pathway for the systemic transport of RNA. Indeed, recent evidence indicates that numerous RNA species travel through plant vasculature, revealing a novel type of systemic signaling. Specifically, PTGS, an innate plant defense mechanism, is probably elicited by such systemic RNA signals (reviewed in Refs 12,13; Fig. 1).

PTGS silences genes in *trans*, following the introduction of either transgenes or viruses. Gene silencing is defined as post transcriptional when the RNA of the silenced gene does not accumulate even though its transcription occurs[12]. When PTGS affects both the transgene and the endogenous gene, it is also termed co-suppression[13]. Pioneering work using grafting procedures demonstrated that silenced tobacco stocks induce PTGS in scions expressing the corresponding transgene, and provided the first indication of the systemic transport of PTGS signals[14].

Recent evidence suggests that the PTGS signal is double stranded (ds) RNA, induced either by antisense transgenes[15] or when transgenes insert in reverse orientation close to an endogenous promoter[16]; this notion is further supported by silencing induced by the direct introduction of dsRNA into animal cells[17]. If dsRNA is the signal, it provokes numerous questions, such as how is it generated and amplified to a degree that allows its transport and activity at distant sites in the plant? In the present context, it is further challenging to consider the mechanics of dsRNA transport into the vasculature, given that most models and relevant data invoke single stranded intermediates for vascular transport. It is notable that plant viruses induce viral resistance by a mechanism similar to systemic silencing in PTGS (Refs 13,15). Thus, in plants, PTGS and virus-induced silencing provided the first indications of a regulatory role for the systemic transport of RNA.

Long distance transport of specific endogenous RNA molecules

Systemic virus infection, PTGS and RNA-mediated virus resistance are based on the transport of RNA molecules of foreign origin (i.e. viral genomes or transgenes). It is now known that endogenous cellular RNAs are also transported through the plant vascular system (Fig. 1). *In situ* hybridization experiments have demonstrated that the mRNA for the leaf sucrose transporter SUT1 is located mainly within sieve elements, whereas its transcription occurs in the adjacent companion cells, indicating that SUT1 mRNA moves through the phloem of potato plants[18]. This transport is consistent with the essential role of SUT1 in phloem loading and long distance transport of sucrose (Ref. 18 and references therein).

Besides SUT1, other endogenous mRNA species have been found in the phloem. CmPp16, a phloem protein from pumpkin (*Cucurbita maxima*), transports RNA between companion cells and sieve elements in a sequence non-specific fashion[19], and *thioredoxin-h* mRNA is detected within phloem sap[20], suggesting that cellular mRNAs can move systemically via the phloem. Recently, >100 cDNA clones were identified following reverse transcription of RNA from pumpkin phloem[21], highlighting that RNA transport occurs more promiscuously than imagined previously. In this study, one cDNA, designated *CmNACP*, belonging to a gene family potentially involved in apical meristem development, was characterized in detail[21].

CmNACP mRNA was detected in companion cells and sieve elements of leaf, stem and root phloem, and its long distance transport was shown using grafting experiments[21], similar to those employed to demonstrate systemic signaling in PTGS (Ref. 14). Importantly, phloem traffic of *CmNACP* mRNA differed from that of most other known cases of systemic RNA transport in its targeting to a specific tissue (i.e. the shoot apex) (Fig. 1).

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How RNA molecules enter the vascular system and then exit into target tissues is unclear. By analogy to nucleic acid transport across nuclear membranes (reviewed in Refs 22,23), specific chaperone-like proteins that associate with the transported RNA molecule[24] might mediate systemic traffic of RNA in plants. Conceptually, viral movement proteins might act as such chaperones because they bind to the transferred RNA, unfold it and provide transport functions (reviewed in Refs 3,22). For transport of endogenous RNA, CmPp16 protein might function as a chaperone that potentiates RNA movement within phloem[19]. Pumpkin phloem sap proteins, which bind RNA without sequence specificity[21], might also chaperone their cognate RNA molecules from companion cells into sieve elements. In addition, for selective targeting of *CmNACP* mRNA (Ref. 21), a protein that recognizes this mRNA specifically and transports it to apical tissues must exist.

The biological 'rationale' for transporting *CmNACP* or other mRNAs long distances, rather than generating this meristem-specific signal within the target tissue or its vicinity, is unclear. For example, another gene product important in meristematic development, the Knotted1 protein, as well as its mRNA, has been suggested to move from cell to cell within the meristem rather than migrate from remote tissues[25]. This short distance signaling circumvents several potential difficulties inherently associated with the long distance transport of signals. First, signal transduction between adjacent cell layers, as in Knotted1 transportation (Ref. 25), is fast, whereas systemic signaling can occur at slower rates. Second, cell-to-cell transport minimizes signal degradation or mistargeting, whereas movement through the entire phloem poses a higher risk of signal decay or delivery to the wrong tissues. Finally, production and transport of important developmental signals, such as *CmNACP* mRNA, should be tightly controlled. Regulatory feedback mechanisms for cell-to-cell transport are easy to envision. By contrast, long distances separating the site of signal production from that of its action obviously complicate regulation, requiring another phloem-moving signal targeted from the meristem back to the *CmNACP* mRNA-producing tissues. Such regulatory mechanisms might be hindered further by the source-to-sink direction of phloem flow, which coincides with the traffic of *CmNACP* mRNA but opposes that of its putative feedback regulator.

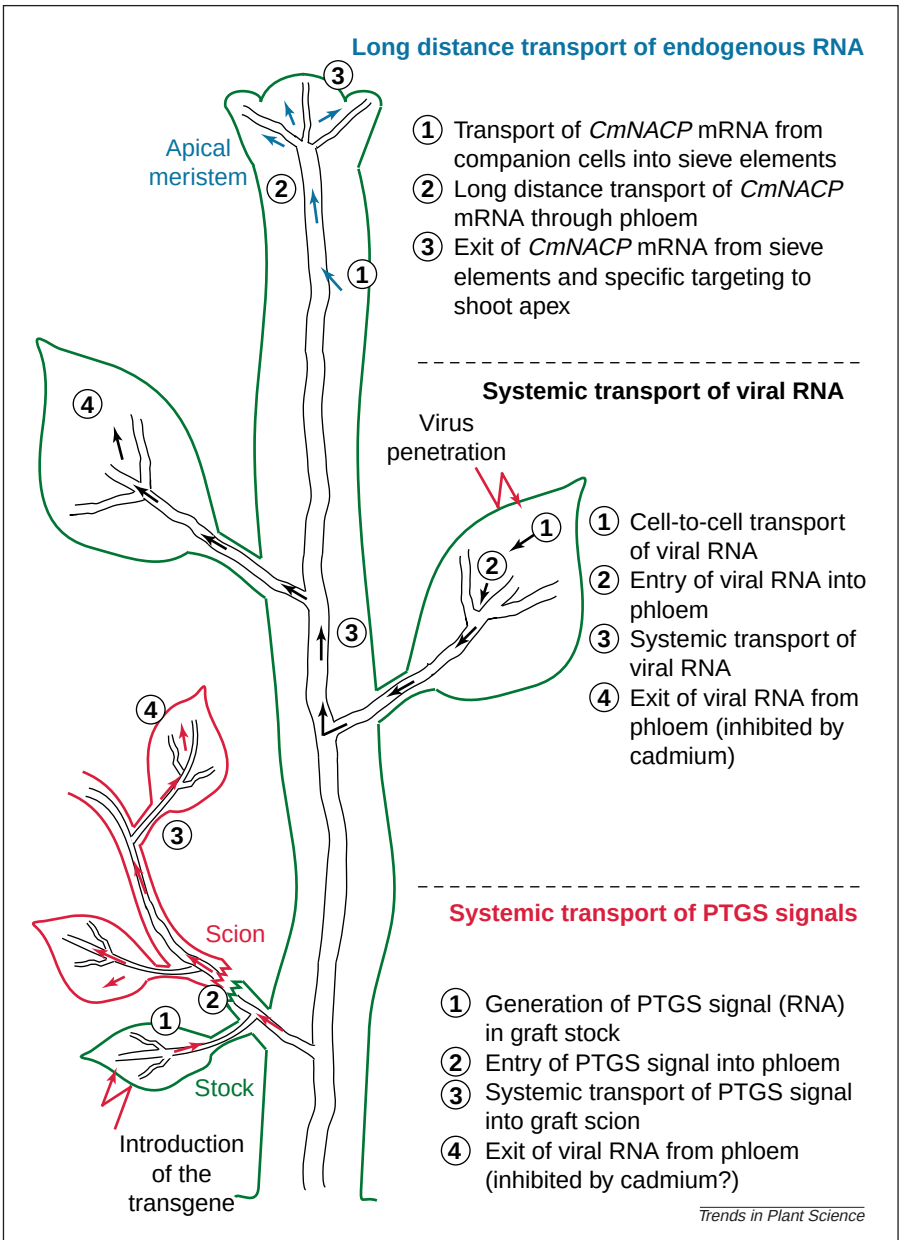

Fig. 1. Three major types of systemic RNA transport in plants. RNA transport involved in viral infection and gene silencing is initiated by the insertion of foreign genetic material (i.e. virus inoculation or introduction of the transgene, respectively). RNA molecules derived from this genetic material are then generated for subsequent transport. Whereas viral RNAs must first travel cell to cell to reach vasculature, post-translational gene silencing (PTGS) RNA signals and endogenous transported RNAs can originate directly in phloem elements, such as companion cells, circumventing the cell-to-cell movement step. For example, although cell-to-cell movement of PTGS signals can occur, it is not essential for the development of PTGS (Ref. 16). All three types of RNA then enter the sieve elements of the vasculature and spread systemically, probably in the source-to-sink direction (i.e. from older to younger leaves). Finally, the signal molecules exit the vasculature in most tissues (viral RNAs and PTGS signals),

Prospects and unanswered questions

The immediate goal of systemic RNA transport studies in plants is to identify cellular factors that mediate and, importantly, control this process. In addition, the role of RNA signals in PTGS should be demonstrated directly.

Questions for the future include:

- Do phloem targeting signals exist, and are they protein- or RNA-based?
- Is RNA transport into the phloem an active process associated with increases in plasmodesmal permeability?
- Is RNA exit from the phloem mediated by specific signals, and what are they?
- What distinguishes PTGS signals that load into the phloem irrespective of their nucleotide sequence from selective endogenous RNA (e.g. *CmNACP* mRNA) transport into the phloem?
- Why are PTGS signals and viral genomes unloaded from the phloem in most tissues and organs of the plant whereas *CmNACP*

mRNA appears to unload only in the apical meristem?

With the further development of genetic, biochemical and biological tools to dissect systemic RNA transport, critical experiments that unravel the mechanisms of this process should follow.

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