

## Virus Transmission—Getting Out and In

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**Abstract** Logically, most plant viruses being vector-transmitted, the majority of viral transport mechanisms associated to the transmission step have been approached through the study of virus-vector relationships. However, in the case of non-vector vertical transmission through the seeds, some viruses have evolved specific patterns to colonize either the gametes or the embryo, thereby connecting viral transport within the plant to that in between plants. Moreover, though it may appear counter intuitive and has been largely overlooked, some specific virus accumulation within cells or organs, as well as specific control of multiple infections of single cells, can also directly affect the success and efficiency of vector transmission, again connecting viral transport mechanisms inside and outside the host plants. This work summarizes the data available on viral transport outside the plant in various vectors, and also highlights a few available examples and proposes hypotheses for illustrating the concept that some viral trafficking within plants is specifically intended to prepare ulterior acquisition by the vectors.

### 1

#### Introduction

Besides replicating in cells and trafficking from cell-to-cell and long distance, when invasion of the host plant is completed, viruses have found very diverse ways to move on and jump into the outside world, seeking another host plant. This adventure involves various steps and sophisticated modes of transport, not only for travelling safely in the big outdoors, but also, before and after, for preparing to leave and securing efficient installation, respectively. In contrast to intracellular or symplastic intercellular trafficking within plants, viral transport between plants implies one additional major difficulty: the repeated passage through cell walls, both for getting out of an infected plant and back into a healthy one. While some very rare viruses can autonomously and passively exit and enter adjacent plants from wounds via non-specific mechanical transmission, the vast majority have adopted a strategy that uses plant-feeding invertebrates as transport devices, which easily ensures the passage through cell walls and also allows the virus to cover considerable distances between host plants in the environment.

Because of its tremendous impact on epidemiology, virus transmission has been intensely studied for nearly a century (Doolittle and Walker 1928) in

different scientific disciplines (for reviews see Nault 1997; Gray and Banerjee 1999; Van den Heuvel et al. 1999; Blanc 2004). The development of molecular biology marked a big turning point in this scientific field, allowing the identification and characterization of the numerous viral determinants involved in transmission, and a few counterpart “receptors” in the corresponding vectors. In the near future, cell biology and imaging also promise great returns in this field; despite their limited use to date, they have already informed on some mechanisms of viral transport within the vector and even within plants, that are clearly specific to the step of transmission.

The transport of virus particles or viral proteins that is related to plant-to-plant passage includes specific within-plant phenomena allowing the colonization of embryos in vertical seed-transmission, and efficient interaction with specific vectors in horizontal transmission. In the latter, the virus can have a steady interaction with vectors, “sticking” somewhere and waiting for release when an appropriate destination is reached, but can sometimes also traffic through the vector cells, implying mechanisms different from those existing in the plant cells that are described in other chapters of the present volume. Still related to vector-transmission, a largely overlooked phenomenon is being uncovered: viruses can develop interactions with the host plant, involving protein or viral particle transport processes, that are specifically destined to prepare and optimize acquisition by the vector in the infected source plant or facilitate the initiation of *de novo* infection in the inoculated healthy plant.

This work reviews known molecular mechanisms and cellular processes, occurring in either plants or vectors, that contribute to the successful transport of viruses from one host plant to the next. While some aspects have long been investigated and deserve continued research efforts, others are just being discovered and will be highlighted as they represent promising future prospects.

## 2

### **Virus Transport Involved in Non-Vector Transmission**

Vertical transmission through seeds is a phenomenon relevant to about 15% of plant virus species (Hull 2001). A tremendous amount of data is available concerning the list of virus-host combinations where seed transmission can occur, as well as on the dramatic variations in the percentage of infected seeds observed either with different virus isolates in a given host, or with a single isolate in different host species or ecotypes (Mink 1993).

With the exception of TMV, and presumably other tobamoviruses, which externally contaminate the seed coat and are later transmitted mechanically to the germinating plants (Broadbent 1965), the most frequent case is infection of the embryo, via two distinct but sometimes co-existing pathways.

Embryo infection can occur indirectly before fertilization, by infection of the gametes, or after fertilization by direct invasion of the seed tissues (Maule and Wang 1996). Both pathways are summarized and discussed below, as both could rely on specific transport mechanisms.

## 2.1

### Indirect Embryo Colonization by Early Infection of Gametes

Several virus species, for instance cryptic viruses (Kassanis et al. 1978), some tobnaviruses (Wang et al. 1997) and nepoviruses (Hull 2001), readily infect gametes, and this is believed to be positively correlated to a rather uncommon property in plant viruses, i.e. the capacity to invade meristematic cells (Maule and Wang 1996). It would be interesting to understand what specific mechanisms allow or prevent a viral presence in meristem cells subsequently leading to gamete infection and vertical transmission.

Meristem exclusion of some RNA viruses has been indirectly related to post-transcriptional gene silencing (PTGS) (Foster et al. 2002), and this was recently confirmed for Potato virus X (PVX) in *Nicotiana bentamiana* (Schwach et al. 2005). The authors of this latter study have shown that virus accumulation in meristematic cells is prevented by the action of the RDR6 cellular RNA-dependent RNA-polymerase. In the same report, RDR6 is proposed to relay the long-distance silencing signal reaching the apical growing points, by promoting rapid production of a secondary siRNA at the site of virus entry. From these data, we could reason that the ability of some viruses to infect gametes depends not on specific mechanisms of viral transports into the meristem, but rather on circumvention of PTGS in this tissue. The case of *Barley stripe mosaic virus* (BSMV), which is known to indirectly infect embryos by early colonizing of gametes (Maule and Wang 1996), and where the viral determinant of seed-transmission was shown to be the protein  $\gamma$ b (Edwards 1995), a protein later characterized as a PTGS suppressor (Yelina et al. 2002), is consistent with this scenario (for detailed information on PTGS, see the work by T. Hohn et al., in this volume).

This PTGS-related mechanism of meristem exclusion, however, may not apply to all virus species, as inspired by a recent work on the early development of the *Arabidopsis thaliana* embryo (Kim et al. 2005). In this work, the authors demonstrate the rapid establishment of specific boundaries that separate symplastic sub-domains prefiguring shoot apex, cotyledons, hypocotyls and roots. Interestingly, they also observed that the movement protein of TMV (P30) cannot dilate embryonic plasmodesmata and overcome these boundaries between subdomains. One could imagine that a similar putative boundary around the meristematic symplastic domain could later prevent TMV entry. This provides another hypothetical mechanism of meristem exclusion that could apply to TMV, which interestingly is not affected by the RDR6-related PTGS discussed above (Schwach et al. 2005). This putative

meristem boundary could possibly be overcome by some gamete-infecting viruses, implying unknown specific mechanisms of viral transport at this level.

## 2.2

### Direct Infection of the Embryo by Invasion of Seed Tissues

Besides the early infection of gametes, another pathway for embryo colonization occurs after fertilization by sequential virus movement into the seed, from the micropylar region of the maternal testa, to the endosperm, suspensor and finally the embryo. This route is also used by the above-mentioned BSMV, and is the exclusive mode of seed transmission for the best-studied case, *Pea seed borne mosaic virus* [PsBMV, (Wang and Maule 1992)].

One major conceptual problem long discussed in this pathway of direct embryo colonization centres on the fact that the virus can reach the micropylar region of the testa by genuine cell-to-cell movement in a symplastic maternal tissue (reviewed in Hull 2001). The same is true for movement from the suspensor to the embryo, as the suspensor derives from early embryonic cell divisions, and symplastic connections also exist at this level. The problem is passage of the virus from maternal to embryonic cells, between which symplastic connections are severed early during meiosis. This barrier was believed to allow the passage of small nutrient molecules by apoplastic transport at the maternal-filial interface, where transfer cell wall projections were observed in the endosperm (Tegeeder et al. 1999, 2000). Thus, there was no possible anatomically based explanation for the passage of virus from testa to endosperm, and from endosperm to suspensor cells, until the question was carefully re-investigated by electron microscopy specifically targeting the ultrastructure of the micropylar region (Roberts et al. 2003). In this study, the cylindrical inclusions induced by PsBMV infection were used as markers of putative symplastic connections, as the same authors had previously shown that these were positioned in the close vicinity of plasmodesmata (Roberts et al. 1998). Cylindrical inclusion bundles, arranged perpendicular to cell walls separating maternal testa and endosperm, were clearly visible and labelled by a PsBMV antiserum. Although proper plasmodesmata could not be observed, the authors interpreted occasional distortion of the cell wall, near the cylindrical inclusions, as reminiscent of plasmodesmal cavities. This result suggests a possible means of virus transfer between maternal tissues and endosperm that requires further investigation to decide whether these symplastic connections are constitutive or specifically induced by seed-transmitted viruses (Roberts et al. 2003). The last problematic barrier to be elucidated is that between endosperm and suspensor cells. The same authors described regions of the embryo sheath, at the base of the suspensor cells, which are discontinued and punctuated with pore-like structures, putatively allowing the transfer of large molecular weight complexes, including viruses. These “pore-like” connections were previously

unknown, and whether viral transport at this level is passive or requires specific active processes, remains to be investigated.

### 3

## **Virus Transport Involved in Vector-Transmission**

Unlike animal viruses, where hosts are mobile and often come into contact with each other, plant viruses need to cover the often large distances separating their fixed hosts. Hitch-hiking with the invertebrate parasites of plants provides both rapid transportation and secure housing. While the majority of plant viruses rely simply on controlling the timely retention in, and release from, a specific unique location in the vector, a few others have developed a more intricate relationship that also involves specific transport processes as part of a dynamic cycle within the vector body. The mechanisms of virus-vector relationships are logically most often studied outside the plant, and reviews on the subject are published frequently (Nault 1997; Van den Heuvel et al. 1999; Gray and Banerjee 1999; Harris et al. 2001; Pirone and Perry 2002; Blanc 2004). However, the viral processes that occur within the plant, before and after the vector intervention, to prepare for efficient acquisition and ensure successful inoculation, have been largely ignored, though some specific transport events may play an important role. This section will first summarize the diversity of the strategies encountered in virus-vector interactions leading to plant-to-plant transport of viruses, and then highlight the few data available on within-plant mechanisms preceding the way out and accompanying the way in.

### 3.1

## **Transport in Vectors**

### 3.1.1

## **Transport of Circulative Viruses**

The term “circulative” was first introduced by Sylvester (1956) and again by Harris (1977) to describe viruses that undergo part of their life cycles within the body of the vector. The term applies to viruses transmitted by arthropod vectors such as mites and mostly insects. Circulative viruses are acquired by vectors feeding upon infected plants. The viruses then traverse the gut epithelium at the midgut or hindgut level (for examples see Reinbold et al. 2003; de Assis Filho et al. 2005), and are released into the haemolymph. The viruses can then adopt various pathways to join and enter the salivary glands, where they are released in the saliva and finally inoculated into healthy hosts, initiating new infection. The latent period—the time required for the virus to complete this cycle—depends on the virus-vector pair and numerous other