MPMI Vol. 9, No. 6, 1996, pp. 435-442. Publication no. M-1996-0506-01O.

Current Review

Vascular Movement of Plant Viruses

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Received 16 October 1995. Accepted 29 March 1996.

Systemic infection of a host plant by a virus is a complex phenomenon entailing viral movement and/or replication in a diverse array of tissues. It has generally been considered to be composed of two steps (Hull 1991; Dawson and Hilf 1992; and references in both). First, the virus enters a wounded cell and the viral proteins are synthesized. One of the nonstructural viral proteins, the movement protein, allows the infectious particles to move from cell to cell until infection reaches the vascular tissues. It does so by potentiating its own movement through plasmodesmata (Waigmann and Zambryski 1995; Fujiwara et al. 1993) and this is sometimes reflected by an increase in the size exclusion limit (SEL) of the plasmodesmata that link mesophyll cells (Wolf et al. 1989; Ding et al. 1992). This first step is commonly referred to as cell-to-cell movement. During the second step, the entire plant becomes infected. To achieve this, the infectious particles must enter, circulate within, and leave the vascular tissues. Most viruses are believed to circulate through the phloem (Leisner and Turgeon 1993; Lucas and Gilbertson 1994), although movement through the xylem has been reported, in particular for viruses transmitted by beetles (Gergerich et al. 1988). The process of systemic plant invasion by viruses has most frequently been referred to as long-distance movement. However, this designation poorly reflects the necessity of the vascular tissue for this movement and can be confused with systemic infection resulting from slow cell-to-cell movement. We therefore favor the designation "vascular movement," and this latter term will be adopted in this review.

Vascular movement is poorly understood, partly because the connections and interactions between the various cell types that compose the vascular system, and between these and other cell types, have not been studied and described in detail (Leisner and Turgeon 1993). In addition, invasion of a plant by a virus requires both cell-to-cell and vascular movement. Hence the two steps leading to systemic infection are tightly linked. As a consequence, the participation of the movement protein in vascular movement is difficult to distinguish from its obligatory role in cell-to-cell movement. For these reasons, more reviews have been devoted to cell-to-cell movement than to vascular movement. Our aim is to present an overall view of recent data on this latter aspect of virus infection.

After vascular movement has been characterized and the general approaches used to study it outlined, two aspects are presented: first, the virus elements reported to be involved in vascular movement; and second, the interactions between virus elements and host elements that affect vascular movement. Since the information provided in this short review cannot be exhaustive, the reader is advised to turn to other recent reviews that have given some attention to vascular movement (Hull 1991; Dawson and Hilf 1992; Leisner and Turgeon 1993; Lucas and Gilbertson 1994; and references therein).

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Characteristics of vascular movement.

Three stages of vascular movement can be distinguished: entry into, translocation through, and exit from the vascular system. These stages have as yet not been characterized. Entry could take place through the plasmodesmata along the side of vascular bundles or through the end of minor veins. Translocation could be from sieve element to sieve element and/or via companion cells. Exit, being the reverse of entry, could also take place across the sides of veins or at the ends.

Viruses move symplastically between mesophyll cells and phloem cells within the vascular tissues of minor veins. Indeed, plasmodesmata are present at every interface from mesophyll cells to vascular cells, although their number and structure vary greatly between different cell types and plant species (Lucas and Wolf 1993; Lucas and Gilbertson 1994; see Figure 1 for a schematic representation). Moreover, lack of protein synthesis in sieve elements implies that viral factors needed for translocation through and exit from the vascular system must follow the virus or the viral genome into the sieve tube.

The structure of plasmodesmata linking various cell types in transgenic tobacco plants expressing the tobacco mosaic tobamovirus (TMV) movement protein has been examined by means of cryofixed sections observed via transmission electron microscopy, and the modification of the structure caused by the movement protein examined by immunogold labeling and dye coupling. The movement protein increases the SEL of plasmodesmata between mesophyll cells. However, it cannot do so in the plasmodesmata linking the bundle-sheath and phloem-parenchyma cells, even though it accumulates in the secondary plasmodesmata linking these cell types (Ding et al. 1992). To circulate in the sieve elements within the vascular tissues and to migrate from these tissues to mesophyll cells, virus and/or host elements are certainly required but no information is available concerning these migration processes.

Vascular movement.

Different approaches can be used to monitor movement of the virus through the vasculature. One of them involves scor-

ing the symptoms observed on the inoculated leaf and on the whole plant to establish directly if vascular movement has occurred. Other assays to determine the extent of vascular movement by the virus include the detection of viral proteins and/or nucleic acids in distinct parts of the plant by Western blots (immunoblots), Northern (RNA) blots, tissue prints, immunocytochemistry at the light or electron microscopy levels, and in situ hybridization. Detection is usually performed on the inoculated leaf and on leaves above the point of infection.

Study of vascular movement is aided if the site within the vasculature where virus movement is impaired can be examined. Here a reporter gene such as β-glucuronidase (GUS) is introduced into the mutated viral genome and infection followed by the detection of the product of the reporter gene. The reporter gene makes it possible to locate the virus within the vascular tissues and to correlate the site at which virus movement has been blocked with the mutations carried by the virus (Cronin et al. 1995). The jellyfish green fluorescent protein (GFP) has also been used as a reporter to study virus infection (Baulcombe et al. 1995) and should prove useful to examine vascular movement in detail. This strategy can be adopted only if the presence of the reporter gene does not impair infection by the virus. Unfortunately not all viruses are easily amenable to such modifications.

An important approach to study viral elements involved in vascular movement is site-directed mutagenesis of a cDNA clone of a virus from which infectious transcripts can be obtained. The infectious transcript containing the mutated sequence is used to infect plants and the consequences of the mutation on vascular movement examined. It can be assumed that a given protein or viral sequence is involved in vascular movement if the mutations introduced do not prevent replication of the viral RNA in protoplasts and cell-to-cell movement within the inoculated leaf, yet prevent invasion of the plant by the virus (Bransom et al. 1995). A related approach involves natural mutants or closely related viruses with specific defects in vascular movement as source material to begin such studies. These viruses supply sequences that already contain specific mutations affecting vascular movement.

This first approach can be complemented by a second one that utilizes transgenic plants. Such plants are used to test whether the expressed transgene-derived protein can complement a mutated virus defective in systemic spread, and restore vascular movement (Dolja et al. 1994).

The behavior of a given virus upon infection of two different plants can be examined. It should for instance be possible to screen a collection of *Arabidopsis thaliana* T-DNA tagged mutants for a defect in vascular movement upon infection with a virus encoding a reporter gene such as the GFP, and to determine at which step the virus is blocked. The mutated gene(s) of the *A. thaliana* mutant(s) would then be characterized. If successful, these experiments could lead to the identification of the host factor(s) involved in vascular movement. A first step in this direction was reported by Simon et al. (1992). The *A. thaliana* ecotype Dijon possesses a resistance gene that restricts vascular movement of turnip crinkle carmovirus (TCV). Such restriction was not observed in ecotype Col-0. Identifying the resistance gene should provide a clue

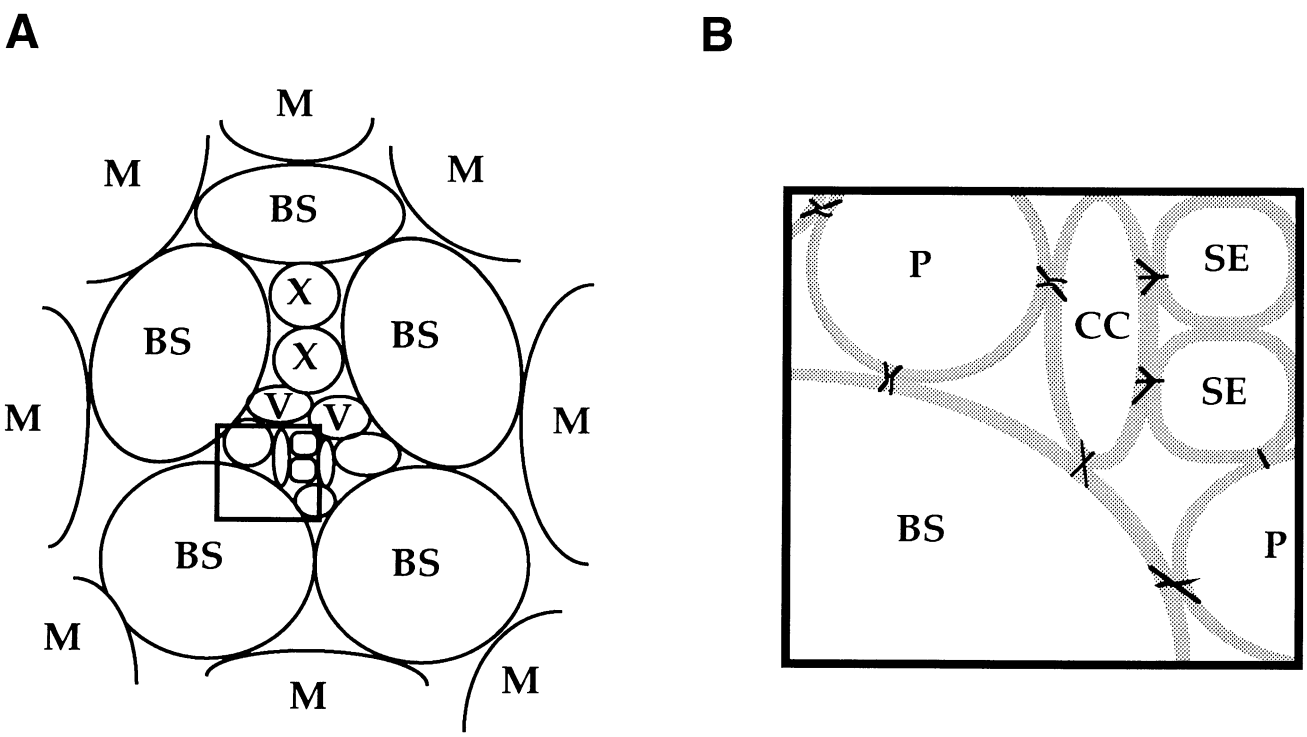

Fig. 1. A, Schematic representation of the cross-section of a vascular tissue. Mesophyll (M) and bundle sheath (BS) cells surround a minor vein comprising xylem (X), vascular parenchyma cells (V), and companion cells and sieve elements (framed). **B,** Schematic representation of the conducting cells (enlargement of frame presented in **A**). Phloem parenchyma cells (P), companion cells (CC), and sieve elements (SE) are linked by plasmodesmata (black lines) whose numbers differ between the various cells within the vein. The plasmodesmata linking the companion cells and sieve elements possess deltoid-shaped structures.

concerning the nature of the host factor involved in vascular movement.

In most of the experiments described below, viruses are mechanically inoculated into the plant. Therefore they may not enter the same cells as when they are introduced by their natural vector (generally an insect). However, once in the inoculated leaf, it can be assumed that the virus will follow its natural route to the vascular system and to the leaves above the site of infection. Hence, mechanical inoculation seems valid as a means to study vascular movement. Agroinoculation is less valid since it does not allow distinction of a defect in systemic infection due to a block of cell-to-cell movement or vascular movement.

Developmental conditions of the host.

Culture conditions and the developmental stage of the host plant can have a strong impact on vascular movement of certain viruses. The involvement of the developmental stage of the host plant in systemic infection has been mainly examined with cauliflower mosaic caulimovirus (CaMV) in turnip (Leisner et al. 1992) or in *A. thaliana* (Leisner et al. 1993). It was demonstrated that CaMV moves passively throughout the plant within the phloem, following the flow of photoassimilates from source to sink leaves. This flow fluctuates depending on the growth and developmental conditions of the plant (reviewed in Leisner and Turgeon 1993). Further studies with *A. thaliana* (Leisner et al. 1993) have demonstrated that as the plant ages, fewer rosette leaves (other than those initially inoculated) become infected by vascular transport of the virus. This correlates with a decline in flow of photoassimilates to mature leaves.

It was also shown for CaMV that light plays a role in systemic infection (Wintermartel et al. 1993). The authors identified domains within gene VI of CaMV that influence systemic infection of *Nicotiana bigelovii* in a light-dependent manner. They postulate that light could change the cellular environment in such a way that the product of gene VI would be unable to interact properly with host components either for post-transcriptional transactivation, vascular movement, or elicitation of a defense response.

The growth conditions of the host plant also affect the vascular movement of red clover necrotic mosaic dianthovirus (RCNMV). In *N. benthamiana* the RCNMV coat protein (CP) is required for systemic infection when the plants are grown at 25°C but not when they are grown at 15°C (Xiong et al. 1993). The molecular mechanism underlying this difference in virus movement is not understood. Moreover, in *N. clevelandii* the CP is indispensable for vascular movement at both temperatures.

The fact that vascular movement is strongly influenced, or even controlled, by the physiological status of the host plants and that a single virus mutant might show two opposing phenotypes on different host plants means that some caution must be exerted before reaching firm conclusions about the role of particular viral gene products in virus movement.

Involvement of virus elements.

CP. The CP is the viral protein to which a function in vascular movement has been most frequently ascribed. It appears to be a fundamental element in the vascular movement of most viruses but it influences vascular movement in different ways depending on the virus.

For some viruses, the CP is not required for cell-to-cell-movement but is an essential co-factor for vascular movement. Indeed, deletions and/or site-directed mutageneses performed in the CP open reading frame (ORF) of a number of viruses have shown that the resulting mutants are unable to move throughout the plant. This is the case with turnip yellow mosaic tymovirus: mutants that result in truncated CP or absence of CP expression can move from cell to cell, but their vascular movement is abolished (Bransom et al. 1995). It is generally accepted that TMV requires the CP to efficiently move throughout the plant (Takamatsu et al. 1987; Dawson et al. 1988; and references in both), but that the CP is not needed for cell-to-cell movement. It has been proposed that the CP must be capable of assembling into virions, and therefore that the origin of virus assembly must be present for vascular movement to occur (Saito et al. 1990). This conclusion was reached by introducing various mutations into the CP ORF or in the assembly origin, and performing bioassays, immunoblots, or sucrose gradients to examine CP accumulation or virion accumulation in extracts of inoculated leaves or upper leaves. However, the possibility that an informosome-like ribonucleoprotein complex that would include viral RNA and CP, but not in the form of a virion (Dorokhov et al. 1983; Dorokhov et al. 1984), has not been totally disproved as a movement form.

In other instances, the CP is required for both cell-to-cell and vascular movement. The involvement of the CP in these different processes can be resolved by mutation of the CP or co-inoculation with a second virus. This applies to beet necrotic yellow vein furovirus (BNYVV; Quillet et al. 1989). A fortuitous point mutation in the CP gene of a recombinant clone of BNYVV RNA2 did not affect infectivity of the transcribed RNA, but prevented virus encapsidation and vascular movement, probably as a result of packaging deficiency. In the case of tobacco etch potyvirus (TEV), the CP is required for vascular movement (Dolja et al. 1994; Dolja et al. 1995). Indeed, vascular movement of a TEV mutant defective in the CP can be rescued by a transgenic plant expressing the CP. The variable N- and C-terminal regions of the CP, believed to be exposed on the virion surface, are necessary for vascular transport. Removal of either region abolishes vascular movement, but still allows slow cell-to-cell movement. It has been postulated that these terminal regions interact with host factors during migration of the virus. However, the precise step at which such interactions occur has not been established. The common bean can be systemically infected by sunn-hemp mosaic tobamovirus (SHMV), but is resistant to infection by the cowpea strain of southern bean mosaic sobemovirus (SBMV-C). Co-inoculation of the two viruses allows SBMV-C to spread in the inoculated leaf, yet not throughout the plant, and no SBMV-C capsid is present in the vascular tissues. Lack of efficient assembly of SBMV-C virions appears to be responsible for the inability of the virus to spread throughout the plant even though it does not prevent cell-to-cell movement (Fuentes and Hamilton 1993).

There are also examples in which the CP is required for both cell-to-cell and vascular movements, but for which CP mutants affecting vascular movement also affect cell-to-cell movement; this means that the two processes cannot be resolved. In cucumber mosaic cucumovirus (CMV), a small internal deletion in the N-terminal region of the CP still allows

cell-to-cell movement of the virus yet abolishes vascular movement, suggesting that viral assembly may be required for translocation throughout the plant. Larger deletions also abolish cell-to-cell movement (Suzuki et al. 1991). Similarly, deletion of the N-terminal 25 amino acids of the brome mosaic bromovirus (BMV) CP prevents vascular movement and packaging of the viral RNA in protoplasts (Sacher and Ahlquist 1989). To demonstrate that the CP of TCV is involved in both cell-to-cell and vascular movement (Heaton et al. 1991; Laakso and Heaton 1993), two families of mutants were produced. Infection of plants with members of one family, containing mutations in the hinge between the protruding and the shell domain of the CP, showed that RNA accumulation was efficient in the inoculated leaves and protoplasts (Heaton et al. 1991), indicating that replication and cell-to-cell movement of these viruses were mostly unaffected. With members of the other family, bearing mutations in a putative calcium-binding site of the CP, RNA accumulation was dramatically reduced in the inoculated leaves of systemic hosts even though virus accumulation in protoplasts was comparable in the mutant and wild-type virus (Laakso and Heaton 1993). With members of either family, vascular movement was abolished. A comparable situation is observed with CP mutants of rice yellow mottle sobemovirus. Such mutants did not develop symptoms, and neither virus particles nor the truncated CP could be detected in inoculated leaves or in upper noninoculated leaves. Replication of the viral RNA, however, did occur in inoculated leaves. These results underline the importance of the CP for vascular movement and possibly also for cell-to-cell movement (Brugidou et al. 1995). Requirement for the movement protein as well as the CP has been investigated using cowpea chlorotic mottle bromovirus (CCMV; Allison et al. 1990). When cowpea plants were inoculated with wild-type RNA1 and RNA2, and two types of deletion mutants of RNA3, in the 3a protein and the CP ORF, respectively, systemic infection proceeded normally due to recombination between the mutated RNAs. Consequently, the combination of functional 3a protein and CP has a selective advantage for vascular movement. It has been proposed that the 3a protein and the CP actively participate in the transport of the infectious material, and/or participate in bypassing or overcoming active host defenses. Certain viruses such as cowpea mosaic comovirus (Wellink and van Kammen 1989) and potato potexvirus X (Chapman et al. 1992) require the CP to move from cell to cell. Although no data is available concerning their vascular spread, it seems very likely that at this step also the CP is required.

On the other hand, for some viruses the CP is not mandatory for vascular movement, implying that the infectious particle moving through the vascular tissues is a ribonucleoprotein. Among geminiviruses, for instance, those with a bipartite DNA genome do not require the CP for systemic spread of the virus. In African cassava mosaic geminivirus, large deletions in the CP gene are tolerated for vascular movement, provided the size of the mutated DNA is maintained (Etessami et al. 1989). Total removal of the CP gene from tomato golden mosaic geminivirus does not affect systemic spread of the virus (Gardiner et al. 1988). In contrast, for geminiviruses with a monopartite genome, the CP is indispensable for virus movement (Boulton et al. 1989). RNA viruses for which mutants with large deletions in the CP gene were produced, such as for cucumber necrosis tombusvirus (McLean et al. 1993) and Cymbidium ringspot tombusvirus (Dalmay et al. 1993), still infect host plants systemically albeit to a considerably lower extent than wild-type virus. When barley (but not *N. benthamiana*) plants are inoculated with wild-type RNA α and γ of barley stripe mosaic hordeivirus (BSMV) and RNA β deleted in the CP gene, vascular movement is not affected (Petty and Jackson 1990). In tomato bushy stunt tombusvirus (TBSV), CP expression is dispensable for cell-to-cell and vascular movement. The symptoms produced in the absence of intact CP are similar to those produced by TBSV containing the intact CP (Scholthof et al. 1993). In tobacco rattle tobravirus (TRV) the CP cistron is dispensable for systemic infection (Harrison and Robinson 1986). This was demonstrated with the NM-type isolates, which only contain RNA1. In the absence of RNA2, no virus particles can be formed, yet infection spreads throughout the plant and induces typical symptoms. If, as has been postulated by Harrison and Robinson (1986), invasion of the plant does not occur via phloem sieve tubes, then TRV NM-type infections probably do not involve phloem loading. It is not clear in this case whether the systemic movement is vascular movement or fast cell-to-cell movement.

Replicase protein. The masked strain of TMV (M-TMV) is a very peculiar strain that produces mild symptoms in infected *N. tabacum* due to impeded phloem-dependent accumulation (Nelson et al. 1993). The difference between common TMV and M-TMV resides in the ORF encoding the 126K protein and the 183K readthrough protein, in and between the domains that code for the putative methyltransferase and helicase (Holt et al. 1990). This constitutes indirect evidence for the involvement of the replicase protein or the encoding ORF in vascular movement. A recent study (Ding et al. 1995b) has demonstrated that when plants are inoculated with transcripts derived from M-TMV cDNA, progeny virus accumulates in fewer vascular parenchyma and companion cells than when plants are inoculated with transcripts derived from the common TMV strain; this reflects a cell type–specific delay that is correlated with the differences in the 126K ORF of these two strains. Vascular movement of M-TMV seems to be impaired at the level of the entry of the virus into the vascular tissues, and vascular parenchyma and companion cells could be considered potential barriers for systemic infection. Thus, impeded entry of the virus into these cells rather than its inability to replicate could account for the absence of systemic infection of M-TMV.

CMV RNA1 has long been thought to be involved only in virus replication. However, a comparison of the rates of accumulation of two strains of CMV in a given host plant has now shown that sequences on RNA1 can regulate the rate of movement of the virus throughout the plant (Gal-On et al. 1994). For the BMV 2a protein, which is one of the two nonstructural proteins involved in replication, mutations in regions that still support strong RNA replication in protoplasts prevented the production of symptoms on inoculated or upper noninoculated leaves of barley plants. However, accumulation of virus in inoculated leaves analyzed by Northern blots was somewhat inhibited compared with the wild-type virus (Traynor et al. 1991). Therefore, for BMV and CMV vascular transport is inhibited, but the impact of inhibited cell-to-cell movement displayed by the mutant viruses must be considered.

In the case of BSMV the αa protein plays a role in vascular movement and in host specificity (Weiland and Edwards 1994). Two BSMV strains that differ in their effect on oat, one being pathogenic and the other nonpathogenic, were used to show that the determinants responsible for this difference reside in RNAα. Recombinants between RNAα of the two strains support the conclusion that the αa protein sequence may dictate whether or not the protein can interact with host components to allow cell-to-cell or vascular movement in oats.

Other nonstructural proteins. Other nonstructural proteins that do not participate in replication can be involved in vascular movement. This is the case for the helper component proteinase (HC-Pro) of TEV (Cronin et al. 1995). This protein, which is required for aphid transmission, is also necessary for systemic infection. Experiments were performed with a construct harboring the GUS reporter gene immediately upstream of the HC-Pro gene. A mutant in the HC-Pro of this construct was impaired in vascular movement but was nevertheless able to move from cell to cell since it could reach the sieve elements. This indicates that the defect in vascular movement was associated with a late step in the movement pathway such as trafficking within sieve elements or exit from the vascular tissues. Since, as mentioned above, the CP of TEV is required for vascular movement, the authors postulated that, as for transmission of the virus by aphids, vascular movement requires interactions between HC-Pro and the CP.

SHMV moves slowly from cell to cell in *N. tabacum* and is unable to invade the whole plant, as opposed to TMV, which systematically infects *N. tabacum*. Chimeric viruses were constructed in which the movement proteins of the two viruses were exchanged (Deom et al. 1994). The general conclusion reached with these chimeras was that the movement protein is not directly involved in the ability or inability of these tobamoviruses to invade the plant but that other virus components are required for systemic infection.

Recently, the expression of an ORF (ORF 2b) that overlaps the 2a ORF in RNA2 of CMV was shown to facilitate vascular movement of the virus in an host-specific manner. However, expression of ORF 2b was not required for cell-to-cell movement of the virus (Ding et al. 1995a).

Other ORFs or leader RNA sequences. A case has been reported of the involvement of short ORFs preceding normal coding regions in vascular movement. RNA γ of the type strain of BSMV contains a short ORF within the 5′ leader sequence preceding the ORF for γa protein. This strain is unable to infect *N. benthamiana* systemically. However, a mutation in the initiator AUG of the short ORF allows vascular movement of the virus (Petty et al. 1990). Lack of vascular movement of the wild-type RNA could be due to the fact that the intact small ORF would decrease translational efficiency of the 5′ proximal γa gene rather than to a direct effect of the 5′ leader sequence.

Involvement of CaMV virus elements. The ability of CaMV to infect members of the Solanaceae is strain specific and involves a variety of gene regions. However, systemic spread of CaMV is extremely complex. The use of chimeras with strains that do or do not infect members of the Solanaceae has revealed that genes I, II, IV, V, and VI as well as the large intergenic region are involved in vascular movement (Qiu and Schoelz 1992; and references therein).

Interactions between virus elements and the host.

Studies performed with chimeric viruses indicate that in some cases, host specificity is correlated with the ability of a virus to invade one host and its restriction to the site of infection in another host.

A well-studied example is that of TMV and Odontoglossum ringspot tobamovirus (ORSV; Hilf and Dawson 1993). Both viruses infect *N. tabacum* but in different ways. TMV produces systemic infection whereas ORSV is restricted to the inoculated leaf. A chimeric virus was constructed in which the ORSV CP was inserted in place of the TMV CP within the genome of TMV. This recombinant virus was unable to infect *N. tabacum* systemically. It thus appears that the CP of tobamoviruses may be required to interact with host components in order to have an impact on vascular movement. Such interactions could occur at the junction between mesophyll cells and vascular cells (reviewed in Lucas and Gilbertson 1994).

Studies on CMV and tomato aspermy cucumovirus (TAV) also show that the role of the CP in vascular movement is linked to host specificity (Taliansky and García-Arenal 1995). CMV and TAV share systemic host plants. However, whereas CMV systemically infects cucumber, TAV does not. Coinoculation with both viruses allows TAV to invade the plant. Inoculation of TAV with one or the other of the CMV genomic RNAs showed that RNA3 is sufficient to complement vascular movement in cucumber. Introduction of mutations in either the 3a protein or the CP gene of CMV RNA3 demonstrated that the CP is responsible for host-specific vascular movement of the virus.

As discussed earlier, the CP and 3a protein are required for systemic spread of CCMV (Allison et al. 1990). Construction of viral hybrids in which the 3a proteins of BMV and CCMV were exchanged led to the conclusion that the 3a protein plays a crucial role in host specificity (Mise et al. 1993). The hybrids failed to systemically infect their selective parental host, barley for BMV and cowpea for CCMV, even though they retained their capacity to replicate and to move from cell to cell. On the other hand, a study with different cowpea lines has led to the proposal that systemic movement of CCMV in cowpeas is controlled by a single dominant gene (Kuhn et al. 1981). A recent study on the BMV CP indicates that the CP is also involved in host specificity, dictating whether or not systemic spread of the virus will take place (Flasinski et al. 1995). Deletion of the seven N-terminal amino acids of the BMV CP prevents systemic infection of the virus in *Chenopodium hybridum* but not in barley, both of which are systemic hosts of BMV. These experiments suggest that the BMV CP and host factors are important for vascular movement.

Soybean PI 346307 is resistant to CCMV. No symptoms are expressed and virus concentration in uninoculated leaves is low. This nonnecrotic resistance contrasts with the effects observed with the susceptible soybean cultivar Davis. Immunocytochemical studies of the resistant and susceptible cultivars revealed that cell-to-cell movement of CCMV was the same in both cultivars. However, the virus antigen was virtually absent from the vascular tissues of PI 346307, but present in the Davis cultivar. Consequently, nonnecrotic resistance in PI 346307 is due to restriction of virus entry into and/or out of the vascular tissues (Goodrick et al. 1991).

It should be borne in mind that a virus component could possibly interact with a host component and activate defense

reactions. In such a situation, a decrease in vascular movement would be due to activation of defense rather than to a direct effect of the virus component on vascular movement.

Another explanation for the effects observed could be that delayed vascular movement would indirectly result from reduced replication and/or cell-to-cell movement (Dawson and Hilf 1992; and references therein).

Conclusions.

Because of the apparent simplicity of viruses and the small size of their genome, it is relatively easy to establish which viral elements are involved in a given step of the virus life cycle, and decipher their mechanisms of action. This contrasts with the complexity of the host and explains the difficulties encountered in defining, for a given virus-host couple, the host elements that participate in virus vascular movement.

For example, it seems well established that virus elements such as the CP, nonstructural viral proteins such as those involved in replication, the movement protein and HC-Pro, and even untranslated regions of the genome most certainly interact with host factors during vascular movement. However, no host component has so far been characterized. It is therefore difficult to determine whether interaction between viral and host elements allows vascular movement, or rather if lack of interaction permits vascular movement.

The host factors could, for instance, be plasmodesmatal proteins since movement of some viruses into or out of certain phloem cell types is controlled. They could correspond to receptors able or unable to interact with virus components such as the CP (Hilf and Dawson 1993). If such receptors were encoded by a resistance gene, movement of the virus would be blocked. For instance, the gene(s) involved in nonnecrotic resistance towards CCMV of a particular soybean cultivar may encode altered receptor or plasmodesmatal proteins, such that interaction of the resistance gene product with the virus element would block entry of the virus into the phloem. This would suggest that an active host-specific function of the virus element exists. It would also imply that the plasmodesmata of vascular and mesophyll tissues differ functionally. Ding et al. (1992) and Ding et al. (1995b) have determined, through the analysis of SEL of plasmodesmata and the accumulation characteristics of two TMV strains, respectively, that the connections between the bundle sheath cells and the phloem parenchyma cells of veins in tobacco appear to have different characteristics from those between mesophyll and bundle sheath cells, thus supporting the idea that vascular and mesophyll tissues differ functionally.

The fact that the CP of most viruses is needed for vascular movement suggests that most viruses move in the vascular system as intact virions. In the case of a virus such as TMV, which moves from mesophyll to mesophyll cell as a ribonucleoprotein, encapsidation of the viral RNA would be required to allow movement of the infectious particles between phloem cells or within the sieve elements as intact virions. On the other hand, some viruses do not need the CP for vascular movement; these viruses are thus able to move throughout the vascular tissues, presumably as RNA-movement protein complexes.

At least two reasons can be put forward to explain the gap that exists in our knowledge of the host elements involved in vascular movement.

First, for a given virus-host interaction, it is imperative to distinguish the events occurring at three levels: in infected protoplasts, in the infected leaf, and in leaves above the point of infection. Indeed, one cannot determine if a host element is involved in vascular movement when the study does not include these three levels of examination necessary to pinpoint that vascular movement alone is affected. A survey of the scientific literature unfortunately reveals that one of these three levels of examination is frequently missing, making interpretation of the results more difficult to establish. Such studies should be greatly facilitated by the development of new assay systems that would be independent of exogenous substrates or cofactors. A recent example is provided by GFP whose easy fluorescence-based detection make it a potentially attractive marker to investigate virus movement.

Second, in spite of the pioneering work of several groups, it is clear that a better understanding is required of the structure and function of the various cell types composing the vascular tissues, and of the interactions between them, and between them and mesophyll cells.

The experiments already undertaken to compare the intracellular localization of viruses and/or virus elements in a systemic host and in a resistant host constitute a valuable approach in deciphering host elements. It can be hoped that once similar experiments have been performed with a number of viruses and host plants, we shall better understand the host elements involved in vascular movement.

ACKNOWLEDGMENTS

We are grateful to D. Chriqui for useful discussions. The first author is recipient of a fellowship from the "Ministère de la Recherche et de l'Enseignement Supérieur." The Institut Jacques Monod is an "Institut Mixte, CNRS - Université Paris 7."

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