The silence of the RAMs – viral suppression in Root Apical Meristems

T.A. Valentine, M. Dorward & K.J. Oparka

Systemic plant viruses possess the capacity to move both cell-to-cell (local movement) and also over long distances in the vascular system (systemic movement). Both local and systemic components of the viral movement process appear to be under the control of one or more viral gene products synthesised during the infection process. As a general rule, systemic viruses follow the pathway of assimilate translocation in the plant[1], moving from source tissues to sink tissues. To date, however, the systemic movement of viruses has been studied almost exclusively in leaves, and almost nothing is known of the pattern of virus infection in roots, despite the fact that several viruses invade the root systems of many commercially important crop species.

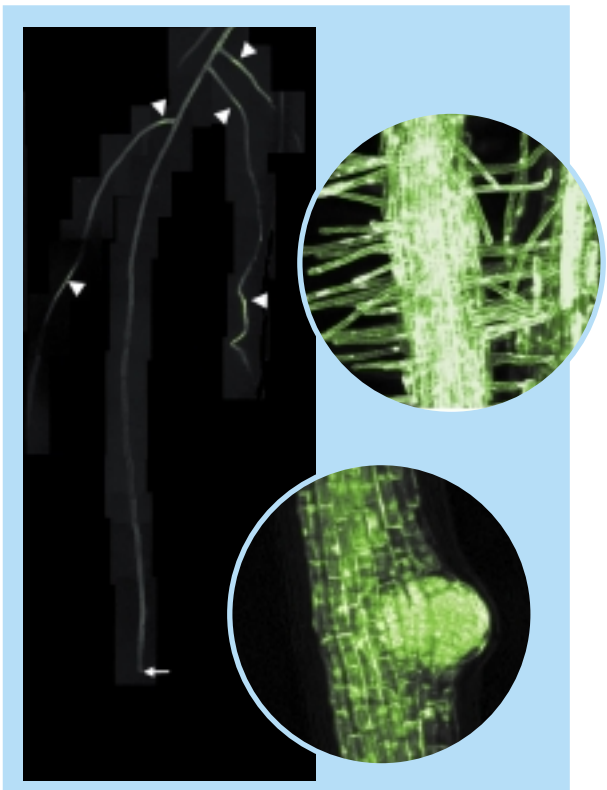

Figure 1 Distribution of TMV.GFP in *N.Benthamiana* root as indicated by GFP. Triangles indicate the first unloading points of TMV from the phloem, which are visible as green flecks. Arrow indicates primary root apex. Upper insert: GFP expression in a root highly infected with TMV.GFP. Lower insert: Developing lateral root primordium highly infected with TMV.GFP.

It appears that, in both roots and shoots, the apical meristem is largely devoid of virus infection. The inability of viruses to invade apical meristems has been exploited commercially and forms the basis of meristem-tip culture, a method by which virus-free clones can be obtained by growing excised shoot tips in tissue culture[2]. In general, a zone close to the apical meristem (usually within 0.1mm) is free of replicating virus. Why do meristems fail to support virus replication? One possibility is that a barrier to infection is established by cells located some distance from the functional meristem, although such a putative barrier has yet to be established[2]. A second hypothesis for the ability of meristems to escape infection is that a mechanism for viral gene silencing occurs in meristematic cells that targets the viral RNA for degradation, allowing newly differentiated tissue to escape infection. There is now substantial evidence for a strong parallel between the phenomenon of post-transcriptional gene silencing (PTGS), in which transgenes are silenced by a sequence-specific RNA degradation process, and the RNA-mediated defence that occurs against plant viruses[3]. In an ongoing collaboration with Large Scale Biology Corporation, we have examined the capacity of a range of systemic plant viruses, tagged with the green fluorescent protein (GFP), to invade the root system of the host plant *Nicotiana benthamiana*. In particular, we were interested in the ability of virus to enter and replicate within the root apical meristem (RAM). The results presented here relate specifically to the movement of tobacco mosaic virus (TMV).

Viral invasion of root systems Plants infected with TMV.GFP were imaged non-invasively within petri dishes using a confocal laser scanning microscope (CLSM). In this way, root systems could be imaged over several consecutive days without affecting their development. Fluorescence within the primary root system, indicating TMV replication, was visible at 5-10 days post-inoculation as intermittent 'streaks' arising from the phloem of the vascular cylinder (Fig. 1). With time these streaks spread longitudinally, and also radially outward into the cortex and root hairs (Fig. 1, inset). Contrary to our expectations, the lateral root primordia that formed within infected roots became highly fluorescent, and were visible as bright plaques of cells within the root stele. As the lateral pri-

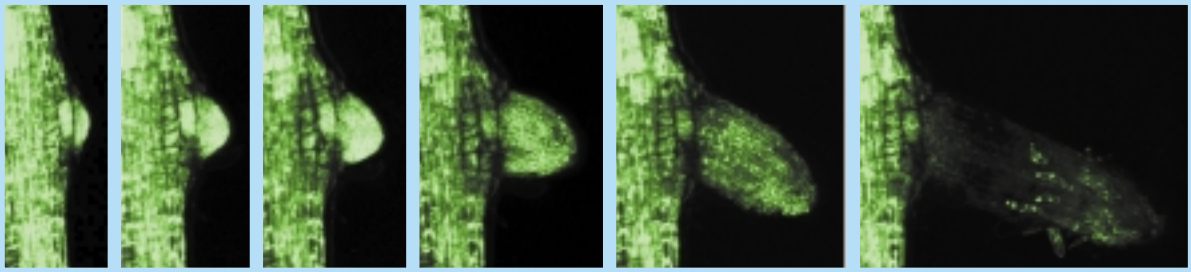

Figure 2 Developmental sequence of a lateral root primordium into a fully functional lateral root. Initially the primordium is highly fluorescent due to infection of cells with TMV.GFP. As the root develops, areas of reduced fluorescence appear near the apex. These areas of viral suppression gradually spread throughout the lateral root. The primary root does not diminish in fluorescence.

mordium emerged, the cells within it remained highly fluorescent due to the presence of replicating virus (Fig. 1, inset).

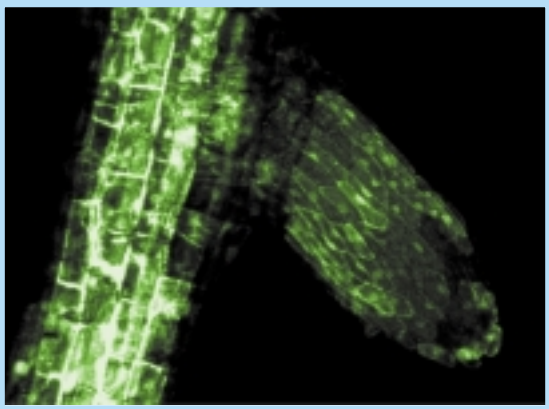

Figure 3 Lateral root beginning to undergo viral suppression. Note the dark non-fluorescent region at the tip of the root.

Viral suppression in emerging lateral roots As the lateral roots began to elongate, they gradually lost fluorescence, despite the maintenance of unaltered levels of fluorescence in the primary root. We imaged, non-invasively, the emergence of over 30 infected lateral roots, and all of these showed an identical pattern of viral suppression. A typical sequence is shown in Figure 2. By the time the lateral roots had reached an approximate length of 200 µm, a distinct zone lacking GFP fluorescence was observed close to the RAM (Fig. 3). With time this 'cone' of suppression spread basipetally along the lateral root up to the point of its connection with the infected primary root. In all suppressed roots, a sharp demarcation was observed between the base of the lateral root and its connection with the primary root. Primary roots did not decrease in fluorescence, indicating that the signal for viral suppression did not spread into mature infected cells of the primary root system.

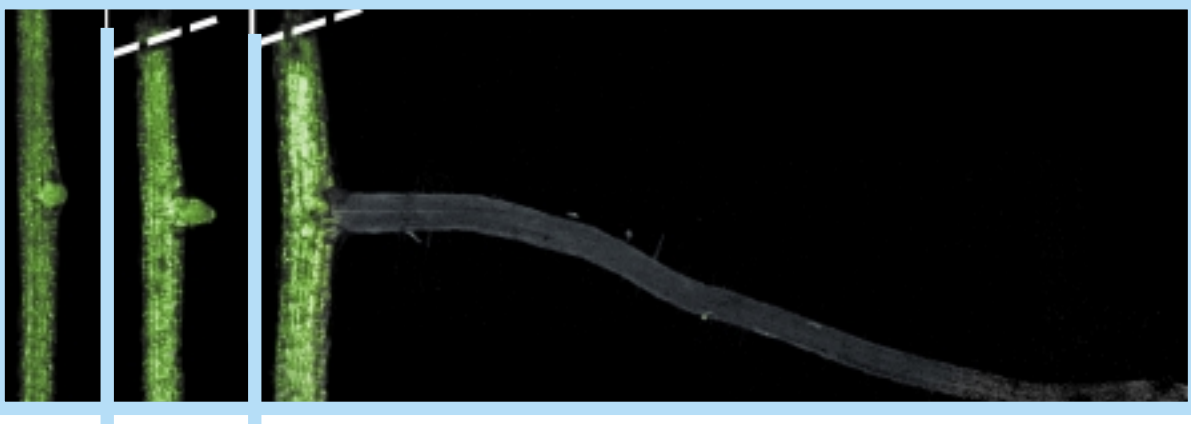

Figure 4 Removal of communication with the shoot. To investigate whether a signal for viral suppression was being transported to developing lateral roots from the shoot, primary roots were cut just above the developing lateral roots prior to the onset of viral suppression (dotted line). Lateral roots continued to undergo viral suppression after this treatment.

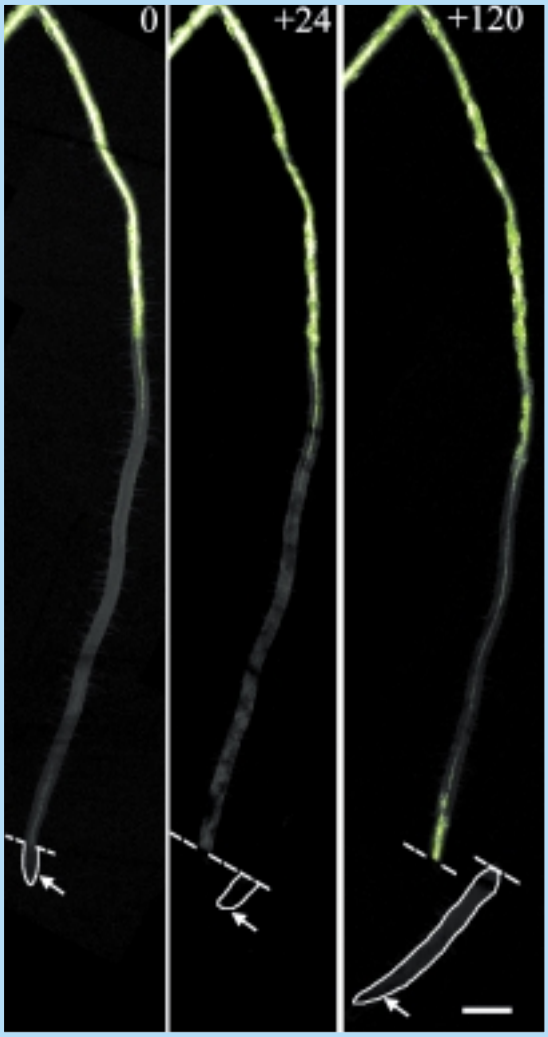

Figure 5 Removal of communication with the root meristem allows virus to replicate up to the excision point. The cut meristem continues to grow but remains uninfected.

The signal for viral suppression originates in the lateral RAM In order to examine the origin of the viral suppression signal, we removed the source tissues of seedlings by severing the primary root directly above the first detectable lateral root initials (Fig. 4). The roots were then observed for a further 3-4 days. During this time, the lateral roots continued to emerge and underwent a progression of viral suppression identical to that observed in intact, infected plants (Fig. 4). These results suggested that the signal for viral suppression did not arise in source tissues, and we concluded that a phloem-mobile signal from the shoot was unlikely to have been the trigger for viral suppression. In a second series of experiments, we surgically removed the apical regions of infected roots, and monitored these roots for a further 4 days. During this time, replicating virus moved into the non-fluorescent cells behind the RAM, up to the point of meristem excision (Fig. 5). However, no virus infection was established in cells apical to the excision point (Fig. 5). Collectively, these data suggested that the onset of viral suppression in the subapical region of the root was brought about by a RAM-derived signal that moved basipetally.

What is the mechanism of viral suppression? We are investigating further the mechanism of viral suppression in developing root systems. Although many of our observations are consistent with a PTGS-like mechanism, we have not shown, unequivocally, that viral suppression results directly from a gene-silencing mechanism in which the viral RNA was detected and

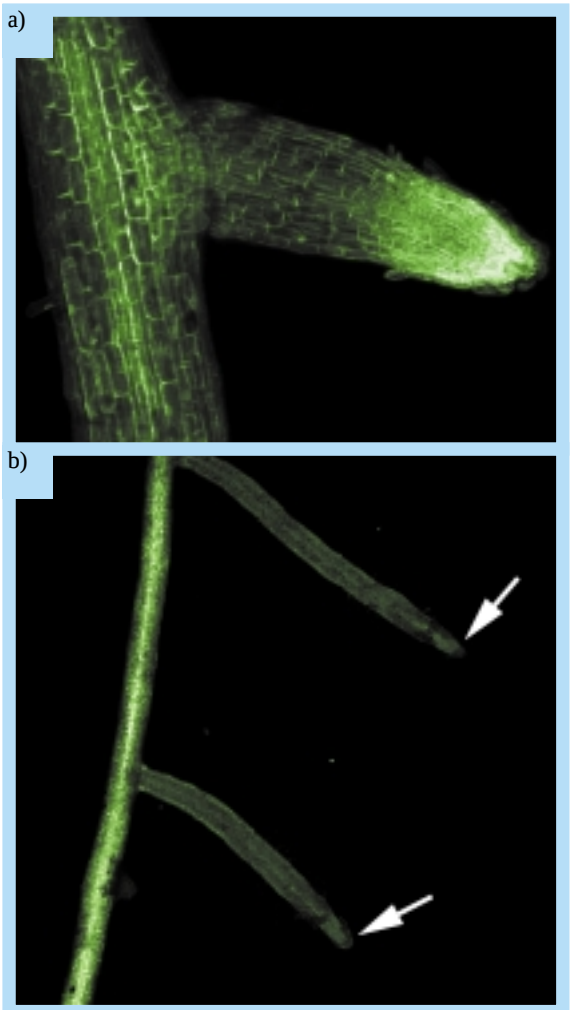

Figure 6 Potential for virus induced gene silencing: (a) Lateral root of a plant expressing GFP from the 35S promoter. (b) Root of a plant expressing GFP from the 35S promoter, infected with TMV.GFP. The lateral roots suppress both the viral GFP and the integrated 35S GFP.

degraded by a host surveillance system[4]. It remains possible that viral replication was impeded by more indirect mechanisms, such as the degradation of one or more viral proteins, or competition of viral RNA with host RNA for translational machinery in meristematic cells. However, we now have evidence that the signal moves through plasmodesmata, and is impeded from entering the primary root due to a symplastic barrier (functional loss of cell-cell communication) at the junction between the lateral and primary root systems (data not shown).

Utility of viral vectors in silencing host-root genes Contrary to published dogma, the above studies have shown that at least some systemic viruses are able to enter the RAM. Regardless of the mechanism of viral suppression, the ability of these viruses to replicate within the RAM, albeit transiently, prompted us to explore the possibility of silencing host-root genes using a virus-vector system. This approach involves the insertion of a host gene sequence into the virus vector and the subsequent silencing of this host gene by a PTGS-like mechanism[4]. This phenomenon, known as virus-induced gene silencing (VIGS), has been described elsewhere for shoot systems[4] and may be a useful tool in plant genomics studies. To examine the potential for VIGS in roots, we utilised transgenic *N. benthamiana* plants that expressed GFP constitutively under a 35S promoter[5]. In these plants, the GFP was conspicuous in the lateral RAM (Fig. 6a). We then inoculated these plants with a TMV vector carrying the complementary *gfp* sequence and examined the plants for subsequent loss of the endogenous GFP signal. At 20 days post inoculation, fluorescence was completely absent in many emerging lateral roots, indicating that silencing of the endogenous *gfp* had occurred (Fig. 6b). Uninfected root systems, or root systems infected with TMV lacking the *gfp* gene, failed to show silencing (data not shown). These observations indicate the potential for analysing root-gene functions using systemic viral vectors.

A developmental link between viral suppression and meristem activation? Why does viral suppression in lateral roots occur some time after the lateral root has emerged? Recent studies on *Arabidopsis* provide a clue. Lateral roots are initiated by anticlinal cell divisions in the pericycle, a single layer of cells found immediately within the root endodermis. One of the first events in lateral-root formation is the onset of anticlinal divisions of individual pericycle cells as these undergo re-entry into the cell cycle[6]. We found that the pericycle initials that give rise to lateral primordia became heavily infected with virus during this early stage in the formation of the lateral root primordium. Malamy and Benfey (1997)[6] have found that, during the emergence phase, the number of cells in the primordium epidermis and root cap remain constant, suggesting that growth during the emergence stage is achieved largely through cell expansion within the primordium. After emergence, the number of cells begins to increase, and new cells appear near the apex. The root then starts to grow via new cell divisions at the apex, concomitant with the formation of a functional lateral RAM. This stage is referred to as 'meristem activation', and occurs when the emerging lateral root has achived a length of approximately 200 µm[6]. This is precisely the point at which we observed viral suppression in the emerging lateral root, suggesting that the signal for viral suppression was initiated in the newly formed lateral RAM. A clear challenge for the future will be to isolate and characterise the host mobile signal(s) that give rise to viral suppression in root systems and to dissect their molecular mode of action.

References

1 Oparka, K.J. & Santa Cruz, S. (2000). *Annual Review of Plant Physiology and Plant Molecular Biology* 51, 323-347.

2 Matthews R.E.F. (1991). *Plant Virology* (3rd Edition). Academic Press, Inc., San Diego.

3 Ratcliff, F., Harrison, B.D. & Baulcombe, D.C. (1997). *Science* 276, 1558-1560.

4 Baulcombe, D.C. (1999). *Current Opinion in Cell Biology* 2, 109-113.

5 Voinnet, O., Vain, P., Angell, S. & Baulcombe, D.C. (1998). *Cell* 95, 177-187.

6 Malamy, J.E. & Benfey, P.N. (1997). *Development* 124, 33-44.