Restricted Movement of a Temperature-sensitive Virus in Tobacco Leaves is Associated with a Reduction in Numbers of Plasmodesmata

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SUMMARY

The LS1 isolate of tomato mosaic virus (a strain of tobacco mosaic virus) replicates and moves from cell to cell in intact tobacco leaves at 22 °C but is restricted in movement when leaves are maintained at 32 °C, although it can replicate at that temperature. Electron microscopy of thin sections revealed a significantly lower number of plasmodesmata between LS1-infected palisade and/or mesophyll cells from leaves held at 32 °C than from those held at 22 °C. There were also substantially fewer plasmodesmata at 32 °C in LS1-infected tissue when compared with tissue infected with the type strain of tomato mosaic virus (L) which moves from cell to cell at the higher temperature. There were no differences in numbers of plasmodesmata in uninfected tissue at either temperature. No qualitative differences in the structure of plasmodesmata were observed under any conditions tested.

It is believed that plant viruses move from cell to cell through plasmodesmata, the protoplasmic strands extending from one cell to another through the cell wall (Fig. 1). This belief is based on evidence that plant viruses cannot pass through intact cell walls, and on the presence of virus particles in plasmodesmata in infected tissue viewed in sections with the electron microscope (Matthews, 1970; Esau, 1968). One virus (dahlia mosaic virus) has been reported to modify the structure of plasmodesmata, presumably facilitating passage of virions through the cell wall (Kitajima & Lauritis, 1969).

Recently, Nishiguchi et al. (1978, 1980) discovered a temperature-sensitive isolate of tomato mosaic virus (a tobacco mosaic virus strain designated LS1) which replicates and moves from cell to cell at a permissive temperature (22 °C), and replicates in cells and protoplasts but cannot move from cell to cell at a restrictive temperature (32 °C).

We investigated the qualitative and quantitative changes in plasmodesmata in tissue infected with LS1 at the restrictive temperature, which might explain the restriction of cell-to-cell movement. Although the ultrastructure of individual plasmodesmata did not change, there was a marked reduction in the numbers of plasmodesmata in tissue infected with LS1 at the restrictive temperature.

The LS1 isolate was obtained from Dr M. Nishiguchi, as was the type strain of tomato mosaic virus (designated L) which moves from cell to cell at either temperature. Fully expanded leaves of Turkish tobacco (Nicotiana tabacum L. cv. Samsun) were manually inoculated with LS1 and L on opposite half-leaves. The plants were incubated in a growth chamber at 22 °C with a 12 h daily light period of 1800 foot-candles (approx. 18 500 lux) for 4 days. Plants were then transferred to another growth chamber with the same light regime but at 32 °C. At the time of transfer, half-leaves inoculated with both isolates exhibited faint chlorotic lesions approx. 3 mm in diam., indicating initial infection centres. Both LS1- and L-induced lesions continued to expand if maintained at 22 °C. However, when transferred to 32 °C LS1-induced lesions ceased to expand while those induced by L continued to develop in size for several days. At 4 days after inoculation (22 °C) and 7 days later (11 days after inoculation, 32 °C), samples were taken from the lesions and surrounding healthy tissue for

Fig. 1. Selected electron micrograph (at higher magnification than those used for the data in Table 1) showing four plasmodesmata between two uninfected tobacco mesophyll cells (opposite the circled stars). The plasmodesmata to the right and left are characteristically branched. W, Cell wall; V, vacuoles. Bar marker represents 1 μm.

Table 1. Average number of plasmodesmata per cell pair

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Isolate</th>
<th>Temperature (°C)</th>
<th>No. of cell pairs</th>
<th>Average no. of plasmodesmata</th>
<th>A*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LS1</td>
<td>32</td>
<td>18</td>
<td>0.33 ± 0.68†</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>32</td>
<td>18</td>
<td>0.88 ± 0.83</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>LS1</td>
<td>32</td>
<td>18</td>
<td>0.33 ± 0.83</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>32</td>
<td>18</td>
<td>1.16 ± 1.41</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>LS1</td>
<td>32</td>
<td>47</td>
<td>0.25 ± 0.43</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>LS1</td>
<td>22</td>
<td>50</td>
<td>0.68 ± 0.92</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>LS1</td>
<td>32</td>
<td>23</td>
<td>0.87 ± 1.29</td>
<td>0.01</td>
</tr>
<tr>
<td>U‡</td>
<td>32</td>
<td>22</td>
<td>2.18 ± 1.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>L</td>
<td>32</td>
<td>23</td>
<td>0.43 ± 0.67§</td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>32</td>
<td>25</td>
<td>0.68 ± 1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M†</td>
<td>32</td>
<td>24</td>
<td>0.71 ± 0.85</td>
<td>NS</td>
</tr>
<tr>
<td>M</td>
<td>22</td>
<td>24</td>
<td>0.75 ± 0.85</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Level of significance in t-test.  
† Standard deviation.  
‡ U, Uninfected tissue of inoculated leaves.  
§ NS, No significant difference.  
†† M, Mock-inoculated leaves.

electron microscopy. Mock-inoculated leaves (M) incubated at both temperatures were also sampled. Tissues were fixed with glutaraldehyde and osmium tetroxide, dehydrated in acetone and embedded in epoxy resin (Milne, 1972). Sections were cut with a diamond knife, stained with lead citrate and uranyl acetate, and examined with an AEI EM6B electron microscope. For measurement of plasmodesmata size, micrographs were taken at a magnification of 7500 and enlarged on photographic paper to 70000. The areas of plasmodesmata were measured with a Zeiss MOP-3 modular system for quantitative digital image analysis. For quantitative determinations, randomly selected micrographs of walls between mesophyll or palisade cells
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(cell pairs) were taken at 7500 magnification and the number of plasmodesmata was counted directly from the electron micrographs. Thirty-six to 97 cell pairs from one leaf were sampled per experiment. Differences in numbers of plasmodesmata in each experiment were analysed by Student's \( t \)-test. Comparisons of numbers of plasmodesmata were only made within individual experiments because of possible differences in the stage of leaf development from one experiment to another, although care was taken in all cases to select uniform leaves.

There were no differences in the size or structure of plasmodesmata in healthy, LS1-infected or L-infected tissues at either temperature. However, there were significantly fewer plasmodesmata (5\% level) in LS1-infected tissue at 32 °C than in L-infected tissue at the same temperature (Table 1, experiments 1 and 2). There was also a highly significant (1\% level) reduction in the number of plasmodesmata per micrograph in LS1-infected tissue at 32 °C compared with tissue infected with the same isolate at 22 °C (Table 1, experiment 3). At 32 °C, LS1-infected tissue had a highly significant reduction (1\% level) in numbers of plasmodesmata compared with uninfected tissue at the same temperature (Table 1, experiment 4). There were no differences in numbers of plasmodesmata in L-infected tissue and uninfected tissue at 32 °C (Table 1, experiment 5) or in healthy tissue incubated at 32 °C compared with healthy tissue at 22 °C (Table 1, experiment 6).

It seems probable that cells infected by LS1 at a restrictive temperature have reduced numbers of plasmodesmata mainly because they lose existing plasmodesmata and not because there is a virus-mediated inhibition of the formation of new ones. For us to identify infected tissues to sample for electron microscopy, LS1-infected plants were first held at a permissive temperature and then the temperature was raised to restrict virus movement. Accordingly, our sampling of LS1-infected tissue consisted principally, if not exclusively, of cells which had been infected at 22 °C but which had been held at 32 °C prior to sampling. Cells thus lost plasmodesmata as a result of the temperature shift.

The reduction in the number of plasmodesmata induced by LS1 at 32 °C would explain in part the inability of the virus genome to spread from cell to cell at that temperature. This provides further evidence that plasmodesmata are indeed the paths of virus movement from cell to cell. Although at the restrictive temperature some plasmodesmata persist, restricted movement could be due to a reduced probability of virus encountering and moving through those remaining.

Plasmodesmata arise during cell wall formation and are thought usually to be persistent throughout the life of the cells. The evidence presented here supports the concept that in some cases they are ephemeral. In some tissues plasmodesmata are occluded during cell differentiation (Carr, 1976). For example, during differentiation of the protodermal tissue of *Phaseolus vulgaris* L., plasmodesmata are readily found between guard mother cells and surrounding epidermal cells. However, in mature tissue, plasmodesmata are lacking between guard cells and surrounding cells (Willmer & Sexton, 1979). It was speculated that their breakdown was due to stretching of the cell wall during stomatal opening. Since we could find no difference in the size of mesophyll or palisade cells among any of our samples, some other mechanism must be responsible for breakdown of plasmodesmata.

Leonard & Zaitlin (1982) have found that *in vitro* translation products of LS1 RNA include a protein which differs slightly from the 30000 mol. wt. protein translated from RNA of the L strain. This suggests that the 30000 mol. wt. protein may have a role in cell-to-cell movement of the L strain and when altered becomes non-functional. Whether the altered protein has any role in breakdown of plasmodesmata remains to be determined.

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