Intracellular distribution of the 126K/183K and capsid proteins in cells infected by some tobamoviruses

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Leaves of plants infected by tobacco mosaic virus (TMV) strain U1, TMV strain M, tomato mosaic virus strain Dahlemense and tobacco mild green mosaic virus strain U2 were examined for the presence and intracellular distribution of their capsid and 126K/183K proteins by immunoblotting and immunogold electron microscopy. The bulk of the capsid protein was found in the virus bundles (crystals), although small amounts were found in the chloroplasts and nuclei of cells infected by some of these tobamoviruses. The 126K protein of TMV-U1 and -M was localized in the X-bodies, whereas in cells infected by the other two viruses which induce no X-bodies, the 126K protein was found to be associated with virus bundles.

The tobamoviruses constitute a group of plant viruses whose type member is tobacco mosaic virus (TMV). A number of strains of TMV as well as several other distinct viruses are included in the tobamovirus group (Gibbs, 1986). Among these are TMV strain U1 (common, Vulgare), masked strain (M), tomato mosaic virus (ToMV) and tobacco mild green mosaic virus (TMGMV). Both ToMV and TMGMV also have a number of strains (Brunt, 1986; Wetter, 1986). These include the Dahlemense strain of ToMV and the U2 strain of TMGMV, both of which were previously regarded as strains of TMV. The genomes of all the viruses described herein, namely TMV-U1, -M, ToMV and TMGMV-U2, have been sequenced (Goelet et al., 1982; Holt et al., 1990; Ohno et al., 1984; Solis & Garcia-Arenal, 1990). All encode genes for the putative helicase/polymerase (126K/183K) and the coat protein (17.5K) as well as genes for other non-structural tobamoviral proteins.

TMV-U1 is known to induce large quantities of the 126K/183K proteins which are mainly located in viroplasms known as X-bodies (Saito et al., 1987; Hills et al., 1987; Wijdeveld et al., 1989). These X-bodies are absent from cells infected by ToMV (Francki et al., 1985) and TMGMV-U2 (Bald & Solberg, 1961), although there is a report of the presence of X-bodies in plants infected by the aucuba strain of TMV (Warmke, 1969). There are no reports of the presence or absence of X-bodies for TMV-M. In this paper, we report attempts to localize the tobamoviral 126K/183K proteins of these strains/viruses in view of the functional importance of these proteins in viral replication. Simultaneously, we studied the distribution of the capsid protein in infected cells in view of conflicting reports about their location in different cell organelles.

All viruses used in this study were obtained from Dr A. Siegel (Wayne State University, Detroit, Mich., U.S.A.). Viruses were propagated in Nicotiana tabacum L. cv. Samsun and 7-day-old systemically infected leaves were used for all studies. Antiserum to the TMV-U1 capsid protein was prepared by injecting rabbits with capsid protein prepared by the acetic acid method (Fraenkel-Conrat, 1957). Antiserum to the recombinant 126K protein was a generous gift from Dr Y. Watanabe (Saito et al., 1987). Antiserum to a synthetic peptide, corresponding to the N-terminal 21 amino acids of the 126K protein of TMV-U1 (N-MAYTQTATSSALLETVRG-NNT-C) was prepared by injecting the peptide directly into rabbits for antibody production. One mg of the peptide was used to immunize rabbits at 2 week intervals.

Immunoblotting experiments with healthy and virus-infected tissue extracts were carried out as described previously (Baunoch et al., 1990). For immunogold labelling, tissue from healthy and virus-infected plants was fixed, dehydrated and embedded in Lowicryl K11M (Polysciences) as described previously (Baunoch et al., 1990). Ultrathin sections were incubated in rabbit antisera specific for the 126K/183K or capsid proteins and immunolabelled using gold (10 nm) -conjugated goat anti-rabbit IgG (Sigma). Samples were viewed and photographed using a Philips 201 or 301 electron microscope.
microscope. These micrographs were then used to count the number of gold particles per unit area.

Preliminary immunoblotting experiments established that our antiserum to the capsid protein of TMV-U1 cross-reacted with the capsid proteins of all four tobamoviruses. With regard to the distribution of the capsid protein in infected cells, the results of these experiments are summarized in Table 1. In all cases, labelling was mainly associated with the virus bundles with very little capsid protein occurring in the cytoplasm. Labelling was also found in nuclei and chloroplasts of TMV-U1- and TMGMV-infected cells. Although some recent investigators have been unable to detect the capsid protein of TMV-U1 in nuclei and chloroplasts (Hills et al., 1987; Wijdeveld et al., 1989), other investigators in the past have recorded the presence of the capsid protein in these organelles (Shalla & Amici, 1966; Pratt, 1969; Shalla et al., 1975). We found that cells of systemically infected leaves tested at 4 days post-inoculation (p.i.) did not contain capsid protein in their nuclei and chloroplasts but cells from older infections (7 and 14 days p.i.) did.

In the immunoblotting experiments, antiserum to the 126K recombinant fusion protein of TMV-U1 cross-reacted with the 126K/183K proteins of all the tobamoviruses tested. However, the antiserum to the N-terminal peptide detected the 126K/183K proteins of TMV-U1, -M and ToMV but not that of TMGMV (data not shown). This is not surprising since there is little amino acid sequence similarity in this region between the 126K proteins of TMV-U1 and TMGMV (Solis & Garcia-Arenal, 1990).

The immunogold electron microscopy studies localized the 126K/183K proteins to the X-bodies in the plants infected by TMV-U1 (Table 2). Similarly, both types of antisera localized the 126K/183K proteins to X-bodies in plants infected by TMV-M (Fig. 1, Table 2). The X-bodies in this case consisted of tube-like structures as in the TMV-U1-infected plants, but the tubes were more loosely arranged than the more compact types found in TMV-U1-infected cells. Furthermore, we found that only about 10% of the cells infected by TMV-M which contained virus particles also contained X-bodies, whereas every cell infected by TMV-U1 containing virus particles also contained one or more X-bodies. Changes in specific amino acids in the 126K/183K proteins of TMV-M have been suggested as possible reasons for the observed symptomatology of TMV-M (Holt et al., 1990). These amino acid differences in the 126K/183K proteins of TMV-M possibly affect the stability of the X-bodies so that only a few are found in the infected cells. In plant cells infected by ToMV and TMGMV, no X-bodies were detected. However, our most interesting finding was that antibodies to the recombinant 126K protein of TMV-U1 localized the 126K/183K proteins of ToMV to virus bundles (Fig. 2, Table 2). Similar results were obtained with TMGMV (Table 2). That this labelling was not due to contaminating capsid antibodies in this serum was shown by the absence of labelling of the capsid by this serum in the immunoblots (data not shown) as well as the lack of labelling of the virus bundles in cells infected by either TMV-U1 or TMV-M (Table 2). Ohno et al. (1984) noted that although there is 90% amino acid sequence similarity between the 126K/183K proteins of TMV-U1 and ToMV(L), there are also several changes clustered at amino acid residues 170 to 195, 498 to 513 and other changes that are scattered. Similarly, the amino acid sequence of the 126K/183K proteins of TMGMV (Solis & Garcia-Arenal, 1990) shows a number of changes when compared to the sequence of these proteins in TMV-U1. Thus, one or more of these changes could prevent the polymerization of these proteins into X-bodies in the ToMV- and TMGMV-infected cells. The possible reason for the association of these proteins with the virus bundles in these strains is intriguing.

Lastly, we observed that the 126K N-terminal peptide serum did not label the 126K/183K proteins in either the ToMV- or TMGMV-infected cells. This is not surprising

Table 1. Distribution of capsid protein in infected cells (7 days p.i.) based on the average number of gold particles per μm² with antiserum to the TMV-U1 capsid protein.

<table>
<thead>
<tr>
<th></th>
<th>TMV-U1</th>
<th>TMGMV</th>
<th>TMV-M</th>
<th>ToMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleus</td>
<td>17</td>
<td>12</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Chloroplast</td>
<td>24</td>
<td>14</td>
<td>5</td>
<td>2</td>
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<tr>
<td>Mitochondria</td>
<td>1-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-body</td>
<td>34</td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Virus bundle</td>
<td>102</td>
<td>96</td>
<td>64</td>
<td>87</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>7</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Cell wall</td>
<td>0-2</td>
<td>0-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vacuole</td>
<td>51</td>
<td>27</td>
<td>9</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 2. Distribution of the 126K/183K proteins in infected cells (7 days p.i.) based on the average number of gold particles per μm².

<table>
<thead>
<tr>
<th></th>
<th>TMV-U1</th>
<th>TMGMV</th>
<th>TMV-M</th>
<th>ToMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-body*</td>
<td>35</td>
<td></td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Virus bundle*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-body†</td>
<td>21</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virus bundle†</td>
<td>0-1</td>
<td>14</td>
<td></td>
<td>15</td>
</tr>
</tbody>
</table>

* Antiserum to 126K peptide.
† Antiserum to 126K recombinant protein.
Fig. 1. Distribution of the 126K/183K proteins and capsid protein in cells infected by TMV-M. (a) Immunogold labelling of the X-body with antiserum to the 126K recombinant protein. Bar marker represents 125 nm. (b) Immunolabelling of the X-body with antiserum to the 126K peptide. Bar marker represents 125 nm. (c) Immunolabelling of the virus bundle with antibodies to the TMV-U1 capsid protein. Bar marker represents 125 nm. x, X-body; v, virus bundle; N, nucleus.
Fig. 2. Distribution of the 126K/183K proteins and capsid protein in cells infected by ToMV. (a and b) Immunogold labelling of the virus bundle with antiserum to the 126K recombinant protein. Bar markers represent 240 nm. (c) Immunolabelling of an infected cell with antibodies to the TMV-U1 capsid protein. Bar marker represents 230 nm. (d) Enlargement of the virus bundle labelled with antibodies to the 126K recombinant protein. Bar marker represents 100 nm. (e) Enlargement of the virus bundle immunolabelled with antiserum to the TMV-U1 capsid protein. Bar marker represents 125 nm. v, Virus bundle; N, nucleus.
in the case of cells infected by TMGMV since there is little sequence similarity between the peptide used to induce antibodies and the sequence of the 126K protein of TMGMV. In the case of the 126K protein of ToMV, however, the lack of labelling appears to be due to the lack of availability of the N-terminal region of this protein in its native form for immunolabelling, since the denatured 126K protein of ToMV does react with this serum in immunoblots.

References


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