Studies of coat protein-mediated resistance to tobacco mosaic virus (TMV). II. Challenge by a mutant with altered virion surface does not overcome resistance conferred by TMV coat protein

W. Gregg Clark,1† John Fitchen,2 Ali Nejidat,1‡ C. Michael Deom3 and Roger N. Beachy1*  

1Department of Biology, Washington University, St Louis, MO 63130, 2Department of Cell Biology, The Scripps Research Institute, La Jolla, CA 92037 and 3Department of Plant Pathology, University of Georgia, Athens, GA 90602, USA

Transgenic tobacco plants expressing the coat protein (CP) gene of the U1 strain of tobacco mosaic virus (TMV) exhibit CP-mediated resistance (CP-MR) against some, but not all, tobamoviruses. To investigate the role of the amino acid sequences on the surface of the challenge virus in CP-MR, mutant strains of U1 TMV were constructed to contain the amino or carboxy termini of the CP of Sunn hemp mosaic tobamovirus (SHMV). The modified virus was unable to overcome CP-MR in transgenic plants that contained the TMV CP. In contrast, TMV in which the CP was replaced by the SHMV CP overcame CP-MR to the same extent as did SHMV. We conclude that CP-MR conferred by TMV CP involves interactions between amino acid sequences of the challenge viruses and the transgene protein other than those on the surface of the challenge virus.

Coat protein-mediated resistance (CP-MR) has been widely used to develop protection in monocots and dicots against a number of different viruses (see reviews by Fitchen & Beachy, 1993; Wilson, 1993). However, the molecular and cellular nature of the resistance has not been fully elucidated for any example of CP-MR. We have focused our studies of CP-MR on protection against tobacco mosaic tobamovirus (TMV) in tomato and tobacco plants. CP-MR conferred by the CP of TMV was highly effective against TMV and closely related tobamoviruses and less effective against the more distantly related Sunn hemp mosaic virus (SHMV; Anderson et al., 1989). The amino acid sequence of the CP of SHMV differs by about 60% from the CP sequence of TMV. It was proposed that the low level of amino acid sequence identity between the CPs of TMV strain U1 and SHMV contributes to the ability of the challenge virus to evade the protection mechanism(s) (Nejidat & Beachy, 1990).

Because a major component of CP-MR against TMV is affected at or before uncoating of the virus (Register & Beachy, 1988; Wu et al., 1990), it was postulated that a cellular component that is involved in virus disassembly may be blocked or occupied by transgenic CP, thereby denying access by TMV and related tobamoviruses. Since transgenic plants accumulate rodlets comprised of TMV CP (Osbourn et al., 1989), it was proposed that such rodlets bind the proposed host component, and that structural features of the rodlets or other forms of capsid multimers are important for CP-MR. It follows that the interaction of challenge virions with the plant component could be significantly influenced by the surface structure of the challenge virus.

To test this hypothesis, we performed experiments to determine if changes in the surface structure of TMV could influence the level of CP-MR in transgenic tobacco plants accumulating TMV strain U1 CP (CP'). It has been determined by X-ray diffraction studies that the surface of TMV includes the amino and carboxy termini of CP subunits (Namba et al., 1989). We constructed infectious, mutant TMV cDNA clones bearing changes in either the amino or carboxy termini of the CP to resemble those of SHMV, as well as TMV in which the complete CP cistron was replaced with that of SHMV. The resulting mutant viruses were used to challenge
transgenic tobacco plants that accumulate the CP of TMV (Abel et al., 1986).

To alter the surface structure of TMV to resemble the surface of SHMV, we made amino acid sequence changes to the amino and carboxy termini of the U1 CP gene to resemble those of SHMV CP (mutants SH-1 and AP; Fig. 1) using oligonucleotide mutagenesis (Kunkel et al., 1987). During one such mutagenesis reaction, a second, inadvertent mutation was created in which a nucleotide was deleted from the 3’ sequence of the CP open reading frame. This deletion caused a shift in the reading frame and a substitution of 12 amino acids for the last amino acid of the TMV CP. The new amino acid sequence of this CP mutant, named CX, was derived from the polylinker region of the cloning vector and lacked significant similarity to any other known tobamovirus CP. The pertinent sequence changes in the mutant CPs were confirmed by DNA sequence analysis and are presented in Fig. 1.

Following mutagenesis, the CP sequences were ligated into a cloned full-length cDNA of TMV RNA from which the CP coding sequence was deleted and replaced by a SnaB1 restriction site (Gafny et al., 1992). A cloned cDNA that encodes the entire CP of SHMV was also inserted as a precise replacement of the TMV CP sequence; this clone was designated pTSCP. A complete description of the construction of pTSCP will be provided elsewhere (C. M. Deom and X. Z. He, unpublished results).

The cloned viral cDNAs are preceded by the T7 polymerase promoter which was used to produce RNA transcripts of the cloned cDNA in vitro (Holt & Beachy, 1991). Transcripts were inoculated to tobacco plants (Nicotiana tabacum cv. Xanthi NN) and progeny virus was recovered as described by Asselin & Zaitlin (1978). Progeny virus was stable and was equally as infectious as wild-type TMV (data not shown). When mutant virus was mechanically inoculated onto N. tabacum cv. Xanthi NN, necrotic local lesions were produced by each of the mutants, although slightly more slowly than lesions induced by U1 TMV.

Immunological analyses were performed to determine whether the changes made at the termini of the TMV CP caused the mutant CPs to take on antigenic characteristics of SHMV. Equal amounts of CP isolated from mutants AP, SH-1 and CX were subjected to SDS-PAGE (Laemmli, 1970), electroblotted to nitrocellulose and reacted with polyclonal antibodies raised against U1 TMV (Towbin et al., 1979) or SHMV. To increase the specificity of the reactions, each antibody was cross-absorbed with the alternate virus before being used in these studies. Each of the proteins tested reacted with the anti-U1 antibodies, although to different degrees (Fig. 2a). When reacted with the polyclonal antibody raised against SHMV, only SHMV and the SH-1 mutant were reactive; as expected the SH-1 CP was significantly less reactive than SHMV on a mass of protein basis (Fig. 2b). This can be explained by the likelihood that only a subpopulation of the antibodies recognize the SHMV sequence present in SH-1 CP. The SHMV antibody did not react with U1 TMV, indicating that the interaction of the antibodies with SH-1 CP was specific. There was no reaction with the AP mutant (Fig. 1), presumably because the antibody did not contain molecules that bind specifically to the amino terminus of the CP molecule. As anticipated, CP produced by TSCP reacted with anti-SHMV antibodies to the same degree as the CP of SHMV (data not shown).

To demonstrate that the SHMV sequence was on the surface of the SH-1 virus, purified virus was placed on EM grids and reacted with anti-SHMV antibody, followed by anti-rabbit antibody conjugated with col-
Fig. 2. Immunoblot of CP released from purified virus and reacted with virus-specific polyclonal antibodies. Purified virus was treated with sample buffer prior to SDS-PAGE. The amount loaded in each lane (in ng) is indicated. Proteins were transferred to nitrocellulose by electroblotting and the blotted proteins were reacted with antibodies and exposed to X-ray film. (a) Results of reaction with anti-U1 TMV antibody; (b) results of reaction with anti-SHMV antibody. The secondary antibody was 125I-labelled goat anti-rabbit antibody.

loidal gold. As shown in Fig. 3, the SH-1 virus, but not TMV, bound anti-SHMV antibodies, confirming that the SHMV protein sequence on SH-1 was exposed and was probably on the surface of the virus as predicted. Purified TMV did not bind anti-SHMV antibody (Fig. 3).

TMV, SHMV and each of the mutant viruses were used to challenge plant line 748 (Nelson et al., 1987), a line of N. tabacum cv. Xanthi NN that accumulates TMV CP. Plant line 748 is highly resistant to TMV but is susceptible to infection by TMV RNA, characteristic of CP-MR of tobamoviruses (see review by Fitchen & Beachy, 1993). Leaves were mechanically inoculated with mutant virus or with U1 TMV at levels of inocula that produced comparable numbers of local lesions on non-transgenic N. tabacum cv. Xanthi NN. As shown in Table 1, virus mutants SH-1, CX and AP, and U1 TMV produced many fewer local lesions on line 748 than on N. tabacum cv. Xanthi NN. Although there were variations in the ratio of lesions produced on CP- and CP+ plants, the level of protection against the mutant viruses was comparable to protection against U1 TMV (Table 1). Consistent with the results of Anderson et al. (1989), plant line 748 exhibited much less resistance against the mutant TSCP. The observation that TSCP, comprised of TMV RNA encapsidated in SHMV CP, overcomes CP-MR in plant line 748 to the same extent as SHMV establishes that the determinants of specificity for CP-MR in tobamoviruses reside in the CP rather than in the genome per se. This is also consistent with the conclusion that the effectiveness of CP-MR depends on the degree of similarity between the transgene-derived CP and the CP of the challenge virus (Nejidat & Beachy, 1990). These results appear to contrast, however, with results from a nearly reciprocal experiment (Wilson, 1993) in which CP-MR in TMV CP-containing plants was shown to be ineffective against SHMV RNA encapsidated in vitro in TMV CP. The results of the two experiments could be reconciled if the SHMV RNA had not been fully encapsidated in the in vitro assembly reactions; it has been previously demonstrated (Register & Beachy, 1988) that TMV that is partially uncoated to expose the 5' end of the RNA overcomes CP-MR.

Furthermore, these results demonstrate that CP-MR conferred by U1 TMV CP was equally effective against U1 TMV and TMV with mutations at the amino or carboxy termini of the CP. As the amino and carboxy termini of each CP subunit are exposed on the virus particle, contribute significantly to the molecular surface and contain the immunodominant epitopes, changes in these regions of the CP would be expected to alter
interactions between the virus particle and a putative cellular factor recognizing the surface of the virus. We conclude that either the differences in the surface structure of the mutants (AP, SH-1, CX) used in this study were insufficient to block interaction with such a cellular factor and that mutant TSCP contained some other critical change, or that the molecular structures that contribute to the specificity of CP-MR reside in internal regions of the CP, presumably at subunit interfaces.

A localization of the specificity determinants at subunit interfaces is entirely consistent with a model for the mechanism of CP-MR (Register et al., 1989; Clark et al., 1995) that postulates direct association of a small number (1 to 6) of transgene-derived CP molecules with the challenge virus during disassembly. Such association would prevent binding of ribosomes to the RNA of the challenge virus, thereby preventing infection. The molecular characteristics of the interface surfaces of the transgene-derived and challenge CP and their association constant would determine whether ribosomes were excluded and resistance affected. Other studies are in progress to elucidate further the role of selected amino acid sequences in conferring CP-MR against the tobamoviruses.

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References

Table 1. Number of necrotic lesions induced by mutant viruses on CP− and CP+ tobacco plants

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Virus strain*</th>
<th>Plant line 748</th>
<th>Protection‡ (%)</th>
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<tr>
<td></td>
<td></td>
<td>NN (CP−)</td>
<td>(CP+)</td>
</tr>
<tr>
<td>1</td>
<td>U1</td>
<td>187 ± 23 (8)</td>
<td>4 ± 1 (8)</td>
</tr>
<tr>
<td>SH-1</td>
<td>251 ± 22 (8)</td>
<td>9 ± 2 (8)</td>
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<tr>
<td>U1</td>
<td>149 ± 16 (10)</td>
<td>7 ± 3 (10)</td>
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</tr>
<tr>
<td>CX</td>
<td>108 ± 13 (10)</td>
<td>17 ± 9 (10)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>U1</td>
<td>189 ± 17 (10)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>SH-1</td>
<td>258 ± 57 (10)</td>
<td>1 ± 1 (5)</td>
<td></td>
</tr>
<tr>
<td>U1</td>
<td>304 ± 34 (10)</td>
<td>68 ± 23 (10)</td>
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<tr>
<td>AP</td>
<td>127 ± 18 (10)</td>
<td>24 ± 13 (10)</td>
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<tr>
<td>CX</td>
<td>125 ± 14 (10)</td>
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<tr>
<td>AP</td>
<td>77 ± 6 (9)</td>
<td>5 ± 3 (10)</td>
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<tr>
<td>5</td>
<td>TSCP</td>
<td>80 ± 20 (10)</td>
<td>32 ± 8 (10)</td>
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<tr>
<td>U1</td>
<td>42 ± 8 (10)</td>
<td>2 ± 1 (10)</td>
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<tr>
<td>SHMV</td>
<td>124 ± 14 (10)</td>
<td>78 ± 20 (10)</td>
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* Virus strain SH-1, AP and CX are described in Fig. 1. TSCP is a derivative of TMV in which the entire CP sequence of SHMV replaced the CP of TMV.
† The number of lesions ±SEM. Numbers in parentheses indicate the number of half leaves inoculated. Homozygous R₀ and R₄ progeny of plant line 748 used for this study are derived from the R₄ parent previously described (Nelson et al., 1987). Plants were grown in a greenhouse to the 6-8 leaf stage, topped and placed in the dark 16-24 h prior to inoculation. Inocula were applied to leaves dusted with carborundum. Local lesions were counted 4-5 days after infection.
‡ Percentage protection was calculated as 100−[(lesions on 748/lesions on NN) x 100].


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