Nucleotide sequence analysis of the movement genes of resistance breaking strains of tomato mosaic virus

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The nucleotide sequences were determined for the movement genes, encoding 30K proteins, of two resistance breaking strains of tomato mosaic virus (ToMV), LII-ToMV and LIIA-ToMV. The putative amino acid sequences of the encoded proteins were compared with L-ToMV and with two previously published resistance breaking strains, Ltb1-ToMV and C32-ToMV. LII-ToMV, a Type 2 strain able to infect tomatoes containing the Tin-2 gene, has four amino acid changes relative to L-ToMV. One of these substitutions, Glu-Lys 133, is also found in the other Type 2 strains, Ltb1-ToMV and C32-ToMV. LIIA-ToMV, a Type 22 strain able to overcome the resistance conferred by the tomato Tm-22 gene, has four amino acid differences from L-ToMV. The Type 22 strain has no substitutions in common with any of the Type 2 strains; however the predominance of amino acid substitutions that involve a change in the local charge (six out of the eight) suggests an electrostatic interaction between a host factor and the 30K protein may be intrinsic to the function of the movement gene and/or resistance breakage.

The cell-to-cell movement of both tobacco mosaic virus (TMV) and tomato mosaic virus (ToMV) is potentiated by the 30K gene product of these two tobamoviruses (Deom et al., 1987; Meshi et al., 1989). Resistance to infection by these viruses is conferred by three genes in tomato: Tm-1, Tm-2 and Tm-22 (Pelham, 1966; Hall, 1980). The latter two are alleles and do not affect virus replication in protoplasts (Motoyoshi & Oshima, 1975, 1977), but appear to operate by preventing the cell-to-cell movement of TMV and ToMV (reviewed by Fraser, 1990). Strains of ToMV that can break the resistance conferred by either the Tm-2 gene (Type 2 strains) or the Tm-22 gene (Type 22 strains) have been identified (Fraser, 1990). Sequence analysis and recombination between the (movement) genes encoding the 30K proteins of a wild-type (Type 0) strain, L-ToMV, and a type 2 strain, Ltb1-ToMV, demonstrated that two amino acid differences between the respective 30K proteins encoded by these viruses were essential to overcome the resistance completely (Meshi et al., 1989). In C32-ToMV, a second Type 2 strain, the Glu to Lys change at position 133 of the 30K protein was still present, but the Cys to Phe change at position 68 in Ltb1-ToMV was replaced by a Glu to Lys change at position 52 in C32-ToMV. Thus, although two changes were required to break the resistance, only one sequence alteration was common to both Type 2 strains (Meshi et al., 1989). This present study was undertaken to determine whether another Type 2 strain, LII-ToMV, showed yet other differences or sequence alterations similar/identical to the ones observed in the 30K proteins of the two Japanese Type 2 strains. In addition, we determined the nucleotide sequence changes in the movement gene of a Type 22 strain, LIIA-ToMV. The latter movement gene is presumed to be involved in resistance breakage of the Tm-22 gene (Schultze & Pfitzner, 1990).

L-ToMV was obtained from Dr M. Zaitlin of this Department, and both the LII- and LIIA-ToMV strains originated from Dr A. Rast (Institute of Phytopathological Research, Wageningen, The Netherlands). All three strains were purified from infected tobacco (Nicotiana tabacum cv. Samsun) as described by Asselin & Zaitlin (1978), and viral RNA was extracted from virions by phenol–chloroform extraction and ethanol precipitation as described by Bruening et al. (1976). The resistance breaking characteristics of the purified strains were confirmed by inoculation to near isogenic tomato (Lycopersicon esculentum cv. Vendor) lines homozygous for the wild-type, the Tm-2 or the Tm-22 genes.

To localize the nucleotide sequence differences in the movement genes of the LII- and LIIA-ToMV RNAs relative to the L-ToMV RNA, the genes were directly sequenced by the dideoxynucleotide chain-termination method (Mierendorf & Pfeffer, 1987; Fichot & Girard, 1990). The primers used were complementary to L-ToMV RNA sequences 5095 to 5109, 5156 to 5171, 5351 to 5366, 5514 to 5528 and 5751 to 5767. In addition, cDNA clones representing all but 140 nucleotides (nt) at
The size of the movement to nucleotides 4817 to 4836 of L-ToMV RNA as determined by colony hybridization using random-primed cDNA (Stratagene) and transformed into pLRB6 and pLRB7, shown. The direction and extent of sequencing from each primer (small circle) is indicated by the arrowed lines. CP is the gene for the coat protein.

The nucleotide numbering (bold numbers) follows that used by Ohno et al. (1984) for L-ToMV. The sizes and positions of the two cDNA clones of LIIA-ToMV, pLRB6 and pLRB7, are shown. The direction and extent of sequencing from each primer (small circle) is indicated by the arrowed lines. CP is the gene for the coat protein.

The putative amino acid sequences of the movement genes of LII- and LIIA-ToMV were sequenced in comparison with that of L-ToMV in Fig. 2. As can be seen, there are only six nt differences between the movement genes of L- and LII-ToMV RNA, and nine nt differences between L- and LIIA-ToMV RNA. In addition, there is another nucleotide substitution in the 5' non-translated region of the movement gene mRNA of LII-ToMV compared to L- ToMV RNA, and nine nt differences between L- and LIIA-ToMV RNA sequences.

The inserts in the ToMV cDNA-containing plasmids was determined by gel electrophoresis of restricted DNA (Sambrook et al., 1989). The cDNA inserts within the vector were sequenced on one strand only (Fig. 1) by the dideoxynucleotide chain-termination method using the protocols described for the Sequenase system by U.S. Biochemicals, with T7 DNA polymerase (Pharmacia) and oligonucleotide primers (see above) synthesized at the Cornell Biotechnology Program Facility. The primers were complementary to either sequences flanking the cDNA insert in the vector, or L-ToMV RNA sequences.

The nucleotide sequences of the gene for the 30K protein and the 5' non-translated region of LIIL-ToMV and LIIA-ToMV aligned with L-ToMV. Hyphens denote nucleotides identical to those in the L-ToMV sequence. The start codon and the stop codon of the 30K protein are underlined. The asterisk denotes the start of the 30K mRNA, the 1p-RNA. The nucleotide numbering follows that described by Ohno et al. (1984).

The 3' end of the movement gene of LIIA-ToMV RNA were sequenced. A cDNA to the movement gene of LIIA-ToMV RNA was synthesized as described by Ahlquist (1986), using two specific primers: one complementary to nucleotides 5679 to 5694 of L-ToMV RNA as the first-strand cDNA primer, and another anticomplementary to nucleotides 4817 to 4836 of L-ToMV RNA as the second-strand primer. The blunt-end cDNAs were ligated into the HincII site of pBluescript SK+ (Stratagene) and transformed into Escherichia coli strain DH5a. The cDNA clones containing ToMV inserts were screened by colony hybridization using random-primed ToMV cDNA (Palukaitis & Symons, 1980). The size of the inserts in the ToMV cDNA-containing plasmids was determined by gel electrophoresis of restricted DNA (Sambrook et al., 1989). The cDNA inserts within the vector were sequenced on one strand only (Fig. 1) by the dideoxynucleotide chain-termination method using the protocols described for the Sequenase system by U.S. Biochemicals, with T7 DNA polymerase (Pharmacia) and oligonucleotide primers (see above) synthesized at the Cornell Biotechnology Program Facility. The primers were complementary to either sequences flanking the cDNA insert in the vector, or L-ToMV RNA sequences.

The nucleotide sequences of the movement genes of LII- and LIIA-ToMV are shown in comparison with that of L-ToMV in Fig. 2. As can be seen, there are only six nt differences between the movement genes of L- and LII-ToMV RNA, and nine nt differences between L- and LIIA-ToMV RNA. In addition, there is another nucleotide substitution in the 5' non-translated region of the movement gene mRNA of LII-ToMV compared to L-ToMV (Fig. 2).

The putative amino acid sequences of the movement genes of L-, LII- and LIIA-ToMV are shown in Fig. 3, together with the putative sequence of the movement genes of two other Type 2 strains, Ltbl- and C32-ToMV.
There were four amino acid differences between either L-ToMV and LII-ToMV, or L-ToMV and LIIA-ToMV (Fig. 3). The other nucleotide differences (Fig. 2) were not reflected at the amino acid level (Fig. 3). Most of the amino acid substitutions between L-ToMV and either resistance breaking strain involve changes in the charge of amino acid, i.e. Glu-Lys<sup>146</sup>, Glu-Lys<sup>133</sup> and Asn-Lys<sup>182</sup> for LII-ToMV, and Lys-Glu<sup>130</sup>, Ser-Arg<sup>238</sup> and Lys-Glu<sup>244</sup> for LIIA-ToMV. Substitutions were not observed at common positions between LIIA-ToMV and any of the Type 2 ToMV strains; however, all three Type 2 strains contained the Glu-Lys<sup>133</sup> substitution.

The 30K proteins of tobamoviruses have been compared and several domains have been described (Saito et al., 1988). Domains I and II (Fig. 3) are highly conserved in their amino acid sequence, whereas domains A, B and C are conserved in charge: A and C are acidic, B is basic. The Glu-Lys<sup>133</sup> substitution common to all Type 2 strains is in domain II. The Lys-Glu<sup>130</sup> substitution in LIIA-ToMV is also in domain II. Previously, a mutant, Lst-ToMV, temperature-sensitive for cell-to-cell movement, was shown to have a Pro-Ser<sup>154</sup> substitution (Meshi et al., 1987), which is also in this domain. A temperature-sensitive mutant of TMV, N12519, also was shown to have a substitution in this domain (Arg-Gly<sup>144</sup>) (Zimmern & Hunter, 1983). These data suggest that domain II is involved in interactions with the host.

Several amino acid substitutions also occur within or N-terminal to (upstream of) domain I. The Cys-Phe<sup>68</sup> substitution in Ltb1-ToMV is within domain I, while the Glu-Lys<sup>52</sup> and the Lys-Glu<sup>46</sup> substitutions in C32-ToMV and LII-ToMV, respectively, occur outside domain I. Domain I has been suggested as a possible nucleotide-binding domain (Saito et al., 1988). This may be relevant to the observation that CAMP can 'complement' the temperature sensitivity of the Lst1-ToMV mutant in tobacco leaf discs, resulting in cell-to-cell movement (Atabekov et al., 1990). Domain I also contains the amino acids (65 to 86) needed for the single-stranded nucleic acid-binding activity of this protein (Citovsky et al., 1990). With the exception of an Ala-Pro<sup>2</sup> substitution in LIIA-ToMV, all but one of the remaining differences between L-ToMV and either LII- or LIIA-ToMV were in or near the domains A, B, or C, and only the Asn-Lys<sup>182</sup> substitution adjacent to domain A of LII-ToMV is in a region essential for the function of the 30K protein (Berna et al., 1991).

Although the sequence alterations required for resistance breakage of the Tm-2 and Tm<sup>22</sup> genes were encoded at different positions, they had the same effect, namely, to alter the local charge of the 30K protein, albeit to opposite polarities. These changes in charge did not alter the computer-assisted, putative secondary structure for the 30K proteins of the various ToMV strains (data not shown). Similar alterations in the local charge of the 126K/183K proteins of Type 1 ToMV strains were observed, leading to resistance breakage of the Tm-1 gene in tomato. The Tm-1 gene inhibits the replication of TMV and ToMV (Meshi et al., 1988). Thus, as previously suggested for the Tm-1 gene, the function of the movement gene and/or resistance breakage of both the Tm-2 and Tm<sup>22</sup> genes may involve electrostatic interactions between the 30K protein and some host factor encoded by the Tm2/Tm<sup>22</sup> locus. Alterations in charge of specific domains of the 30K protein may affect the ability of the host resistance gene product to interact with the viral 30K protein. The nature of these interactions has yet to be determined.

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References


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