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Mapping Analysis of Virus Sequences Affecting Host Range and Movement of Cucumber Mosaic Virus

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Resistance to plant viruses occurs at several stages : activation of hypersensitive response, inhibition of virus replication, inhibition of cell-to-cell (local) movement, and inhibition of leaf-to-leaf (long-distance or systemic) movement. The latter two stages are the most common forms of resistance. Several viral gene products are involved both in promoting local and systemic movement. Plant viruses require viral-encoded protein(s) to facilitate local or systemic movement, and movement occurs via several different mechanisms (reviewed by Carrington et al., 1996). Cucumber mosaic virus (CMV), a type species of the *Cucumovirus* genus, is a positive-sense RNA plant virus with a tripartite genome (reviewed by Palukaitis et al., 1992). Cucumovirus possesses a segmented genome consisting of three single-stranded genomic RNAs and two subgenomic RNAs (Palukaitis et al., 1992). RNAs 1 and 2 encode the nonstructural proteins involved in viral replication (Fig. 1). RNA 3 encodes a movement protein (MP) and the coat protein (CP), which is translated from a subgenomic mRNA, RNA 4, both of which are involved in virus movement (Fig. 1). A fifth open reading frame, the 2b gene protein, is also encoded on 3' terminal region of RNA 2 (Ding et al., 1994), and which is implicated in virus movement and symptom severity (Ding et al., 1995). CMV has the widest host range over 800 species in 85 families among the plant viruses (Palukaitis et al., 1992). CMV is considered as one of the most important viruses infecting economically important crops. The virus is spread naturally by more than 60 aphid species and this property is a potential cause of virus epidemics in crops which are important economically. To date a number of strains of CMV have been reported and characterized (Palukaitis et al., 1992), and can be divided into two main subgroups, subgroup I (S-I) and subgroup II (S-II), by serological data, peptide mapping of the CP, and nucleic acid hybridization (Palukaitis et al., 1992). There are no clear differences in the host range of isolates of the S-I and S-II. The two subgroups are closely related serologically with polyclonal antibodies although monoclonal antibodies raised against S-I and S-II isolates could differentiate the two subgroups. Sequence comparison of a representative strain from each subgroup verified the designations (Palukaitis et al., 1992). Over the past several years a large number of CMV strains have been described and partially sequenced. Percentages of nucleotide sequence homologies of full-length of RNA 3 between S-I and S-II range from 64 to 67%, while within each subgroup they are over 85%. Recent analysis of the CP genes of several subgroup I strains suggests that they can be further divided into two groups, new grouping for the subgroup S-I, designated S-IB (Asian isolates) and S-IA (all other isolates), has been proposed on the basis of sequence data (Roossinck et al., 1999). Recently, the subgroup S-I can be separated into 4 subgroups, denoted as S-I, S-Iα, S-Iβ and S-Iγ, on the basis of electrophoretic patterns of restriction fragments of reverse transcription and polymerase chain reaction (RT-PCR) products digested with *Msp*I restriction enzyme (Anonymous, 1998). More recently, a RT-PCR method has been developed for simultaneous detection of all the three

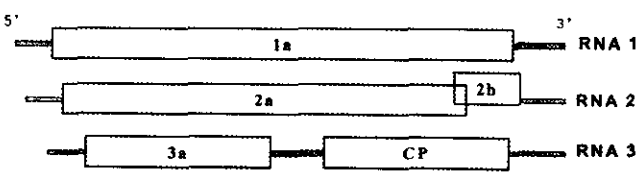

Fig. 1. Genome organization of cucumber mosaic virus.

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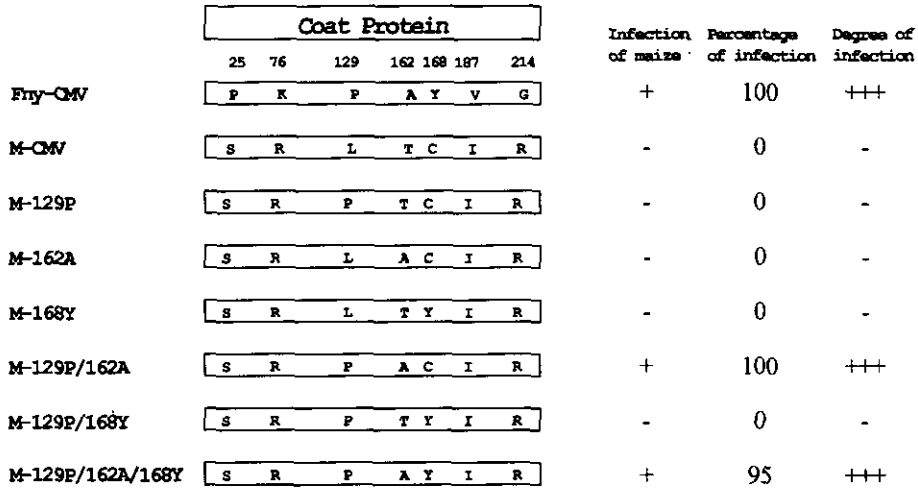

Fig. 2. Infectivity of wild-type (Fny-CMV and M-CMV) and coat protein mutants of cucumber mosaic virus (CMV) on maize. Infectivity was verified by dot blot hybridization. Modified from Ryu et al. (1998).

cucumoviruses (CMV, peanut stunt virus, PSV, and tomato aspermy virus, TAV) from virus-infected plants with a single pair of the genus-specific degenerate primers, and they could be easily differentiated by restriction fragment length polymorphism (RFLP) coupled with the RT-PCR and by nucleotide sequence analysis of the amplified product (Choi et al., 1999).

The following parts describe recent results concerning the viral sequences involved in resistance in maize plant with the nature of the mechanism of the resistance and summarize the viral sequences involved in movement function and other properties with CMV-host interactions system.

Host Range Determinant in Maize-CMV Interactions

Interactions between viruses and either host or nonhost (or resistant plants) involves specific interactions, and in most cases, only a few nucleotide or amino acid changes in the viral genome are involved in altering the host vs. nonhost relationship between a plant species and a given virus strain. The MP of tomato mosaic tobamovirus (ToMV) is the elicitor of two resistance genes in tomato that prevent the local movement of ToMV (Meshi et al., 1989; Weber et al., 1993). The CP of tobamoviruses is involved in promoting their systemic movement via the phloem (Hilf and Dawson 1993). In some cases, the CP is the elicitor of either a hypersensitive response or a general inhibitor of viral RNA replication (Knorr and Dawson 1988; Kohm et al., 1993). In the case of CMV, the CP is required for local movement, and also for systemic movement, and specific regions of the CP also have been delimited that affect systemic infection by CMV in zucchini squash (Palukaitis et al., 1990). Several studies have delimited differences to specific viral RNAs involving the reassortment of the genomic RNAs between strains showing differences in replication functions. Some differences in host range have been ascribed to changes in RNA 2, RNA 1 or RNA 3 of CMV, and these reflect an inability to replicate in the resistant species (reviewed by Palukaitis et al., 1991).

To localize viral sequences involved in host range determinants in host-viral interactions that result in specific pathogenic effects, two strains of CMV, Fny-CMV and M-CMV, have been chosen. These two strains differ in a number of characteristics (reviewed by Palukaitis et al., 1992); 1) Fny-CMV shows typical mosaic symptoms in tobacco, but M-CMV induces yellow chlorosis, and CP amino acid position 129 is involved in chlorosis induction, 2) Fny-CMV can be transmitted by aphid, but M-CMV can not, 3) Fny-CMV infects on squash and maize plants, but M-CMV does not. Infection of maize by the Fny-CMV and resistance against infection by the M-CMV were mapped to the CP gene on RNA 3 of CMV, using biologically active cDNA clones of Fny-CMV RNAs 1, 2 and 3 and RNAs 2 and 3 of M-CMV, as well as chimeras constructed between cDNA clones of M-CMV and Fny-CMV RNA 3 (Ryu et al., 1998). A series of mutants was constructed and tested in the M-CMV RNA 3 to alter the sequences encoding the amino acids at positions 129, 162 and 168 of the M-CMV CP to those found in Fny-CMV (Ryu et al., 1998) (Fig. 2). Changes in the CP gene of M-CMV RNA 3 at both positions 129 (Leu to Pro) and 162 (Thr to Ala) were required to overcome the resistance against M-CMV in maize plant (Fig. 2). Resistance to M-CMV in maize was correlated with an inability to detect virus accumulation in the primary inoculated leaves, and the resistance is due to the inability of the M-CMV CP to promote the cell-to-cell movement of CMV in maize. The resistance of maize to infetion by M-CMV could be operating by one of several mechanisms

(Ryu et al., 1998). (1) The M-CMV CP may be unable to interact with some host component to facilitate the cell-to-cell movement of CMV. (2) The M-CMV CP may elicit a host response or interact with a host protein that the Fny-CMV CP does not. (3) The Fny-CMV CP may suppress a response that normally occurs on infection, and the M-CMV CP fails to do so.

Pseudorecombinants formed between Fny-CMV and LS-CMV (subgroup II) were used to demonstrate that resistance to infection of maize by LS-CMV also mapped to RNA 3, and resistance breakage mapped to the MP gene and not to the CP gene. RNA 3 of CMV was implicated in the restriction of infection in maize using two other pair of strains of CMV (pseudorecombination between D- and R-CMV) and RNA 3 of D-CMV conditioned for resistance in maize (Marchoux et al., 1975). Very recently, Carrere et al. (1999) reports that RNAs 1 and/or 2 are involved in inability to move systemically in maize using R- (subgroup I) and I17F-CMV (subgroup II) strains, and in this case the resistance is due to interference with long-distance movement.

Mapping Functional Domains Involved in Movement Function of CMV

The movement of most plant viruses within their hosts involves several distinct stages. Depending on the virus system, plant viruses require one to four viral-encoded proteins involved in movement function. For some viruses, the CP of the virus as well as MP is involved in the virus movement. Models for the movement of viral genomes from site of replication to adjacent cells have been proposed, but, there remains many questions including why some viruses seem to have elements of different mechanisms of movement. CMV requires the CP along with the MP for viral cell-to-cell movement, in contrast to tobacco mosaic tobamovirus, where only 30K MP is required for the virus movement. Deletions in the N-terminal region of the CMV CP were used to demonstrate that virus particle formation is not essential for movement between cells, but is necessary for movement between leaves (Kaplan et al., 1998). In microinjection experiments, the CMV MP was able to traffic itself and RNA, and promoted the cell-to-cell movement of 10-kDa fluorescent dextran (Ding et al., 1995). The CMV MP was also shown to bind RNA and GTP in vitro (Li and Palukaitis, 1996).

To ascribe particular functions to specific sequences of the CMV 3a MP, we have constructed a series of alanine-scanning mutants in the 3a gene of the Fny strain of CMV (Fny-CMV), and assessed the mutants for their affects on CMV, infectivity and pathogenicity on host plants, the ability of the mutants to be complemented in trans by either a homologous or heterologous MP expressed in transgenic plants, and the ability of the corresponding mutants MPs to bind single stranded RNA and GTP *in vitro*. The data from these nine mutants were combined with earlier data on the effects of other point and deletion mutants on infectivity in different hosts, and recent data mapping the in vitro RNA binding domain (Vaquero et al., 1997), to generate a functional map of the CMV MP. Some MP mutants were unable to promote movement in any of host species. Three MP mutants defective in movement function could be complemented for movement in transgenic tobacco expressing the CMV MP. One MP mutant revealed temperature-sensitive for long-distance movement, but not for cell-to-cell movment. This confirms previous experiments indicating a role for the CMV MP in systemic as well as local movement.

Conclusion

Plants interact with viruses in various ways. Some of these interactions are associated with promoting the replication and systemic movement of the viruses, while others are associated with either resistance or susceptibility. Considerable progress has been made in delimiting viral sequences involved in virus vs. host, virus vs. vector, and virus vs. virus interactions. Various strains of CMV show differences in host range, pathogenecity, symptom expression, transmission by aphid vectors, replication of satellite RNA, and/or replication and translation efficiencies. These properties have been delimited to specific RNAs by pseudorecombinant tests using the genomic RNAs between strains. A similar phenotype can map to different viral genes involved in the movement of CMV, and it seems likely that different plant genes are involved in the different mechanisms of resistance to CMV.

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