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Isolation of mutants of *Arabidopsis thaliana* in which accumulation of tobacco mosaic virus coat protein is reduced to low levels

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Summary. We have found that *Arabidopsis thaliana* is susceptible to infection with a crucifer strain of tobacco mosaic virus (TMV-Cg); the coat protein of TMV-Cg accumulated to a high level in uninoculated rosette leaves several days after inoculation. As a first step in the search for host-coded factors that are involved in virus multiplication, we isolated mutants of *A. thaliana* in which the accumulation of TMV-Cg coat protein was reduced to low levels. Of 6000 M_2 plants descended from ethyl methanesulfonate-treated seeds, two such lines (PD114 and PD378) were isolated. Genetic analyses suggested that the PD114 phenotype was caused by a single nuclear recessive mutation, and that PD114 and PD378 belonged to the same complementation group. The coat protein accumulation of a tomato strain of TMV (TMV-L) was also reduced in PD114 plants compared to that in the wild-type plants. In contrast, PD114 plants infected with turnip crinkle or turnip yellow mosaic viruses, which belong to taxonomic groups other than Tobamovirus, expressed similar levels of these coat proteins as did infected wild-type plants.

Key words: Tobacco mosaic virus – *Arabidopsis thaliana* – Mutant – Resistance

Introduction

It is evident that host-derived factors are essential for virus multiplication. In the case of positive-strand RNA viruses, a number of observations imply that host factors, other than those needed for basic translation of virus-related RNAs, are essential for virus multiplication. For example: (1) viruses can systemically invade only a defined range of plant species. (2) For some host species which are normally susceptible to infection with a virus, resistant lines against the virus exist. Some recessive resistance genes may encode factors that support virus multiplication in wild-type hosts (Fraser 1987). (3) The replicase of the positive-strand RNA phage Qβ contains host-coded polypeptides (Blumenthal et al. 1972; Wahba et al. 1974). In the case of cucumber mosaic virus (CMV), a host-derived polypeptide co-purifies with replicase activity, although it is not yet clear whether or not the polypeptide is involved in the replication of CMV RNA (Hayes and Buck 1990). It seems reasonable, therefore, to assume that some specific host factors are involved in virus multiplication. To elucidate the molecular mechanisms involved in the multiplication process of positive-strand RNA viruses, it is essential to know something about these host factors.

One approach to identify putative host factors is to isolate and characterize genes that affect the multiplication of a virus, e.g. resistance genes against a virus. However, it has been, in general, difficult to isolate such genes. The crucifer *Arabidopsis thaliana* has many advantages for genetic and molecular biological studies and has been used extensively in recent years (for review, see Meyerowitz and Pruitt 1985; Estelle and Sommerville 1986; Bowman et al. 1988; Meyerowitz 1989). For these reasons, we set out to isolate mutants of *A. thaliana* that do not permit multiplication of a virus, and to identify the host factors involved in virus multiplication.

Tobacco mosaic virus (TMV) is a positive-strand RNA virus of plants (van Regenmortel and Fraenkel-Conrat 1986). Its genome encodes four known proteins: the non-structural 130 kDa, 180 kDa and 30 kDa proteins and the coat protein (CP) (Goelet et al. 1982; Ohno et al. 1984). After infecting a systemic host with TMV, it multiplies through three distinct processes: RNA replication, cell-to-cell movement, and long distance movement. The 130 kDa and 180 kDa proteins are involved

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In this paper, we use the term "multiplication (of a virus in a plant)" to mean a substantial increase in virus concentration in the uninoculated leaves of the infected plant. Therefore, the efficiency of each process of invasion of the plant by the virus, uncoating, replication and degradation of the virus genome, formation and degradation of the virus particles, and spreading of the virus in the plant will affect the degree of multiplication

in RNA replication, the 30 kDa protein in cell-to-cell movement, and the CP in long distance movement (for review, see Okada et al. 1990). In addition to the simple genetic organization and relatively well characterized relationship between virus-coded factors and functions, the following characteristics make TMV advantageous for experiments. TMV is stable and infectivity is sustained for weeks in crude leaf sap at room temperature (Gibbs 1977; Li et al. 1983). Infection of TMV is accomplished easily by inoculating the sap of infected tissue using a simple carborundum method. These characteristics enabled us to infect many individuals at a time. In a suitable TMV strain-host combination, TMV accumulates to an extremely high level: the common strain of TMV accumulates to 5–10 mg per g tissue in Samsun tobacco. This makes the detection and quantitation of CP accumulation easy.

Since we have been using mainly TMV-L (a tomato strain) for study (e.g. Meshi et al. 1986), we first tested whether TMV-L infects *A. thaliana* (ecotype Columbia). However, the rate of multiplication and the final level of CP accumulation was low. In this paper, we report the high-level CP accumulation of a crucifer strain of TMV (TMV-Cg) in *A. thaliana*, and the isolation and partial characterization of mutants of *A. thaliana* in which the accumulation of TMV-Cg CP is reduced to low levels.

Materials and methods

Plant materials and growth conditions. Seeds of *A. thaliana* ecotypes Columbia (Col-0) and Bensheim, and the mutant line Landsberg-erecta (La-*er*) were obtained from Dr. S. Naito. All other ecotypes (Landsberg, Niederzenz and Nossen) as well as the mutant line M7 (*ap-1, clv* line in the La-*er* background) were from Prof. A.R. Kranz. Plants were grown on a block of rockwool placed on a Vermiculite layer irrigated with mineral nutrients at 22–25° C under continuous fluorescent illumination.

Viruses and infection conditions. TMV-Cg was obtained from Prof. E. Shikata and Dr. T. Sano, turnip crinkle virus (TCV) from Prof. T.J. Morris, and turnip yellow mosaic virus (TYMV) from Prof. A.-L. Haenni. Each virus was propagated in turnip plants (*Brassica rapa* L. cv. Yorii) and infected leaves were stored at −80° C. Inocula for infection of *A. thaliana* were prepared as follows: frozen infected turnip leaves (5 g) were homogenized in 10 ml of 0.1 M sodium phosphate buffer (pH 7.0) and the resulting homogenate was diluted by adding 90 ml of H_2O, divided into small aliquots, and stored at −80° C before use. Rosette leaves of young *Arabidopsis* plants (15–21 days after sowing) were inoculated using carborundum as an abrasive and a small glass spatula. The inoculum solution was washed from the inoculated leaves with distilled water several minutes after inoculation.

Mutant screening. Ethyl methanesulfonate (EMS) mutagenesis of *A. thaliana* seeds was performed as described by Kranz and Kirchheim (1987). M_2 plants were inoculated with TMV-Cg and the uninoculated rosette leaves were harvested separately at 8–10 days after inoculation. Harvested leaves were placed in the wells of microtiter plates, sealed with plastic tape, and frozen and thawed twice. The leaves were crushed in the wells, and leaf sap was spotted directly onto Zeta-Probe membrane (Bio-Rad). The membrane was dried, rinsed with methanol: chloroform (1:1 mixture) until green materials were washed out, dried, and blocked by incubating in 5% (w/v) skim milk (Difco) in TBST (20 mM TRIS-HCl pH 7.6, 137 mM NaCl, 0.1% (w/v) Tween 20) at 50° C for 16 h. Detection of TMV-Cg antigen on the membrane was performed using anti TMV-Cg rabbit serum (provided by Prof. E. Shikata and Dr. T. Sano, and used at 1/50000 dilution in 5% (w/v) skim milk in TBS (20 mM TRIS-HCl pH 7.6, 137 mM NaCl) and a blotting detection kit (Amersham, RPN 23). From the individuals that gave weak signals, selfed (M_3) seeds were taken and a similar assay was performed for five to ten individuals of M_3 plants per line and all negative lines were selected.

Quantitation of viral coat proteins. A rosette leaf ($\times$ mg) of *Arabidopsis* was homogenized in an equal volume ($\times$ µl) of 250 mM TRIS-HCl pH 7.5, 2.5 mM EDTA, 0.1% (w/v) ascorbic acid, 1 mM phenylmethylsulfonyl fluoride, the resulting homogenate was mixed with 4 volumes ($4 \times$ µl) of 78 mM TRIS-HCl pH 6.8, 3.75% (w/v) 2-mercaptoethanol, 2.5% (w/v) SDS, 0.01% (w/v) bromophenol blue, boiled for 3 min, and then used for protein analysis by SDS-polyacrylamide gel electrophoresis (PAGE) (Laemmli 1970). Quantitation of viral coat proteins separated by SDS-PAGE was performed by the method described by Ball (1986). Briefly, gels were stained by soaking in 0.05% Coomassie brilliant blue R-250 (CBB-R) in 25% (v/v) 2-propanol, 10% (v/v) acetic acid for 2 h at room temperature. Gels were destained completely with 10% (v/v) acetic acid, 10% (v/v) methanol. A segment of the gel in which the viral coat protein band was contained was cut out and CBB-R dye bound to proteins in the gel piece was eluted by soaking in 50% (v/v) 2-propanol, 3% (w/v) SDS in H_2O at 37° C for 24 h and the concentration of CBB-R was quantitated by measuring absorbance at a wavelength of 595 nm. The quantity of viral coat proteins was estimated from a standard curve taking background signals into account. When the accumulation level of viral coat protein was low, immunodetection on Western blots was used to estimate the approximate quantity of coat proteins.

Results

Susceptibility of A. thaliana *to infection with TMV-Cg (a crucifer strain)*

In order to find host factors which are involved in virus multiplication using a genetic approach, a suitable virus-host model system was established. As described in the

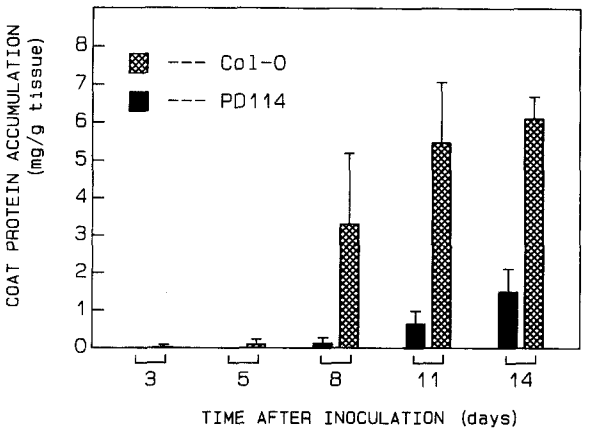

Fig. 1. Time course of tobacco mosaic virus (TMV-Cg) coat protein accumulation in Col-0 and PD114 plants. Col-0 and PD114 (M_5) plants (25 individuals for each line) were inoculated simultaneously with TMV-Cg and rosette leaves from five inoculated individuals were harvested separately at 3, 5, 8, 11, and 14 days after inoculation (each inoculated plant was subjected to sampling only once). The accumulated level of coat protein (CP) in rosette leaves was determined for each individual by the method of Ball (1986) when the accumulation of CP was higher than 0.5 mg/g tissue, or by immunoblot analysis when the accumulation was lower than 0.5 mg/g tissue. The figure includes data from two independent experiments, i.e. *Boxes* show the averages of CP accumulation in ten individuals. Error bars equal one standard deviation

Introduction, *A. thaliana* and TMV individually have many advantageous characteristics suitable for such studies. However, it must be noted that a tomato strain of TMV did not multiply to a high level in *A. thaliana*.

TMV-Cg is a crucifer strain of TMV isolated from garlic in Japan (Li et al. 1983). When *A. thaliana* ecotype Columbia (Col-0) plants were inoculated with TMV-Cg, a large amount of CP accumulated in the uninoculated leaves of the infected plants: about 3 mg/g tissue at 8 days post-inoculation (p.i.), and about 6 mg/g tissue at 14 days p.i. The time course of CP accumulation is shown in Fig. 1. The susceptibility of some other ecotypes or a mutant line of *A. thaliana* (Bensheim, Landsberg, La-*er*, Niederzenz, and Nossen) was tested. All were susceptible to TMV-Cg infection with small differences in the time course of CP accumulation. Considering the advantages of handling the most commonly used ecotype, e.g. availability of probes for restriction fragment length polymorphism (RFLP) mapping (Chang et al. 1988; Nam et al. 1989), we used the ecotype Col-0 for mutant isolation.

When Col-0 plants were inoculated with TMV-Cg, they showed mild disease symptoms: stunting and abnormal curving of flowering stalks and dark-greening of rosette leaves were observed after several days p.i. when young plants (15–20 days after sowing) were inoculated. When older plants (>3 weeks after sowing) were inoculated, the disease symptoms were very weak or undetectable. Because inoculated leaves died within a few days after inoculation due to damage caused by rubbing, we were not able to observe the symptoms on the inoculated leaves. Infected plants produced seeds in the late stages of anthesis although most flowers were infertile in the early stages. All the progeny plants derived from the seeds of infected plants examined were free from TMV-Cg.

Isolation of mutants of A. thaliana *in which accumulation of TMV-Cg coat protein was reduced to low levels*

Selection of mutant *Arabidopsis* plants, in which accumulation of TMV-Cg was impaired, was performed as follows: Plants were inoculated with TMV-Cg and after an appropriate interval (8–10 days), the uninoculated rosette leaves were harvested separately and the accumulation of CP was quantitated. Plants in which CP accumulation was undetectable or low were selected. A dot enzyme-linked immunosorbent assay was used to quantitate CP accumulation in leaves (see Materials and methods).

We examined the susceptibility of 6000 M_2 plants descended from ethyl methanesulfonate (EMS)-treated seeds to infection with TMV-Cg, and selected about 400 candidates in which undetectable or low levels of CP accumulated. These candidates undoubtedly included individuals which were intrinsically susceptible to infection but failed to be infected or did not permit full virus multiplication for unknown reasons, e.g. the physiological conditions of the plants. Each candidate was self-pollinated. M_3 seeds were harvested separately, and M_3 plants were tested for susceptibility to infection (5–10 M_3 individuals per candidate). Among those tested, only two lines, PD114 and PD378, generated M_3 plants which did not permit full accumulation of TMV-Cg CP. PD114 and PD378 were descended from independent M_1 seeds derived from independently mutagenized populations. PD114 and PD378 bred true for the phenotype of the reduced accumulation of TMV-Cg CP in the M_3, M_4 and M_5 generations. In the M_3 generation, PD114 segregated dwarf individuals which were too small to be inoculated. Because the dwarf individuals were about one-fourth of the M_3 plants, it was suggested that the dwarf phenotype was caused by a single recessive mutation and that the PD114 M_2 plant was heterozygous for the mutation. Therefore, we selected a PD114 M_3 line which did not segregate the dwarf phenotype in the next generation.

Figure 1 shows the time course of CP accumulation in PD114 M_5 plants and in the parent Col-0. In PD114 plants, the accumulation of CP was reduced to about one-fourth of that in Col-0 plants at 14 days p.i. In PD378 plants, the time course of CP accumulation was similar to that in PD114 plants (data not shown). The accumulation level of TMV-Cg CP at 14 days p.i. in PD114 plants that had been inoculated with leaf sap obtained from PD114 plants inoculated with TMV-Cg and harvested at 14 days p.i. after confirmation that the CP accumulation reached the level of 1.5 mg/g tissue, was similar to that in PD114 plants inoculated with authentic TMV-Cg (Komatani et al., unpublished results). The result indicated that CP accumulated in PD114 plants in later stages of infection was probably not derived from mutant virus or viruses which were able to multiply in PD114 plants. Rather, this observation sug-

gests that PD114 plants permit a low level of multiplication of TMV-Cg.

Reduced accumulation of TMV-Cg coat protein in PD114 plants is caused by a recessive mutation at a single locus in the nuclear genome

The genetic basis for the phenotype of reduced accumulation of TMV-Cg CP was determined by making several genetic crosses. The F_1 plants resulting from cross of the wild-type (wt) Col-0 with pollen from PD114 or the reciprocal cross were susceptible to infection with TMV-Cg, and the level of CP accumulation was similar to, or slightly reduced in comparison with those seen in wt Col-0 plants (Fig. 2). The disease symptoms shown by infected F_1 plants were similar to those shown by infected Col-0 plants. Furthermore, these results were confirmed by crossing the mutant line M7 (a La-*er* derivative homozygous for the recessive morphological mutations *ap-1* and *clv*) with pollen from PD114. Success of the cross was confirmed by the wt morphology of F_1 plants. The level of accumulation of TMV-Cg CP in the F_1 plants from the ♀M7 × ♂PD114 cross was similar to, or slightly reduced in comparison with that in the F_1 plants from the ♀M7 × ♂Col-0 cross (Fig. 2).

Of the F_2 plants derived from self-pollination of F_1 plants (♀Col-0 × ♂PD114 or ♀PD114 × ♂Col-0) about one-fourth of the total showed a low-level (i.e. similar to that in PD114 plants) of TMV-Cg CP accumulation (Table 1). The 3:1 (high-level, i.e. similar to that in Col-0 plants: low level TMV-Cg CP accumulation) segregation, together with the result that F_1 plants showed a high-level TMV-Cg CP accumulation phenotype, is consistent with the hypothesis that the phenotype of reduced TMV-Cg CP accumulation is caused by a monogenic recessive Mendelian trait. This conclusion was supported by the 1:1 segregation in a test cross in which pollen from PD114 was crossed to a F_1 plant (♀Col-0 × ♂PD114) (Table 1). We call the mutation that causes the phenotype of reduced accumulation of TMV-Cg CP in PD114 *tom1-1* (*To*bamovirus *m*ultiplication) to indicate that it is a recessive allele of the *TOM1* gene.

The growth of PD114 plants in the M_3, M_4 and M_5 generations was slightly slower than that of Col-0 plants, although no differences were detectable under some conditions. The reduced-growth phenotype did not co-segregate with the phenotype of reduced accumulation of TMV-Cg CP in F_2 plants derived from self-pollination of F_1 plants (♀Col-0 × ♂PD114 or ♀PD114 × ♂Col-0), suggesting that this phenotype is independent of the *tom1-1* mutation. We did not identify any morphological changes that segregated with the *tom1-1* mutation. In contrast to Col-0 plants, PD114 plants infected with TMV-Cg showed only slight or undetectable disease symptoms.

In F_1 plants resulting from the crosses between Col-0 and PD378 (♀Col-0 × ♂PD378 and ♀PD378 × ♂Col-0), the accumulation of TMV-Cg CP was similar to that in Col-0 plants (data not shown). In F_1 plants resulting from the crosses between PD378 and PD114 (♀PD378 × ♂PD114 and ♀PD114 × ♂PD378), the accumulation of TMV-Cg CP was similar to those in PD378 and PD114

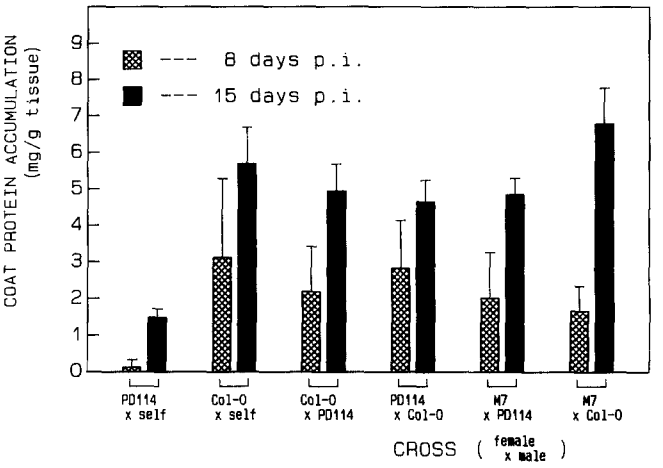

Fig. 2. Comparison of TMV-Cg coat protein accumulation between Col-0, PD114, and F_1 plants resulting from crosses involving PD114, Col-0 or a mutant line M7. Self-fertilized plants (PD114(M_4) × self; 13 individuals), (Col-0 × self; 14 individuals) and F_1 plants resulting from (♀Col-0 × ♂PD114(M_4); 16 individuals), (♀PD114(M_4) × ♂Col-0; 12 individuals), (♀M7 × ♂PD114(M_4); 13 individuals), and (♀M7 × ♂Col-0; 14 individuals) crosses were inoculated with TMV-Cg, from which uninoculated rosette leaves were harvested separately at 8 days and 15 days after inoculation (each inoculated plant was subjected to sampling twice), and the accumulated level of TMV-Cg CP was determined as described in Fig. 1. *Boxes* show the averages of CP accumulation in the indicated numbers of individuals. Error bars equal one standard deviation

plants, i.e. lower than that in Col-0 plants (data not shown). These data suggest the causative mutation in PD378 is recessive and also occurred at the *TOM1* locus. We call the mutation that causes the phenotype of reduced accumulation of TMV-Cg CP in PD378 *tom1-2*. The size of PD378 plants (in the M_3, M_4 and M_5 generations) was slightly smaller than that of the wt Col-0 plants. However, this phenotype was not associated with the *tom1-2* mutation.

Susceptibility of PD114 to infection with viruses other than TMV-Cg

To determine whether or not the *tom1* mutation affected CP accumulation of viruses other than TMV-Cg, we inoculated PD114 M_5 plants with TMV-L, turnip crinkle virus (TCV; Hollings 1972; Carrington et al. 1989), or turnip yellow mosaic virus (TYMV; Matthews 1980; Morch et al. 1988). TMV, TCV and TYMV belong to different taxonomic groups. TCV and TYMV are known to infect *A. thaliana* (Ramachandra et al. 1987). The accumulation of CPs in Col-0 and PD114 plants inoculated with TMV-L, TCV or TYMV was examined by separating the total proteins of uninoculated leaves in SDS-polyacrylamide gels and staining with Coomassie brilliant blue dye. Of 34 Col-0 plants inoculated with TMV-L, 30 accumulated TMV-L CP to the level of 1–2 mg/g tissue at 21 days p.i., but of 37 PD114 (M_5) plants inoculated with TMV-L, only 4 accumulated detectable (but much lower than 0.5 mg/g tissue) amounts of CP. In contrast, similar amounts of CPs accumulated in Col-0 and PD114 (M_5) plants at 14 days after inoculation with TCV or TYMV (for TCV, see panel B in Fig. 3 and

Table 1. Genetic segregation of the phenotype of low-level accumulation of TMV-Cg coat protein

Cross	Number of plants in which TMV-Cg CP accumulated to		χ^{2b}
	High-level[a]	Low-level[a]	
(♀PD114 × ♂Col-0) × self (F_2)	153	51	0.00[c]
(♀Col-0 × ♂PD114) × self (F_2)	159	62	1.10[c]
♀(♀Col-0 × ♂PD114) × ♂PD114 (test cross)	46	54	0.64[c]

[a] Whether an F_2 or test cross plant exhibited a high-level coat protein (CP) accumulation or a low-level CP accumulation phenotype was judged as follows. The F_2 or test cross plants were inoculated with tobacco mosaic virus (TMV-Cg), and the accumulation of CP was examined in SDS-polyacrylamide gels stained with Coomassie brilliant blue dye. Among the plants examined, individuals in which the CP accumulation was significantly lower than in the standard Col-0 plants were selected (hereafter the selected plants are designated L-plants). This L-plant population may have contained individuals which allow TMV-Cg CP accumulation to levels similar to that in Col-0 plants but failed to be infected. The L-plants were then self-pollinated and TMV-Cg CP accumulation was examined again in the next generation. For each L-plant, five individuals of the next generation were inoculated with TMV-Cg, leaves were harvested from them, mixed, and the accumulation level of TMV-Cg was examined. Finally, those L-plants in which the accumulation level of TMV-Cg CP was similar to that in PD114 plants in a further (next generation of L-plant) assay were assigned to the low-level accumulation class and the other F_2 or test-cross plants were counted as showing the high-level accumulation phenotype.

[b] The chi-square values were calculated on the basis of the expected ratio of 3 high-level:1 low-level and 1 high-level:1 low-level for the F_2 and test cross, respectively

[c] Probability >0.05

compare with the results for TMV-Cg in panel A). These observations suggest that the *tom1-1* mutation acts specifically on the CP accumulation of Tobamoviruses and not on those of TCV or TYMV.

Discussion

On the assumption that positive-strand RNA viruses multiply using specific host factors as well as factors encoded by virus genomes, we attempted to identify such host factors using a genetic approach. Using the *Arabidopsis*-TMV-Cg system, we attempted to isolate *Arabidopsis* mutants which could not support TMV-Cg multiplication, or supported it with an efficiency different from that in wt plants. Among 6000 M_2 plants descended from EMS-treated seeds, we were able to isolate two mutants in which TMV-Cg CP accumulation was reduced. The causative mutations in both mutants were monogenic, recessive and allelic, occurring in the nuclear gene *TOM1*.

It has been shown that mutations resulting in loss of function can be recovered by screening approximately 2000 M_2 plants derived from EMS mutagenesis (Estelle and Sommerville 1986). Assuming there are additional genes which are involved in TMV multiplication (*TOM* genes), there are several possible reasons why *tom* mutants other than *tom1* lines were not isolated in this study: Other *TOM* genes may be essential for normal plant growth, and the loss of one of the functional *TOM* genes would be lethal or would result in plants that are too small to be inoculated. In order to isolate mutants in which the products of such *TOM* genes have lost the ability to support virus multiplication, but still retain their ability to support normal growth of the host, it

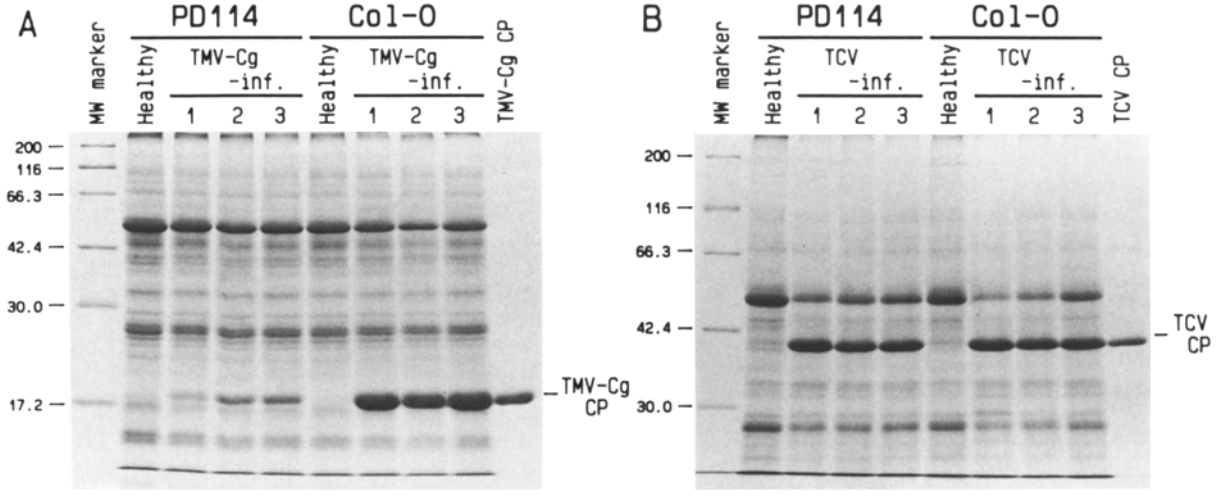

Fig. 3. Accumulation of coat proteins of TMV-Cg (panel A) and turnip crinkle virus (TCV; panel B) in Col-0 and PD114 plants. Inoculation experiments were repeated three times. The numbers 1, 2, and 3 above the lanes indicate independent experiments. In each experiment, five Col-0 and PD114 (M_5) plants were inoculated with TMV-Cg and TCV, rosette leaves were harvested from them separately at 14 days after inoculation, total leaf proteins were prepared and equal volumes of total leaf proteins were mixed and analyzed. Total proteins from 0.67 mg of leaves or coat proteins from 2.5 μg of virus particles of TMV-Cg or TCV were separated in SDS-11% (containing 0.55% bisacrylamide; panel A) or 9% (containing 0.27% bisacrylamide; panel B) polyacrylamide gels and stained with Coomassie brilliant blue dye. Total leaf proteins from healthy plants were concurrently electrophoresed. The positions of coat proteins of TMV-Cg and TCV are indicated at the right of the gels. The positions of molecular weight markers (myosin, 200 kDa; β-galactosidase, 116 kDa; bovine serum albumin, 66.3 kDa; aldolase, 42.4 kDa; carbonic anhydrase, 30.0 kDa; myoglobin, 17.2 kDa) are shown at the left of the gels

will be necessary to screen many more M_2 plants, or to search for conditional mutants. Alternatively, other *TOM* genes may each occur in multiple copies or independently encoded factors could substitute for one another during the various steps of virus multiplication.

The *tom1* mutation had a specific effect on the CP accumulation of TMV-Cg and TMV-L, but not on that of TCV and TYMV. This implies that, in the *tom1* lines, the accumulation of TMV CP is lowered due to the acquisition or the loss of a specific interaction, direct or indirect, between TMV-derived molecule(s) and the *TOM1* gene product, rather than by some non-specific effects. The recessiveness of the *tom1* mutation implies that in wt plants a functional interaction between TMV-encoded molecule(s) and the *TOM1* gene product is required to support full accumulation of TMV CP. However, other possibilities are conceivable. For example, the *TOM1* gene product might be a repressor of a putative function which inhibits multiplication of TMV. If the *TOM1* gene product supports the multiplication of TMV, the observation that TMV-Cg was able to multiply at low levels in *tom1* lines can be interpreted in any of the following ways. (1) The function of the *TOM1* gene product may not be completely destroyed in either of the two *tom1* lines. (2) Factor(s) encoded by gene(s) other than *TOM1* may act in place of the *TOM1* gene product with reduced but significant efficiency. (3) The *TOM1* gene product may act as an enhancer of TMV CP accumulation. Considering that the PD114 and PD378 lines, which are independent *tom1* mutants, showed a similar decrease in the multiplication of TMV-Cg, the first possibility seems to be the least likely.

It is likely that, in *tom1* lines, the lesion(s) in the infection process occur(s) after the uncoating of virus particles because the *tom1* mutation affected the level of TMV-Cg CP accumulation. However, it is as yet unknown what is the *TOM1* gene product functionally acting (protein, RNA, or other molecules), or which step in the accumulation of TMV-Cg CP is impaired in the *tom1* lines (replication or degradation of genomic and/or complementary RNAs, transcription or degradation of CP mRNA, translation or degradation of CP, cell-to-cell or long-distance movement of virus). In order to reveal the mechanism of the inhibition of TMV-CP accumulation in *tom1* plants, further experimentation is necessary to examine the multiplication process in protoplasts isolated from wt and *tom1* plants.

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