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Complete Inhibition of Tobamovirus Multiplication by Simultaneous Mutations in Two Homologous Host Genes

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The *TOM1* gene of *Arabidopsis thaliana* encodes a putative multipass transmembrane protein which is necessary for the efficient multiplication of tobamoviruses. We have previously shown that mutations severely destructive to the *TOM1* gene reduce tobamovirus multiplication to low levels but do not impair it completely. In this report, we subjected one of the *tom1* mutants (*tom1-1*) to another round of mutagenesis and isolated a new mutant which did not permit a detectable level of tobamovirus multiplication. In addition to *tom1-1*, this mutant carried a mutation referred to as *tom3-1*. Positional cloning showed that *TOM3* was one of two *TOM1*-like genes in *Arabidopsis*. Based on the similarity between the amino acid sequences of TOM1 and TOM3, together with the results of a Sos recruitment assay suggesting that both TOM1 and TOM3 bind tobamovirus-encoded replication proteins, we propose that TOM1 and TOM3 play parallel and essential roles in the replication of tobamoviruses.

A variety of host factors are suggested to be involved in the intracellular multiplication of viruses. For eukaryotic positive-strand RNA viruses, host proteins that are associated with viral RNA-dependent RNA polymerases and those which specifically bind virus-related RNAs have been identified and implicated in viral RNA replication (reviewed in references 6 and 14). More recently, host mutants have been isolated in which the intracellular multiplication of a virus is suppressed (10, 12, 15, 17, 19), and the wild-type genes corresponding to some of these mutations have subsequently been cloned. Such genetic approaches have led to the identification of *Saccharomyces cerevisiae* genes that are necessary for efficient RNA replication and translation of brome mosaic virus (7, 15, 17) and an *Arabidopsis thaliana* gene, *TOM1*, that is necessary for efficient multiplication of tobamoviruses (25). These accumulating data suggest that viruses recruit many host components for their own intracellular multiplication, including gene expression and replication.

Tobamovirus is a representative member of the alpha-like virus supergroup of positive-strand RNA viruses (6). It replicates in a membrane-bound replication complex containing the virally encoded protein with an approximate molecular weight of 130,000 (130K protein), the 180K protein (read-through product of the 130K protein), and host-derived proteins (20, 23). We have previously identified the *Arabidopsis TOM1* gene, which encodes a putative multipass transmembrane protein necessary for the efficient multiplication of tobamoviruses (25). The 291-amino-acid TOM1 polypeptide likely interacts with the tobamovirus-encoded 130K-180K proteins via the conserved helicase domain and has been suggested elsewhere to function as a membrane anchor for the tobamovirus replication complex in vivo (25). The function of TOM1 in the host remains to be revealed.

Despite the anticipated important role of TOM1 in tobamovirus multiplication, none of the three *tom1* mutations (*tom1-1* is a point mutation at a splice acceptor site, *tom1-2* is a nonsense mutation at amino acid residue 68, and *tom1-3* is a frameshift mutation at amino acid residue 147) repressed tobamovirus multiplication completely (Fig. 1) (25). This residual activity supporting tobamovirus multiplication might depend on one or more *TOM1*-like genes in *Arabidopsis*. Alternatively, *TOM1* might function primarily to enhance the replication of tobamoviruses but might not be absolutely required for it. In the study reported in this paper, we mutagenized one of the *tom1* mutants and isolated a new double mutant in which the multiplication of tobamoviruses could not be detected. Positional cloning identified *TOM3* as the causal mutation gene, which is one of the two *TOM1*-like genes in *Arabidopsis*. The results suggested that TOM1 and TOM3 have parallel and essential roles in the multiplication of tobamoviruses.

MATERIALS AND METHODS

Plants, viruses, and DNA clones. *A. thaliana* (L.) Heynh. ecotype Columbia (Col-0) was used as the wild-type strain. The *tom1-1* and *tom1-3* mutants have been previously described (12, 25). TMV-Cg, a crucifer-infecting tobamovirus (24), and TMV-L, a tomato mosaic tobamovirus (18), were used for the inoculation of *Arabidopsis* plants. The former virus is closely related to Chinese rape mosaic virus (1), which was independently characterized. Cucumber mosaic virus (CMV) strain Y and turnip crinkle virus (TCV) strain B (12) were also used in this study. Tobamoviruses and CMV belong to the alpha-like virus supergroup, whereas TCV belongs to the carmo-like virus supergroup (6). The conditions of plant growth, inoculation with the viruses, mutagenesis of *A. thaliana* seeds with ethyl methanesulfonate, and screening for mutants with reduced TMV-Cg multiplication were set or performed as described previously (12). Isolation of the *tom3-1* single mutant from the *tom1-1 tom3-1* double mutant was carried out by first crossing the *tom1-1 tom3-1* double mutant with wild-type Col-0. Genomic DNA was then separately purified from the F_2 plants and used for derived

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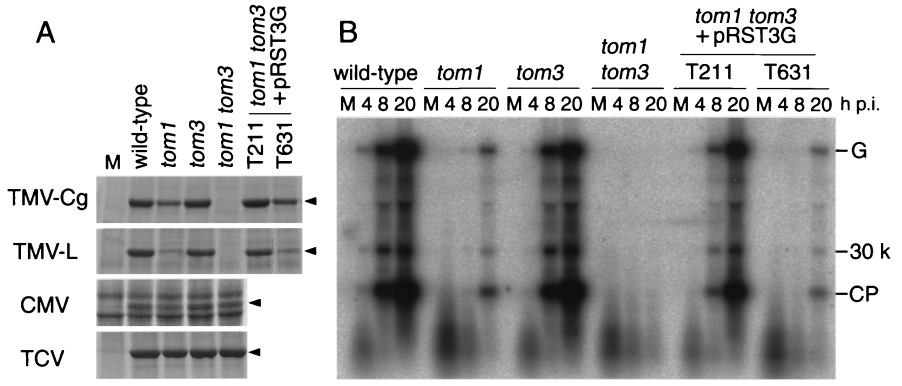

FIG. 1. Tobamovirus multiplication in the *tom1-1 tom3-1* double mutant. (A) Accumulation of viral CPs in inoculated plants. Wild-type (Col-0), mutant, and T_3 transgenic line T211 and T631 plants were inoculated with the viruses TMV-Cg, TMV-L, CMV-Y, and TCV-B as indicated. T211 and T631 were derived from a *tom1-1 tom3-1* double mutant transformed with the T-DNA clone pRST3G, and each carried the transgene at a single locus homozygously. At 16, 21, 10, and 10 days after inoculation with TMV-Cg, TMV-L, CMV-Y, and TCV-B, respectively, total protein was prepared from aerial parts of the inoculated plants, separated by SDS-PAGE, and stained with Coomassie blue. M, mock-inoculated wild-type plants. Arrowheads show the positions of viral CPs. (B) Accumulation of TMV-Cg-related RNA in protoplasts. Protoplasts were inoculated with TMV-Cg virion RNA by electroporation and cultured for 4, 8, or 20 h, and the accumulation of TMV-Cg-related RNAs was examined by Northern blot hybridization. The positions of the genomic RNA (G) and the subgenomic mRNAs for 30-kDa protein (30 k) and CP are indicated at the right. M, mock-inoculated protoplasts harvested at 20 h postinoculation (h p.i.). The quality of protoplasts in each preparation was confirmed by similar levels of accumulation of TCV-B-related RNAs after inoculation (data not shown). A representative result of three independent repeats is shown.

cleaved amplified polymorphic sequence analysis (16) to determine the genotype at the *TOM1* and *TOM3* loci (T. Yamanaka, S. Naito, and M. Ishikawa, unpublished data). The *tom3-1* single mutant (wild type at *TOM1* locus) was selected and self-pollinated, and the resulting F_3 seeds were used for analysis. Yeast artificial chromosome (YAC) and bacterial artificial chromosome (BAC) *Arabidopsis* genomic clones and expressed sequence tag (EST) clones were obtained from the Arabidopsis Biological Resource Center (Ohio State University, Columbus).

Isolation of *TOM3* and *THH1* cDNAs and sequence analysis. For *TOM3*, we screened an *A. thaliana* 5′-STRETCH cDNA library (Clontech) containing 10^5 plaques and identified cDNA clones hybridizing with the EST clone 86D11 (GenBank accession no. T20522). Inserts of the cDNA clones were amplified by PCR and sequenced. Primers used to amplify and sequence the cDNA inserts were 5′-CGCCTCCATCAACAAACTTTCTTG-3′ and 5′-GTTCTGGTAAAA AGCGTGGTC-3′. For *THH1*, the mRNA sequence covering the coding region was identified by assembling the sequence of the EST clone 701681346 (GenBank accession no. AI997527) together with that determined by 5′ rapid amplification of cDNA ends (RACE) using a SMART PCR cDNA synthesis kit (Clontech). A 377 DNA sequencer (Applied Biosystems) and BigDye Terminator Sequencing kit (Perkin-Elmer) were used for sequencing.

Complementation analysis of the *tom3-1* mutation. The T-DNA clone pRST3G was constructed by inserting the 7.4-kb *Xba*I-*Xho*I fragment encompassing the *TOM3* mRNA region from the BAC genomic clone F5O4 (GenBank accession no. AC005936) into the T-DNA vector pCLD04541 (8). pRST3G was electroporated into *Agrobacterium tumefaciens* C58C1(pGV2260) and was used to transform *A. thaliana* plants by vacuum infiltration (5).

Protein and RNA analysis. The accumulation of viral coat proteins (CPs) was examined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) followed by Coomassie blue staining or immunoblot analysis as previously described (12). Preparation of protoplasts, introduction of viral RNAs into the protoplasts by electroporation, and subsequent RNA analysis were performed as described previously (11). For the analysis of *TOM3* mRNA, total RNA was extracted and purified from frozen plant tissues by using an RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Northern blotting and hybridization were performed as described previously (11). To prepare a probe, the open reading frame (ORF) sequence of *TOM3* was amplified from *Arabidopsis* SMART cDNA by PCR and cloned into pET3b (Novagen, Madison, Wis.). ^{32}P-labeled DNA probe was prepared with a gel-purified DNA fragment corresponding to the *TOM3* ORF sequence with a Multiprime or Megaprime DNA labeling system (Amersham Pharmacia Biotech). Preparation of a DNA probe for 18S rRNA has been described previously (25).

The Sos recruitment system. The *S. cerevisiae cdc25-2* strain and plasmids to express TOM1, 5′Sos-CgHel (referred to as "5′Sos-Hel" in reference 25), TOM1-5′Sos, and p110-5′Sos, have been previously described (4, 25). TOM3-5′Sos is a fusion of the full-length TOM3 (amino acids 1 to 303) to the N terminus of 5′Sos (amino acids 1 to 1066). 5′Sos-LHel is a fusion of the C-terminal part of the TMV-L 130K protein (amino acids 648 to 1116) (18) to the C terminus of 5′Sos. The *S. cerevisiae ADH1* promoter (3) was used to drive expression on 2μm plasmid vectors (9).

Nucleotide sequence accession number. The sequences reported in this paper have been deposited in the GenBank database (accession no. AB036427 and AB057678).

RESULTS

Isolation of an *Arabidopsis* mutant in which tobamovirus multiplication is undetectable. In order to investigate why tobamovirus multiplication was not completely impaired by mutations severely destructive to *TOM1*, *tom1-1* seeds were mutagenized with ethyl methanesulfonate and the M_2 plants were screened for mutants in which the multiplication of TMV-Cg was further reduced. TMV-Cg multiplication was determined by using the dot enzyme-linked immunosorbent assay method to measure the accumulation of TMV-Cg CP at 12 days after inoculation (12). From a total of 6,100 M_2 plants, one mutant was isolated in which the multiplication of TMV-Cg was undetectable. Genetic analyses suggested that the mutant harbored a recessive or semidominant mutation in addition to *tom1-1* (data not shown). This new mutation was referred to as *tom3-1*. The *tom1-1 tom3-1* double mutant grew a little slower than, and the plants were slightly smaller than, the wild-type plants. The CP of TMV-Cg was not detected in inoculated *tom1-1 tom3-1* double mutant plants by either Coomassie blue staining (Fig. 1A) or immunoblot analysis (data not shown) of total proteins separated by SDS-PAGE. When *tom1-1 tom3-1* double mutant protoplasts were inoculated with TMV-Cg RNA by electroporation, neither genomic nor subgenomic TMV-Cg RNA could be detected by Northern analysis (Fig. 1B). Comparison with serial dilutions of an RNA sample from TMV-Cg-inoculated wild-type protoplasts indicated that the level of TMV-Cg RNA accumulation in the double mutant protoplasts was less than 0.1% of the level detected in the wild-type protoplasts at 20 h postinoculation (data not shown).

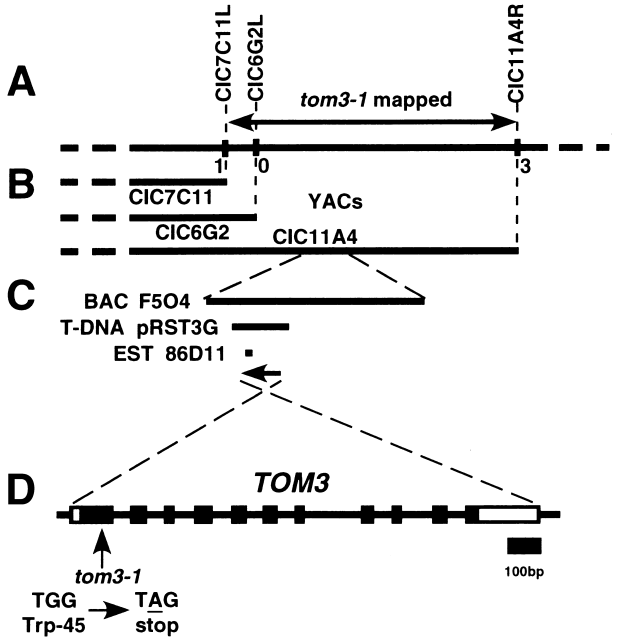

FIG. 2. Genetic mapping and positional cloning of *TOM3*. (A) Genetic map around the *TOM3* locus on chromosome 2. Vertical bars represent YAC end probes. Each number represents the number of recombined chromatids between the marker and the *TOM3* locus among the 12 *tom1 tom3* F_2 mapping lines (see Materials and Methods). (B) YAC contig around *TOM3* locus. (C) The positions of the BAC clone F5O4, T-DNA clone pRST3G, and EST clone 86D11. The T-DNA clone pRST3G was tested for complementation of the TMV-Cg multiplication phenotype in the *tom1 tom3* double mutant. The position and orientation of the *TOM3* gene are shown by the arrow. (D) Intron-exon organization of the *TOM3* gene and the *tom3-1* mutation. Exons are indicated by boxes. Open boxes indicate noncoding regions, and closed boxes indicate coding regions. The *tom3-1* mutation is shown below the intron-exon structure.

The multiplication of TMV-L, a tomato mosaic tobamovirus, was also undetectable in the *tom1-1 tom3-1* double mutant plants (Fig. 1A). On the other hand, CMV (a cucumovirus) and TCV (a carmovirus) both multiplied in the *tom1-1 tom3-1* double mutant plants as efficiently as in the wild-type plants (Fig. 1A). In *tom3-1* single mutant plants, which were obtained by back-crossing the double mutant with wild-type Col-0, TMV-Cg, TMV-L, CMV, and TCV all multiplied as efficiently as in the wild-type plants (Fig. 1A). Accumulation of TMV-Cg RNA in *tom3-1* single mutant protoplasts was also similar to that in wild type (Fig. 1B).

Identification of *TOM3*. The *tom3-1* mutation was first roughly mapped by selecting 12 F_2 lines carrying a *tom1 tom3* double mutation from a cross between a *tom1-3* single mutant (ecotype Wassilewskija background) and the *tom1-1 tom3-1* double mutant (Col-0 background). Genotypes of DNA markers were determined for each line, and this indicated that the *tom3-1* mutation was located on chromosome 2 within a region covered by the YAC clone CIC11A4 (Fig. 2) (The Arabidopsis Information Resource [http://www.arabidopsis.org/]).

A BLAST search (2) of the *Arabidopsis* genome database (The Arabidopsis Information Resource [http://www.arabidopsis.org/]) revealed two expressed genes closely related to *TOM1*. One of the EST clones, 86D11 (GenBank accession no. T20522), was interestingly derived from the region of chromosome 2 covered by both the BAC clone F5O4 (GenBank accession no. AC005936) and the YAC clone CIC11A4, in which the *tom3-1* mutation was mapped. cDNA cloning using 86D11 DNA as a probe and the 5′- and 3′-RACE methods revealed a 1,229-nucleotide mRNA sequence containing an ORF encoding a 303-amino-acid protein (GenBank accession no. AB036427) (Fig. 3). The *tom3-1* mutant had a nonsense mutation in the first exon of this gene (Fig. 2). Accumulation of the mRNA corresponding to this gene was reduced in *tom3-1* and *tom1-1 tom3-1* mutant plants compared to that in plants carrying the wild-type *TOM3* gene (Fig. 4), possibly reflecting a nonsense-mediated mRNA decay-like phenomenon. These results suggested that this gene was *TOM3*.

To further confirm whether the above mRNA sequence represented *TOM3*, a 7.4-kb wild-type genomic DNA fragment from the BAC clone F5O4 encompassing the mRNA sequence (Fig. 2C) was subcloned into a T-DNA vector and used to stably transform the *tom1-1 tom3-1* double mutant. In the resulting 15 T_2 plants, TMV-Cg multiplied to various degrees, ranging up to a level observed in the wild-type plants. In the transgenic line T211, TMV-Cg multiplied almost as efficiently as in the wild-type plants, whereas in the line T631 the multiplication level was similar to the level in the *tom1-1* single mutant plants (Fig. 1). These two transgenic lines carried the transgene at a single locus homozygously. Northern blot hybridization analyses showed that the accumulation level of *TOM3* mRNA in T211 and T631 was higher than and similar to that in the wild-type plants, respectively (Fig. 4). These results confirmed that the *TOM1*-like gene on BAC F5O4 is *TOM3* and demonstrated that overexpression of *TOM3* fully complements the *tom1-1 tom3-1* double defect.

Characterization of the other *TOM1*-like gene, *THH1*. The other EST encoding a *TOM1*-like sequence was 701681346 (GenBank accession no. AI997527), which was derived from a region on chromosome 1 covered by the BAC clone T5E2 (GenBank accession no. AC005936). Reverse transcription-PCR and 5′-RACE analyses, combined with the sequence data for the EST clone, identified a 1,218-nucleotide mRNA sequence (GenBank accession no. AB057678) containing an ORF encoding 293 amino acids (Fig. 3). We named this gene *THH1* (for TOM three homolog), because the predicted amino acid sequence of THH1 resembles that of TOM3 more than that of TOM1. Based on the alignment shown in Fig. 3A, TOM1 and TOM3 share 56% identity, TOM1 and THH1 share 58% identity, and TOM3 and THH1 share 88% identity.

The amino acid sequences of TOM1, TOM3, and THH1 contained several highly hydrophobic regions (Fig. 3). A computer program that predicts transmembrane segments based on multiple sequence alignment (TMAP [21]) suggested that these three proteins were seven-pass transmembrane proteins (Fig. 3A). As was the case for TOM1 (25), database searches for proteins with amino acid sequences similar to that of TOM3 or THH1 did not list significant scores with any other proteins whose function has already been revealed.

Possible interaction between TOM3 and the tobamovirus-encoded replication proteins. In our previous report, we demonstrated a possible interaction between the membrane-associated TOM1 protein and the helicase domain polypeptide of

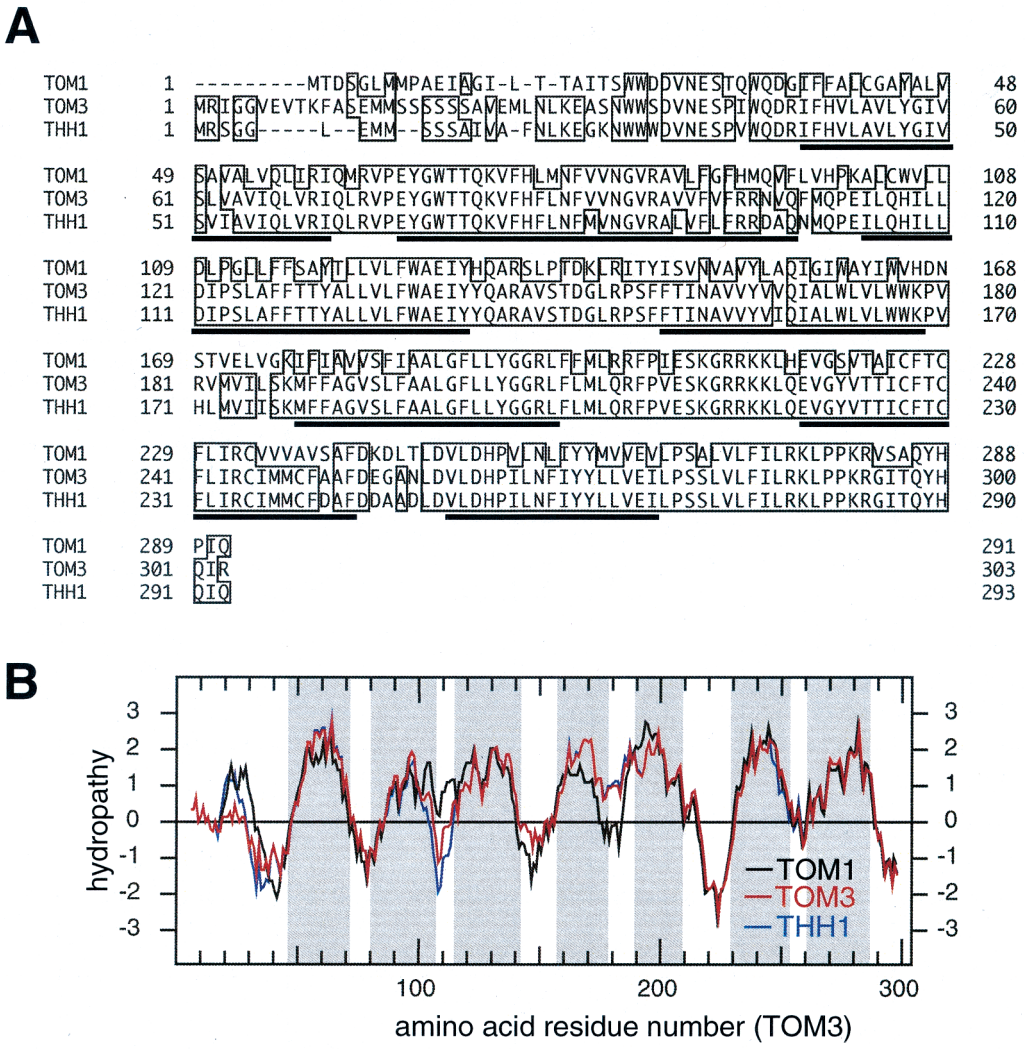

FIG. 3. Structure of TOM3. (A) An alignment of the deduced amino acid sequences of TOM1, TOM3, and THH1. Underlined amino acid residues represent those predicted by the TMAP program (21) to be in the membrane-spanning regions. Identical amino acid residues are boxed. (B) Hydropathy plot for the deduced amino acid sequences of TOM1 (black), TOM3 (red), and THH1 (blue). The hydropathy plot was created using the method described by Kyte and Doolittle (13). The regions predicted to be the membrane-spanning regions in panel A are shadowed.

the TMV-Cg-encoded replication proteins by using the Sos recruitment system in yeast (25) (Fig. 5). This system is based on the observation that the N-terminal fragment of the Ras guanine nucleotide exchange factor (5′Sos), the human homolog of *Drosophila melanogaster* Son of Sevenless protein, suppresses temperature-sensitive growth caused by the *cdc25-2* mutation in *S. cerevisiae* if 5′Sos is targeted to the plasma membrane in the vicinity of Ras (Fig. 5B) (4). To investigate the membrane association of TOM3 and possible interactions with the replication proteins of tobamoviruses, the same test was performed for TOM3 in this study.

Expression of a TOM3-5′Sos fusion protein but not the TOM3 protein alone suppressed the *cdc25-2* temperature sensitivity in *S. cerevisiae* (Fig. 5A), confirming that TOM3 is actually expressed and that it is targeted, at least in part, to the plasma membrane in yeast cells with its C terminus exposed to the cytoplasm (Fig. 5B). The extent of suppression was slightly weaker than that observed for TOM1-5′Sos, suggesting that the amount of TOM3-5′Sos expressed and subsequently localized to the plasma membrane was less than that of TOM1-5′Sos. Alternatively, it is also possible that the position of the 5′Sos moiety in TOM3-5′Sos on the plasma membrane was not suitable for full complementation of the *cdc25-2* mutation. Unlike TOM1, TOM3 did not exhibit an activity to enhance cell growth of *cdc25-2* yeast expressing a fusion protein between 5′Sos and the helicase domain of TMV-Cg (5′Sos-CgHel [Fig. 5A]). In contrast, the growth of *cdc25-2* yeast expressing a fusion protein between 5′Sos and the corresponding helicase domain of TMV-L (5′Sos-LHel) was enhanced by the expression of TOM3, although it was still weaker than the growth enhanced by the expression of TOM1 (Fig. 5A). This enhancement was not observed for a TOM3 derivative with an internal deletion (data not shown). Taken together, it is likely that TOM3 is able to interact with the helicase domain polypeptide of TMV-L (Fig. 5B).

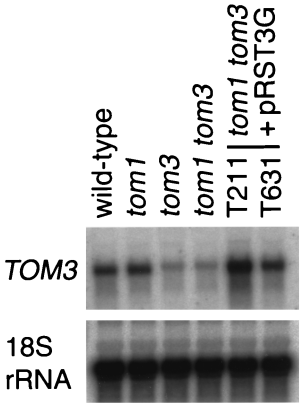

FIG. 4. Northern blot hybridization analysis of the mRNA for *TOM3*. The accumulation of *TOM3* mRNA and 18S rRNA in wild-type and mutant plants was examined. Total RNA was extracted from aerial tissues of 26-day-old noninoculated wild-type (Col-0), mutant (*tom1-1* and *tom3-1*), and transgenic (lines T211 and T631) plants. Details of the transgenic lines T211 and T631 are given in the Fig. 1 legend. For the detection of *TOM3* mRNA and 18S rRNA, 10 and 3 μg of the total RNA samples, respectively, were denatured by glyoxal, separated by 1% agarose gel electrophoresis, and blotted onto a nylon membrane. Blots were probed with ^{32}P-labeled DNAs hybridizing with either *TOM3* or 18S rRNA sequences. To prepare the *TOM3*-specific probe, a DNA fragment corresponding to the predicted ORF was amplified by PCR from cDNA clones and gel purified. This probe did not cross-hybridize with *TOM1* RNA but did cross-hybridize slightly with *THH1* RNA. However, the extent of cross-hybridization is negligible for the interpretation of the result (data not shown). The hybridization signals were detected with a Bio Imaging Analyzer (BAS1000; Fuji Photo Film, Tokyo, Japan). Results were confirmed in an independent experiment.

DISCUSSION

Here, we have shown that simultaneous mutations in the two homologous host genes *TOM1* and *TOM3*, likely encoding seven-pass transmembrane proteins, completely suppress tobamovirus multiplication in *Arabidopsis*. The combination of mutations in two different genes sometimes results in a more enhanced phenotype than that caused by the mutation in either one of the genes. Such genetic interaction, referred to as "synthetic enhancement," is known to be caused by numerous mechanisms but is often observed when the genes are associated with parallel or related pathways controlling the same function (22). Since the predicted amino acid sequences of TOM1 and TOM3 share a high degree of similarity (56% identical), it is plausible that they do share a parallel and essential function in tobamovirus multiplication. In keeping with this possibility, overexpression of TOM3 in the *tom1-1 tom3-1* double mutant restored tobamovirus multiplication to the wild-type level.

Multiple host factors are known to be involved in the replication process of a virus (reviewed in references 6 and 14), and the data presented in this report show that some of these host factors, such as TOM1 and TOM3, are not only involved but also essential for this replication process. Based on our observations of tobamovirus multiplication in the *tom1-1*, *tom3-1*, and *tom1-1 tom3-1* mutants, we propose the following model regarding the effect on overall virus multiplication efficiency if the activity of an essential host factor is altered by mutation or another process. If the activity of an essential host factor is lower than a certain level (hereafter referred to as threshold I), host defense functions or degradation of virus genome will nullify the production of virus genome and no substantial amplification of the virus genome will occur. On the other hand, if the activity is higher than another specific level (hereafter threshold II), virus multiplication will no longer be affected because the activity of another essential host factor will limit the overall efficiency of multiplication. If the activity is between thresholds I and II, the overall efficiency of virus multiplication will be dependent on the activity of the host factor in question.

In accordance with the above model, the activity of genomic *TOM1* (plus *THH1*) would be above threshold II, since tobamovirus multiplication was normal (wild-type level) in the *tom3-1* single mutant. On the other hand, the genomic *TOM3* activity (plus *THH1*) would be between thresholds I and II, since the *tom1* single mutations reduced the tobamovirus multiplication to a low but still detectable level. The fact that tobamovirus multiplication was not detected in the *tom1-1 tom3-1* double mutant suggests that the activity of genomic *THH1* would be below threshold I.

Considering the nature of TOM1 as a membrane protein and its ability to interact with tobamovirus-encoded replicase components, it seems likely that TOM1 participates in the formation of the replication complex in the infected cells by serving as a membrane anchor (25). In the Sos recruitment assay, coexpression of TOM3 enhanced the growth of *cdc25-2* yeast harboring 5′Sos-LHel at 36°C, suggesting that TOM3, as well as TOM1, interacts with the helicase domain of TMV-L replication proteins. Although coexpression of TOM3 did not enhance the growth of *cdc25-2* yeast cells expressing 5′Sos-CgHel, the demonstrated in vivo activity of TOM3 to support both TMV-Cg and TMV-L multiplication in place of TOM1 in *Arabidopsis* plants, together with the amino acid sequence similarity between TOM1 and TOM3, suggests that an interaction does likely occur between TOM3 and TMV-Cg replication proteins in plant cells.

We have previously found that coinoculation with CMV results in reduced multiplication of TMV-Cg in *tom1-1* protoplasts but not in wild-type protoplasts (11). In contrast, coinoculation with TCV did not reduce TMV-Cg multiplication in either *tom1-1* or wild-type protoplasts (11). A possible explanation for these observations is that CMV may also utilize TOM3 for its multiplication and compete with TMV-Cg for TOM3 in the coinoculated *tom1-1* protoplasts. If this is true, CMV may also utilize THH1. The normal multiplication of CMV in the *tom1-1 tom3-1* double mutant could therefore indicate that the activity of THH1 to support CMV multiplication is above threshold II. Inactivation of *THH1* in the *tom1-1 tom3-1* double mutant will test this possibility.

To our knowledge, this is the first demonstration of a complete inhibition of intracellular virus multiplication by inactivation of host genes essential for the multiplication of a virus in eukaryotes. While more than 10 host proteins have so far been implicated in the multiplication of positive-strand RNA viruses, most of them also have essential roles in maintaining viability of the hosts (7, 14, 15, 17). Thus, although virus multiplication could be inhibited by suppressing the expression of host genes essential for virus multiplication, it might be a difficult prerequisite to lower the activity of the target gene to at least below threshold II, preferably below threshold I, with-

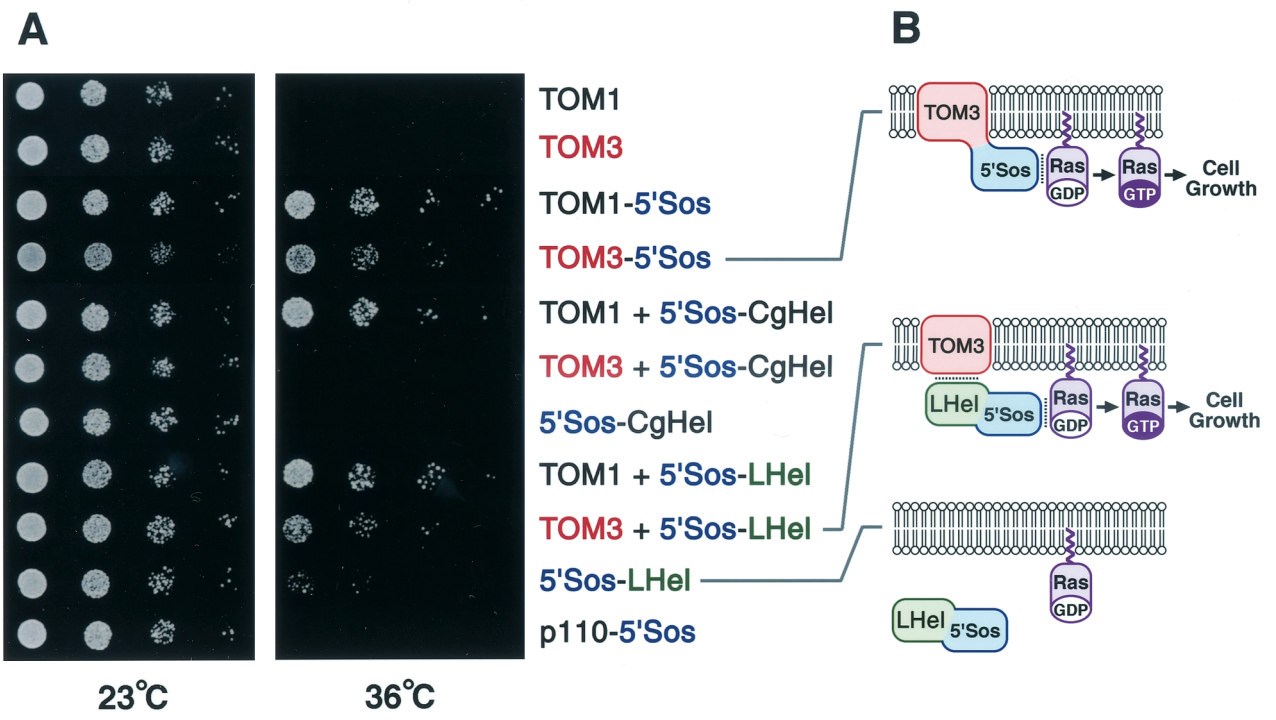

FIG. 5. Interaction between TOM3 and the TMV-L-encoded replication proteins. (A) *cdc25-2* yeast strains harboring plasmids designed to constitutively express the indicated proteins were diluted in sterile water to absorbances at 600 nm of 0.2, 0.025, 0.003, and 0.0004 (eightfold serial dilutions). Two microliters of each dilution was spotted onto yeast extract-peptone-dextrose plates and cultured at 23°C for 56.5 h or at 36°C for 54.5 h. Part of p110 β, a subunit of phosphatidylinositol-3-phosphate kinase, fused to 5′Sos (p110-15′Sos), was used as a negative control for suppression of *cdc25-2* temperature sensitivity (4). For comparison, similar constructs with TOM1 in place of TOM3 were simultaneously analyzed. (B) Models explaining the results of panel A for TOM3. Lipid bilayers indicate plasma membranes with the lower sides cytoplasmic. Noncovalent interactions are indicated by dotted lines. Covalent linkage of 5′Sos polypeptide with TOM3 or a noncovalent interaction between TOM3 and the helicase domain of TMV-L-encoded replication proteins in 5′Sos-LHel recruits 5′Sos to the plasma membrane to activate Ras signaling.

out affecting host viability. In this sense, the *tom1-1 tom3-1* double mutant represents a unique case where mutations in host genes seriously affect tobamovirus multiplication with only a minimal, if any, effect on plant growth. We have recently identified *TOM1*- and *TOM3*-like genes in tobacco and tomato plants (unpublished data), raising the possibility that immunity against tobamovirus infection could also be engineered in these plant species by simultaneous inactivation of these genes. Although the effect of the *tom1-1 tom3-1* double mutation is limited to tobamovirus multiplication, this is a significant finding in that it also indicates that a similar antivirus strategy could be applied to other viruses once appropriate host genes essential for the multiplication of the respective viruses are identified.

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