

The Eukaryotic Translation Initiation Factor 4E Controls Lettuce Susceptibility to the Potyvirus *Lettuce mosaic virus*¹

Valérie Nicaise, Sylvie German-Retana, Raquel Sanjuán, Marie-Pierre Dubrana, Marianne Mazier, Brigitte Maisonneuve, Thierry Candresse, Carole Caranta, and Olivier LeGall*

Interactions Plante-Virus, Institut de Biologie Végétale Moléculaire, Institut National de la Recherche Agronomique, Boîte Postale 81, F-33883 Villenave d'Ornon cedex, France (V.N., S.G.-R., M.-P.D., T.C., O.L.G.); and Unité de Génétique et d'Amélioration des Fruits et Légumes, Institut National de la Recherche Agronomique, Boîte Postale 94, F-84143 Montfavet cedex, France (R.S., M.M., B.M., C.C.)

The *eIF4E* and *eIF(iso)4E* cDNAs from several genotypes of lettuce (*Lactuca sativa*) that are susceptible, tolerant, or resistant to infection by *Lettuce mosaic virus* (LMV; genus *Potyvirus*) were cloned and sequenced. Although *Ls-eIF(iso)4E* was monomorphic in sequence, three types of *Ls-eIF4E* differed by point sequence variations, and a short in-frame deletion in one of them. The amino acid variations specific to *Ls-eIF4E*¹ and *Ls-eIF4E*² were predicted to be located near the cap recognition pocket in a homology-based tridimensional protein model. In 19 lettuce genotypes, including two near-isogenic pairs, there was a strict correlation between these three allelic types and the presence or absence of the recessive LMV resistance genes *mo1*¹ and *mo1*². *Ls-eIF4E*¹ and *mo1*¹ cosegregated in the progeny of two separate crosses between susceptible genotypes and an *mo1*¹ genotype. Finally, transient ectopic expression of *Ls-eIF4E* restored systemic accumulation of a green fluorescent protein-tagged LMV in LMV-resistant *mo1*² plants and a recombinant LMV expressing *Ls-eIF4E*^o from its genome, but not *Ls-eIF4E*¹ or *Ls-eIF(iso)4E*, accumulated and produced symptoms in *mo1*¹ or *mo1*² genotypes. Therefore, sequence correlation, tight genetic linkage, and functional complementation strongly suggest that *eIF4E* plays a role in the LMV cycle in lettuce and that *mo1*¹ and *mo1*² are alleles coding for forms of *eIF4E* unable or less effective to fulfill this role. More generally, the isoforms of *eIF4E* appear to be host factors involved in the cycle of potyviruses in plants, probably through a general mechanism yet to be clarified.

Disease is a remarkable but exceptional outcome of the interaction between a plant and a microorganism: In most cases, microorganisms are excluded by active or passive host defenses. Two well-known active defense mechanisms of plants against pathogen attack are the hypersensitive reaction (Pontier et al., 1998) and, in the case of viruses, RNA-mediated defense (Ratcliff et al., 1997; Voinnet, 2001). However, in the most common situation, a given plant is simply not a suitable biological substrate for the multiplication of a given microorganism, resulting in passive resistance. In the case of obligatory parasites such as viruses, absence or inadequacy of a single host factor may lead to the inability for the pathogen to multiply in the host or to systemically invade it (Ishikawa et al., 1997; Yamanaka et al., 2000). Such a mechanism implies that the dominant alleles of the host genes involved would be associated with susceptibility and the recessive alleles encoding nonfunctional versions of this host factor with resistance. Identification of

recessive genes implicated in plant resistance to viruses, therefore, should provide a better knowledge of the molecular mechanisms involved in compatible interactions between a host plant and a virus.

There are many instances where recessive resistance genes are used to control agronomically important pathogens, especially potyviruses for which they have even been estimated to represent about 40% of the known resistance genes (Provvidenti and Hampton, 1992). *Potyvirus* is the largest and most diverse genus of plant viruses and causes severe losses to many crops, particularly to vegetables (Shukla et al., 1994). Potyviruses are efficiently transmitted by aphids, and some of them are also seed borne. Their flexuous particles contain a single genomic RNA molecule of about 10,000 nucleotides, encoding a polyprotein that is processed by three virus-encoded proteinases (Reichmann et al., 1992). The viral RNA is polyadenylated at its 3' end, and its 5' end is not capped but covalently linked to a virus-encoded protein of about 25 kD, named VPg (Murphy et al., 1990).

In several potyviruses, the central domain of the VPg is involved in the ability to overcome resistances associated with recessive genes. However, the phenotypes of the corresponding resistances differ depending on the host and potyvirus partners. For instance, the central domain of VPg has been shown

¹ This work was partially supported by the Etablissement Public Régional Aquitaine (ref. no. 20000307004).

*Corresponding author; e-mail legall@bordeaux.inra.fr; fax 33-557-122-384.

Article, publication date, and citation information can be found at <http://www.plantphysiol.org/cgi/doi/10.1104/pp.102.017855>.

to be involved in the control of *Pea seedborne mosaic virus* accumulation in single cells by the pea (*Pisum sativum*) *sbm-1* gene (Keller et al., 1998), in the prevention of *Tobacco vein mottling virus* cell-to-cell movement by the tobacco (*Nicotiana tabacum*) *va* gene (Gibb et al., 1989), and in the block of *Tobacco etch virus* (TEV) long-distance movement by two recessive genes present in the tobacco genotype V20 (Schaad and Carrington, 1996; Schaad et al., 1996).

Whatever the mechanism(s) involved in these different resistance phenotypes, it has been speculated that VPg carries out some of its functions in the viral cycle by interacting with host factors. In the yeast two-hybrid system and in other in vitro assays, the VPg of *Turnip mosaic virus* (TuMV) and of TEV interact with the eukaryotic translation initiation factor eIF4E and its isoform eIF(iso)4E from Arabidopsis, tomato (*Lycopersicon esculentum*), and tobacco (Wittmann et al., 1997; Léonard et al., 2000; Schaad et al., 2000). eIF4E is a cap-binding protein, and its main role in eukaryotic mRNA translation is to recruit the eIF4F complex near the 5' cap structure and allow additional translation factors to be recruited, resulting in mRNA circularization and translation initiation (Pestova et al., 2001). In Arabidopsis, eIF4E is 52% identical in amino acid sequence to eIF(iso)4E, and both are about 70% identical to their respective wheat (*Triticum aestivum*) homologs (Rodriguez et al., 1998). Differences in transcription patterns (Rodriguez et al., 1998) and in binding affinities (Carberry et al., 1991) suggest that these isoforms might have complementary biological roles.

Conceptually, as it does for cellular mRNAs, eIF4E could participate in potyvirus RNA translation by interacting with the 5' VPg that replaces a 5' cap structure in these RNAs. A point mutation in the TuMV VPg that abolishes its interaction with eIF(iso)4E is associated with a loss of viral infectivity (Léonard et al., 2000). Furthermore, it has been shown recently that disruption of the *eIF(iso)4E* gene of Arabidopsis results in resistance to three potyviruses (TEV, TuMV, and LMV; Duprat et al., 2002; Lellis et al., 2002) and that the natural recessive resistance to TEV and *Potato virus Y* (PVY) in pepper (*Capsicum annuum*) conferred by the recessive gene *pvv2* is associated with sequence variation in *eIF4E* (Ruffel et al., 2002).

In lettuce (*Lactuca sativa*), the recessive *mo1¹* and *mo1²* genes are associated with reduced accumulation and lack of symptoms (tolerance) or absence of accumulation (resistance) of common isolates of the potyvirus *Lettuce mosaic virus* (LMV; Ryder, 1970; Dinant and Lot, 1992). The issue of the interaction, resistance or tolerance, depends on the virus isolate and genetic background (Pink et al., 1992; Revers et al., 1997), but *mo1¹* is generally associated with resistance and *mo1²* with tolerance (Bos et al., 1994; Revers et al., 1997). The genes *mo1¹* and *mo1²* are tightly linked and probably alleles (Ryder, 1970; Pink et al.,

1992). The ability of some LMV isolates to successfully infect and produce symptoms in lettuce genotypes with *mo1¹* or *mo1²* is associated to a central region of the viral genome encoding the C terminus of the CI protein, VPg, and the N terminus of the NIa proteinase (Redondo et al., 2001).

We have investigated the possible role of eIF4E and eIF(iso)4E in the compatibility between lettuce and LMV. For this purpose, *eIF4E* and *eIF(iso)4E* cDNAs were sequenced from a set of susceptible, tolerant, or resistant lettuce genotypes, the genetic cosegregation between *mo1¹* and *eIF4E* was tested, and the ability of ectopically expressed eIF4E to functionally restore full LMV susceptibility in tolerant or resistant lettuce genotypes was evaluated.

RESULTS

Cloning and Sequence Analysis of the Lettuce *eIF4E* and *eIF(iso)4E* cDNAs

A multiple alignment of the nucleotide sequences of the *eIF4E* coding regions from different plant species and of their human (*Homo sapiens*) and murine (*Mus musculus*) counterparts revealed a higher degree of conservation in the central domain of each of these proteins than in the N- and C-terminal regions (data not shown). The central region of the *eIF4E* cDNA from the susceptible lettuce genotype Salinas was PCR amplified using oligonucleotides 4E193f and 4E408r (all oligonucleotide sequences are given in the "Materials and Methods" section), designed in reference to seven plant *eIF4E* sequences, including three sequences in Arabidopsis. This product was cloned, and all of the eight clones sequenced yielded the same 169-bp sequence. This sequence information was used to design the oligonucleotides Ls4E250f and Ls4E255r for 3'- and 5'-RACE amplification of the cDNA ends, respectively. Finally, the oligonucleotides Ls4E3f and Ls4E813r were used to PCR amplify the nearly full-length *eIF4E* cDNA, including the entire coding region, before cloning in pGEM-T Easy and sequencing. The assembled *eIF4E* cDNA nucleotide sequence was determined from at least five independent clones at each position. No variability was observed between the cDNA clones sequenced. The full-length sequence (GenBank accession no. AF530162) was 1,032 nucleotides in length, with a single open reading frame from positions 21 to 710, encoding a protein with a calculated molecular mass of 26.1 kD (Fig. 1). The closest matches obtained after a BLAST search in GenBank with the full-length nucleotide sequence were the *eIF4E* cDNAs from tomato (accession no. AF259801, $E = 5.10^{-27}$), rice (*Oryza sativa*; U34597, $E = 5.10^{-27}$) and Arabidopsis (Y10548, $E = 8.10^{-26}$), and the closest matches with the predicted translation product were the eIF4E amino acid sequences from Arabidopsis ($E = 3.10^{-81}$), tomato ($E = 3.10^{-80}$), and maize (*Zea mays*; AF076954, $E = 4.10^{-78}$), confirming that the cDNA

Table 1. Sequence differences in the *eIF4E* cDNAs of 19 lettuce genotypes

For each genotype, the nucleotides that differed from the sequence of *eIF4E* of the genotype Salinas (as a reference: GenBank accession no. AF530162) are indicated with the corresponding amino acid changes. Nucleotides and amino acids identical to those of genotype Salinas at a given position are shown with hyphens. For the genotypes labeled with an asterisk, the nucleotide sequence was determined only between positions 109 and 412 and, therefore, not at the positions marked with two asterisks. Nucleotide 730 is in the 3'-non-coding region; therefore, designation of the corresponding amino acid does not apply (n.a.). On the basis of these amino acid differences, the sequences were classified in three types as indicated on the left.

Genotypes	Nucleotide/Amino Acid Positions											
	228/70		299/93		344-349/108-110		576**/186		730**/n.a.			
	Nucleotides	Amino acids	Nucleotides	Amino acids	Nucleotides	Amino acids	Nucleotides	Amino acids	Nucleotides	Amino acids	Nucleotides	Amino acids
Type 0												
Salinas, Fiona*, Girelle*, Jessy*, Mariska*, Trocadéro*, Vanguard 87-20M	G	Ala	C	Phe	AGGAGC	QGA	G	Ala	C	n.a.		
Type 1												
Alizé*, Classic*, Floribibb, Malika, Mantilia, Oriana*, Presidio*	-	-	-	-	-	-	-	-	-	T	-	-
Type 2												
Autumn Gold*, Desert Storm*, Salinas 88, Vanguard 75	C	Pro	-	-	-	-	-	-	-	T	-	-

gests that eIF(iso)4E sequence variations are not directly correlated with the *mo1*-related phenotypes.

Predicted Three-Dimensional (3D) Structure of the Ls-eIF4E Protein

A 3D model of the Ls-eIF4E protein was predicted based on the known 3D structures of human (Hs-eIF4E) and murine (Mm-eIF4E) cap-bound eIF4E molecules (Marcotrigiano et al., 1999; Tomoo et al., 2002). The central domain of Ls-eIF4E°, from beta-sheet 2 to beta-sheet 6 (Fig. 1), was 50% identical in sequence to that of Mm-eIF4E (Fig. 1) and Hs-eIF4E (data not shown), suggesting that a homology-based model of the Ls-eIF4E folding would be valid within the range of 1 Å (Chothia and Lesk, 1986). Two independent algorithms (Guex and Peitsch, 1997; Bates et al., 2001) yielded structurally similar predictions (data not shown). Using Swiss-model and Mm-eIF4E as a template, the Ls-eIF4E° central domain tightly matched that of Mm-eIF4E (root mean square [RMS] deviation = 0.74 Å) and Hs-eIF4E (RMS = 1.4 Å). When Hs-eIF4E was used as the modeling template, RMS deviation values obtained with Ls-eIF4E were 1.23 Å for Hs-eIF4E and 1.35 Å for Mm-eIF4E, respectively. Using the same procedure to predict the folding of Hs-eIF4E based on its homology with Mm-eIF4E yielded a model with a 0.96-Å RMS deviation compared with its actual x-ray diffraction structure, providing a landmark for comparison of RMS deviation values.

Figure 2 shows the predicted 3D structure of Ls-

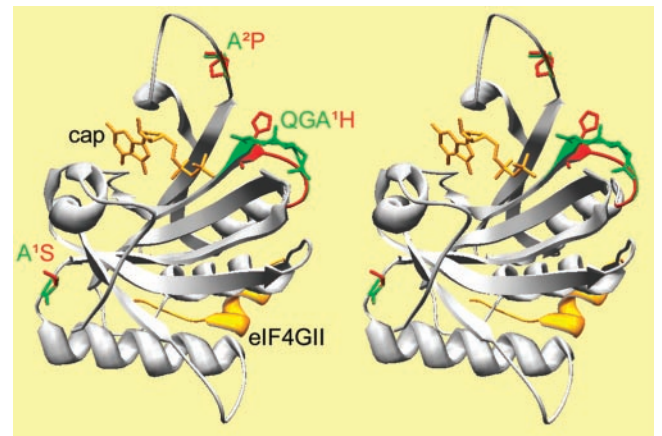


Figure 2. Stereo view of the predicted 3D structure of Ls-eIF4E. The structure of Ls-eIF4E° was predicted based on its homology with Mm-eIF4E, using Swiss-model (Guex and Peitsch, 1997) and shown in a ribbon representation. The positions of the cap analog 7-methyl-GDP (cap, yellow) and an eIF4G-derived peptide (eIF4GII, yellow) are derived from those observed in a cocrystal of these molecules with the murine eIF4E (Marcotrigiano et al., 1999). At variable positions, the side chains of the amino acid present in Ls-eIF4E° are displayed in green, and those in Ls-eIF4E¹ or Ls-eIF4E² are in red (labeled X¹Y and X²Y, respectively, where X is the residue in Ls-eIF4E° and Y in Ls-eIF4E¹ or Ls-eIF4E²). At the variable positions, the ribbon is colored using the same code.

eIF4E^o and, superimposed to visualize the predicted structural differences, those of Ls-eIF4E¹ and Ls-eIF4E². According to this model, the amino acids that differ between the three Ls-eIF4E types were all predicted to be at or near the surface of the protein. All mapped near the cap recognition pocket, on the face of eIF4E opposite to the eIF4G-binding site (Fig. 2). Ala-70-Pro, the only amino acid different between Ls-eIF4E^o and Ls-eIF4E², was predicted to be part of the loop between beta-sheets 1 and 2 (Marcotrigiano et al., 1997), which protrudes at the rim of the cap-recognition pocket itself (Fig. 2). The three amino acids Gln-108-Gly-109-Ala-110, replaced by a single His in Ls-eIF4E¹, are located in the neighboring loop (Figs. 1 and 2), between alpha-helix 1 and beta-sheet 3. Although the replacement of three amino acids by a single one in Ls-eIF4E¹ was predicted to have a strong effect on the folding of this loop (Fig. 2), the Ala-70-Pro replacement in Ls-eIF4E² was only predicted to have a minor impact because of the already existing sterical constraints on the chain in this region (Fig. 2). Similarly, the Ala-186-Ser replacement in the loop between beta-sheet 6 and alpha-helix 3 in Ls-eIF4E¹ was predicted to cause no detectable effect on the predicted folding (Fig. 2).

Genetic Cosegregation between the *mo1*¹ and *Ls-eIF4E*¹ Loci

With the objective to strengthen the correlation between sequence variation in the *eIF4E* cDNA and LMV resistance controlled by *mo1*¹, the genetic linkage between *mo1*¹ and the six-nucleotide deletion found at positions 344 to 349 in *Ls-eIF4E*¹ was studied in two F₂ progenies Mariska (susceptible) × Mantilia (*mo1*¹) and Girelle (susceptible) × Mantilia (*mo1*¹). A cleaved amplified polymorphic sequence (CAPS) marker, eIF4E-PagI, was defined on the *Ls-eIF4E* cDNA in relation with the presence of a *PagI* restriction site generated by the six-nucleotide deletion in the *Ls-eIF4E*¹ cDNA. Ten to 15 d after inoculation of 36 F₂ plants with LMV-0, 10 plants showed no symptoms, whereas the remaining 26 presented typical mosaic symptoms caused by LMV. This ratio, 10 resistant to 26 susceptible, is consistent with the segregation of a single recessive gene coding for LMV resistance ($\chi^2 = 0.15$, $P = 70\%$). Twenty-five of these F₂ plants, eight without symptoms and 17 with typical LMV symptoms, were further analyzed for segregation of the CAPS marker eIF4E-PagI. A cDNA fragment of 442 to 448 bp was PCR generated using oligonucleotides Ls4E250f and Ls4E697r, designed on both sides of the six-nucleotide deletion present in *Ls-eIF4E*¹. Electrophoretic analysis of *PagI*-digested PCR products revealed three distinct restriction patterns (Fig. 3; data not shown). All eight resistant plants showed a complete digestion of the PCR product, as in the Mantilia parent, and, thus, were determined to be homozygous for the presence of eIF4E-

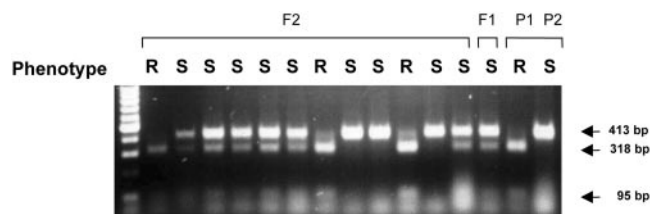


Figure 3. Cosegregation of the LMV resistance phenotype and *Ls-eIF4E*¹ in the F₂ progeny from a cross between Mantilia (*mo1*¹) and Mariska (susceptible). PCR products of 448 bp (442 bp for *Ls-eIF4E*¹) were generated by reverse transcriptase (RT)-PCR from RNAs isolated from leaves of the F₂, F₁, and the parental lines Mantilia (P1) and Mariska (P2) using oligonucleotides Ls4E250f and Ls4E697r. These cDNA fragments were digested by *PagI* and resolved in a 1.5% (w/v) agarose gel. Arrows indicate the molecular weights of the various fragments. The resistant (R) or susceptible (S) phenotype of each individual plant was scored in parallel 10 to 15 d after inoculation with LMV-0.

PagI. The five susceptible plants showed no detectable *PagI* digestion, as in the Mariska and Girelle parents, and, thus, were homozygous for the absence of eIF4E-PagI. Finally, the remaining 12 susceptible plants showed a partial digestion, as in the F₁ plant, which was characteristic of individual plants heterozygous for eIF4E-PagI. Thus, a complete map cosegregation was observed between *mo1*¹-associated LMV resistance and the CAPS marker eIF4E-PagI.

Agro-Infiltration of *Ls-eIF4E* Restores LMV Systemic Accumulation in *mo1*² Plants

Leaf infiltration with a suspension of *Agrobacterium tumefaciens* transformed with a binary plasmid containing an expression cassette between T-DNA borders (agro-infiltration) allows localized, transient expression of a foreign protein in plant tissues (Bechtold et al., 1993; Schöb et al., 1997). This strategy was used to evaluate the ability of *Ls-eIF4E* to restore LMV susceptibility in *mo1*² plants.

We have shown previously that *mo1*² prevents systemic accumulation of LMV-0-GFP, a green fluorescent protein-tagged LMV-0 (German-Retana et al., 2000; Candresse et al., 2002), but, unlike *mo1*¹, does not completely block replication in the inoculated leaf (German-Retana et al., 2000) and phloem loading (S. German-Retana, unpublished data). In our assay, lettuce plants were inoculated with LMV-0-GFP, and a few days later, their apical noninoculated leaves were agro-infiltrated with constructs designed to locally express *Ls-eIF4E*^o, *Ls-eIF4E*¹, or *Ls-eIF4E*². A construct allowing the expression of the β -glucuronidase (GUS) was co-agro-infiltrated as an internal control. Six days after agro-infiltration, systemic accumulation of LMV-0-GFP, i.e. GFP accumulation in the noninoculated agro-infiltrated areas, was scored under UV light (Fig. 4).

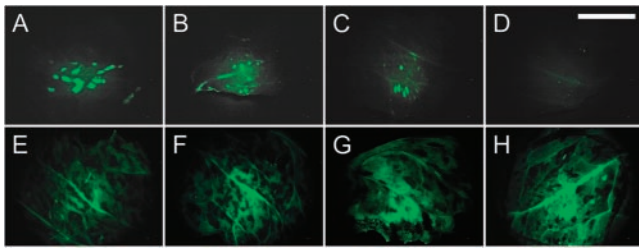


Figure 4. Functional complementation of the systemic accumulation of LMV-0-GFP in *mo1*² plants by transient Ls-eIF4E expression. GFP fluorescence stereomicroscopy was visualized in leaf areas of an *mo1*² (Salinas 88, A–D) and a susceptible (Salinas, E–H) genotype after co-agro-infiltration with constructs allowing expression of the GUS and Ls-eIF4E. A and E, Areas agro-infiltrated with a construct allowing expression of Ls-eIF4E⁰. B and F, Areas agro-infiltrated with a construct allowing expression of Ls-eIF4E¹. C and G, Areas agro-infiltrated with a construct allowing expression of Ls-eIF4E². D and H, Areas agro-infiltrated with the GUS construct only. Bar = 1 cm.

The pattern and timing of viral accumulation were not detectably changed in susceptible plants of the genotype Salinas in leaf areas upon agro-infiltration with any of the three types of Ls-eIF4E. Stereomicroscopic observation under UV light showed comparable GFP fluorescence intensity in Salinas leaves agro-infiltrated with the Ls-eIF4E⁰, Ls-eIF4E¹, or Ls-eIF4E² constructs (Fig. 4, E–G). GUS activity was readily detected in all infiltrated areas (data not shown), confirming the success of agro-infiltration for foreign protein expression.

In Salinas 88 (*mo1*²), no systemic spread of GFP-tagged LMV-0 was observed up to 2 weeks after inoculation, as expected from previous results (Candresse et al., 2002). However, GFP fluorescence was observed in leaves distant from the inoculation sites that had been agro-infiltrated with any of the three forms of Ls-eIF4E (Fig. 4, A–C). The GUS construct alone did not restore the ability of LMV-0-GFP to infect these leaves (Fig. 4D). The size difference of the GFP spots that is apparent in Figure 4, A to C, was not considered significant because it was not reproduced from one leaf to another.

All three forms of Ls-eIF4E restored to some extent the ability of LMV-0-GFP to spread into agro-

infiltrated areas distant from the inoculation site. To determine whether the Ls-eIF4E alleles differed quantitatively in this ability, we counted the GFP spots in each agro-infiltrated area (Table II). The analysis of the number of fluorescent spots confirmed the visual observation that all three types of Ls-eIF4E resulted in complementation for LMV-0-GFP systemic accumulation, both when the numbers of spots per agro-infiltrated area and the number of areas containing fluorescent spots were considered. For these two parameters, the χ^2 probability for the random hypothesis was lower than 0.01%. However, the Ls-eIF4E types differed quantitatively in their abilities to restore the systemic accumulation of LMV-0-GFP. Although GFP accumulation was overall less efficient in agro-infiltrated areas of Salinas 88 leaves than in Salinas (Fig. 4), the number of GFP spots was higher after agro-infiltration with Ls-eIF4E⁰ than it was with Ls-eIF4E¹ or Ls-eIF4E² (Table II). More areas were GFP positive after agro-infiltration with Ls-eIF4E⁰ than with Ls-eIF4E¹ ($P = 3.05\%$ for the random hypothesis) or Ls-eIF4E² ($P = 6.06\%$), and a significantly higher total number of spots was counted with Ls-eIF4E⁰ than with Ls-eIF4E¹ ($P = 0.04\%$) or Ls-eIF4E² ($P = 2.91\%$). The apparent differences in efficiency of Ls-eIF4E¹ and Ls-eIF4E² to restore systemic viral accumulation (Table II) were not statistically supported ($P = 75\%$ for the number of GFP-positive areas and $P = 21\%$ for the number of spots). In summary, agro-infiltration of all three forms of Ls-eIF4E restored the LMV-0-GFP systemic accumulation in the *mo1*² genotype Salinas 88, and Ls-eIF4E⁰ appeared to be more efficient than Ls-eIF4E¹ and Ls-eIF4E².

Expression of Ls-eIF4E⁰, But Not Ls-eIF4E¹, Restores the Infectivity of a Recombinant LMV in *mo1*¹ or *mo1*² Plants

To test the ability of Ls-eIF4E to restore LMV susceptibility in *mo1*¹ genotypes of lettuce, the infectivity of LMV-0-4E⁰ was evaluated. This viral construct is derived from the nonresistance breaking isolate LMV-0 and contains the Ls-eIF4E⁰ coding region as a

Table II. Ls-eIF4E ectopic expression complements LMV-0-GFP accumulation in a *mo1*² context

After LMV-0-GFP inoculation, noninoculated upper leaves were agro-infiltrated with the constructs indicated on the left in addition to a GUS construct used as an internal control. The no. of areas containing GFP spots and the no. of GFP spots in each agro-infiltrated area, both reflecting LMV-0-GFP systemic accumulation, were counted using fluorescence stereomicroscopy. The results of three independent experiments were pooled.

Construct	Agro-Infiltrated Areas			GFP Spots	
	Total	No. containing GFP spots	Percent containing GFP spots	Total no.	Per agro-infiltrated area
pGreenII	48	1	2	1	0.02
Ls-eIF4E ⁰	67	35	52	136	2.0
Ls-eIF4E ¹	55	18	33	33	0.6
Ls-eIF4E ²	59	21	36	51	0.9

translational fusion between the viral P1 and Hc-Pro domains that is proteolytically processed in vivo to yield the free proteins. Similarly, LMV-0-4E¹ and LMV-0-iso4E contain the Ls-eIF4E¹ and the Ls-eIF(iso)4E coding regions, respectively.

LMV-0-4E⁰, LMV-0-4E¹, and LMV-0-iso4E were inoculated to three susceptible genotypes (Trocadéro, Salinas, and Vanguard), three *mo1*¹ genotypes (Floribibb, Malika, and Mantilia) and two *mo1*² genotypes (Salinas 88 and Vanguard 75), and symptoms were scored visually (Fig. 5; data not shown). As expected, LMV-0 caused symptoms only in the susceptible varieties. This was also the case of LMV-0-4E¹ and LMV-0-iso4E. However, symptoms appeared in all plants inoculated with LMV-0-4E⁰. The timing of symptoms appearance in *mo1*¹ and *mo1*² genotypes inoculated with LMV-0-4E⁰ was similar to that in susceptible plants infected with LMV-0: A faint vein clearing became evident in emerging leaves 10 to 12 d after inoculation, followed by mosaic symptoms 2 to 3 d later (data not shown).

Accumulation of LMV-0-4E⁰, LMV-0-4E¹, and LMV-0-iso4E was assayed by ELISA (Table III; data

not shown). The accumulation of nonrecombinant LMV was not detected in *mo1*¹ genotypes and was strongly reduced in *mo1*² genotypes compared with susceptible genotypes, consistent with the general idea that *mo1*¹ is associated with stronger LMV resistance than *mo1*². The same situation was observed for LMV-0-4E¹ and LMV-iso4E, except no virus accumulation was detected in *mo1*² genotypes. On the other hand, LMV-0-4E⁰ accumulated in all three categories of genotypes. These results, and the persistence of the Ls-eIF4E insert in the replicating virus during the course of the experiments, were confirmed by back inoculation and by RT-PCR (data not shown). LMV-0-4E⁰ had a similar or slightly decreased accumulation in susceptible plants compared with nonrecombinant LMV, indicating that its ability to infect *mo1*¹ and *mo1*² plants was probably not due to a nonspecific enhancement of virus accumulation by the Ls-eIF4E⁰ insert.

Together, these results indicate that expression of Ls-eIF4E⁰ rendered LMV able to accumulate and produce symptoms in *mo1*¹ and *mo1*² genotypes, unlike that of Ls-eIF4E¹ or Ls-eIF(iso)4E.

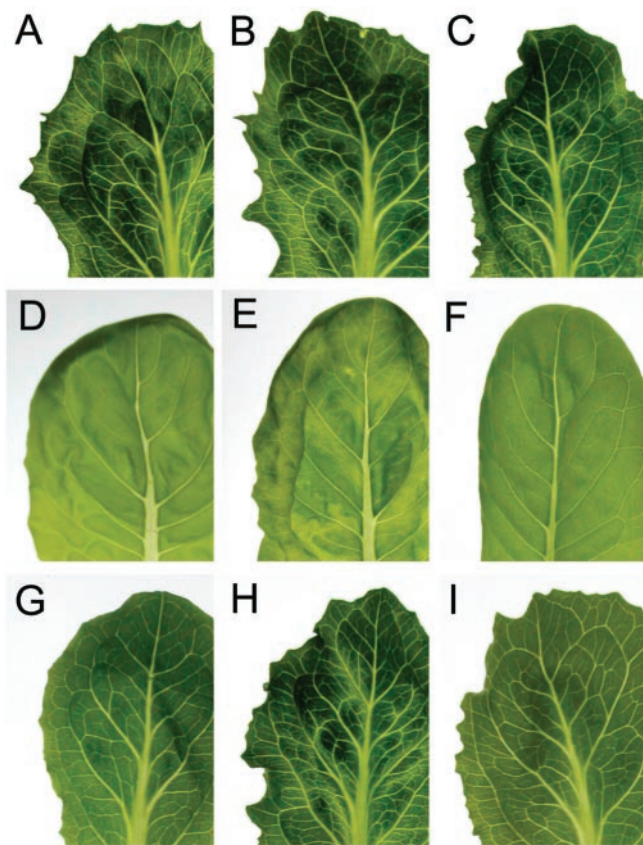


Figure 5. LMV-0-4E⁰ and LMV-0-4E¹ symptoms in lettuce *mo1*¹ or *mo1*² genotypes. Lettuce seedlings were rub inoculated with LMV-0, LMV-0-4E⁰, or LMV-0-4E¹, and detached leaves were photographed 3 weeks later. Lettuce genotypes are: A to C, Salinas (susceptible); D to F, Mantilia (*mo1*¹); and G to I, Salinas 88 (*mo1*²). LMV constructs are: A, D, and G: LMV-0; B, E, and H: LMV-0-4E⁰; and C, F, and I: LMV-0-4E¹.

DISCUSSION

In this paper, we report the isolation of three alleles of the lettuce translation initiation factor eIF4E in their cDNA form. Sequence covariation, genetic cosegregation of one of these alleles, and functional complementation using two independent assays make evidence that two of these alleles correspond to the recessive LMV resistance genes *mo1*¹ and *mo1*², respectively. A direct role of *Ls-eIF(iso)4E* sequence variations in lettuce *mo1*-regulated response to LMV was ruled out because no variation in sequence could be observed in this gene when lettuce genotypes differing in their behaviors against LMV where examined, and Ls-eIF(iso)4E ectopic expression did not restore LMV susceptibility. The immediate consequence of this conclusion is to confirm that *mo1*¹ and *mo1*² are alleles of a single gene as it has been suggested (Ryder, 1970; Dinant and Lot, 1992).

The involvement of eIF4E in the ability of LMV to successfully infect and produce symptoms in lettuce is reminiscent to the recent demonstration that the homologous protein is involved in recessive PVY resistance in pepper (Ruffel et al., 2002). In Arabidopsis, disruption of *eIF(iso)4E* either by point mutations (Lellis et al., 2002) or by T-DNA insertion (Duprat et al., 2002) was associated with resistance to three different potyviruses (TEV, TuMV, and LMV). Therefore, according to the plant-potyvirus pair considered, one or another eIF4E isoform appears to be involved in virus resistance.

The translation initiation factor 4E is an essential component of the eukaryotic mRNA translation machinery and possibly also has a role in other processes of the cell cycle (Strudwick and Borden, 2002).

Table III. Expression of *Ls-eIF4E^o*, but not *Ls-eIF4E¹*, restores LMV accumulation in *mo1¹* or *mo1²* plants

Virus accumulation was assayed by ELISA 3 weeks after inoculation with LMV-0 containing the *Ls-eIF4E^o*, *Ls-eIF4E¹*, or *Ls-eIF(is)4E* coding sequence. Results not shown, obtained after serial dilutions indicated that in this particular experiment, the relationship between antigen concentration and OD₄₀₅ was linear for OD values below 0.6.

Genotype	LMV-0-clv	LMV-0-4E ^o	LMV-0-4E ¹	LMV-0-iso4E
Salinas (susceptible)	0.856 ± 0.052	0.800 ± 0.074	0.840 ± 0.118	0.783 ± 0.098
Mantilia (<i>mo1¹</i>)	0.019 ± 0.003	1.017 ± 0.092	0.017 ± 0.005	0.014 ± 0.002
Salinas 88 (<i>mo1²</i>)	0.274 ± 0.122	0.707 ± 0.064	0.017 ± 0.015	0.010 ± 0.002

Therefore, the viability of any eIF4E mutant must be associated either with a lack of deleterious functional effects or in functional takeover by other proteins, chiefly eIF4E isoforms. The domains of eIF4E involved in cap binding and VPg binding are probably close but distinct because binding of TuMV VPg to the Arabidopsis eIF4E competes with, but does not completely block, that of a cap analog (Léonard et al., 2000). *Ls-eIF4E¹* and *Ls-eIF4E²* differed from *Ls-eIF4E^o* in two different loops contiguous in the 3D structure of eIF4E at the rim of the cap-binding pocket. The Gln-108-Gly-109-Ala-110 triplet substituted by a His in *Ls-eIF4E¹* is located next to Asp-111, an amino acid involved in structural stabilization of the cap-binding pocket in the murine protein (Marcotrigiano et al., 1997). Our homology-based model of the lettuce eIF4E, although not confirmed experimentally, suggests that the position of the side chain of this Asp is not significantly affected by the deletion observed in *Ls-eIF4E¹* (Fig. 2; data not shown). Interestingly, the sequence variations in the pepper *eIF4E* gene found to be related with PVY resistance mapped to amino acids in these same two loops (Ruffel et al., 2002).

Although in Arabidopsis eIF(iso)4E defects block several potyviruses (Lellis et al., 2002) including LMV (Duprat et al., 2002), apparently no other potyviruses than LMV are controlled by *mo1¹* or *mo1²* in lettuce (Provvidenti and Hampton, 1992; Montecclaros et al., 1997). This difference may be related to the fact that the eIF(iso)4E defects associated with potyvirus resistance in Arabidopsis are the result of large truncations of the gene product by premature translation termination (Lellis et al., 2002). Such C-terminally truncated proteins are likely to be more broadly affected than point sequence variants such as *Ls-eIF4E¹* or *Ls-eIF4E²* for any function played in the viral cycle, therefore providing a base for a plurispesific antiviral effect. Conceptually, other sequence variations could also result in a plurispesific effect, as in the case of the PVY (Ruffel et al., 2002) and TEV (S. Ruffel, unpublished data) resistance gene *pvr2* in pepper.

Functional complementation for systemic accumulation of LMV-0-GFP in *mo1²* plants was readily observed after agro-infiltration of each of the three *Ls-eIF4E* allelic types. However, *Ls-eIF4E^o*, the allele found in susceptible lettuce genotypes, was more efficient in that than were *Ls-eIF4E¹* or *Ls-eIF4E²*. The

ability of *Ls-eIF4E¹* to complement for LMV susceptibility in *mo1²* plants when expressed by agro-infiltration but not from the viral genome may reflect essential differences in the two expression systems used (agro-infiltration allows expression before virus replication) and properties assayed (systemic download versus the whole process of infection).

The ability of *Ls-eIF4E²* to complement systemic LMV accumulation upon agro-infiltration in an *mo1²* background suggests that quantitative expression can at least partially overcome the qualitative effect of mutations. A quantitative effect of *Ls-eIF4E* sequence variations in the LMV cycle can be related to the tolerance rather than resistance phenotype observed against LMV-0 in *mo1²* plants (Dinant and Lot, 1992; Revers et al., 1997). Similarly, in at least one of the Arabidopsis loss-of-susceptibility eIF(iso)4E mutants, rare infection foci were sometimes observed (Lellis et al., 2002), indicating that resistance is strong but not absolute even in the presence of an eIF(iso)4E protein completely lacking its C-terminal half. The quantitative stimulatory effect of eIF4E expression from the TEV genome in protoplasts prepared from a susceptible host (Schaad et al., 2000) also suggests a quantitative role of eIF4E in the potyvirus cycle. The ability of *Ls-eIF4E^o*, upon expression from the viral genome, to restore not only systemic LMV accumulation in normally resistant *mo1¹* plants, but also symptom expression in normally tolerant *mo1²* plants, suggests a functional link between tolerance (symptom-less virus multiplication) and resistance. Therefore, at least in the lettuce/LMV system, these two distinct phenotypes might represent two outcomes of a same mechanism playing at quantitatively different intensities.

How eIF4E and/or its isoform are involved in the cycle of potyviruses in plants is not understood currently. Candidate pathways include initiation of viral RNA translation, circularization of viral RNA before replication, or eIF4E sequestration related to host gene shut off (Aranda and Maule, 1998). In addition, because a significant fraction of both VPg and eIF4E is nuclear (Shukla et al., 1994; Strudwick and Borden, 2002), it is also possible that the VPg-eIF4E interaction takes place in the nucleus and disturbs one of the increasingly better known functions of eIF4E within this cell compartment, such as mRNA processing or even nuclear translation (Strudwick and Borden, 2002). Finally, eIF4E could be involved, through its

interaction with VPg, in the control of the successive fates encountered by the viral RNA (translation, replication, intracellular and cell-to-cell transport, encapsidation, etc).

In conclusion, although an increasing number of resistance genes against plant pathogens are being cloned and characterized, most of them are dominant and related to the hypersensitive reaction or to extreme resistance, a phenotype related to the hypersensitive reaction and leading to virtual immunity to viruses in plants (Bendahmane et al., 1999). Host genes whose mutations impair the cycle of plant viruses have been identified, and some of them have even been isolated (Diez et al., 2000; Yamanaka et al., 2000; Lellis et al., 2002). However *eIF4E* is the first natural recessive virus resistance gene that has been isolated in plants: pepper (Ruffel et al., 2002) and now lettuce (this study). The function of *eIF4E* and/or *eIF(iso)4E* in the potyvirus cycle is largely unknown, but the negative effect of sequence variations in these factors on the accumulation of various potyviruses in various host plants indicates that it is probably a conserved one. It is tempting to speculate that some of the mechanisms involved may even be shared with other viral genera having a VPg at the 5' end of their genome; for instance, nepoviruses, for which interaction of the VPg-Pro proteolytic precursor with *eIF(iso)4E* has been described recently (Léonard et al., 2002).

MATERIALS AND METHODS

Plant Material and Viral Constructs

All plants were grown under greenhouse conditions. The LMV-susceptible lettuce (*Lactuca sativa*) genotypes Fiona, Girelle, Jessy, Mariska, Salinas, Vanguard, Trocadéro, and the early flowering accession 87-20M (Ryder, 1996), the *mo1*¹ genotypes Alizé, Classic, Floribibb, Malika, Mantilia, Oriana, and Presidio, and the *mo1*² genotypes Autumn Gold, Desert Storm, Vanguard 75, and Salinas 88 (Candresse et al., 2002) were used. The susceptible genotype Trocadéro was routinely used to propagate LMV. Two independent pairs of lettuce genotypes included in this analysis, namely Salinas/Salinas 88 and Vanguard/Vanguard 75, are nearly isogenic for *mo1*² (Ryder, 1979, 1991). The use of genetic markers showed that the region introgressed around *mo1*² in the genome of Salinas 88 and Vanguard 75 is small (Irwin et al., 1999).

All experiments were made with LMV-0, an LMV isolate unable to produce symptoms in *mo1*¹ and *mo1*² plants (Revers et al., 1997). The recombinant construct LMV-0-GFP carries an insertion of the *Aequorea victoria gfp* gene between the regions coding for P1 and Hc-Pro of the viral genome, with an engineered Nla-Pro cleavage site, so that the GFP protein is processed from Hc-Pro in vivo (S. German-Retana, unpublished data). LMV-0-4E⁰, LMV-0-4E¹, and LMV-0-iso4E were constructed similarly by inserting the *Ls-eIF4E*⁰, *Ls-eIF4E*¹, and *Ls-eIF(iso)4E* coding regions, respectively, in frame between the P1 and Hc-Pro domains of LMV, with an Nla-Pro cleavage site resulting in the addition of eight amino acids (PG-DEVYHQ) at the C terminus of the *eIF4E* sequence and five amino acids (SDVPG) at its N terminus. LMV-0-clv is an empty viral vector with only these additional amino acids but no *eIF4E* or other gene inserted. Lettuce plants primarily inoculated by biolistics (Helios Gene-Gun, Bio-Rad, Hercules, CA) were homogenized in 25 mM Na₂HPO₄ containing 2% (w/v) diethyldithiocarbamate and used to rub inoculate plants as described previously (Revers et al., 1997). Detection of the viral progeny by RT-PCR was performed as described previously (Krause-Sakate et al., 2002). Double-antibody sandwich ELISA was performed as described after homogenization of plant tissues in 4 volumes of extraction buffer (Revers et al., 1997).

F₁ hybrids between Mantilia (*mo1*¹/*mo1*¹) and the susceptible genotypes Mariska and Girelle were self-pollinated to obtain F₂ progenies. The parental lines, the F₁ hybrids, and the F₂ individuals were evaluated under greenhouse conditions for LMV resistance. Plants were rub inoculated 3 weeks after sowing at the five- to six-leaves stage. Symptoms were recorded 10 to 15 d after inoculation. These conditions ensured symptom appearance in 100% of Mariska and Girelle, whereas no symptoms were visible in Mantilia.

RT-PCR Amplification, Cloning, and Sequencing of cDNAs

Total RNA was extracted from 100 to 200 mg of leaf tissues using TRI Reagent (Sigma-Aldrich, St. Louis). Total cDNA was synthesized from 5 µg of total RNA using 15 units of AMV RT (Amersham Biosciences, Uppsala) and 1 µM oligo(dT) in a total volume of 50 µL, incubated for 1 h at 42°C.

PCR amplification was routinely performed using 1 µL of the total cDNAs in 50-µL reactions containing 0.5 units of Extra-Pol I *Taq* DNA polymerase (Eurobio, Les Ulis, France) or Platinum *Taq* DNA Polymerase High Fidelity (Invitrogen), using 1 µM oligonucleotide primers. For these reactions, 40 cycles (30 s of denaturation at 95°C, 30 s of annealing at different temperatures according to the primers used, and 1 min of elongation at 72°C) were performed in a thermal cycler (iCycler, Bio-Rad) after an initial denaturation of the RNA-cDNA duplex at 95°C for 2 min. The SMART RACE cDNA amplification kit (CLONTECH) was used according to the recommendations provided by the supplier to amplify the cDNA 5' and 3' ends of *eIF4E*, and, similarly, the 3'- and 5'-RACE Systems (Invitrogen) were used to amplify the 5' and 3' ends of *eIF(iso)4E* cDNA. The oligonucleotides used are listed in Table IV.

The pGEM-T Easy vector system (Promega, Madison, WI) was used to clone cDNAs after PCR amplification. Automated DNA sequencing of cloned fragments or PCR products was performed at Genaxis (St-Cloud, France) or Genome Express (Grenoble, France), using ABI PRISM 373XL or 377 sequencers (Applied Biosystems, Foster City, CA).

Generation of the CAPS Marker *eIF4E*-PagI

Total RNA was extracted from leaves of genotypes Mantilia, Mariska, and from the F₁ hybrids and the F₂ progenies as described above. From each RNA preparation, an *eIF4E* cDNA fragment of about 442 to 448 bp was amplified by RT-PCR using the oligonucleotides Ls4E250f and Ls4E697r (Table IV). The amplified cDNAs were subjected to digestion by the *PagI* endonuclease (Fermentas, Vilnius, Lithuania). The digestion products were resolved by electrophoresis in a 1.5% (w/v) agarose gel. Digestion at an additional *PagI* site present in all three types of *Ls-eIF4E* sequences provided an internal control for *PagI* activity by generating a 35-bp fragment not

Table IV. Oligonucleotides used in this work

The name of each oligonucleotide is given, with "f" as a suffix for forward oligonucleotides with respect to the mRNA polarity and "r" for reverse oligonucleotides. In the name of each oligonucleotide, the no. before the suffix indicates the position of the 5'-most nucleotide along the *Ls-eIF4E* sequence. Oligonucleotides provided with the RACE kits (CLONTECH, Palo Alto, CA; Invitrogen, Carlsbad, CA) are not shown.

Name	Sequence (5' → 3')
Ls4E3f	GGGGGGTGGGAAGAAATA
Ls4E83f	GGAGAAGAAGATGAACAACTGGAAGAGG
4E198f	TCATGGACWTTYTGTTYGATAAY
4E193f	CCAGAATTCCTGGACCTTYTGGTTCCA
Ls4E250f	TTGGGGTAGTTCATGCGCCCTA
Ls4E255r	CCCCAAGCGACTTGCTTGGCACTTAGCAGAGGG
4E408r	GCACAGATAGGTYTCCCA
Ls4E442r	TTTGGTAAAGGTCATAGTCCACTTCCA
Ls4E697r	TTTAGCACTTCTATCAAGAG
Ls4E813r	GCAGAATTGTAGCATAAATCGGG
Ls(iso)4E680r	GCTGCAAATTGTTCTAGG

visualized in this electrophoresis system and a 407- to 413-bp fragment further digested in Ls-eIF4E¹ to yield two fragments of 312 and 95 bp.

Functional Complementation Assay Using Agro-Infiltration

For transient expression in planta by agro-infiltration (Bechtold et al., 1993; Schöb et al., 1997), cDNAs were inserted in a 35S-nos expression cassette in the binary vector pGreenII (Hellens et al., 2000). *Agrobacterium tumefaciens* C58C1 was transformed by electroporation and grown overnight at 28°C in Luria-Bertani medium containing 5 µg mL⁻¹ tetracycline, 50 µg mL⁻¹ kanamycin, 10 mM MES, and 20 µM acetosyringone. The bacteria were collected by centrifugation and resuspended in 10 mM MES and 10 mM MgCl₂ containing 150 µM acetosyringone to an OD₆₀₀ of 0.6. They were then incubated at room temperature for 4 h and infiltrated using a syringe in apical leaves of plants that had been inoculated 5 to 6 d earlier with LMV-0-GFP on their lower leaves. Agro-infiltration took place when GFP fluorescence began to be visible under UV light near the veins in upper noninoculated leaves in the susceptible genotypes. Four to 6 d after infiltration, GFP expression was monitored as described by German-Retana et al. (2000) and using a fluorescence stereomicroscope (MZ FLIII, Leica Microsystems, Heerburg, Switzerland) equipped with a filter with an excitation window at 470 ± 20 nm and an arrest window at 525 ± 25 nm.

Sequence Analysis

The following eIF4E cDNA and protein sequences were retrieved from GenBank: human (*Homo sapiens*; NM_004846, XM_017925), mouse (*Mus musculus*) (M61731), *Spodoptera frugiperda* (AF281654), rice (*Oryza sativa*; U34597), maize (*Zea mays*; AF076954), wheat (*Triticum aestivum*; Z12616), tomato (*Lycopersicon esculentum*; AF259801), and Arabidopsis (Y10548, NM_102695 and NM_102699). The eIF(iso)4E sequences were: rice (U34598), maize (AF076955), and Arabidopsis (Y10547). Homology-based database searches were made in GenBank using the program BLAST (Altschul et al., 1990). The nucleotide and amino acid sequences were aligned using ClustalW (Thompson et al., 1994), which generates and uses a distance dendrogram (Saitou and Nei, 1987) to construct multiple sequence alignments, or with Align (Myers and Miller, 1988) for pair-wise alignments.

The 3D structures of cap-bound eIF4E of human (Hs-eIF4E) and mouse (Mm-eIF4E) were retrieved from the Protein Data Bank (<http://www.rcsb.org/pdb>) with the codes 1IPB and 1EJH, respectively. Comparative protein modeling was elaborated online using Swiss-model and Swiss-PdbViewer (Guex and Peitsch, 1997) and 3D-Jigsaw (Bates et al., 2001). The fit between 3D structure models was evaluated in Swiss-PdbViewer by calculating the RMS deviation (Chothia and Lesk, 1986) after iterative fitting.

ACKNOWLEDGMENTS

We are grateful to the excellence of Thierry Mauduit for plant care, to Kathryn Mayo for improving the English of the manuscript, and to Sandrine Ruffel and Drs. Thierry Delaunay, Thierry Michon, Frédéric Revers, and Christophe Robaglia for their stimulating comments and discussions.

Received November 18, 2002; returned for revision January 30, 2003; accepted March 14, 2003.

LITERATURE CITED

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215: 403–410
- Aranda M, Maule AJ (1998) Virus-induced host gene shutoff in animals and plants. *Virology* 243: 261–267
- Bates PA, Kelley LA, MacCallum RM, Sternberg MJ (2001) Enhancement of protein modeling by human intervention in applying the automatic programs 3D-JIGSAW and 3D-PSSM. *Proteins Suppl* 5: 39–46
- Bechtold N, Ellis J, Pelletier G (1993) In planta *Agrobacterium* mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. *C R Acad Sci Ser III Sci Vie* 316: 1194–1199

- Bendahmane A, Kanyuka K, Baulcombe DC (1999) The Rx gene from potato controls separate virus resistance and cell death responses. *Plant Cell* 11: 781–792
- Bos L, Huijberts N, Cuperus C (1994) Further observations on variation of lettuce mosaic virus in relation to lettuce (*Lactuca sativa*), and a discussion of resistance terminology. *Eur J Plant Pathol* 100: 293–314
- Candresse T, Le Gall O, Maisonneuve B, German-Retana S, Redondo E (2002) The use of green fluorescent protein-tagged recombinant viruses greatly simplifies *Lettuce mosaic virus* resistance testing in lettuce. *Phytopathology* 92: 169–176
- Carberry SE, Darzynkiewicz E, Goss DJ (1991) A comparison of the binding of methylated cap analogues to wheat germ protein synthesis initiation factors 4F and (iso)4F. *Biochemistry* 30: 1624–1627
- Chothia C, Lesk AM (1986) The relation between the divergence of sequence and structure in proteins. *EMBO J* 5: 823–826
- Diez J, Ishikawa M, Kaido M, Ahlquist P (2000) Identification and characterization of a host protein required for efficient template selection in viral RNA replication. *Proc Natl Acad Sci USA* 97: 3913–3918
- Dinant S, Lot H (1992) *Lettuce mosaic virus*. *Plant Pathol* 41: 528–542
- Duprat A, Caranta C, Revers F, Menand B, Browning KS, Robaglia C (2002) The Arabidopsis eukaryotic initiation factor (iso)4E is dispensable for plant growth but required for susceptibility to potyviruses. *Plant J* 32: 927–934
- German-Retana S, Candresse T, Alias E, Delbos R, Le Gall O (2000) Effects of GFP or GUS tagging on the accumulation and pathogenicity of a resistance breaking LMV isolate in susceptible and resistant lettuce cultivars. *Mol Plant-Microbe Interact* 13: 316–324
- Gibb KS, Hellmann GM, Pirone TP (1989) Nature of resistance of a tobacco cultivar to tobacco vein mottling virus. *Mol Plant-Microbe Interact* 2: 232–239
- Guex N, Peitsch MC (1997) SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis* 18: 2714–2723
- Hellens RP, Edwards EA, Leyland NR, Bean S, Mullineaux PM (2000) pGreen: a versatile and flexible binary Ti vector for *Agrobacterium*-mediated plant transformation. *Plant Mol Biol* 42: 819–832
- Irwin SV, Kesseli RV, Waycott W, Ryder EJ, Cho JJ, Micheltore RW (1999) Identification of PCR-based markers flanking the recessive LMV resistance gene *mo1* in an intraspecific cross in lettuce. *Genome* 42: 982–986
- Ishikawa M, Diez J, Restrepo-Hartwig M, Ahlquist P (1997) Yeast mutations in multiple complementation groups inhibit brome mosaic virus RNA replication and transcription and perturb regulated expression of the viral polymerase-like gene. *Proc Natl Acad Sci USA* 94: 13810–13815
- Keller KE, Johansen IE, Martin RR, Hampton RO (1998) Potyvirus genome-linked protein (VPg) determines *Pea seed-borne mosaic virus* pathotype-specific virulence in *Pisum sativum*. *Mol Plant-Microbe Interact* 11: 124–130
- Krause-Sakate R, Le Gall O, Fakhfakh H, Peypelut M, Marrakchi M, Varveri C, Pavan MA, Souche S, Lot H, Zerbini FM et al. (2002) Molecular characterization of *Lettuce mosaic virus* field isolates reveals a distinct and widespread type of resistance-breaking isolate: LMV-Most. *Phytopathology* 92: 563–572
- Lellis AD, Kasschau KD, Whitham SA, Carrington JC (2002) Loss-of-susceptibility mutants of *Arabidopsis thaliana* reveal an essential role for eIF(iso)4E during potyvirus infection. *Curr Biol* 12: 1046–1051
- Léonard S, Chisholm J, Laliberté JF, Sanfaçon H (2002) Interaction *in vitro* between the proteinase of *Tomato ringspot virus* (genus *Nepovirus*) and the eukaryotic translation initiation factor iso4E from *Arabidopsis thaliana*. *J Gen Virol* 83: 2085–2089
- Léonard S, Plante D, Wittmann S, Daigneault N, Fortin MG, Laliberté JF (2000) Complex formation between potyvirus VPg and translation eukaryotic initiation factor 4E correlates with virus infectivity. *J Virol* 74: 7730–7737
- Marcotrigiano J, Gingras AC, Sonenberg N, Burley SK (1997) Cocystal structure of the messenger RNA 5' cap-binding protein (eIF4E) bound to 7-methyl-GDP. *Cell* 89: 951–961
- Marcotrigiano J, Gingras AC, Sonenberg N, Burley SK (1999) Cap-dependent translation initiation in eukaryotes is regulated by a molecular mimic of eIF4G. *Mol Cell* 3: 707–716
- Montesclaros L, Nicol N, Ubalijoro E, Leclerc-Potvin C, Ganivet L, Laliberté JF, Fortin MG (1997) Response to potyvirus infection and genetic

- mapping of resistance loci to potyvirus infection in *Lactuca*. *Theor Appl Genet* **94**: 941–946
- Murphy JF, Rhoads RE, Hunt AG, Shaw JG** (1990) The VPg of tobacco etch virus RNA is the 49-kDa proteinase or the N-terminal 24-kDa part of the proteinase. *Virology* **178**: 285–288
- Myers EW, Miller W** (1988) Optimal alignments in linear space. *Comput Appl Biosci* **4**: 11–17
- Pestova TV, Kolupaeva VG, Lomakin IB, Pilipenko EV, Shatsky IN, Agol VI, Hellen CU** (2001) Molecular mechanisms of translation initiation in eukaryotes. *Proc Natl Acad Sci USA* **98**: 7029–7036
- Pink DAC, Lot H, Johnson R** (1992) Novel pathotypes of lettuce mosaic virus: breakdown of a durable resistance? *Euphytica* **63**: 169–174
- Pontier D, Balague C, Roby D** (1998) The hypersensitive response. A programmed cell death associated with plant resistance. *C R Acad Sci Ser III Sci Vie* **321**: 721–734
- Provvidenti R, Hampton RO** (1992) Sources of resistance to viruses in the *Potyviroidea*. *Arch Virol Suppl* **5**: 189–211
- Ratcliff F, Harrison BD, Baulcombe DC** (1997) A similarity between viral defense and gene silencing in plants. *Science* **276**: 1558–1560
- Redondo E, Krause-Sakate R, Yang SJ, Lot H, Le Gall O, Candresse T** (2001) *Lettuce mosaic virus* (LMV) pathogenicity determinants in susceptible and tolerant lettuce varieties map to different regions of the viral genome. *Mol Plant-Microbe Interact* **14**: 804–810
- Reichmann JL, Lain S, Garcia JA** (1992) Highlights and prospects of potyvirus molecular biology. *J Gen Virol* **73**: 1–16
- Revers F, Lot H, Souche S, Le Gall O, Candresse T, Dunez J** (1997) Biological and molecular variability of *Lettuce mosaic virus* isolates. *Phytopathology* **87**: 397–403
- Rodriguez CM, Freire MA, Camilleri C, Robaglia C** (1998) The *Arabidopsis thaliana* cDNAs coding for eIF4E and eIF(iso)4E are not functionally equivalent for yeast complementation and are differentially expressed during plant development. *Plant J* **13**: 465–473
- Ruffel S, Dussault MH, Palloix A, Moury B, Bendahmane A, Robaglia C, Caranta C** (2002) A natural recessive resistance gene against potato virus Y in pepper corresponds to the eukaryotic initiation factor 4E (eIF4E). *Plant J* **32**: 1067–1075
- Ryder EJ** (1970) Inheritance of resistance to common lettuce mosaic. *J Am Soc Hortic Sci* **95**: 378–379
- Ryder EJ** (1979) Vanguard 75 lettuce. *Hortscience* **14**: 284–286
- Ryder EJ** (1991) Salinas 88 lettuce. *Hortscience* **26**: 439–440
- Ryder EJ** (1996) Ten lettuce genetic stocks with early flowering genes *Ef-1ef-1* and *Ef-2ef-2*. *Hortscience* **31**: 473–475
- Saitou N, Nei M** (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**: 406–425
- Schaad MC, Anderberg RJ, Carrington JC** (2000) Strain-specific interaction of the *Tobacco etch virus* NIa protein with the translation initiation factor eIF4E in the yeast two-hybrid system. *Virology* **273**: 300–306
- Schaad MC, Carrington JC** (1996) Suppression of long-distance movement of tobacco etch virus in a nonsusceptible host. *J Virol* **70**: 2556–2561
- Schaad MC, Haldeman-Cahill R, Cronin S, Carrington JC** (1996) Analysis of the VPg-proteinase (NIa) encoded by tobacco etch potyvirus: effects of mutations on subcellular transport, proteolytic processing, and genome amplification. *J Virol* **70**: 7039–7048
- Schöb H, Kunz C, Meins F Jr** (1997) Silencing of transgenes introduced into leaves by agroinfiltration: a simple, rapid method for investigating sequence requirements for gene silencing. *Mol Gen Genet* **256**: 581–585
- Shukla DD, Ward CW, Brunt AA** (1994) *The Potyviroidea*. CAB International, Wallingford, UK
- Strudwick S, Borden KL** (2002) The emerging roles of translation factor eIF4E in the nucleus. *Differentiation* **70**: 10–22
- Thompson JD, Higgins DG, Gibson TJ** (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**: 4673–4680
- Tomoo K, Shen X, Okabe K, Nozoe Y, Fukuhara S, Morino S, Ishida T, Taniguchi T, Hasegawa H, Terashima A et al.** (2002) Crystal structures of 7-methylguanosine 5'-triphosphate (m(7) GTP)- and P(1)-7-methylguanosine-P(3)-adenosine-5',5'-triphosphate (m(7) GpppA)-bound human full-length eukaryotic initiation factor 4E: biological importance of the C-terminal flexible region. *Biochem J* **362**: 539–544
- Voinnet O** (2001) RNA silencing as a plant immune system against viruses. *Trends Genet* **17**: 449–459
- Wittmann S, Chatel H, Fortin MG, Laliberté JF** (1997) Interaction of the viral protein genome linked of *Turnip mosaic potyvirus* with the translational eukaryotic initiation factor (iso) 4E of *Arabidopsis thaliana* using the yeast two-hybrid system. *Virology* **234**: 84–92
- Yamanaka T, Ohta T, Takahashi M, Meshi T, Schmidt R, Dean C, Naito S, Ishikawa M** (2000) TOM1, an *Arabidopsis* gene required for efficient multiplication of a tobamovirus, encodes a putative transmembrane protein. *Proc Natl Acad Sci USA* **97**: 10107–10112