FEBS Letters 581 (2007) 1041–1046

Coordinated and selective recruitment of eIF4E and eIF4G factors for potyvirus infection in *Arabidopsis thaliana*

Valérie Nicaise[a], Jean-Luc Gallois[b], Faccila Chafiai[a], Leah M. Allen[c], Valérie Schurdi-Levraud[a,d], Karen S. Browning[c], Thierry Candresse[a], Carole Caranta[b], Olivier Le Gall[a], Sylvie German-Retana[a,*]

[a] *Interactions Plante-Virus (IPV), UMR 1090, INRA Bordeaux 2, BP 81, F-33883 Villenave d'Ornon, France*
[b] *Genetics and Breeding of Fruits and Vegetable, INRA Avignon, BP 94, F-84143 Montfavet, France*
[c] *Department of Chemistry and Biochemistry and the Institute for Cell and Molecular Biology, The University of Texas at Austin, Austin, TX 78712, USA*
[d] *AGRO.M, 2 place Viala, F-34060 Montpellier Cedex 1, France*

Received 26 January 2007; accepted 1 February 2007

Available online 14 February 2007

Edited by Michael R. Sussman

This work is dedicated to the memory of F. Chafiai

Abstract The translation initiation factors eIF4E and eIF (iso)4E play a key role during virus infection in plants. During mRNA translation, eIF4E provides the cap-binding function and is associated with the protein eIF4G to form the eIF4F complex. Susceptibility analyses of *Arabidopsis* mutants knocked-out for *At-eIF4G* genes showed that eIF4G factors are indispensable for potyvirus infection. The colonization pattern by a viral recombinant carrying GFP indicated that eIF4G is involved at a very early infection step. Like eIF4E, eIF4G isoforms are selectively recruited for infection. Moreover, the eIF4G selective involvement parallels eIF4E recruitment. This is the first report of a coordinated and selective recruitment of eIF4E and eIF4G factors, suggesting the whole eIF4F recruitment.

Keywords: Recessive resistance; eIF4E; eIF4G; Plant potyvirus

1. Introduction

As obligatory parasites and because their genome encodes only a limited number of proteins, viruses have to reroute the host cellular machinery to their own advantage. Therefore, the success of the viral infection cycle depends on complex interplays between functions encoded by the viral and host genomes [1].

Eukaryotic translation initiation factors (eIF4E and eIF (iso)4E) are key determinants of the interactions between plants and several RNA viruses [2,3]. Viruses requiring these factors mainly belong to the genus *Potyvirus*, the largest and the most diverse genus of plant viruses [4,5]. Their positive single-stranded RNA genome of about 10 kb is 3′-polyadenylated and covalently linked at the 5′ end to a virus-encoded protein (VPg) [6]. The potyvirus RNA encodes a polyprotein which is processed into ca. 10 mature proteins by viral proteinases [7].

In eukaryotes, eIF4E belongs to the eIF4F complex which recruits ribosomes and the cellular mRNAs to initiate protein synthesis. eIF4F includes eIF4E, which binds the cap structure at the 5′ end of mRNAs, eIF4A, a DEAD-box RNA helicase that unwinds the mRNA 5′UTR to facilitate the ribosome binding, and eIF4G, a scaffold protein which interacts with many other translation machinery components [8]. In plants, there is a second eIF4F complex, eIF(iso)4F, containing the isoforms eIF(iso)4E and eIF(iso)4G [9].

Potyviruses recruit selectively eIF4E isoforms. Indeed, the disruption of *At-eIF(iso)4E* gene in *Arabidopsis* results in resistance to *Turnip mosaic virus* (TuMV), *Lettuce mosaic virus* (LMV) and *Plum pox virus* (PPV) while the disruption of *eIF4E* gene results in resistance to *Clover yellow vein virus* (ClYVV) [10–12].

In addition to its central role in cellular translation, eIF4G is involved in the cap-independent translation process of viruses, including picornaviruses (animal RNA viruses with a 5′ VPg and a 3′ polyA, like potyviruses) and the potyvirus *Tobacco etch virus* (TEV), which is mediated only by the association between a viral IRES element, eIF4G and ribosomes [13,14]. Therefore, eIF4G was chosen as a candidate which could be involved in plant–potyvirus interactions. In the genome of *A. thaliana*, three genes encode members of eIF4G family: *At-eIF4G*, *At-eIF(iso)4G1* and *At-eIF(iso)4G2*. To address the involvement of eIF4G factors in potyvirus infection, the susceptibility of *A. thaliana* plants disrupted in *eIF4G* genes to several potyviruses (ClYVV, LMV, PPV and TuMV) was evaluated.

2. Materials and methods

2.1. Plant material

A. thaliana Columbia-0 plants were grown in a greenhouse or in growth chambers at 25 °C with a 16-h light period. Susceptibility assays were performed on T-DNA insertion lines ordered from the SALK collection: *eIF4G* (At3g60240) mutant = SALK112882,

*Corresponding author. Fax: +33 557 122 384.
E-mail address: german@bordeaux.inra.fr (S. German-Retana).

0014-5793/$32.00
doi:10.1016/j.febslet.2007.02.007

eIF(iso)4G1 (At5g57870) mutant = SALK009905 and *eIF(iso)4G2* (At2g24050) mutant = SALK076633. The T-DNA insertion and the homozygous state of the mutant lines were tested by PCR of genomic DNA using primers defined on the SALK web site (http://signal.salk.edu/tdnaprimers.2.html). Homozygous mutants were backcrossed once to wild-type plants and the F2 progeny screened again for the homozygous state. Double mutants were obtained by crossing the relevant single mutants and selfing the F1 hybrid to obtain F2 progenies that were screened by PCR for the double homozygous state. The phenotypic analysis of these mutants which do not have obvious phenotype in growth or flowering will be described elsewhere, with data on absence of mRNA and protein expression (L. Allen, A. Lellis and K. Browning, unpublished data).

2.2. Virus inoculation and detection

The isolates PPV-NAT [15], PPV-PS [16] and PPV-R3-GFP [17] were used in this work. A recombinant LMV, LMV-ExbaAF [18], was selected for its capacity to systemically infect Columbia-0. The ClYVV isolate PV-0367 was obtained from the DSMZ Plant Virus Collection (Germany). The TuMV isolates used were CDN1 and UK1 [19,20]. Except for PPV-R3-GFP, viruses were inoculated mechanically [10]. Virus accumulation in inoculated and apical non-inoculated leaves (above the rosette leaves) was analyzed by ELISA and/or RT-PCR. Five microliters of total RNAs [21] were used for RT-PCR using Abgene-reverse transcriptase and the DyNAzymeEXT® DNA polymerase (Finnzyme), in the presence of virus-specific primers (Table 1). PPV-R3-GFP was inoculated as described previously by infiltration with *Agrobacterium tumefaciens* C58C1 cells carrying the infectious clone pBINPPV-NK-GFP construct [17,22]. GFP fluorescence was monitored using a fluorescence stereomicroscope (MZ FLIII, Leica Microsystems) equipped with a filter with an excitation window at 470 ± 20 nm and an arrest window at 525 ± 25 nm.

2.3. Functional complementation

The *At-eIF(iso)4G1* cDNA was inserted in the binary vector pFP108 [23] to yield pFP108-iso4G1 and introduced in *A. tumefaciens* C58C1. Cultures carrying the PPV-R3-GFP infectious clone and the pBin61-35S:P19 plasmid [24] were mixed (v/v/v) with or without the culture carrying pFP108-iso4G1 in order to perform complementation experiments as described [22].

3. Results

3.1. eIF(iso)4G1 is necessary for PPV infection

The involvement of eIF4G in the PPV–*Arabidopsis* interaction was evaluated by analyzing the susceptibility of *A. thaliana* T-DNA insertion mutants disrupted in each of the three *eIF4G* genes. Knock-out (KO) mutant lines were inoculated with PPV-PS in four independent experiments. The presence of PPV was evaluated by RT-PCR in non-inoculated tissues at 21 days post-inoculation (dpi). In all experiments, PPV-PS was detected in the *At-eIF4G* and *At-eIF(iso)4G2* KO mutants as well as in wild-type plants (WT), but never in the *At-eIF(iso)4G1* mutant (Fig. 1). The same behavior was observed with two other PPV isolates, PPV-NAT and PPV-R3-GFP (Fig. 1).

To determine at which level the block in the PPV infection occurred in the *At-eIF(iso)4G1* mutant, virus accumulation was monitored using a recombinant PPV isolate expressing GFP, PPV-R3-GFP. In the *At-eIF4G* and *At-eIF(iso)4G2* mutants as well as in WT, infection foci on inoculated leaves

Table 1
Oligonucleotides used in this work

Name	Sequence (5′ → 3′)	Fragment amplified
PPV-F	CAGACTACAGCCTCGCCAGA	a 243-bp fragment of PPV CP sequence
PPV-R	ACCGAGACCACTACACACTCCC	
LMV-F	ACAATCTCAAACTTCTCCAT	a 483-bp fragment of LMV CI-6K2 sequence
LMV-R	GCAGCTCCAAAATAGTGTTCCA	
ClYVV-F	GAGTGGACAATGATGGATGG	a 429-bp fragment of PPV CI sequence
ClYVV-R	AATTTAWWGACGGATACTCTA	
TuMV-F	TATGGATGGTTGTTCAACAC	a 686-bp fragment of TuMV VPg sequence
TuMV-R	TTGTGAGATGACAAATGTTG	

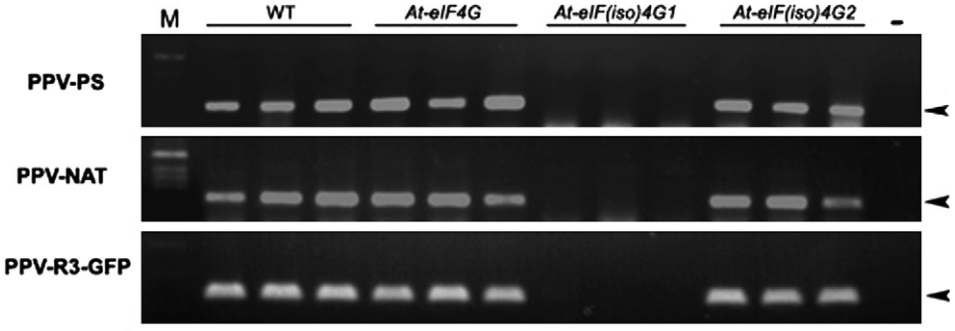

Fig. 1. Infection of the wild-type and *eIF4G Arabidopsis* mutants with three PPV isolates. Virus accumulation was assayed by RT-PCR. M, molecular weight marker; "−", negative control. The expected position of the RT-PCR fragment is arrowed. Three plants were assayed for each genotype.

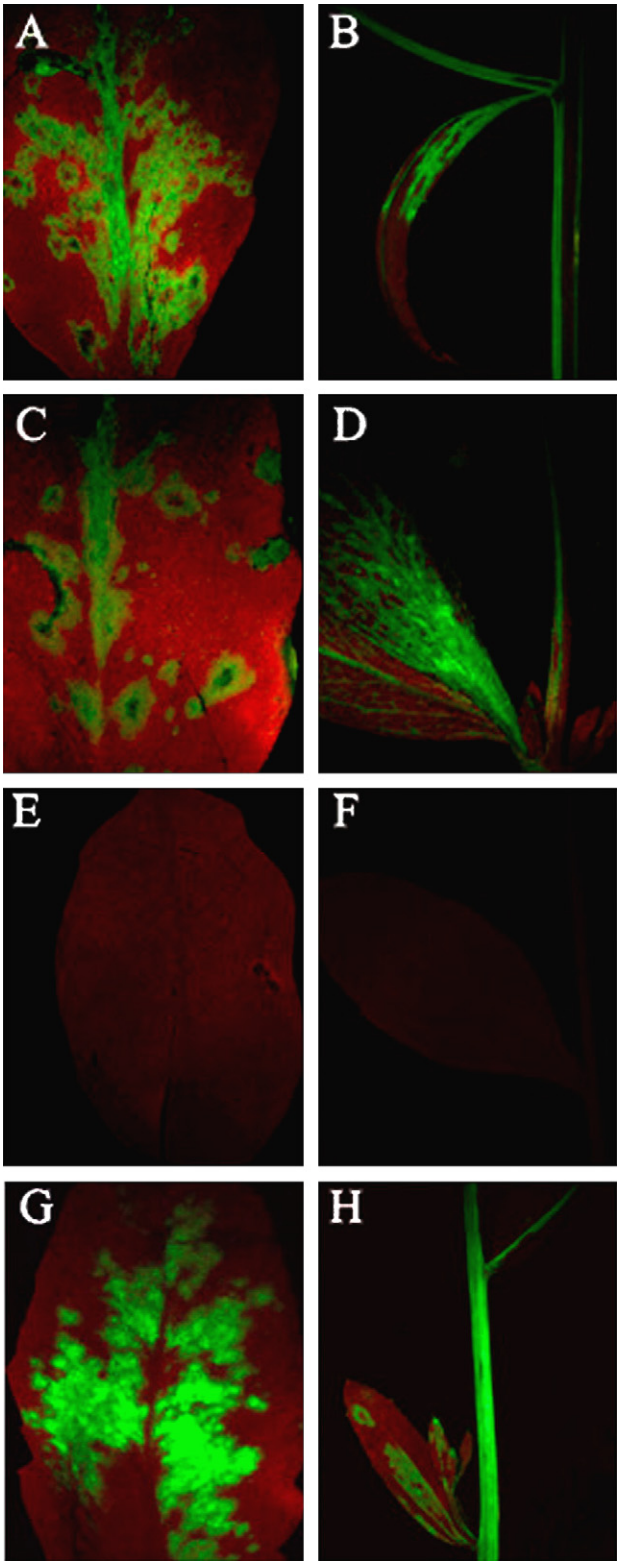

Fig. 2. PPV-R3-GFP infection pattern in *eIF4G* mutants. GFP fluorescence was visualized in wild-type (A, B), *At-eIF4G* (C, D), *At-eIF(iso)4G1* (E, F) and *At-eIF(iso)4G2* (G, H) mutants in inoculated (A, C, E and G) and non-inoculated tissues (B, D, F and H).

could be visualized by fluorescence microscopy, starting at 5 dpi. At 14 dpi, GFP fluorescence was observed in non-inoculated leaves, in stems (Fig. 2), flowers and roots (not shown). In contrast, no GFP fluorescence was detected in inoculated or non-inoculated tissues, up to 2 months after inoculation in the *At-eIF(iso)4G1* mutant (Fig. 2).

In order to demonstrate that eIF(iso)4G1 functions as a susceptibility factor for PPV infection, complementation experiments were performed in the *At-eIF(iso)4G1* mutant. *Agrobacterium*-mediated transient expression of *eIF(iso)4G1* was performed in PPV-inoculated leaves in the presence of the silencing suppressor P19 [24]. At 8 dpi, PPV-R3-GFP fluorescence was detected in agroinfiltrated leaves of both WT and complemented-*At-eIF(iso)4G1* plants (Fig. 3A). In contrast, no GFP fluorescence was observed in the non-complemented *At-eIF(iso)4G1* mutant. These results were confirmed by RT-PCR (Fig. 3B).

Therefore, eIF(iso)4G1 is specifically required for the PPV cycle in *A. thaliana*. Since PPV-R3-GFP fluorescence was never observed in *eIF(iso)4G1* mutants, even at the single cell level, it is likely that eIF(iso)4G1 plays a role in the early events of the infection process.

3.2. eIF4G isoforms are not equivalent for potyvirus infection

To accomplish their infectious cycle, different potyviruses recruit selectively eIF4E or eIF(iso)4E isoforms [11]. In order to test if a selective involvement also occurs for eIF4G family members, the susceptibility of *eIF4G* mutants to three other potyviruses was assayed (ClYVV, LMV and TuMV). Virus accumulation was analyzed by RT-PCR in non-inoculated tissues at 21 dpi. As for PPV, LMV did not accumulate in the *At-eIF(iso)4G1* mutant. In contrast, ClYVV accumulated in the *At-eIF(iso)4G1* mutant but not in the *At-eIF4G* mutant. All three mutants were susceptible to TuMV (Fig. 4). Comparable results were obtained with the virus inoculated leaves (not shown).

3.3. TuMV infection requires both eIF(iso)4G1 and eIF(iso)4G2

The observation that TuMV is able to multiply in the three *eIF4G* mutants could indicate that either eIF4G factors are not needed for TuMV infection, or that TuMV is able to recruit several isoforms of this factor, complementing the absence of one of them by the recruitment of another one. In order to test

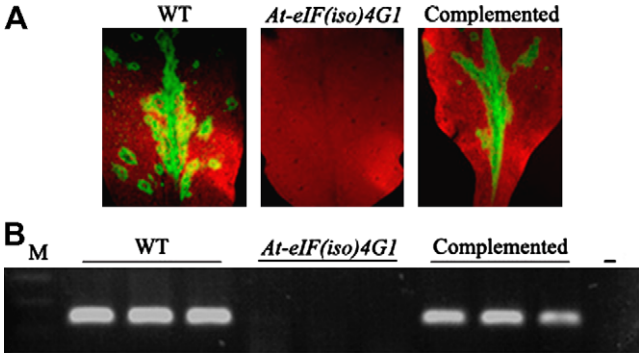

Fig. 3. *At-eIF(iso)4G1* mutant complementation. (A) GFP fluorescence in wild-type and *At-eIF(iso)4G1* plants agroinfiltrated with PPV-R3-GFP. Complemented plants correspond to the *At-eIF(iso)4G1* mutant agroinfiltrated with the mix [PPV-R3-GFP + eIF(iso)4G1]. (B) The presence of PPV was assayed by RT-PCR. M, molecular weight marker; "–", negative control. Three plants were assayed for each genotype.

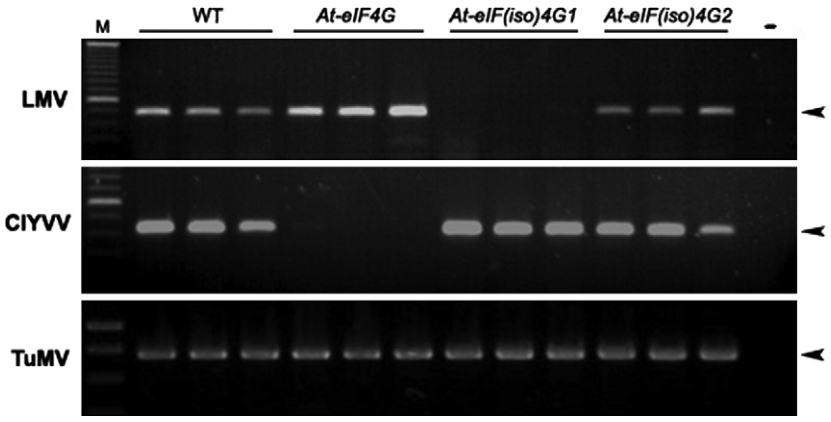

Fig. 4. Infectivity of LMV, ClYVV and TuMV in wild-type and *eIF4G* mutants. Virus accumulation was assayed by RT-PCR. M, molecular weight marker; "−", negative control. The expected position of the RT-PCR fragments are arrowed. Three plants were assayed for each genotype.

this, the susceptibility of double mutants to TuMV was analyzed. Despite repeated efforts, the *At-eIF4G* × *At-eIF(iso)4G2* double mutant could not be obtained (L. Allen and K. Browning, unpublished data), indicating that the simultaneous inactivation of these two isoforms is probably lethal in *A. thaliana*. The *At-eIF4G* × *At-eIF(iso)4G1* double mutant was susceptible to TuMV-CDN1 (not shown). In contrast, all 40 plants of the *At-eIF(iso)4G1* × *At-eIF(iso)4G2* double mutant were ELISA negative at 10 and 20 dpi. These results were confirmed by RT-PCR at 5, 7, 10 and 20 dpi, showing that TuMV does not accumulate in this double mutant (Fig. 5A). Similar resistance was observed with the TuMV-UK1 isolate (not shown). In order to confirm the role of eIF(iso)4G1 and eIF (iso)4G2 in TuMV infection, the genetic linkage between the resistance phenotype and the simultaneous disruption of the genes *eIF(iso)4G1* and *eIF(iso)4G2* was studied. Among the 160 F2 plants of an *At-eIF(iso)4G1* KO × *At-eIF(iso)4G2* KO cross, eight plants did not accumulate TuMV and were ELISA negative at 20 dpi. This ratio of 152 susceptible to eight resistant fits with the segregation of two recessive factors involved in TuMV resistance (χ^2 [1R:15S] = 0.426; P = 51.4%). All F2 plants were genotyped for *eIF(iso)4G1* and *eIF(iso) 4G2* and only the 8 F2 plants resistant to TuMV were found to be double homozygous mutants (Fig. 5B and C).

These results indicate that the simultaneous disruption of the *eIF(iso)4G1* and *eIF(iso)4G2* genes lead to lack of susceptibility to two TuMV isolates, indicating that either of these factors can be recruited during TuMV infection in *A. thaliana* and that the disruption in either one of these genes can be complemented by the other one.

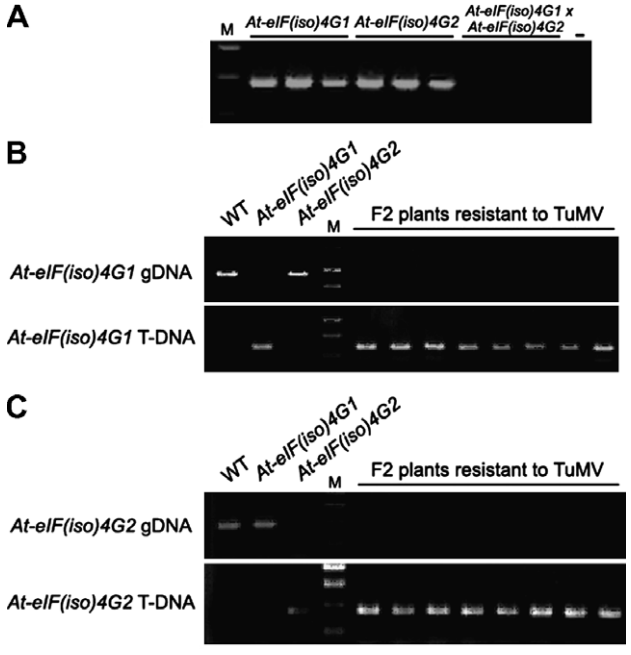

Fig. 5. TuMV infectivity in *At-eIF(iso)4G1* and *At-eIF(iso)4G2* single and double mutants. (A) Detection of TuMV by RT-PCR. M, molecular weight marker; "−", negative control. (B, C) Genotyping for *eIF(iso)G1* (B) and *eIF(iso)G2* (C) of F2 plants resistant to TuMV compared to WT and single mutants.

4. Discussion

Although mechanisms of plant infection remain poorly understood, recent studies have demonstrated the key role of eIF4E factors during potyvirus infection [1,2,12]. Moreover, a physical interaction between eIF4E and the VPg of potyviruses has been demonstrated in several plant–potyvirus systems [25–29].

The present work demonstrates a crucial role of the eIF4G factors during *A. thaliana* infection by potyviruses since the *At-eIF4G* mutant is resistant to ClYVV, the *At-eIF(iso)4G1* mutant is resistant to PPV and LMV and the *At-eIF(iso)4-G1* × *At-eIF(iso)4G2* double mutant is resistant to TuMV. The resistance of the *At-eIF(iso)4G1* mutant to PPV-R3-GFP is effective at the single cell level in inoculated leaves, suggesting that the eIF4G proteins are required at a very early step of the infection process. Recently, the analyses of the rice *rymv1* and *Arabidopsis cum2* resistance genes, conferring the resistance to *Rice yellow mottle sobemovirus* and *Cucumber mosaic cucumovirus*, were shown to encode eIF4G factors [30,31], suggesting that eIF4G contributes to a general mechanism of plant susceptibility to viruses.

How eIF4G factors are involved in potyvirus infection is not understood currently. As eIF4E and eIF4G are both needed for host–virus compatibility, the obvious hypothesis is that

Table 2
Involvement of eIF4E and eIF4G family members in the cycle of potyviruses

	WT	*At-eIF4E1*	*At-eIF(iso)4E*	*At-eIF4G*	*At-eIF(iso)4G1*	*At-eIF(iso)4G2*	*At-eIF(iso)4G1 × At-eIF(iso)4G2*
PPV	+	+[a]	−[c]	+	−	+	−
LMV	+	+[a]	−[d]	+	−	+	nt
ClYVV	+	−[b]	+[b]	−	+	+	nt
TuMV	+	+[b]	−[d]	+	+	+	−

Susceptibility of *eIF4E* and *eIF4G* mutants to several potyviruses: PPV, LMV, ClYVV and TuMV. +, virus accumulation; −, no virus detected; nt, not tested.
[a](Nicaise V., unpublished data).
[b]Ref. [11].
[c]Ref. [12].
[d]Ref. [10].

potyviruses recruit the whole eIF4F complex for their cycle. However, in two different families of RNA viruses with a 5′ VPg including potyviruses, cap-independent translation mediated only by the association between an IRES element, eIF4G and ribosomes was described [13,14], suggesting that the role of eIF4G in infection could be eIF4E-independent.

The existence of two eIF4F complexes to initiate protein synthesis is a peculiarity of plants. Although the eIF4F and eIF (iso)4F complexes are considered equivalent for *in vitro* translation of some mRNAs, they may differ functionally in cellular translation. Indeed, they differ in their expression pattern *in vivo* and show some specificity for different capped mRNAs [32]. Here, we demonstrate that potyviruses selectively use members of eIF4G family in a fashion that parallels their selective recruitment of eIF4E isoforms [11]: ClYVV recruits eIF4E and eIF4G while PPV, LMV and TuMV recruit eIF(iso)4E and one or several eIF(iso)4G factors (Table 2). Therefore, it seems that the potyvirus cycle requires the same eIF4E–eIF4G complexes, which are functional for cellular translation. This is the first report bringing to light a collaborative recruitment of both eIF4E and eIF4G factors during the potyvirus infection. Thus, this is in favor of a possible role of both eIF4E and eIF4G through the eIF4F complex recruitment.

To date however, the function of eIF4E and eIF4G during the infection process remains to be elucidated. Roles in the early events of infection are the main candidate hypotheses: viral RNA translation and/or replication, circularization of viral RNA, host protein sequestration, or virus movement from infected to uninfected cells.

Acknowledgements: We thank the Salk Institute for the heterozygous *A. thaliana* mutants, S. Winter (DSMZ, Germany) for the ClYVV isolate, T. Mauduit and M. Roncoroni for plant care, M. Fortin and P. Dufresne for stimulating discussions (McGill University, Canada). VN was supported by an A.R.N. fellowship and JLG by a post-doctoral fellowship from the French Ministry of Research. This work was supported by grants from GENOPLANTE (NEWVIR Gener56), the French National Agency for Research (Poty4E, ANR-05-BLAN-0302-01) and the National Science Foundation (MCB-0214996).

References

[1] Thivierge, K., Nicaise, V., Dufresne, P.J., Cotton, S., Laliberte, J.-F., Le Gall, O. and Fortin, M.G. (2005) Plant virus RNAs. Coordinated recruitment of conserved host functions by (+) ssRNA viruses during early infection events. Plant Physiol. 138, 1822–1827.
[2] Robaglia, C. and Caranta, C. (2006) Translation initiation factors: a weak link in plant RNA virus infection. Trends Plant Sci. 11, 40–45.
[3] Nieto, C. et al. (2006) An eIF4E allele confers resistance to an uncapped and non-polyadenylated RNA virus in melon. Plant J. 48, 1–11.
[4] Adams, M.J., Antoniw, J.F. and Beaudoin, F. (2005) Overview and analysis of the polyprotein cleavage sites in the family *Potyviridae*. Mol. Plant Pathol. 6, 471–487.
[5] Shukla, D.D., Ward, C.W. and Brunt, A.A. (1994) The *Potyviridae*, CAB International, Wallingford, UK.
[6] Murphy, J.F., Rychlik, W., Rhoads, R.E., Hunt, A.G. and Shaw, J.G. (1991) A tyrosine residue in the small nuclear inclusion protein of tobacco vein mottling virus links the VPg to the viral RNA. J. Virol. 65, 511–513.
[7] Reichmann, J.L., Lain, S. and Garcia, J.A. (1992) Highlights and prospects of potyvirus molecular biology. J. Gen. Virol. 73, 1–16.
[8] Gingras, A.C., Raught, B. and Sonenberg, N. (1999) eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. Annu. Rev. Biochem. 68, 913–963.
[9] Browning, K.S. (2004) Plant translation initiation factors: it is not easy to be green. Biochem. Soc. Trans. 32, 589–591.
[10] Duprat, A., Caranta, C., Revers, F., Menand, B., Browning, K.S. and 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.
[11] Sato, M., Nakahara, K., Yoshii, M., Ishikawa, M. and Uyeda, I. (2005) Selective involvement of members of the eukaryotic initiation factor 4E family in the infection of *Arabidopsis thaliana* by potyviruses. FEBS Lett. 579, 1167–1171.
[12] Decroocq, V. et al. (2006) Multiple resistance traits control Plum pox virus infection in *Arabidopsis thaliana*. Mol. Plant-Microb. Interact. 19, 541–549.
[13] Gallie, D.R. (2001) Cap-independent translation conferred by the 5′ leader of tobacco etch virus is eukaryotic initiation factor 4G dependent. J. Virol. 75, 12141–12152.
[14] Michel, Y.M., Borman, A.M., Paulous, S. and Kean, K.M. (2001) Eukaryotic initiation factor 4G-poly(A) binding protein interaction is required for poly(A) tail-mediated stimulation of picornavirus internal ribosome entry segment-driven translation but not for X-mediated stimulation of hepatitis C virus translation. Mol. Cell Biol. 21, 4097–4109.
[15] Maiss, E., Timpe, U., Brisske, A., Jelkmann, W., Casper, R., Himmler, G., Mattanovich, D. and Katinger, H.W. (1989) The complete nucleotide sequence of plum pox virus RNA. J. Gen. Virol. 70, 513–524.
[16] Saenz, P., Cervera, M.T., Dallot, S., Quiot, L., Quiot, J.B., Riechmann, J.L. and Garcia, J.A. (2000) Identification of a pathogenicity determinant of Plum pox virus in the sequence encoding the C-terminal region of protein P3+6K(1). J. Gen. Virol. 81, 557–566.
[17] Jimenez, I., Lopez, L., Alamillo, J.M., Valli, A. and Garcia, J.A. (2006) Identification of a plum pox virus CI-interacting protein from chloroplast that has a negative effect in virus infection. Mol. Plant-Microb. Interact. 19, 350–358.
[18] Krause-Sakate, R. et al. (2005) Molecular mapping of the viral determinants of systemic wilting induced by a Lettuce mosaic virus (LMV) isolate in some lettuce cultivars. Virus Res. 109, 175–180.
[19] Jenner, C.E., Sanchez, F., Nettleship, S.B., Foster, G.D., Ponz, F. and Walsh, J.A. (2000) The cylindrical inclusion gene of Turnip mosaic virus encodes a pathogenic determinant to the Brassica

resistance gene TuRB01. Mol. Plant-Microb. Interact. 13, 1102–1108.
[20] Lehmann, P., Petrzik, K., Jenner, C.E., Greenland, A.J., Spak, J., Kozubek, E. and Walsh, J.A. (1997) Nucleotide and amino acid variation in the coat protein coding region of turnip mosaic virus isolates and possible involvement in the interaction with the brassica resistance geneTuRB01. Physiol. Mol. Plant Pathol. 51, 195–208.
[21] Bertheau, Y., Frechon, D., Toth, I.K. and Hyman, L.J. (1998) DNA amplification by polymerase chain reaction (PCR) (Perombelon, M.C.M. and van der Wolff, J.M., Eds.), Methods for the Detection and Quantification of *Erwinia carotovora* subsp. atroseptica on Potatoes, Vol. 10, pp. 39–59, Scottish Crop Research Institute Occasional Publication.
[22] Nicaise, V. et al. (2003) The eukaryotic translation initiation factor 4E controls lettuce susceptibility to the potyvirus *Lettuce mosaic virus*. Plant Physiol. 132, 1272–1282.
[23] To, A., Valon, C., Savino, G., Guilleminot, J., Devic, M., Giraudat, J. and Parcy, F. (2006) A network of local and redundant gene regulation governs *Arabidopsis* seed maturation. Plant Cell 18, 1642–1651.
[24] Voinnet, O., Rivas, S., Mestre, P. and Baulcombe, D. (2003) An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. Plant J. 33, 949–956.
[25] Leonard, S., Plante, D., Wittmann, S., Daigneault, N., Fortin, M.G. and Laliberte, J.F. (2000) Complex formation between potyvirus VPg and translation eukaryotic initiation factor 4E correlates with virus infectivity. J. Virol. 74, 7730–7737.
[26] Leonard, S., Chisholm, J., Laliberte, J.F. and Sanfacon, 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.
[27] Wittmann, S., Chatel, H., Fortin, M.G. and Laliberté, J.F. (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.
[28] Schaad, M.C., Anderberg, R.J. and Carrington, J.C. (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.
[29] Michon, T., Estevez, Y., Walter, J., German-Retana, S. and Le Gall, O. (2006) The potyviral virus genome-linked protein VPg forms a ternary complex with the eukaryotic initiation factors eIF4E and eIF4G and reduces eIF4E affinity for a mRNA cap analogue. FEBS J. 273, 1312–1322.
[30] Yoshii, M., Nishikiori, M., Tomita, K., Yoshioka, N., Kozuka, R., Naito, S. and Ishikawa, M. (2004) The *Arabidopsis* Cucumovirus multiplication 1 and 2 loci encode translation initiation factors 4E and 4G. J. Virol. 78, 6102–6111.
[31] Albar, L., Bangratz-Reyser, M., Hebrard, E., Ndjiondjop, M.N., Jones, M. and Ghesquiere, A. (2006) Mutations in the eIF(iso)4G translation initiation factor confer high resistance of rice to Rice yellow mottle virus. Plant J. 47, 417–426.
[32] Rodriguez, C.M., Freire, M.A., Camilleri, C. and 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.