Review

Eukaryotic translation initiation factor 4E-mediated recessive resistance to plant viruses and its utility in crop improvement

AIMING WANG* AND SOWMYA KRISHNASWAMY

Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, 1391 Sandford St., London, ON, Canada, N5V 4T3

SUMMARY

The use of genetic resistance is considered to be the most effective and sustainable approach to the control of plant pathogens. Although most of the known natural resistance genes are monogenic dominant *R* genes that are predominant against fungi and bacteria, more and more recessive resistance genes against viruses have been cloned in the last decade. Interestingly, of the 14 natural recessive resistance genes against plant viruses that have been cloned from diverse plant species thus far, 12 encode the eukaryotic translation initiation factor 4E (eIF4E) or its isoform eIF(iso)4E. This review is intended to summarize the current state of knowledge about eIF4E and the possible mechanisms underlying its essential role in virus infection, and to discuss recent progress and the potential of *eIF4E* as a target gene in the development of genetic resistance to viruses for crop improvement.

INTRODUCTION

Plant pathogens are a major constraint to agriculture and threaten global food security (Dodds, 2010). The use of genetic resistance is considered to be the most effective and sustainable strategy to control plant pathogens in agricultural practice, as it is environmentally friendly and target specific, and provides reliable protection against pathogens without additional labour or material costs (Kang *et al.*, 2005a; Maule *et al.*, 2007). On the basis of inheritance, resistance genes that confer qualitative traits can be classified into two types: dominant and recessive. The majority of dominant *R* genes confer resistance to bacteria and fungi. *R* gene-mediated resistance, often associated with extreme resistance (ER) and a hypersensitive response (HR), is triggered by either direct or indirect interactions between the *R* gene-encoded protein of the host and the avirulence factor produced by the correspond-

ing avirulence (*Avr*) gene of the invading pathogen (Bonas and Lahaye, 2002; Soosaar *et al.*, 2005). Interestingly, published data suggest that recessive resistance is more frequently found for plant viruses than for other plant pathogens (Truniger and Aranda, 2009).

Viruses have small genomes that encode a very limited number of proteins. For instance, the genome of potyviruses, the largest group of plant viruses that includes many agriculturally important viruses, is a single-stranded, positive-sense RNA molecule of approximately 10 kb encoding a single polyprotein and a truncated frameshift peptide (Chung *et al.*, 2008; Shukla *et al.*, 1994). Therefore, viruses must depend on host factors to complete their infection cycle. Loss or mutation of an essential host factor required by the virus may induce recessive resistance to this virus (Diaz-Pendon *et al.*, 2004). To date, all recessive genes involved in plant–virus interactions that have been characterized encode eukaryotic translation initiation factors (eIFs), mainly eIF4E, eIF4G and their isoforms. These include at least 14 natural recessive resistance genes, e.g. *mo1* in lettuce (*Lactuca sativa*); *nsv* in melon (*Cucumis melo*); *pot-1* in tomato (*Solanum lycopersicum*); *pvr1*, *pvr2* and *pvr6* in pepper (*Capsicum annuum* and *C. chinense*); *rym4*, *rym5* and *rym6* in barley (*Hordeum vulgare*); *sbm1*, *wlv* and *cyv2* in pea (*Pisum sativum*); and *tsv1* and *rymv-1* in rice (*Oryza sativa*). Except for *tsv1* and *rymv-1*, which encode a mutated form of eIF4G or a defective form of eIF(iso)4G, and confer resistance to Rice tungro spherical virus (RTSV, *Waikavirus*) and Rice yellow mottle virus (RYMV, *Sobemovirus*) (Albar *et al.*, 2006; Lee *et al.*, 2010), all others encode either eIF4E or eIF(iso)4E. Given the unique status of eIF4E, both as a key regulator of cellular translation and a controller of resistance/susceptibility to plant viruses, a better understanding of the molecular mechanisms underlying eIF4E-mediated resistance may assist in the development of novel strategies against plant pathogens. In the past several years, excellent reviews have been devoted to plant *R* and/or recessive resistance genes, including those encoding eIFs (Kang *et al.*, 2005a; Le Gall *et al.*, 2011; Maule *et al.*, 2007; Robaglia and Caranta, 2006; Truniger and Aranda, 2009). In this review, we focus on *eIF4E* and

*Correspondence: Email: aiming.wang@agr.gc.ca

Reproduced with the permission of the Minister of Agriculture and Agri-Food Canada.

eIF4E-mediated resistance, and its deployment through advanced biotechnology for application in agriculture.

BIOLOGICAL FUNCTIONS OF eIF4E

eIF4E is a cap-binding protein that plays an essential role in the initiation of cap-dependent mRNA translation

eIF4E was originally named the 'cap-binding protein' as it interacts specifically with the 5'-terminal cap of mRNA (Sonenberg *et al.*, 1978). Now, it is best known for its essential function in the initiation of mRNA translation, a rate-limiting step of protein synthesis (see the recent review by Jackson *et al.*, 2010). In eukaryotes, mRNA translation is predominantly cap dependent, with an exception for a small portion of mRNAs whose translation is mediated by internal ribosome entry sites (IRESs). Cap-dependent translation initiation is a multistep process requiring the assembly of an mRNA-protein complex by different eIFs. The first step is the binding of eIF4E to the 5'-7mGpppN-cap of mRNAs. Subsequently, eIF4E recruits eIF4G (the scaffold protein) and eIF4A (the DEAD-box RNA helicase), leading to the formation of the eIF4F protein complex (Goodfellow and Roberts, 2008). Translation initiates on recruitment of the eIF4F complex to the small (40S) ribosomal subunit carrying eIF3 and the ternary initiation tRNA-eIF2-GTP complex through the interaction of eIF3 with eIF4G. Then, 40S starts to scan the mRNA in the 5' to 3' direction to the initiation codon. The scaffold protein eIF4G interacts with the 3'-bound poly(A)-binding protein (PABP), bringing the mRNA ends into proximity for efficient translation. The formation of the complete translation initiation complex requires the eIF5B-bridged coupling of the 40S with the 60S ribosomal subunit (Kawaguchi and Bailey-Serres, 2002).

eIF4E is also a nuclear protein and is involved in the nuclear export of mRNA

In addition to its essential function in protein synthesis in the cytoplasm, approximately 68% of all eIF4E is present in the nucleus of mammalian cells (Iborra *et al.*, 2001). In plants, the distribution of eIF4E seems to be dynamic. The *Arabidopsis* eIF4E has been shown to accumulate in the nucleus in quiescent cells, but is preferentially present in the cytoplasm in proliferating cells (Bush *et al.*, 2009). As a nuclear protein, eIF4E is involved in the nuclear export of a subset of mRNAs containing eIF4E-sensitivity elements. eIF4E is targeted to the nucleus via interactions with the 4E-transporter (4E-T), an eIF4E-binding protein (Dostie *et al.*, 2000). In the nucleus, a number of homeodomain proteins can bind to eIF4E to regulate its cap binding and mRNA export capacity (see the review by Culjkovic *et al.*, 2007).

Plants have a second form of eIF4E, termed eIF(iso)4E, and both forms have similar activities, but may have distinct physiological functions

In contrast with other eukaryotes, plants have a second form of eIF4F, named eIF(iso)4F (Bush *et al.*, 2009). The eIF(iso)4F complex is composed of the corresponding isoforms of eIFs, including eIF(iso)4E and eIF(iso)4G. eIF(iso)4F and eIF4F complexes have complementing activities, but their respective components are differentially expressed, suggesting divergent roles during development or in response to different environmental (biotic or abiotic) cues (Dinkova *et al.*, 2011; Gallie *et al.*, 1998; Rodriguez *et al.*, 1998;). Functional redundancy has been observed between eIF4E and eIF(iso)4E isoforms under regular growth conditions (Combe *et al.*, 2005; Duprat *et al.*, 2002; Lellis *et al.*, 2002). For example, *Arabidopsis thaliana* has three *eIF4E* genes, i.e. *eIF4E1* (At4g18040, commonly designated *eIF4E*), *eIF4E2* (At1g29690) and *eIF4E3* (At1g29550), and one eIF(iso)4E gene (At5g35620) (Robaglia and Caranta, 2006). In contrast with eIF4E, which is differentially subcellularly distributed dependent on the cell type, eIF(iso)4E localizes to both the cytoplasm and nucleus in either quiescent or proliferating cells (Bush *et al.*, 2009). In *Arabidopsis*, no phenotypic or fertility difference is evident among *eIF4E* or *eIF(iso)4E* mutants and wild-type plants (Duprat *et al.*, 2002; Lellis *et al.*, 2002; Sato *et al.*, 2005), demonstrating that *eIF4E* or *eIF(iso)4E* is dispensable for plant growth and development by being complemented by the other form. In *Arabidopsis* eIF(iso)4E knockout (KO) mutants, an increase in the concentration of the eIF4E protein was observed, suggesting the existence of a cellular mechanism that regulates eIF4E expression to compensate for the loss of eIF(iso)4E (Duprat *et al.*, 2002). Antisense depletion of either *eIF4E* or two copies of *eIF(iso)4E* in tobacco plants does not alter the phenotype of the plant, whereas antisense depletion of both *eIF4E* and *eIF(iso)4E* results in a semi-dwarf phenotype and reduces polysome loading, demonstrating their complementary and additive effect in plant growth (Combe *et al.*, 2005).

DISCOVERY OF eIF4E AS A HOST FACTOR IN VIRUS INFECTION

Potyviruses encode a genome-linked viral protein, VPg, which is covalently linked to the 5' end of the genomic RNA (Murphy *et al.*, 1996). This viral protein is absolutely required for virus infection; host proteins physically associating with VPg are likely to play an essential role in virus infection. Wittmann *et al.* (1997) pioneered the research on the identification of VPg-interacting host proteins by conducting a yeast two-hybrid screen. The *Turnip mosaic virus* (TuMV) VPg was used as a bait to screen a cDNA expression library derived from *A. thaliana*. eIF(iso)4E was identified as an

interacting partner of the viral protein (Wittmann *et al.*, 1997). Point mutagenesis of the VPg domain that binds to eIF(iso)4E suggested that the VPg–eIF(iso)4E interaction is essential to preserve virus infectivity *in planta* (Léonard *et al.*, 2000). Lellis *et al.* (2002) screened a mutant population of *A. thaliana* for decreased susceptibility to TuMV and found three loss-of-susceptibility mutants. Map-based cloning identified *eIF(iso)4E* as the gene responsible for the susceptibility change in all three mutants. Inspired by these studies, Ruffel *et al.* (2002) used a candidate gene approach to clone the recessive resistance gene *pvr2* in pepper, which confers resistance to *Potato virus Y* (PVY). Restriction fragment length polymorphism (RFLP) analysis on the PVY-susceptible and PVY-resistant pepper cultivars showed a perfect map co-segregation between a polymorphism in the *eIF4E* gene and the *pvr2* alleles (Ruffel *et al.*, 2002). Susceptibility of the *pvr2*-genotype cultivar was restored by transient expression of eIF4E from a susceptible cultivar, providing conclusive evidence that eIF4E is a host factor required for PVY infection and that *pvr2* encodes an eIF4E variant that confers recessive resistance to PVY (Ruffel *et al.*, 2002). The *pvr2* gene became the first natural recessive resistance gene to be cloned in plants.

NATURAL RECESSIVE RESISTANCE GENES ENCODE eIF4E OR eIF(iso)4E

Cloned natural recessive genes encoding eIF4E

There are two amino acid substitutions in the *pvr2*-encoded eIF4E (Ruffel *et al.*, 2002). Later, it was found that the *pvr2* alleles are actually alleles at the *pvr1* locus which also encode eIF4E variants (Kang *et al.*, 2005b). The *pvr2* or *pvr1* alleles also confer resistance to other potyviruses, including *Pepper vein mottle virus* (PVMV) and *Tobacco etch virus* (TEV) (Charron *et al.*, 2008; Kang *et al.*, 2005b; Ruffel *et al.*, 2006). Recently, five new *pvr2* variants conferring PVY resistance have been identified in *Capsicum* species (Ibiza *et al.*, 2010). Shortly after the cloning of *pvr2*, the lettuce recessive gene *mo1*, which confers resistance to *Lettuce mosaic virus* (LMV), was identified using the same candidate gene approach (Nicaise *et al.*, 2003). It encodes an eIF4E mutant with amino acid variations specifically located near the predicted mRNA cap recognition pocket.

An orthologue of *pvr2* in tomato, *pot-1*, was isolated using comparative genomic mapping (Ruffel *et al.*, 2005). Complementation assays confirmed that small amino acid mutations in the *pot-1*-encoded eIF4E protein account for resistance to PVY and TEV. Almost at the same time, two other groups reported independently that recessive resistance genes *rym4* and *rym5* in barley are alleles of *eIF4E* (Kanyuka *et al.*, 2005; Stein *et al.*, 2005). A few amino acid substitutions in barley eIF4E can alter the outcome of

infections by *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV) from susceptibility to resistance (Kanyuka *et al.*, 2005; Stein *et al.*, 2005). Three-dimensional (3D) modelling of the barley eIF4E suggests that all the polymorphic residues are located near the mRNA cap-binding pocket (Kanyuka *et al.*, 2005). This group also determined that *rym6* is an *eIF4E* mutant (Kanyuka *et al.*, 2005).

In pea, *sbm1*-mediated recessive resistance to *Pea seed-borne mosaic virus* (PSbMV) is also linked to an *eIF4E* allele (Gao *et al.*, 2004a, b). Based on the predicted 3D structure, mutations in *sbm1*-encoded eIF4E potentially disrupt both translational and trafficking functions (Gao *et al.*, 2004b). In two other independent reports, resistance genes *wlv* and *cyv2* in pea, conferring resistance to *Bean yellow mosaic virus* (BYMV) and *Clover yellow vein virus* (CIYVV), respectively, were shown to correspond to the *sbm1* allele of *eIF4E* (Andrade *et al.*, 2009; Bruun-Rasmussen *et al.*, 2007). In common bean (*Phaseolus vulgaris*), another legume species, recessive resistance to *Bean common mosaic virus* (BCMV), controlled by the recessive gene *bc-3*, co-segregates with an eIF4E variant (Naderpour *et al.*, 2010). This variant contains a set of mutations closely resembling a pattern of *eIF4E* mutations determining potyvirus resistance described above. Therefore, *bc-3* is very probably an allele of eIF4E.

In melon, the recessive resistance to *Melon necrotic spot virus* (MNSV), a member of the genus *Carmovirus*, family *Tombusviridae*, is conferred by *nsv* (Nieto *et al.*, 2006). A combination of positional cloning and microsynteny analysis concluded that *nsv* is an eIF4E variant in melon. Further complementary experiments suggested that a single amino acid change at position 228 of the protein is responsible for MNSV resistance (Nieto *et al.*, 2006).

Cloned natural recessive genes encoding eIF4(iso)E

Comparative genetic mapping demonstrated that recessive gene *pvr6* in pepper corresponds to the *eIF(iso)4E* locus (Ruffel *et al.*, 2006). In comparison with the wild-type eIF(iso)4E transcript, *pvr6* contains a deletion of 82 nucleotides (Ruffel *et al.*, 2006). The deletion starts from nucleotide 89 of the eIF(iso)4E cDNA and modifies the open reading frame by a frame shift to introduce a stop codon after amino acid 51 (Ruffel *et al.*, 2006). Conditional on the presence of *pvr1* or *pvr2*, *pvr6* confers resistance to PVMV and *Chilli vein mottle virus* (ChiVMV) (Hwang *et al.*, 2009; Rubio *et al.*, 2009; Ruffel *et al.*, 2006). Consistently, simultaneous mutations in both *eIF4E* and *eIF(iso)4E* are required for ChiVMV and PVMV resistance in pepper (Hwang *et al.*, 2009; Rubio *et al.*, 2009; Ruffel *et al.*, 2006). A genetic study has been carried out to examine whether other *eIF4E* alleles, in combination with *pvr6*, could confer resistance to PVMV. Among 12 *eIF4E* alleles cloned and tested, three alleles have a complementary effect with *eIF(iso)4E* for resistance (Rubio *et al.*, 2009).

MOLECULAR MECHANISMS UNDERLYING eIF4E/eIF(iso)4E-DEPENDENT INFECTION

Recruitment of eIF4E/eIF(iso)4E as a host factor in virus infection

On the basis of the molecular identification of these natural recessive genes, eIF4E and eIF(iso)4E act as host factors for a number of viruses, including both VPg-containing and VPg-free viruses, with the former as a major group. It is worth mentioning that human calicivirus also has a VPg which interacts with eIF4E, and such an interaction is required for the viral genome translation *in vitro* (Goodfellow *et al.*, 2005). In the case of VPg-free viruses, in addition to MNSV (*Tombusviridae*) (Nieto *et al.*, 2006), *Cucumber mosaic virus* (CMV; *Bromoviridae*) also requires eIF4E for infection (Yoshii *et al.*, 2004). The accumulation of CMV is strongly reduced in an *Arabidopsis* eIF4E1-defective mutant (Yoshii *et al.*, 2004). Unlike potyviruses, the genomic RNAs of these two viruses are capped at their 5' termini and do not have a poly(A) tail at the 3' ends. For such viruses, translation initiation is conferred at the 5' proximal AUG by direct base pairing of a stem loop in the 3' untranslated region (UTR) with the corresponding structure in the 5' UTR. The recruitment of eIF4E by MNSV or CMV probably occurs through the 3' UTR (Nieto *et al.*, 2006; Yoshii *et al.*, 2004).

For the cloned recessive resistance genes to VPg-containing viruses, which are exclusive in the family *Potyviridae*, the requirement of eIF4E in virus infection is highly virus specific, and a given virus may prefer one form of eIF4E to the other, or use both for compatible infections (Nicaise *et al.*, 2007). For example, *Arabidopsis* eIF(iso)4E-defective mutants are resistant to TuMV, LMV, TEV and *Plum pox virus* (PPV), whereas mutations of eIF4E1 in *Arabidopsis* inhibit CIYVV infection (Decroocq *et al.*, 2006; Duprat *et al.*, 2002; Lellis *et al.*, 2002; Sato *et al.*, 2005). *Arabidopsis* eIF(iso)4E mutants resistant to TuMV and LMV are susceptible to CIYVV, but the eIF4E mutants resistant to CIYVV have no resistance to TuMV (Sato *et al.*, 2005). The preference for a specific form of eIF4E to the other by viruses can also vary with the host. LMV depends on eIF4E to infect lettuce, but switches to eIF(iso)4E in *Arabidopsis* (Duprat *et al.*, 2002; Nicaise *et al.*, 2003; Ruffel *et al.*, 2005). Both eIF4E and eIF(iso)4E are involved in ChiVMV and PVMV resistance in pepper (Hwang *et al.*, 2009; Rubio *et al.*, 2009; Ruffel *et al.*, 2006). TuMV can use both eIF4E and eIF(iso)4E of *Brassica rapa* for replication in the *Arabidopsis* eIF(iso)4E KO mutant (Jenner *et al.*, 2010). Taken together, these findings suggest that, although eIF4E and eIF(iso)4E can compensate for their function in plant growth and development, such a functional redundancy does not necessarily extend to virus infection (Robaglia and Caranta, 2006).

Apparently, the recruitment of eIF4E or eIF(iso)4E by VPg-containing viruses is through its direct interaction with VPg. The physical interaction of VPg and eIF4E/eIF(iso)4E correlates with

compatible infections in many potyvirus–plant pairs (Charron *et al.*, 2008; Gao *et al.*, 2004b; Grzela *et al.*, 2006; Kang *et al.*, 2005b; Yeam *et al.*, 2007). For instance, in the pepper *pvr1*–TEV, *pvr1*–PVY and *pvr2*–PVY pathosystems, a few amino acid mutations within the *pvr1*- or *pvr2*-encoded eIF4E abolish or impair VPg–eIF4 interactions and inhibit virus infection (Charron *et al.*, 2008; Yeam *et al.*, 2007). Interestingly, among the eIF4E variants encoded by recessive genes from seven different crop species, most of the amino acid substitutions are located in two regions near the cap recognition pocket (Charron *et al.*, 2008; Robaglia and Caranta, 2006; Truniger and Aranda, 2009) and probably mediate interactions of eIF4E with VPg. Consistently, variations in the VPg domain may counter-compromise the resistance conferred by recessive genes encoding defective forms of eIF4E/eIF(iso)4E. In most resistance-breaking potyviral isolates that break down recessive genes, the virulence determinant is mapped to VPg (Borgstrom and Johansen, 2001; Bruun-Rasmussen *et al.*, 2007; Charron *et al.*, 2008; Duprat *et al.*, 2002; Gallois *et al.*, 2010; Grzela *et al.*, 2006; Moury *et al.*, 2004). Most mutations in these VPgs are located in the central region that is involved in the interaction with eIF4E (Roudet-Tavert *et al.*, 2007). These data establish the status of the VPg and eIF4E/eIF(iso)4E interaction as a key determinant of virulence or avirulence in many, but not all, potyvirus infections.

Indeed, the pea *sbm1* gene encoding a defective form of eIF4E confers resistance to PSbMV (Gao *et al.*, 2004a, b). No detectable interaction was found between the PSbMV VPg and eIF4E from a susceptible pea genotype (Gao *et al.*, 2004b). More recently, Gallois *et al.* (2010) performed a systematic yeast two-hybrid assay to examine interactions between all the *Arabidopsis* eIF4Es and virulent VPgs responsible for overcoming resistance in eIF(iso)4E KO *Arabidopsis* mutants. No significant interactions were found between eIF4Es and virulent/avirulent VPgs, other than between eIF(iso)4E and virulent/avirulent VPgs. In these cases, how the eIF4F complex is recruited for viral genome translation has yet to be investigated (Gallois *et al.*, 2010).

Proposed roles of eIF4E/eIF(iso)4E in virus infection

A favourable model is that VPg functions as an analogue of the cap structure of mRNA to recruit the translation complex for viral genome translation (Beauchemin *et al.*, 2007; Khan *et al.*, 2008; Michon *et al.*, 2006). Thus, the VPg and eIF4E/eIF(iso)4E interaction is pivotal to virus infection. This model is supported by several observations (Fig. 1A). First, viral VPg competes and binds to its preferential form of eIF4E more rapidly than does the m7G cap (Khan *et al.*, 2006; Miyoshi *et al.*, 2006). The VPg-binding site is located within or near the cap-binding region (Ashby *et al.*, 2011; Miyoshi *et al.*, 2006). Second, the presence of eIF4G/eIF(iso)4G, another component of the eIF4F complex, enhances VPg–eIF4E/eIF(iso)4E interactions (Khan *et al.*, 2006). eIF4G is also an essential host factor for potyvirus infections (Albar *et al.*, 2006;

Fig. 1 Proposed roles of eukaryotic translation initiation factor 4E (eIF4E) during potyvirus infection. (A) eIF4E binds to the genome-linked viral protein (VPg) and recruits the translation initiation apparatus for viral genome translation. (B) eIF4E, cylindrical inclusion (CI) protein and eIF4G may form a complex that binds to VPg to mediate intracellular trafficking of the viral genome for targeting to plasmodesmata for cell-to-cell movement and, further, for systemic infection. (C) The VPg-eIF4E complex may be involved in the suppression of eIF4E-mediated transport of mRNA from the nucleus to the cytoplasm for translation and in the disturbance of siRNA and microRNA processing in the nucleus. eIF4E, P1, VPg and HC-Pro (helper component-proteinase) may form a complex that functions as an RNA-silencing suppressor to safeguard virus translation/replication in the cytoplasm. 4A, 4E, 4G, 2, 3 and 5 represent eIF4A, eIF4E, eIF4G, eIF2, eIF3 and eIF5, respectively. P1, first protein; PABP, poly(A)-binding protein.

Decroocq *et al.*, 2006; Nicaise *et al.*, 2007). The recruitment of the form of eIF4G and eIF4E by potyviruses is coordinated (Nicaise *et al.*, 2007). Third, viral replicase proteins, eIF4E/eIF(iso)4E and other eIFs, such as eEF1A and PABP, are present in the virus translation/replication complex (Beauchemin *et al.*, 2007; Thivierge *et al.*, 2008; Wei *et al.*, 2010a). A limitation of this model is that it cannot explain why mutations in the eIF4G-binding area do not affect the ability of eIF4E to restore LMV susceptibility in *mol¹* plants (German-Retana *et al.*, 2008a). If VPg is a cap substitute, VPg should promote translation of VPg-linked RNAs and inhibit translation of the capped mRNAs, given that eIF4E is the rate-limiting factor of translation in the cell (Jackson *et al.*, 2010).

In wheat germ extracts, the presence of VPgs indeed inhibits cap-dependent translation, but stimulates translation of uncapped IRES-containing RNAs (including viral RNA) (Khan *et al.*, 2008). The 5' leader of the TEV genomic RNA confers cap-independent translation in an eIF4G-dependent manner (Gallie, 2001). In agreement with this finding, *Potato virus A* (PVA) VPg represses the expression of the capped mRNA and upregulates the expression of both replicating and nonreplicating PVA RNAs (Eskelin *et al.*, 2011). Enhancement of viral RNA expression by VPg functions through the 5' UTR. Therefore, it cannot be excluded that potyviral genome translation initiation may occur at the 5' UTR, independent of the VPg-eIF4E interaction.

In addition to VPg, the potyviral cylindrical inclusion (CI) protein acts as a virulence determinant responsible for overcoming eIF4E-mediated recessive resistance (Abdul-Razzak *et al.*, 2009). The CI protein, having RNA-binding, RNA helicase and ATPase activities, has been shown to be essential in virus intra- and intercellular movement and virus replication (Carrington *et al.*, 1998; Kekalainen *et al.*, 2002; Wei *et al.*, 2010b). In lettuce, the LMV-0 isolate is restricted for systemic infection in *mol¹*-genotype lettuce, but systemically infects the near-isogenic variety without the resistance gene (German-Retana *et al.*, 2003, 2008b). The LMV-E isolate breaks down *mol¹*- or *mol²*-mediated resistance. Replacement of the VPg of LMV-0 with that of LMV-E confers the recombinant virus ability to overcome *mol¹*-mediated resistance, whereas the CI protein of the isolate LMV-E allows both eIF4E alleles *mol¹* and *mol²* to be overcome (Abdul-Razzak *et al.*, 2009). The LMV CI protein interacts with the VPg and the lettuce eIF4E (Roudet-Tavert *et al.*, 2007, 2012). Such interactions are stronger for LMV-E than for LMV-0 (Roudet-Tavert *et al.*, 2012). Therefore, it is possible that the eIF4E-mediated resistance may be associated with the impairment of virus intracellular trafficking, cell-to-cell movement or long distance movement via its interaction with VPg, CI protein and eIF4G (Fig. 1B). This proposal is supported by previous findings that PVY and PSbMV cell-to-cell movement is inhibited in *pvr2*-genotype pepper and *sbm1*-genotype pea, respectively (Arroyo *et al.*, 1996; Gao *et al.*, 2004b).

In view of the nuclear localization of both eIF4E and VPg, it is tempting to speculate a possible role of VPg in the suppression of eIF4E-mediated mRNA transport from the nucleus to cytoplasm for translation (Fig. 1C). This is consistent with the observation that VPg inhibits translation of the capped mRNAs (Eskelin *et al.*, 2011; Khan *et al.*, 2008). It is also possible that the eIF4E-VPg complex is involved in RNA silencing, a counter-defensive mechanism that enables systemic infection. The potyviral protein HC-Pro (helper component-proteinase) is the major RNA-silencing suppressor with the first protein (P1) and VPg as an accessory factor (Kasschau and Carrington, 1998; Rajamäki and Valkonen, 2009). VPg is a nuclear- and nucleolar-targeting protein; mutations that reduce nuclear and nucleolar localization compromise its RNA-silencing ability, possibly through disturbance of siRNA and

microRNA processing in the nucleus, and prevent or diminish virus replication (Rajamäki and Valkonen, 2009). *eIF4E* may be involved in this process via its interaction with VPg (Fig. 1C).

P1 exhibits nonspecific RNA-binding activity and enhances HC-Pro-mediated suppression of RNA silencing (Brantley and Hunt, 1993; Kasschau and Carrington, 1998; Rajamäki *et al.*, 2005). In the pea–CIYVV pathosystem, a point mutation in P1 is responsible for breaking down the resistance conferred by *cyv2* (an *eIF4E* allele) (Nakahara *et al.*, 2010). VPg, P1, HC-Pro and *eIF4E* are interacting partners (Ala-Poikela *et al.*, 2011; Merits *et al.*, 1999; Nakahara *et al.*, 2010; Roudet-Tavert *et al.*, 2007). P1 localizes in the cytoplasm (Nakahara *et al.*, 2010). In infected cells, HC-Pro is present in proximity to the *eIF4E*- and VPg-containing virus replication complex (Ala-Poikela *et al.*, 2011). It would be interesting to determine whether P1 physically associates with the replication complex in virus-infected cells and forms, with VPg, HC-Pro and *eIF4E*, a functional complex to suppress RNA silencing and safeguard virus replication in the cytoplasm (Fig. 1C).

GENERATION OF RECESSIVE RESISTANCE THROUGH BIOTECHNOLOGY

Although the molecular mechanisms underlying recessive gene-mediated resistance to viral pathogens and susceptibility to resistance-breaking isolates are not fully understood, many recessive genes, such as the pepper *pvr1*, have been used successfully for several decades. On the basis of the aforementioned discussion, genetic resistance may be developed by silencing a host factor, such as a specific *eIF4E* or *eIF4G* member essential for viral infection. The resulting 'recessive resistance' is actually controlled by a dominant transgene. For instance, *eIF(iso)4E* is required for PPV infection in plum, and transgenic plum plants in which *eIF(iso)4E* is silenced by the expression of a short hairpin RNA specifically targeting *eIF(iso)4E* are highly resistant to PPV (X. Wang *et al.*, unpublished, Agriculture and Agri-Food Canada, London, Ontario). Genetic resistance can also be generated by the overexpression of recessive genes identified for the target virus (Cavatorta *et al.*, 2011; Kang *et al.*, 2007; Yan *et al.*, 2010; Yeam *et al.*, 2007). One example is that ectopic expression of the recessive *Capsicum* gene *pvr1*, an *eIF4E* allele, in tomato results in dominant broad-spectrum resistance to TEV and *Pepper mottle virus* (PepMoV) (Kang *et al.*, 2007).

The advent of new technologies, such as Targeting-Induced Local Lesions IN Genome (TILLING), high-resolution melting (HRM), KeyPoint and next-generation sequencing, has opened up a new era for the development of genetic resistance in crops (Hofinger *et al.*, 2009; Piron *et al.*, 2010; Rigola *et al.*, 2009). These technologies allow the identification of target gene mutants from an artificially induced mutation population (Fig. 2). For instance, TILLING was employed to screen for *eIF4E* or *eIF4G* mutants in an ethyl methanesulphonate (EMS)-induced tomato mutant popula-

Fig. 2 A flowchart showing the process of generation of recessive-based resistance in crops to a target virus via artificially induced mutagenesis and subsequent screening using new technologies, i.e. TILLING (Targeting-Induced Local Lesions IN Genome) and next-generation sequencing. EMS, ethyl methanesulphonate.

tion (Piron *et al.*, 2010). A splicing mutant *Sl-eIF4E1* (G1485A) was identified to be immune to PVY and PepMoV. The mutated gene *Sl-eIF4E1* (G1485A) encodes a truncated protein that is impaired in cap-binding activity (Piron *et al.*, 2010). A TILLING-based method to identify natural nucleotide diversity, termed EcoTILLING, has also been employed successfully to identify allelic variants of *eIF4E* against MNSV in melon (Nieto *et al.*, 2007) and against PVY in *Capsicum* species (Ibiza *et al.*, 2010). This approach may be particularly effective for heterozygous species in which recessive alleles may exist, but cannot be screened out by the conventional phenotypic resistance assay.

CONCLUDING REMARKS

In the past 15 years, significant progress has been made in the understanding of genetic resistance mediated by recessive genes, particularly *eIF4E* and *eIF4G*. This makes it possible to manipulate them for the control of relevant viruses. However, there still remain difficulties for the use of recessive resistance in some crop species, such as those strictly clonally propagated crops, highly allogamous species and perennial plants with a long life cycle. Moreover, genetic diversity resulting from error-prone replication and RNA recombination in the existing and continuously evolving viral population, and the selection force acting on this variability, lead to the occurrence of resistance-breaking isolates, posing a serious challenge to the deployment of *eIF4E*- and *eIF4G*-mediated resistance. It is worth noting that the recessive genes that have been cloned so far only account for a very small portion of the known recessive genes (Kang *et al.*, 2005a; Truniger and Aranda, 2009). As suggested by Le Gall *et al.* (2011), the predominant overrepresentation of *eIF4E/eIF(iso)4E* in the cloned recessive genes may be a result of the fact that the first cloned recessive gene against a plant virus was *eIF4E*, which prompted the isolation of other recessive genes using a candidate gene approach. The cloning and characterization of additional recessive genes against diverse viruses will certainly help to gain a better understanding of recessive

sive resistance and to more efficiently deploy recessive resistance into breeding programmes against plant viruses as well as other pathogens.

ACKNOWLEDGEMENTS

We are grateful to Jean-François Laliberté and Helene Sanfaçon for critical reviews of the manuscript. This work was supported by Agriculture and Agri-Food Canada and the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

- Abdul-Razzak, A., Guiraud, T., Peypelut, M., Walter, J., Houvenaghel, M.C., Candresse, T., Le Gall, O. and German-Retana, S. (2009) Involvement of the cylindrical inclusion (CI) protein in the overcoming of an eIF4E-mediated resistance against *Lettuce mosaic potyvirus*. *Mol. Plant Pathol.* **10**, 109–113.
- Ala-Poikela, M., Goytia, E., Haikonen, T., Rajamäki, M.-L. and Valkonen, J.P.T. (2011) Helper component proteinase of the genus *Potyvirus* is an interaction partner of translation initiation factors eIF(iso)4E and eIF4E and contains a 4E binding motif. *J. Virol.* **85**, 6784–6794.
- Albar, L., Bangratz-Reyser, M., Hébrard, E., Ndjondjop, M.N., Jones, M. and Ghesquière, 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.
- Andrade, M., Abe, Y., Nakahara, K.S. and Uyeda, I. (2009) The *cyv-2* resistance to *Clover yellow vein virus* in pea is controlled by the eukaryotic initiation factor 4E. *J. Gen. Plant Pathol.* **75**, 241–249.
- Arroyo, R., Soto, M.J., Martínez-Zapater, J. and Ponz, F. (1996) Impaired cell-to-cell movement of *Potato virus Y* in pepper plants carrying the *y²(pr²)* resistance gene. *Mol. Plant–Microbe Interact.* **9**, 314–318.
- Ashby, J.A., Stevenson, C.E.M., Jarvis, G.E., Lawson, D.M. and Maule, A.J. (2011) Structure-based mutational analysis of eIF4E in relation to *sbm1* resistance to *Pea seed-borne mosaic virus* in pea. *PLoS ONE*, **6**, e15873.
- Beauchemin, C., Boutet, N. and Laliberté, J.-F. (2007) Visualization of the interaction between the precursor of VPg, the viral protein genome-linked to the genome of *Turnip mosaic virus*, and the translation initiation factor iso 4E in *planta*. *J. Virol.* **81**, 775–782.
- Bonas, U. and Lahaye, T. (2002) Plant disease resistance triggered by pathogen-derived molecules: refined models of specific recognition. *Curr. Opin. Microbiol.* **5**, 44–50.
- Borgstrom, B. and Johansen, I.E. (2001) Mutations in *Pea seed-borne mosaic potyvirus* genome-linked protein VPg alter pathotype specific virulence in *Pisum sativum*. *Mol. Plant–Microbe Interact.* **14**, 707–714.
- Brantley, J.D. and Hunt, A.G. (1993) The N-terminal protein of the polyprotein encoded by the potyvirus *Tobacco vein mottling virus* is an RNA-binding protein. *J. Gen. Virol.* **74**, 1157–1162.
- Bruun-Rasmussen, M., Møller, I.S., Tulinus, G., Hansen, J.K.R., Lund, O.S. and Johansen, I.E. (2007) The same allele of translation initiation factor 4E mediates resistance against two *Potyvirus* spp. in *Pisum sativum*. *Mol. Plant–Microbe Interact.* **20**, 1075–1082.
- Bush, M.S., Hutchins, A.P., Jones, A.M.E., Naldrett, M.J., Jarmolowski, A., Lloyd, C.W. and Doonan, J.H. (2009) Selective recruitment of proteins to 5' cap complexes during the growth cycle in *Arabidopsis*. *Plant J.* **59**, 400–412.
- Carrington, J.C., Jensen, P.E. and Schaad, M.C. (1998) Genetic evidence for an essential role for potyvirus CI protein in cell-to-cell movement. *Plant J.* **14**, 393–400.
- Cavatorta, J., Perez, K.W., Gray, S.M., Van Eck, J., Yeam, I. and Jahn, M. (2011) Engineering virus resistance using a modified potato gene. *Plant Biotechnol. J.* **9**, 1014–1021.
- Charron, C., Nicolai, M., Gallois, J.-L., Robaglia, C., Moury, B., Palloix, A. and Caranta, C. (2008) Natural variation and functional analyses provide evidence for co-evolution between plant eIF4E and potyviral VPg. *Plant J.* **54**, 56–68.
- Chung, B.Y., Miller, W.A., Atkins, J.F. and Firth, A.E. (2008) An overlapping essential gene in the *Potviridae*. *Proc. Natl. Acad. Sci. USA* **105**, 5897–5902.
- Combe, J.P., Petracek, M.E., van Eldik, G., Meulewaeter, F. and Twell, D. (2005) Translation initiation factors eIF4E and eIFiso4E are required for polysome formation and regulate plant growth in tobacco. *Plant Mol. Biol.* **57**, 749–760.
- Culjkovic, B., Topisirovic, I. and Borden, K.L.B. (2007) Controlling gene expression through RNA regulons. *Cell Cycle*, **6**, 65–69.
- Decroocq, V., Sicard, O., Alamillo, J.M., Lansac, M., Eyquard, J.P., García, J.A., Candresse, T., Le Gall, O. and Revers, F. (2006) Multiple resistance traits control *Plum pox virus* infection in *Arabidopsis thaliana*. *Mol. Plant–Microbe Interact.* **19**, 541–549.
- Diaz-Pendon, J.A., Truniger, V., Nieto, C., Garcia-Mas, J., Bendahmane, A. and Aranda, M.A. (2004) Advances in understanding recessive resistance to plant viruses. *Mol. Plant Pathol.* **5**, 223–233.
- Dinkova, T.D., Marquez-Velazquez, N.A., Aguilar, R., Lazaro-Mixteco, P.E. and de Jimenez, E.S. (2011) Tight translational control by the initiation factors eIF4E and eIF(iso)4E is required for maize seed germination. *Seed Sci. Res.* **21**, 85–93.
- Dodds, P.N. (2010) Genome evolution in plant pathogens. *Science*, **330**, 1486–1487.
- Dostie, J., Ferraiuolo, M., Pause, A., Adam, S.A. and Sonenberg, N. (2000) A novel shuttling protein, 4E-T, mediates the nuclear import of the mRNA 5' cap-binding protein, eIF4E. *EMBO J.* **19**, 3142–3156.
- 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.
- Eskelin, K., Hafrén, A., Rantalainen, K.I. and Mäkinen, K. (2011) Potyviral VPg enhances viral RNA translation and inhibits reporter mRNA translation in *planta*. *J. Virol.* **85**, 9210–9221.
- Gallie, D.R., Le, H., Tanguay, R.L. and Browning, K.S. (1998) Translation initiation factors are differentially regulated in cereals during development and following heat shock. *Plant J.* **14**, 715–722.
- Gallie, R.G. (2001) Cap-independent translation conferred by the 5' leader of *Tobacco etch virus* is eukaryotic initiation factor 4G-dependent. *J. Virol.* **75**, 12 141–12 152.
- Gallois, J.-L., Charron, C., Sanchez, F., Pagny, G., Houvenaghel, M.-C., Moretti, A., Ponz, F., Revers, F., Caranta, C. and German-Retana, S. (2010) Single amino acid changes in the *Turnip mosaic virus* viral genome-linked protein (VPg) confer virulence towards *Arabidopsis thaliana* mutants knocked out for eukaryotic initiation factors eIF(iso)4E and eIF(iso)4G. *J. Gen. Virol.* **91**, 288–293.
- Gao, Z.H., Eyers, S., Thomas, C.L., Ellis, N. and Maule, A.J. (2004a) Identification of markers tightly linked to *sbm* recessive genes for resistance to *Pea seed-borne mosaic virus*. *Theor. Appl. Genet.* **109**, 488–494.
- Gao, Z.H., Johansen, E., Eyers, S., Thomas, C.L., Ellis, N. and Maule, A.J. (2004b) The *Potyvirus* recessive resistance gene, *sbm1*, identifies a novel role for translation initiation factor eIF4E in cell-to-cell trafficking. *Plant J.* **40**, 376–385.
- German-Retana, S., Redondo, E., Taver-Roudet, G., Le Gall, O. and Candresse, T. (2003) Introduction of a Nla proteinase cleavage site between the reporter gene and HC-Pro only partially restores the biological properties of GUS- or GFP-tagged LMV. *Virus Res.* **98**, 151–162.
- German-Retana, S., Walter, J., Doublet, B., Roudet-Tavert, G., Nicaise, V., Lecampion, C., Houvenaghel, M.-C., Robaglia, C., Michon, T. and Le Gall, O. (2008a) Mutational analyses of plant cap-binding protein eIF4E reveals key amino acids involved in biochemical functions and potyvirus infection. *J. Virol.* **82**, 7601–7612.
- German-Retana, S., Walter, J. and Le Gall, O. (2008b) *Lettuce mosaic virus*: from pathogen diversity to host interactors. *Mol. Plant Pathol.* **9**, 127–136.
- Goodfellow, I., Chaudhry, Y., Gioldasi, I., Gerondopoulos, A., Naton, A., Labrie, L., Laliberté, J.-F. and Roberts, L. (2005) Calcivirus translation initiation requires an interaction between VPg and eIF4E. *EMBO Rep.* **6**, 968–972.
- Goodfellow, I.G. and Roberts, L.O. (2008) Eukaryotic initiation factor 4E. *Int. J. Biochem. Cell Biol.* **40**, 2675–2680.
- Grzela, R., Stokovska, L., Andrieu, J.-P., Dublet, B., Zagorski, W. and Chroboczek, J. (2006) Potyvirus terminal protein VPg, effector of host eukaryotic initiation factor eIF4E. *Biochimie*, **88**, 887–896.
- Hofinger, B.J., Jing, H.-C., Hammond-Kosack, K.E. and Kanyuka, K. (2009) High-resolution melting analysis of cDNA-derived PCR amplicons for rapid and cost-effective identification of novel alleles in barley. *Theor. Appl. Genet.* **119**, 851–865.
- Hwang, J., Li, J., Liu, W.-Y., An, S.-J., Cho, H., Her, N.H., Yeam, I., Kim, D. and Kang, B.-C. (2009) Double mutations in *eIF4E* and *eIFiso4E* confer recessive resistance to *Chilli vein mottle virus* in pepper. *Mol. Cells* **27**, 329–336.
- Ibiza, V.P., Cañizares, J. and Nuez, F. (2010) EcoTILLING in *Capsicum* species: searching for new virus resistances. *BMC Genomics*, **11**, 631.
- Iborra, F.J., Jackson, D.A. and Cook, P.R. (2001) Coupled transcription and translation within nuclei of mammalian cells. *Science*, **293**, 1139–1142.
- Jackson, R.J., Hellen, C.U.T. and Pestova, T.V. (2010) The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat. Rev. Mol. Cell Biol.* **10**, 113–127.

- Jenner, C.E., Nellist, C.F., Barker, G.C. and Walsh, J.A. (2010) Turnip mosaic virus (TuMV) is able to use alleles of both *elf4E* and *elf(iso)4E* from multiple loci of the diploid *Brassica rapa*. *Mol. Plant-Microbe Interact.* **23**, 1498–1505.
- Kang, B.-C., Yeam, I. and Jahn, M.M. (2005a) Genetics of plant virus resistance. *Annu. Rev. Phytopathol.* **43**, 581–621.
- Kang, B.-C., Yeam, I., Frantz, D.J., Murphy, J.F. and Jahn, M.M. (2005b) The *pvr1* locus in pepper encodes a translation initiation factor *elf4E* that interacts with tobacco etch virus VPg. *Plant J.* **42**, 392–405.
- Kang, B.-C., Yeam, I., Li, H., Perez, K.W. and Jahn, M.M. (2007) Ectopic expression of a recessive resistance gene generates dominant *Potyvirus* resistance in plants. *Plant Biotechnol. J.* **5**, 526–536.
- Kanyuka, K., Druka, A., Caldwell, D.G., Tymon, A., McCallum, N., Waugh, R. and Adams, M.J. (2005) Evidence that the recessive bymovirus resistance locus *rym4* in barley corresponds to the eukaryotic translation initiation factor *4E* gene. *Mol. Plant Pathol.* **6**, 449–458.
- Kasschau, K.D. and Carrington, J.C. (1998) A counterdefensive strategy of plant viruses: suppression of posttranscriptional gene silencing. *Cell*, **95**, 461–470.
- Kawaguchi, R. and Bailey-Serres, J. (2002) Regulation of translational initiation in plants. *Curr. Opin. Plant Biol.* **5**, 460–465.
- Kekarainen, T., Savilahti, H. and Valkonen, J.P.T. (2002) Functional genomics on *Potato virus A*: virus genome-wide map of sites essential for virus propagation. *Genome Res.* **12**, 584–594.
- Khan, M.A., Miyoshi, H., Ray, S., Natsuaki, T., Suehiro, N. and Goss, D.J. (2006) Interaction of genome-linked protein (VPg) of Turnip mosaic virus with wheat germ translation initiation factors *elf4E* and *elf(iso)4E*. *J. Biol. Chem.* **281**, 28 002–28 010.
- Khan, M.A., Miyoshi, H., Gallie, D.R. and Goss, D.J. (2008) *Potyvirus* genome-linked protein, VPg, directly affects wheat germ *in vitro* translation: interactions with translation initiation factors *elf4E* and *elf(iso)4E*. *J. Biol. Chem.* **283**, 1340–1349.
- Le Gall, O., Aranda, M.A. and Caranta, C. (2011) Plant resistance to viruses mediated by translation initiation factors. In: *Recent Advances in Plant Virology* (Caranta, C., Aranda, M.A., Tepfer, M. and López-Moya, J., eds), pp. 177–194. Wymondham, Norfolk: Caister Academic Press.
- Lee, J.-H., Muhsin, M., Atienza, G.A., Kwak, D.-Y., Kim, S.-M., De Leon, T.B., Angeles, E.R., Coloquio, E., Kondoh, H., Satoh, K., Cabunagan, R.C., Cabauatan, P.Q., Kikuchi, S., Leung, H. and Choi, I.-R. (2010) Single nucleotide polymorphisms in a gene for translation initiation factor (*elf4G*) of rice (*Oryza sativa*) associated with resistance to Rice tungro spherical virus. *Mol. Plant-Microbe Interact.* **23**, 29–38.
- Lellis, A.D., Kasschau, K.D., Whitham, S.A. and Carrington, J.C. (2002) Loss-of-susceptibility mutants of *Arabidopsis thaliana* reveal an essential role for *elf(iso)4E* during *Potyvirus* infection. *Curr. Biol.* **12**, 1046–1051.
- Leonard, S., Plante, D., Wittmann, S., Daigneault, N., Fortin, M.G. and Laliberté, J.-F. (2000) Complex formation between potyvirus VPg and translation eukaryotic initiation factor 4E correlates with virus infectivity. *J. Virol.* **74**, 7730–7737.
- Maule, A.J., Caranta, C. and Boulton, M.I. (2007) Sources of natural resistance to plant viruses: status and prospects. *Mol. Plant Pathol.* **8**, 223–231.
- Merits, A., Guo, D., Jarvekulg, L. and Saarma, M. (1999) Biochemical and genetic evidence for interactions between potato A potyvirus-encoded proteins P1 and P3 and proteins of the putative replication complex. *Virology*, **263**, 15–22.
- 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 *elf4E* and *elf4G* and reduces *elf4E* affinity for a mRNA cap analogue. *FEBS J.* **273**, 1312–1322.
- Miyoshi, H., Suehiro, N., Tomoo, K., Muto, S., Takahashi, T., Tsukamoto, T., Ohmori, T. and Natsuaki, T. (2006) Binding analyses for the interaction between plant virus genome-linked protein (VPg) and plant translational initiation factors. *Biochimie*, **88**, 329–340.
- Moury, B., Morel, C., Johansen, E., Guilbaud, L., Souche, S., Ayme, V., Caranta, C., Palloix, A. and Jacquemond, M. (2004) Mutations in *Potato virus Y* genome-linked protein determine virulence toward recessive resistances in *Capsicum annuum* and *Lycopersicon hirsutum*. *Mol. Plant-Microbe Interact.* **17**, 322–329.
- Murphy, J.F., Klein, P.G., Hunt, A.G. and Shaw, J.G. (1996) Replacement of the tyrosine residue that links a potyviral VPg to the viral RNA is lethal. *Virology*, **220**, 535–538.
- Naderpour, M., Lund, O.S., Larsen, R. and Johansen, E. (2010) Potyviral resistance derived from cultivars of *Phaseolus vulgaris* carrying *bc-3* is associated with the homozygotic presence of a mutated *elf4E* allele. *Mol. Plant Pathol.* **11**, 255–263.
- Nakahara, K.S., Shimada, R., Choi, S.H., Yamamoto, H., Shao, J. and Uyeda, I. (2010) Involvement of the P1 cistron in overcoming *elf4E*-mediated recessive resistance against Clover yellow vein virus in Pea. *Mol. Plant-Microbe Interact.* **23**, 1460–1469.
- Nicaise, V., German-Retana, S., Sanjuán, R., Dubrana, M.-P., Mazier, M., Maisonneuve, B., Candresse, T., Caranta, C. and Le Gall, O. (2003) The eukaryotic translation initiation factor 4E controls lettuce susceptibility to the potyvirus *Lettuce mosaic virus*. *Plant Physiol.* **132**, 1272–1282.
- Nicaise, V., Gallois, J.-L., Chafiai, F., Allen, L.M., Schurdi-Levraud, V., Browning, K.S., Candresse, T., Caranta, C., Le Gall, O. and German-Retana, S. (2007) Coordinated and selective recruitment of *elf4E* and *elf4G* factors for *Potyvirus* infection in *Arabidopsis thaliana*. *FEBS Lett.* **581**, 1041–1046.
- Nieto, C., Morales, M., Orjeda, G., Clepet, C., Monfort, A., Sturbois, B., Puigdomènech, P., Pitrat, M., Caboche, M., Dogimont, C., Garcia-Mas, J., Aranda, M.A. and Bendahmane, A. (2006) An *elf4E* allele confers resistance to an uncapped and non-polyadenylated RNA virus in melon. *Plant J.* **48**, 452–462.
- Nieto, C., Piron, F., Dalmais, M., Marco, C.F., Moriones, E., Gómez-Guillamón, M.L., Truniger, V., Gómez, P., García-Mas, J., Aranda, M.A. and Bendahmane, A. (2007) EcoTILLING for the identification of allelic variants of melon *elf4E*, a factor that controls virus susceptibility. *BMC Plant Biol.* **7**, 34.
- Piron, F., Nicolai, M., Minoia, S., Piednoir, E., Moretti, A., Salgues, A., Zamir, D., Caranta, C. and Bendahmane, A. (2010) An induced mutation in tomato *elf4E* leads to immunity to two potyviruses. *PLoS ONE*, **5**, e11313.
- Rajamäki, M.-L. and Valkonen, J.P.T. (2009) Control of nuclear and nucleolar localization of nuclear inclusion protein a of picorna-like *Potato virus A* in *Nicotiana* species. *Plant Cell*, **21**, 2485–2502.
- Rajamäki, M.-L., Kelloniemi, J., Alminaita, A., Kekarainen, T., Rabenstein, F. and Valkonen, J.P.T. (2005) A novel insertion site inside the potyvirus P1 cistron allows expression of heterologous proteins and suggests some P1 functions. *Virology*, **342**, 88–101.
- Rigola, D., van Oeveren, J., Janssen, A., Bonné, A., Schneiders, H., van der Poel, H.J.A., van Orsouw, N.J., Hogers, R.C.J., de Both, M.T.J. and van Eijk, M.J.T. (2009) High-throughput detection of induced mutations and natural variation using KeyPoint(tm) technology. *PLoS ONE*, **4**, e4761.
- Robaglia, C. and Caranta, C. (2006) Translation initiation factors: a weak link in plant RNA virus infection. *Trends Plant Sci.* **11**, 40–45.
- Rodriguez, C.M., Freire, M.A., Camilleri, C. and Robaglia, C. (1998) The *Arabidopsis thaliana* cDNAs coding for *elf4E* and *elf(iso)4E* are not functionally equivalent for yeast complementation and are differentially expressed during plant development. *Plant J.* **13**, 465–473.
- Roudet-Tavert, G., Michon, T., Walter, J., Delaunay, T., Redondo, E. and Le Gall, O. (2007) Central domain of a potyvirus VPg is involved in the interaction with the host translation initiation factor *elf4E* and the viral protein HC-Pro. *J. Gen. Virol.* **88**, 1029–1033.
- Roudet-Tavert, G., Abdul-Razzak, A., Doublet, B., Walter, B., Delaunay, T., German-Retana, S., Michon, T., Le Gall, O. and Candresse, T. (2012) The C terminus of lettuce mosaic potyvirus cylindrical inclusion helicase interacts with the viral VPg and with lettuce translation initiation factor 4E. *J. Gen. Virol.* **93**, 184–193.
- Rubio, M., Nicolai, M., Caranta, C. and Palloix, A. (2009) Allele mining in the pepper gene pool provided new complementation effects between *pvr2-elf4E* and *pvr6-elf(iso)4E* alleles for resistance to Pepper vein mottle virus. *J. Gen. Virol.* **90**, 2808–2814.
- Ruffel, S., Dussault, M.H., Palloix, A., Moury, B., Bendahmane, A., Robaglia, C. and Caranta, C. (2002) A natural recessive resistance gene against *Potato virus Y* in pepper corresponds to the eukaryotic initiation factor 4E (*elf4E*). *Plant J.* **32**, 1067–1075.
- Ruffel, S., Gallois, J.L., Lesage, M.L. and Caranta, C. (2005) The recessive potyvirus resistance gene *pot-1* is the tomato orthologue of the pepper *pvr2-elf4E* gene. *Mol. Gen. Genomics* **274**, 346–353.
- Ruffel, S., Gallois, J.L., Moury, B., Robaglia, C., Palloix, A. and Caranta, C. (2006) Simultaneous mutations in translation initiation factors *elf4E* and *elf(iso)4E* are required to prevent pepper vein mottle virus infection of pepper. *J. Gen. Virol.* **87**, 2089–2098.
- 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.
- Shukla, D.D., Ward, C.W. and Brunt, A.A. (1994) *The Potyviridae*. Wallingford, Oxfordshire: CAB International.
- Sonenberg, N., Morgan, M.A., Merrick, W.C. and Shatkin, A.J. (1978) A polypeptide in eukaryotic initiation factors that cross links specifically to the 5'-terminal cap in mRNA. *Proc. Natl. Acad. Sci. USA* **75**, 4843–4847.

- Soosaar, J.L., Burch-Smith, T.M. and Dinesh-Kumar, S.P. (2005) Mechanisms of plant resistance to viruses. *Nat. Rev. Microbiol.* **3**, 789–798.
- Stein, N., Perovic, D., Kumlehn, J., Pelli, B., Stracke, S., Streng, S., Ordon, F. and Graner, A. (2005) The eukaryotic translation initiation factor 4E confers multiallelic recessive *Bymovirus* resistance in *Hordeum vulgare* (L.). *Plant J.* **42**, 912–922.
- Thivierge, K., Cotton, S., Dufresne, P.J., Mathieu, I., Beauchemin, C., Ide, C., Fortin, M.G. and Laliberté, J.-F. (2008) Eukaryotic elongation factor 1A interacts with *Turnip mosaic virus* RNA-dependent RNA polymerase and VPg-Pro in virus-induced vesicles. *Virology*, **377**, 216–225.
- Truniger, V. and Aranda, M.A. (2009) Recessive resistance to plant viruses. *Adv. Virus Res.* **75**, 119–159.
- Wei, T., Huang, T.S., McNeil, J.R., Laliberté, J.-F., Hong, J., Nelson, R.S. and Wang, A. (2010a) Sequential recruitment of the endoplasmic reticulum and chloroplasts for plant potyvirus replication. *J. Virol.* **84**, 799–809.
- Wei, T., Zhang, C., Hong, J., Xiong, R., Kasschau, K.D., Zhou, X., Carrington, J.C. and Wang, A. (2010b) Formation of complexes at plasmodesmata for potyvirus intercellular movement is mediated by the viral protein P3N-PIPO. *PLoS Pathog.* **6**, e1000962.
- 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.
- Yan, P., Wang, S., Shen, W., Gao, X., Wu, J. and Zhou, P. (2010) Simple construction of chimeric hairpin RNA for virus resistance in plants. *J. Virol. Methods* **166**, 101–105.
- Yeam, I., Cavatorta, J.R., Ripoll, D.R., Kang, B.-C. and Jahn, M.M. (2007) Functional dissection of naturally occurring amino acid substitutions in eIF4E that confers recessive potyvirus resistance in plants. *Plant Cell*, **19**, 2913–2928.
- 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.