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BRIEF COMMUNICATION

Potyviral VPg and HC-Pro Proteins and the Cellular Translation Initiation Factor eIF(iso)4E Interact with Exoribonuclease Rrp6 and a Small α-Heat Shock Protein

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Abstract The helper-component proteinase (HC-Pro) of potyvirus is a multifunctional protein involved in many mechanisms of viral life cycle. In addition, HC-Pro protein was the first identified suppressor of RNA silencing in plants. However, the identities and functions of direct targets toward the pathways of RNA-silencing suppression mediated by HC-Pro are still to be determined. Here, a yeast two-hybrid search for potyviral HC-Pro interacting tobacco proteins was done to identify host partners and potential silencing suppressors. Two interacting cDNA clones were isolated. One of them encodes an Rrp6-like protein, a subunit of the exosome complex that belongs to the RNase D family of the DEDD superfamily of 3′–5′ hydrolytic exoribonucleases. The other clone codes for a small α-heat shock protein (α-Hsp). The interactions were validated by cross interaction assays with other potyviral HC-Pro proteins. Moreover, both identified clones also interacted with pathogenic viral protein-linked genomes (VPgs) and with translation eukaryotic initiation factors (iso) 4E (eIF(iso)4E) which are host determinants of resistance or susceptibility to potyvirus infections. All together, these findings emphasize the role of the potyviral HC-Pro and VPg proteins and the translation initiation factor eIF(iso)4E, as key players of the plant–virus interplay, where the exoribonuclease Rrp6 and a small α-heat shock protein appear as novel sharing targets.

Keywords Potyvirus · Exosome · Nucleotidylylation · RNA silencing · RNA decay · RNA granules

Abbreviations

HC-Pro	Helper-component proteinase
VPg	Viral protein-linked genome
PVY	Potyvirus Y
PVA	Potyvirus A
TuMV	Turnip mosaic virus
TEV	Tobacco etch virus
ClYVV	Clover yellow vein virus
eIF(iso)4E	Eukaryotic initiation factor (iso) 4E
P bodies	Processing bodies
SGs	Stress granules
α-Hsp	Alpha heat shock protein

Introduction

Potyviruses are members of genus *Potyvirus* and belong to the picornavirus superfamily of RNA plus-stranded virus. The RNA genome of potyviruses codes for a single polyprotein that is processed by viral-encoded proteases into ten mature multifunctional proteins (Urcuqui-Inchima et al. 2001) and a small open reading frame called PIPO inserted in the P3 sequence (Chung et al. 2008). The accomplishment of such plethora of functions results from the availability of the potyviral proteins to interact with different protein factors expressed by the host plant.

Recently, translation initiation factors of the *eIF4E* gene family have emerged as key determinants of the interaction between plants and potyviruses (Robaglia and Caranta 2006; Truniger and Aranda 2009). These proteins are involved in

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translation initiation by directly binding the cap structure at the 5′ end of mRNAs.

Translation initiation factors 4E have been shown to interact with both potyviral viral protein-linked genome (VPg) and helper-component proteinase (HC-Pro) proteins as demonstrated by using yeast two-hybrid assays. The interaction between the VPg of *turnip mosaic virus* and the translation eukaryotic initiation factor (iso) 4E (eIF(iso)4E) of *Arabidopsis* was reported by Wittmann et al. (1997). Similarly, the identification of host proteins that interact with the viral NIa (precursor of VPg) of *tobacco etch virus* recovered the translation factor eIF4E of tomato (Schaad et al. 2000). Recently, Gallois et al. (2010) showed that despite strong homologies between the translation initiation factors of *Arabidopsis*, turnip mosaic virus (TuMV) VPg interacted solely with eIF(iso)4E. On the other hand, HC-Pro of *potyvirus A* (PVA) was found to bind through a 4E-binding motif of both eIF4E and eIF(iso)4E in yeast and in planta (Ala-Poikela et al. 2011). Moreover, host mutations in these translation initiation factors have been shown to confer resistance to numerous potyviruses (Lellis et al. 2002; Ruffel et al. 2002; Duprat et al. 2002; Nicaise et al. 2003; Gao et al. 2004; Kang et al. 2005).

The genomic RNA of viruses of the genus *Potyvirus* lacks a cap structure. Instead, VPg is covalently attached at the 5′ end to the RNA (Riechmann et al. 1989; Murphy et al. 1991; Sadowy et al. 2001) that may serve as an analogue of the cap structure. The molecular consequence of eIF4E–VPg interaction is essential for virus infection, and it seems to be involved in the inhibition of host protein synthesis at a very early stage of initiation (Miyoshi et al. 2008; Khan et al. 2008).

HC-Pro is involved in many important mechanisms of the virus life cycle: aphid-mediated transmission, systemic movement, proteolytic processing of the polyprotein, and viral genome amplification (Urcuqui-Inchima et al. 2001; Syller 2006). In addition, HC-Pro is an important regulator of suppression of RNA-silencing mechanism (Roth et al. 2004). RNA silencing is a sequence-specific epigenetic mechanism that controls gene expression based on RNA extinction process via the generation of short interfering RNAs and microRNAs. RNA silencing is involved in a wide variety of functions such as development, RNA surveillance, adaptive response to stresses, cellular defense, and antiviral responses (Vance and Vaucheret 2001; Voinnet 2005).

HC-Pro protein was the first identified suppressor of RNA silencing in plants (Anandalakshmi et al. 1998; Brigneti et al. 1998; Kasschau and Carrington 1998). It was hypothesized that potyviral HC-Pro might suppress the host defense mechanism of gene silencing via an interaction with components of silencing machinery or regulators of posttranscriptional gene silencing.

A decade ago, a protein interaction assay allowed the identification of a calmodulin-related protein in tobacco interacting with the viral suppressor P1/HC-Pro of TEV. The identified protein was shown to be a cellular suppressor of posttranscriptional gene silencing when overexpressed in *Nicotiana benthamiana* (Anandalakshmi et al. 2000). The screen was done in yeast with the P1/HC-Pro (TEV) and a cDNA library from a mixture of mRNAs isolated from a tobacco suspension cell culture of *N. benthamiana* treated and untreated with parasiticein, an elicitor of hypersensitive response.

More recently, it was found that the HC-Pro of PVA interacts with the RING finger protein HIP1 in *Solanum tuberosum* (Guo et al. 2003); HC-Pro of *lettuce mosaic virus* (LMV) interacts with the 20S proteasome complex of cauliflower (*Brassica oleracea*) (Ballut et al. 2005; Dielen et al. 2011), whereas the HC-Pro of *potyvirus Y* (PVY) interacts with three *Arabidopsis* 20S proteasome subunits (Jin et al. 2007a), with the chloroplast division-related factor NtMinD of tobacco (Jin et al. 2007b), and HC-Pro of *sugar cane mosaic virus* binds a chloroplast precursor of ferredoxin-5 in maize (Cheng et al. 2008).

Here, to provide further insight into the molecular mechanism(s) of potyviral infection involving host and viral components, protein interaction approaches based on yeast two-hybrid assays were performed with the following key points. PVY HC-Pro was used as bait against a leaf cDNA library from untreated *Nicotiana tabacum* cv. Xanthi NN plants to identify cellular partners and potential host-silencing suppressors. This procedure permitted the isolation of two interacting clones: one coding for an Rrp6-like protein, a subunit of the multimeric exosome complex, and another that encodes for a small heat shock protein. To obtain additional information about the isolated clones in the screening, they were tested in protein interaction assays against VPg(s), as virulence pathogenic effectors and with translation initiation factors eIF(iso)4E which are host essential determinants of resistance or susceptibility to potyvirus infections.

Material and Methods

Plasmids and cDNA Library

The bait plasmid pLexA PVY (N) HC-Pro was a gift of C. Tourneur (Institute Jean-Pierre Bourgin, INRA Versailles, France). It contains the PCR-amplified cDNA fragment coding the HC-Pro of *potato virus Y*, (N strain) (Robaglia et al. 1989) fused in frame with the LexA DNA-binding domain of plasmid pBTM116 (Bartel et al. 1993).

The plasmid pLexA PVY (LYE84) HC-Pro (*potato virus Y*, LYE84 strain) reported by Urcuqui-Inchima et al. (1999) was a gift of J. Walter (INRA Bordeaux, France).

The plasmid pLexA PVA HC-Pro (*potato virus A*, HC-Pro) and pLexA iso 4E tobacco (translation initiation factor (iso) 4E)

were obtained by cutting the plasmids GBKT7-HC-Pro PVA (strain B11) and GBKT7-iso 4E tobacco reported by Ala-Poikela et al. (2011) (gifts of J.P.T. Valkonen, Department of Agricultural Sciences, University of Helsinki, Finland) with *Bam*HI/*Sal*I and *Bam*HI/*Pst*I, respectively, and fused in the pLexA plasmids cut with the same enzymes.

The plasmid pLexA TuMV VPg (*turnip mosaic virus*, isolate CDN1), pLexA TEV (HAT) VPg (*tobacco etch virus*, high aphid transmitted), and pLexA ClYVV VPg (*clover yellow vein virus*, V107A) were obtained by cutting the plasmids GBKT7-TuMV VPg reported by Gallois et al. (2010), GBKT7-TEV (HAT) VPg, and GBKT7-ClYVV VPg (gifs of J-L Gallois, INRA Avignon, France) with *Eco*RI/*Pst*I, *Eco*RI, and *Eco*RI/*Bam*HI, respectively, and were inserted into pLexA plasmids cut with the same enzymes.

C-terminal coding regions of *Arabidopsis* AtRrp6L1 (At1G54440; from amino acid (aa) 408 to the end, aa 637), AtRrp6L2 (At5G35910; from aa 553 to the end, aa 870), and AtRrp6L3 (At2G32415; from aa 557 to the end, aa 892) were amplified by PCR from plasmids containing the full-length cDNAs reported by Lange et al. (2008) (gifts of D. Gagliardi, Institut de Biologie Moleculaire de Plantes, Strasbourg, France) with primers that included the appropriate restriction sites for cloning in the pGAD3S2X-digested vector (derivative of pGAD 1318, Benichou et al. 1994).

All constructed plasmids and oligonucleotides used in this study are listed in Supplementary Tables S1 and S2, respectively.

The leaf cDNA library from tobacco was obtained from T. Elmayan (Institut Jean-Pierre Bourgin, INRA, Versailles, France) and described by Kieffer et al. 2000. Basically, the cDNA synthesis was performed with random primers and with lock-docking oligo(dT)25 from poly A plus mRNAs extracted from 55-day-old leaves of tobacco plants (*N. tabacum* cv. Xanthi NN). The cDNAs were ligated to an *Eco*RI-*Not*I-*Sal*I adaptor and inserted into the pGAD3S2X *Eco*RI-digested vector.

Yeast Two-Hybrid Screen and Assays

The two-hybrid screen (Fields and Song 1989) was performed as described by Freire et al. 2000 in the yeast strain L40, (*MAT*a *trp1-901 leu2-3*, *112 his3*-D*200 ade2 LYS2::*(*LexAop*)*4 HIS3*, *URA3::*(*LexAop*)*8 LacZ*) (Vojtek et al. 1993) which has two reporter genes: yeast *HIS3* and *Escherichia coli LacZ*. The plasmid pLexA PVY (N) HC-Pro alone or in combination with pGAD3S2X vector does not activate the transcription of reporter genes *HIS*3 and *Lac*Z allowing the two-hybrid screening. For the screening, a tobacco leaf cDNA library built in the plasmid pGAD3S2X was introduced into the yeast strain L40 by co-transformation with the pLexA PVY (N) HC-Pro plasmid. About 300 thousand transformants were plated on medium lacking tryptophan, leucine, and histidine and screened for the activation of *HIS3* reporter gene. The histidine prototroph colonies were recovered and further tested for beta-galactosidase activity by filter lift assay (Breeden et al. 1985). The plasmids were isolated from selected yeast transformants and transferred to *E. coli* DH5a strain by chemical transformation. The amplified plasmids were isolated and tested for reproducibility and specificity by retransformation of L40 with pLexA PVY (N) HC-Pro and with pLexA Lamin used as an unrelated control protein.

The isolated cDNA clones and constructed plasmids were further characterized by using yeast protein interaction assays with potyviral HC-Pro and VPg proteins and translation initiation factors eIF(iso)4E expressed in the appropriate yeast two-hybrid vectors. The transformants were plated on yeast nitrogen-based minimal medium without amino acids, supplemented with glucose and adenine. Patches of the histidine prototroph colonies were tested for β-galactosidase activity.

Results

Potyviral HC-Pro-Interacting Proteins from Tobacco

In order to identify any cellular proteins interacting with PVY (N) HC-Pro protein, a tobacco leaf cDNA library in the plasmid pGAD2S3X where the inserts are fused to the GAL4 activation domain was introduced by co-transformation into the reporter yeast strain L40 with the plasmid pLex-HC-Pro. About 300 thousand transformants were screened for histidine prototrophy. Among the interacting clones that showed reproducibility and specificity by retransformation of L40, there were two cDNA clones: one designated GAD HC14 and another GAD HC18. GAD HC14 encodes a polypeptide that showed 75 % of homology with a predicted protein of *Solanum lycopersicum* (XP_004248555.1) with high similarity to Rrp6 protein. The Rrp6 protein is a subunit of the exosome complex that belongs to the DEDD superfamily of 3′–5′ hydrolytic exoribonucleases (cd06147 domain) and to the RNase D family because it contains the helicase and RNase D C-terminal (HRDC) domain (cl02578) (Zuo and Deutscher 2001). The predicted tomato Rrp6 protein contains these characteristic domains. The alignment of the protein sequence encoded by the GAD HC14 clone with the tomato Rrp6 protein is shown in Fig. 1a. GAD HC14 codes for the C-terminal region of an Rrp6-like protein, within which there is no putative conserved domain.

The other interacting clone GAD HC18 codes for a polypeptide with 92 % of homology with the C-terminal region of a small alpha heat shock protein (α-Hsp) of *S. lycopersicum* (XP_004236751.1) that includes the characteristic alpha-crystallin domain (ACD) (cd06464). The family of small heat shock proteins that contains the ACD domain act as ATP-independent molecular chaperones that prevent misfolding

a

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GAD HC14       EEIQVQQIKSSVSLPFHAFSGRTEQLQQAATAPAKTLQINHREE-PVATNSKLDVITLDT
               EEIQVQQIKS VS PFH FSG  EQ+QQAAT PAK L+INHREE PVA N K DVIT++T
Tom Rrp6 623   EEIQVQQIKSLVSFPFHPFSGSIEQIQQAATVPAKPLEINHREEEPVAANCKTDVITVET  682

GAD HC14       DSDDGKSLKGELSIGEQENSFAMPVATSKLEDVILLDTDSDLEGSVKDDSEAANNPPECG
               DSDDG+S+KGELS G QENS AMPV TSK EDVILL+TDSD E S KDDSE  NN PECG
Tom Rrp6 683   DSDDGESVKGELSTGGQENSSAMPVVTSKSEDVILLETDSDCEESGKDDSEVTNNQPECG  742

GAD HC14       ENKIAGSAEEMDEGDENMSLCDLSSSFKKCFHSISQKSKAQLTEKAQAHEGQLKVQPFDY
               ENK   S  EMDEG ENM L            SI++KSK +L EK QAHEG+LKV+ FDY
Tom Rrp6 743   ENKNVLSV-EMDEGGENMPL------------SINKKSKGKLAEKPQAHEGELKVEGFDY  789

GAD HC14       EAARKQVLFGEDPGKKKPETEGDEHRRSRTgkgdkkkdlllGQPPNIEGTAEFQQGRRRQ
               EAARKQV+FGEDPGK++ E EGDE RRSR  KG+ KKDLLLGQPP  IE A+FQQGRRRQ
Tom Rrp6 790   EAARKQVVFGEDPGKQQAEREGDESRRSRNEKGN-KKDLLLGQPPKIEEAADFQQGRRRQ  848

GAD HC14       AFPATGNRSYTFR
               AFPA+GNRSYTFR
Tom Rrp6 849   AFPASGNRSYTFR  861
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b

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GAD HC18       VDILSVCNKRKKNGFSGGNNDDCCLPNSQHP--------VSPPWLGAFSGAMQMASGPVT
               ++ILSVCNKRKKNGF+ GNNDDCCLPNSQHP        +SPPWL  FSGAMQ+ASGPVT
Tom α–Hsp 310  LNILSVCNKRKKNGFTRGNNDDCCLPNSQHPERLTDIHPISPPWLRDFSGAMQVASGPVT 369

GAD HC18       AAKTIYEDDTAFLILVSMPFVDLKSVKVTWRNTISHGIVKISCISTGCIPFIKRQNRTFK
               AAKTIYEDD  FLILVSMPFVDLKSVKVTWRNTISHGIVKISC+STGCIP IKRQNRTFK
Tom α–Hsp 370  AAKTIYEDDAGFLILVSMPFVDLKSVKVTWRNTISHGIVKISCVSTGCIPMIKRQNRTFK 429

GAD HC18       LADPAPEHCPSGEFVREIPLRTRIPEDAKLEAYGDETGTMLEILVPKQRVGPEEHEVLVC
               L+DPAPEHCPSGEFVREIPLRTRIPEDAKLEAYGDETGTMLEI VPKQRVGPEEHEVLVC
Tom α–Hsp 430  LSDPAPEHCPSGEFVREIPLRTRIPEDAKLEAYGDETGTMLEIHVPKQRVGPEEHEVLVC 489

GAD HC18       LRPSPWTTHAYVDLTEAMA
               LRPSPWTTHAYVDLTEAMA
Tom α–Hsp 490  LRPSPWTTHAYVDLTEAMA 508
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Fig. 1 Alignment of the coding sequences of GAD HC14 and GAD HC18 clones with predicted tomato proteins. The sequences were aligned using the program BLASTX 2.2.28 (Altschul et al. 1997) at NCBI website with default parameter settings. **a** Alignment of the coding sequence of GAD HC14 clone with the predicted tomato Rrp6 protein (XP_004248555). **b** Alignment of polypeptide encoded by GAD HC18 clone with the tomato small heat shock protein (XP_004236751). The alpha-crystallin domain (cd06464) is *underlined*. Both cDNA clones encode the C-terminal regions including the last amino acids and the stop codons present in the respective homolog tomato sequences

and irreversible protein aggregation. They form large oligomeric complexes consisting of multiple subunits (Narberhaus 2002). The alignment is shown in Fig. 1b.

Potyviral HC-Pro Proteins Interact with Rrp6-Like Proteins and a Small α-Heat Shock Protein

Three Rrp6-like proteins (AtRrp6L1, nucleoplasm and nucleolar vacuole; AtRrp6L2, nucleoli; and AtRrp6L3, cytoplasm) were identified in the *Arabidopsis thaliana* genome (Lange et al. 2008). These proteins have the characteristic 3′–5′ exoribonuclease domain and the HRDC motifs. So, to investigate more about the interaction between potyviral HC-Pro and Rrp6 proteins, the C-terminal regions of the three Rrp6 proteins of *Arabidopsis* were tested against the HC-Pro in two-hybrid assays. The C-terminal region of AtRrp6L2 protein is the closet homolog (48 %) of GADHC14. Surprisingly, AtRrp6L1 showed high reproducibility in the interaction

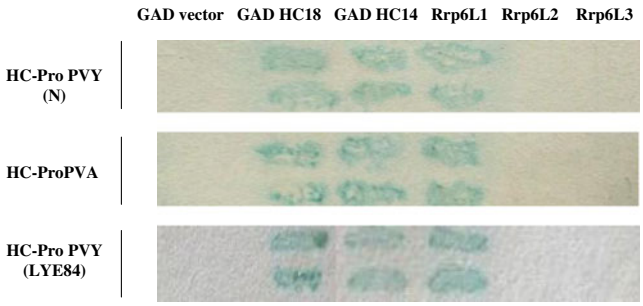

Fig. 2 Potyviral HC-Pro proteins interact with the Rrp6-like proteins and α-Hsp in yeast two-hybrid assays. The reporter yeast strain L40 was co-transformed with the helper-component proteinases of PVY (N strain), PVY (LYE84), or PVA potyviruses expressed in pLexA (DNA-binding domain) with the isolated tobacco clones GAD HC18 coding for C-terminal region of a small heat shock protein, GAD-HC14 encoding the C-terminus of an Rrp6-like protein, or the clones in pGAD3S2X (activation domain) expressing the C-terminal regions of the three Rrp6 (L1, L2, and L3) proteins of *Arabidopsis*. Patches of histidine prototroph colonies were further tested for beta-galactosidase activity by filter lift assay

Fig. 3 Potyviral VPg proteins interact with Rrp6-like proteins and α-Hsp in yeast two-hybrid assays. The yeast strain L40 was co-transformed with VPg proteins from TuMV (CDN1), TEV (HAT), and ClYVV (V107A) viruses expressed in pLexA plasmids with GAD HC18 and GAD HC14 clones or the three C-terminal regions of Rrp6-like proteins from *Arabidopsis*. *LacZ* reporter gene activity of histidine prototrophs was analyzed using the β-galactosidase filter assay

assays, while no interaction was observed with the AtRrp6L2 or AtRrp6L3 (Fig. 2). A similar unexplained finding was reported in yeast complementation assays. AtRrp6L2 is the closet homolog of yeast Rrp6 but did not restore the thermosensitive growth phenotype of the Rrp6Δ yeast strain, while AtRrp6L1 supported the growth at nonpermissive conditions (Lange et al. 2008). One possibility is that AtRrp6L2 might have a negative effect on yeast growth.

Full-length HC-Pro from *potato virus A* (PVA HC-Pro) which is 50 % identical to PVY (N strain) HC-Pro and the HC-Pro from PVY LYE84 strain (95 % homolog to PVY N) were also tested for interactions with GAD HC14 clone and with the C-terminal regions of Rrp6 proteins of *Arabidopsis*, together with the GAD HC18 clone coding for the α-Hsp. Results were similar to those obtain with PVY(N) HC-Pro indicating that Rrp6-like proteins and α-Hsp may be common targets of potyviral helper components.

Potyviral VPg Proteins Interact with Rrp6-Like Proteins and a Small α-Heat Shock Protein

Potyviral VPg proteins are covalently linked at the 5′ end to the genomic RNA of potyvirus by a phosphodiester bond. VPg(s) have been shown to interact with several proteins that exhibit RNA-binding properties such as DEAD box RNA helicase-like protein, translation elongation factor eEF1A, PABP, fibrillarin, and translation initiation factors among others (Jiang and Laliberté 2011). These data suggest that VPg(s) are involved in RNA mechanisms. Moreover, VPg interacts with the viral HC-Pro (Roudet-Tavert et al. 2007; Yambao et al. 2003). Then, to further investigate possible interaction patterns of VPg(s) with exoribonuclease Rrp6-like proteins and α-Hsp, a protein interaction assay was performed. The results in Fig. 3 showed that the VPg(s) from TuMV, TEV, and ClYVV also interacted with the Rrp6-like proteins and α-Hsp. Taken together, these observations suggest that potyviral VPg and HC-Pro and cellular Rrp6 and α-Hsp proteins share interaction commonalities that may be critical to provide a functional platform involved in RNA metabolism.

Interaction of eIF(iso)4E Factors with Rrp6-Like Proteins and α-Hsp

Plant translation initiation factors are key determinants of the interaction between plants and several potyviruses (Robaglia and Caranta 2006). It has been found that VPg of the *lettuce mosaic virus* interacted with the host translation initiation factors eIF4E and the viral HC-Pro (Roudet-Tavert et al. 2007). Moreover, PVA HC-Pro interacts with both eIF4E and eIF(iso)4E translation factors (Ala-Poikela et al. 2011). Therefore, the next step was to examine whether eIF(iso)4E from *Arabidopsis* and tobacco directly interact with the Rrp6-like proteins and α-Hsp. The interactions were indeed found between the translation initiation factors and Rrp6-like proteins and α-Hsp (Fig. 4).

Fig. 4 Plant translation initiation factors eIF(iso)4E interact with Rrp6-like proteins and α-Hsp. *Arabidopsis* and tobacco translation initiation factors eIF(iso)4E expressed in pLexA vector were co-transformed in the yeast reporter strain L40 with GAD HC18 and GAD HC14 clones and the three clones encoding the C-terminal regions of Rrp6L1, Rrp6L2, and Rrp6L3 proteins of *Arabidopsis*. The histidine prototroph colonies were analyzed by β-galactosidase filter assay

Discussion

Many proteins have been reported to interact with potyviral HC-Pro proteins. Nevertheless, the identities and functions of direct targets toward the pathways of RNA silencing suppression mediated by HC-Pro are still to be determined. Here, a two-hybrid screen with the PVY (N) HC-Pro allowed the isolation of two cDNA clones of tobacco: GAD HC14 encodes the C-terminal region of an Rrp6-like protein, a subunit of exosome complex, member of the RNase D family of 3′–5′ hydrolytic exoribonucleases, and the other clone GAD HC18 codes for the C-terminus of a small heat shock protein. The functionality of interactions was supported by cross interaction assays with two other potyviral HC-Pro proteins and an Rrp6 protein from *Arabidopsis*. In addition, both GAD HC14 and GAD HC18 clones were found to be able to interact with three potyviral VPg proteins and translation initiation factors eIF(iso)4E of *Arabidopsis* and tobacco.

All together, these findings confirm the role of the potyviral HC-Pro and VPg proteins and the translation initiation factor eIF(iso)4E, as key players of an integrated model of plant–virus interactions (Elena and Rodrigo 2012), where the exoribonuclease Rrp6 and a small α-heat shock protein appear as novel sharing targets.

Rrp6p in yeast and polymyositis-scleroderma 100 antigen (PM/Scl-100) in humans are similar to bacterial exonuclease RNase D and belong to the DEDD superfamily (Zuo and Deutscher 2001). Members of this family are required for nucleic acid degradation in the 3′–5′ direction via a distributive hydrolytic reaction mechanism. Rrp6p is an auxiliary factor of the exosome complex. The exosome is a eukaryotic evolutionary conserved complex of hydrolytic and phosphorolytic 3′–5′ exoribonucleases existing in both the nucleus and the cytoplasm. It is involved in mRNA turnover, rRNA maturation, and processing the 3′ extremities of a variety of noncoding RNAs and degradation of RNAs with structural defects through the nonsense-mediated decay pathways (NMD) and elimination of mRNAs cleaved by the RNA-induced silencing complex (RISC) (Houseley et al. 2006; Schmid and Jensen 2008).

A decade ago, the first report on the characterization of the *Caenorhabditis elegans MUT-7* gene showed the identification of a gene involved in RNA interference and transposon silencing. The gene codes for a putative exonuclease homologous of human Werner syndrome helicase and member of the RNase D family (Ketting et al. 1999).

Later, by using a candidate gene search approach in *A. thaliana*, it was shown that a member of RNase D exonuclease-like protein related to the *C. elegans* MUT-7 protein was required for posttranscriptional silencing (Glazov et al. 2003).

More recently, it was found that the geminiviral transactivator AC2 protein from Mungbean *yellow mosaic virus-Vigna* may suppress RNA silencing probably by altering the host transcriptome of *N. benthamiana*. Among the AC2-inducible genes, it was the one coding for a Werner exonuclease-like 1 protein (Trinks et al. 2005). It was hypothesized that an excessive expression level of this gene can lead to dominant-negative effects by competing with positive effectors of silencing by an interaction with the core silencing machinery (Voinnet 2005).

In *N. benthamiana* plants, it was shown that HC-Pro targets transgenes with a nonsense codon, suggesting that RNA silencing and NMD may be partially overlapped. It was proposed that HC-Pro may suppress silencing by inhibiting RISC complex by an unknown molecular mechanism involved in miRNA and siRNA inactivation (Kasschau et al. 2003).

A significant portion of the isolated miRNAs contains a few posttranscriptionally added nucleotidyl acids preferentially A or U tails at the 3′ end. The adenylated miRNAs showed a certain stability (Lu et al. 2009), whereas the uridylated ones have a reduced stability (Li et al. 2005). The 3′-terminal 2′-*O*-methylation by the HEN1 methyltransferase appears to protect miRNAs and siRNAs from uridylation and subsequent 3′–5′ exonucleolytic degradation (Ramachadran and Chen 2008).

HC-Pro has been shown to interfere with 3′-end methylation of miRNA (Yu et al. 2006) and viral-derived siRNAs (Ebhardt et al. 2005; Lózsa et al. 2008) probably by competing with HEN1 methyltransferase for binding to these small RNAs and interfering with their incorporation into RISC which mediates posttranscriptional gene silencing.

In the alga *Chlamydomonas*, a terminal nucleotidyl transferase (MUT68) is involved in the 3′ untemplated uridylation of miRNAs and siRNAs in vivo (Ibrahim et al. 2006). MUT68 cooperates with the exosomal Rrp6 protein in vitro to degrade small RNAs, while Rrp6 depletion resulted in the accumulation of miRNAs and siRNAs (Ibrahim et al. 2010).

In yeast, polyadenylation of aberrant RNAs catalyzed by the TRAMP complex is a favorable substrate for degradation by the exosome (Vanacova and Stefl 2007), where TRAMP significantly enhances the activity of the exosome component Rrp6 (Callahan and Butler 2010).

In *Arabidopsis*, mutation of *AtRrp6L2* leads to the accumulation of rRNA byproducts as polyadenylated transcripts, suggesting that the Rrp6L2 protein is involved in the degradation of poly(A)-extended RNAs (Lange et al. 2008), whereas the loss-of-function of AtRrp6L1 enhanced sense transgene posttranscriptional gene silencing (Moreno et al. 2013).

With regard to VPg nucleotidylylation, it was shown that the recombinant PVA VPg protein can be uridylylated in an in vitro reaction by the recombinant Nib that is the potyviral RNA-dependent RNA polymerase (Puustinen and Mäkinen 2004). By analogy to picornavirus, it was proposed that the VPg linked to an uridylyl residue(s) may serve as a primer for genome replication of potyvirus. So, concerning the VPg–Rrp6 interaction, it may play a role in modulating the uridylylation degree of VPg which has also been involved in

the downregulation of host gene expression and upregulation of viral RNAs (Eskelin et al. 2011).

Unexpectedly, the potyviral VPg and HC-Pro proteins interact with translation initiation factors eIF(iso)4E, Rrp6-like proteins, and α-Hsp. However, it is known that viral infections subvert host posttranscriptional gene regulation counteracting RNA granule function of processing bodies protein (P bodies) and stress granules (SGs) to promote synthesis of viral proteins (Reineke and Lloyd 2013). Stress granules are storage sites of mRNAs stalled in translation initiation that consist of a subset of translation initiation factors (eIF3, eIF4E, eIF4G), the 40S ribosomal subunit, and many RNA-binding proteins (Anderson and Kedersha 2008). P bodies contain the basic components of mRNA decay and miRNA repression: deadenylases, decapping factors, and endoribonucleases. In multicellular organisms, some components were found in both structures, for example, the 5′-3′ Xrn exoribonuclease and argonaute proteins involved in miRNA and siRNA function (Decker and Parker 2012).

Stress granules were first observed in plant cells formed during long-term heat shock stress as aggregates of large cytoplasmic complexes, mainly composed of small heat shock proteins tightly associated with untranslated mRNAs (Nover et al. 1989). However, it is a matter of debate if these heat shock stress granules are storage ribonucleoprotein particles (Weber et al. 2008). Further immunofluorescence studies in plant cells provided a clear-cut distinction between cytoplasmic structures of P bodies and SGs by detection of specific marker proteins. Among them, it was observed that there was accumulation of both eIF4E and eIF(iso)4E translation factors in the stress granules with similar kinetics (Weber et al. 2008). In this sense, potyviruses which exploit different members of translation initiation factors 4E for successful infections (Robaglia and Caranta 2006; Truniger and Aranda 2009) may induce the formation of SG(s) as part of the host response to reduce viral replication by sequestration of those limiting factors. Nevertheless, it should be noted that even though key components of SG(s) and P bodies are considered to be involved primarily in limiting viral infection, there is emerging evidence suggesting that these structures can be important both for viral life cycle and for host antiviral defense (Beckham and Parker 2008).

Much more work is needed to establish the functional role in vivo of the each interaction pathway. It also remains to elucidate if potyviruses through HC-Pro and VPg proteins target eIF(iso)4E factor, Rrp6, and α-Hsp to sustain the viral life cycle requirements and/or as a strategy to subvert the host defense mechanisms.

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