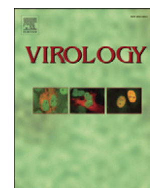
Virology 380 (2008) 165–169

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro



Rapid Communication

The ribosomal protein L10/QM-like protein is a component of the NIK-mediated antiviral signaling

Carolina S. Rocha, Anésia A. Santos, João Paulo B. Machado, Elizabeth P.B. Fontes *

Departamento de Bioquímica e Biologia Molecular, BIOAGRO, Universidade Federal de Viçosa, 36570.000 Viçosa, MG, Brazil

ARTICLE INFO

Article history:
Received 9 June 2008
Returned to author for revision 13 July 2008
Accepted 2 August 2008
Available online 11 September 2008

Keywords:
Begomovirus
Antiviral signaling
NIK receptor
Ribosomal protein L10
Receptor-like kinase

ABSTRACT

The NIK (NSP-interacting kinase)-mediated antiviral signaling pathway was identified as a virulence target of the begomovirus nuclear shuttle protein (NSP). Here, we further characterized this layer of plant innate defense by identifying the ribosomal protein L10 (rpL10), a QM-like protein, as a downstream effector of the antiviral signaling. Although both ribosomal proteins rpL10 and rpL18 were found to associate with NIK1 through yeast two-hybrid screening, the NIK receptors specifically phosphorylated rpL10 *in vitro*. Furthermore, loss of rpL10 function significantly increased susceptibility to begomovirus infection, recapitulating the phenotype of nik knockout lines. Our results genetically linked rpL10 to the NIK-mediated antiviral signaling.

© 2008 Elsevier Inc. All rights reserved.

Introduction

The *Geminiviridae* family is considered one of the largest and most widespread families of plant viruses. Members infect a wide range of crops, particularly in tropical and subtropical regions. The family is currently divided into four genera, named *Mastrevirus*, *Curtovirus*, *Begomovirus* and *Topocuvirus*, on the basis of the biological and genetic properties of the viral species and their insect vector (Rojas et al., 2005). The single-stranded DNA genome of begomoviruses may be organized in either single or double-component configuration. Typically, both genomic components of bipartite begomoviruses, designated DNA-A and DNA-B, are required for systemic infection. DNA-A encodes proteins involved in DNA replication (Rep and REn), transcriptional activation of viral genes (TrAP), encapsidation of the viral genome (CP) and suppression of RNAi defense functions (AC4 and TrAP; Vanitharani et al., 2005; Wang et al., 2005). The genes on DNA-B (NSP and MP) provide functions required for systemic movement of the viral genome within infected plants (reviewed by Gafni and Epel, 2002; Lazarowitz and Beachy, 1999; Rojas et al., 2005). The nuclear shuttle protein (NSP) facilitates the intracellular trafficking of viral DNA between the nucleus and the cytoplasm, whereas MP acts as the classic viral movement protein and potentiates the cell-to-cell movement of viral DNA. In addition to interacting with host factors required for basic compatibility functions (Florentino et al., 2006, Carvalho et al., 2008), NSP has also been shown to act as a virulence factor to suppress the kinase activity of transmembrane receptor NIKs (NSP-Interacting Kinases) which are thought to mediate an antiviral signaling response (Mariano et al., 2004; Fontes et al., 2004).

Receptor-like kinases play a crucial role in perception and signal transduction mechanisms that allow eukaryotic organisms to interact with their environment in a regulated and adaptive way. In plants, they comprise a super family of receptor-like serine/threonine kinases, which are represented by approximately 450 sequences in the *Arabidopsis* genome (revised in Shiu and Bleecker, 2001). These receptors are involved in several cellular basic processes, such as cell division, metabolism, hormone perception and signaling as well as plant defense against pathogens. The receptor NIKs belong to the plant defense group of the leucine-rich repeat (LRR) receptor-like kinase (RLK) sub-family constituted by members that harbor five LRRs at the extracellular domain (Zhang et al., 2006). These transmembrane receptors have been initially identified as specific partners of the geminivirus nuclear shuttle protein, NSP (Mariano et al., 2004). NIK has been described as a transmembrane signaling receptor that mediates an antiviral defense response based on the biochemical properties of the kinase, such as autophosphorylation-induced activation and its inhibition by the geminivirus NSP, as well as the enhanced susceptibility phenotype to geminivirus infection of knock-out lines (Fontes et al., 2004). Despite the relevance of this antiviral pathway as a potential layer of the innate plant defense, the underlying mechanism for a NIK-mediated defense response remains to be deciphered. Here we identified through yeast two-hybrid

* Corresponding author. Fax: +55 31 3899 2864.
E-mail address: bbfontes@mail.ufv.br (E.P.B. Fontes).

0042-6822/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2008.08.005

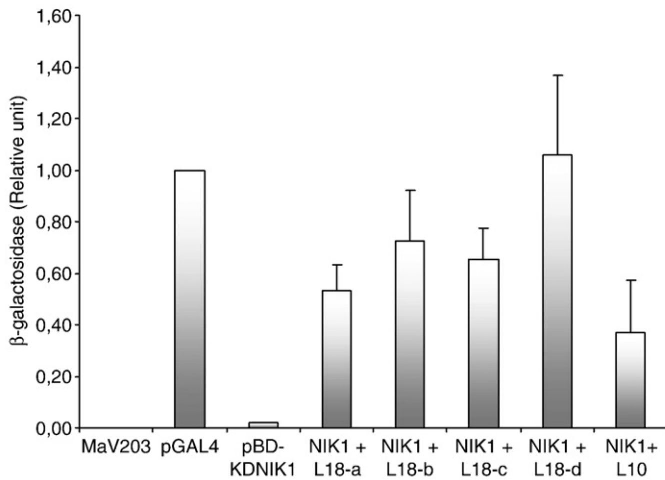


Fig. 1. Interactions of NIK1 from *Arabidopsis* with the ribosomal proteins L18 and L10. The bait protein KDNIK1 was expressed as a GAL4 DNA-binding domain fusion, and the prey proteins (L18 and L10) were expressed as GAL4 activation domain fusions in yeast. KD corresponds to the C-terminal kinase domain of NIK1. Interactions between the indicated proteins were confirmed by measuring the β-galactosidase activity in total protein extracts from yeast transformants grown on selective medium. Four different isolated L18 (a–d) clones were assayed. A value of 1 was arbitrarily assigned to full-length *gal4*. Values for activity are the mean±SD from four replicates.

screening the ribosomal protein L10 as a specific partner and substrate for the kinase domain of NIK. We also provided genetic evidence that rpL10 may function as a downstream effector of the NIK-mediated antiviral response.

Results

To identify potential substrates for NIK1 we performed yeast two-hybrid screens with the kinase domain and an *Arabidopsis* cDNA library (Florentino et al., 2006). From 5×10^6 yeast clones screened, five positive clones showed His and Ura prototrophy. One of them contained a full-length cDNA from the At1g14320 gene which encodes a ribosomal L10 protein (rpL10A), also annotated as a QM-like protein. The four remaining clones harbored a full-length *rpL18* cDNA (At2g34480). These interactions were further confirmed by monitoring β-galactosidase activity in yeast protein extracts (Fig. 1). We next examined whether the ribosomal proteins might be substrates for NIKs. Both ribosomal proteins as well as the kinase domain of NIKs were expressed in *E. coli* as GST fusions (Fig. 2A). The purified recombinant ribosomal proteins were then incubated with bacterially expressed GST-fused NIK-kinase domains under phosphorylation conditions. The Fig. 2B shows that NIK1 phosphorylated efficiently rpL10 but not rpL18. There was no measurable ^{32}P incorporation into GST when GST alone was incubated with KDNIK1 and [λ^{32}P]ATP (date not shown; Fontes et al., 2004). These results also indicated that NIK1 exhibits auto- and substrate phosphorylation activities (Figs. 2C and D). We also examined whether rpL10 and rpL18 served as a substrate for other NIK1-related LRRII-RLK proteins (Fig. 2B). The substrate phosphorylation activity of NIKs was quantified and presented as average from four independent experiments (Fig. 2D). NIK2, the NIK1 most related protein in terms of sequence conservation (69% sequence identity), also phosphorylated rpL10. In contrast, phosphorylation of rpL10 by NIK3 was barely detectable (Figs. 2B and D), although the bacterially purified GST-KDNIK3 also showed a very low autophosphorylation activity (Fig. 2C). These *in vitro* results implicated rpL10 as a potential, specific substrate for the NIK proteins.

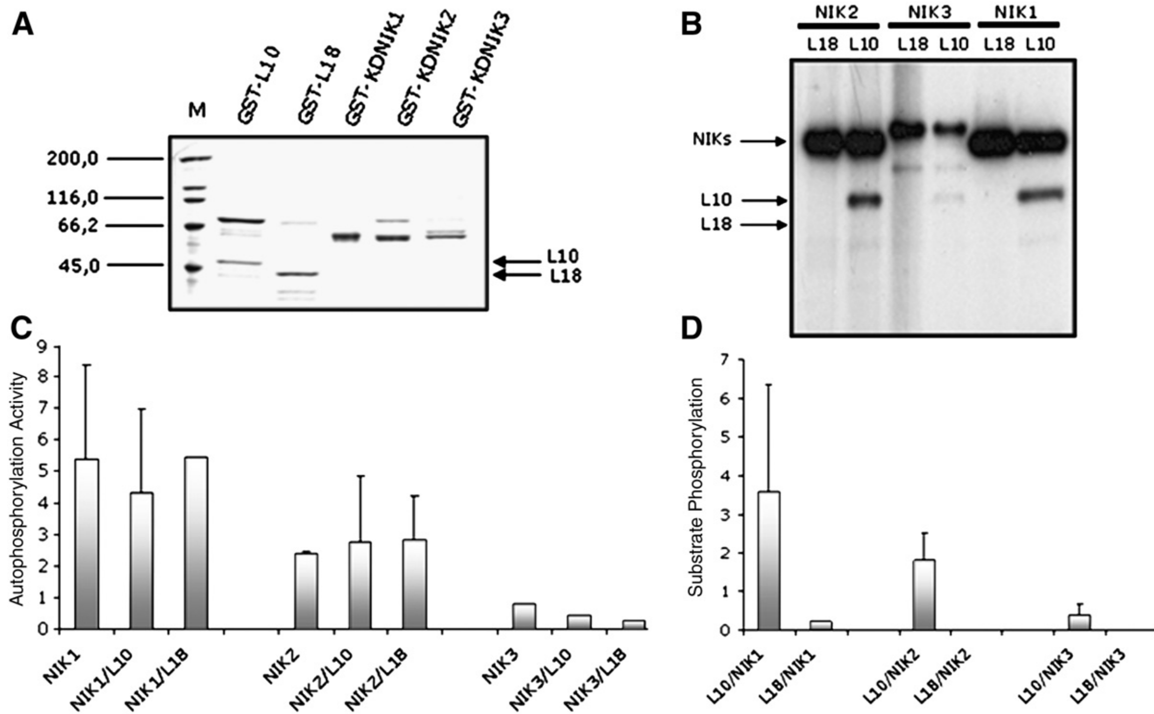


Fig. 2. NIK proteins phosphorylate rpL10, but not rpL18, *in vitro*. (A) SDS-PAGE of *E. coli*-produced GST fusions. GST-fused to rpL10 (GST-L10) and to rpL18 (GST-L18) as well as to the C-terminal kinase domain of NIKs (GST-KDNIK1, GST-KDNIK2 and GST-KDNIK3) were produced in *E. coli*, affinity-purified, separated by SDS/PAGE and stained with Coomassie brilliant blue. Molecular mass markers (kDa) are shown on the left. The position of L10 and L18 is shown by the arrows on the right. (B) rpL10 serve as a substrate for NIK proteins. Purified GST-NIK fusions (as indicated on the top of the lanes) were incubated with equal amounts of GST-rpL18 (L18) or GST-rpL10 (L10) in the presence of [λ-^{32}P]ATP and separated by SDS-PAGE. Phosphoproteins were visualized by autoradiography using a phosphoimager. (C) Autophosphorylation activity and (D) substrate phosphorylation activity of NIKs. Purified GST fusions (as indicated) were incubated with equal amounts of GST-L10 or GST-L18 in the presence of [γ-^{32}P]ATP and separated by SDS-PAGE. The activity of autophosphorylation (C) of the NIK receptors was quantified using a phosphoimager and expressed as relative unit (cpm$\times10^5$)/μg enzyme/min and substrate phosphorylation activity (D) as relative unit (cpm$\times10^4$)/μg enzyme/μg substrate/min. Values for activity are the mean±SD from three replicates.

We have previously demonstrated that loss of *NIK* function increases susceptibility to geminivirus infection (Fontes et al., 2004). In response to geminivirus infection, *nik1* knockout lines develop more severe symptoms and display a higher infection rate as compared to Col-0. To assess whether rpL10 was genetically linked to NIK pathway, we selected both *rpl10* and *rpl18* knockout lines (Figs. 3A and B) and assayed for geminivirus infection. The *rpl18* knockout line was included in the assay as a control because, like rpL10, rpL18 is expected to play a ribosomal role. Thus, the *rpl18* null alleles would allow us to monitor possible variations in susceptibility to virus infection due to perturbations in protein translation. Furthermore, rpL18 did not serve as a substrate for NIK1 and hence is not likely to function directly in the NIK1-mediated antiviral pathway.

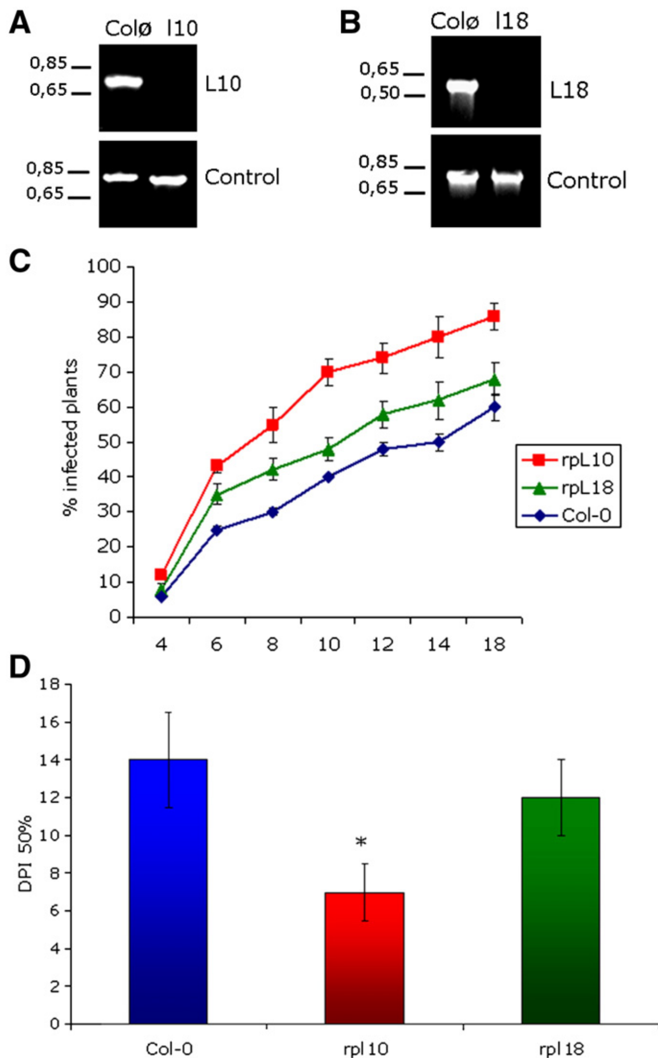


Fig. 3. Loss of rpL10 function, but not rpL18, enhances susceptibility to geminivirus infection. (A) Knockout lines for rpL10. RT-PCR was performed on leaf RNA samples from wild-type (Col-0) and *rpl10* plants with rpL10-specific primers (L10) and actin-specific primers (control). (B) Knockout lines for rpL18. RT-PCR was performed on leaf RNA samples from wild-type (Col-0) and *rpl18* plants with rpL18-specific primers (L18) and actin-specific primers (control). (C) The onset of infection is accelerated in *rpl10* knockout line. Ecotype Col-0, *rpl10* and *rpl18* lines were infected with CaLCuV DNA by the biolistic method. Values represent the percent of systemically infected plants at different days post-inoculation (DPI). Experiments included 20 plants per treatment. (D) Infection rates in *rpl10* null alleles. The infection rate was expressed as DPI to get 50% of infected plants. Values for $DPI^{50\%}$ are the mean±standard deviation from four replicas. In each independent experiment, 20 plants of each line were inoculated with 2 μg of tandemly repeated DNA-A plus DNA-B per plant. Asterisks indicate values significantly different ($P<0.05$, *t*-test).

The virulence function of NSP by targeting and inhibiting the NIK1 kinase activity is likely to contribute for the capacity of CaLCuV to overcome the NIK resistance and hence to infect *Arabidopsis* efficiently. Nevertheless, we have demonstrated previously that removal of coat protein sequences from CaLCuV DNA-A attenuated the virus in wild-type Col-0 plants but not in the *nik1* null background (Fontes et al., 2004). In this case, disease symptoms varied in severity from extreme stunting with severe epinasty and chlorosis in *nik1* knockout to mild stunting with epinasty and moderate chlorosis in Col-0 lines. Therefore, we used this attenuated form of the virus to analyze the course of infection in the *rpl10* and *rpl18* knockout lines by biolistic inoculation (Fontes et al., 2004; Florentino et al., 2006). While *rpl10* displayed severe symptoms resembling those exhibited by infected *nik1* mutant, the symptoms developed in *rpl18* knockout line was mild as in Col-0 (data not shown). The accumulation of viral DNA was detected in all symptomatic plants by PCR with viral DNA-specific primers (data not shown). During the course of infection Col-0 and *rpl18* KO lines displayed similar curve for infection rate (Fig. 3C) reaching a maximum of 80% infected plants about 25–27 days post-inoculation (data not shown). In contrast, the course of infection in *rpl10* null alleles was accelerated (Fig. 3C) and by 20 days post-inoculation 100% of plants were infected (data not shown). These results were confirmed by independent experiments in which the efficiency of virus infection, expressed as $DPI^{50\%}$ (days post-inoculation to reach 50% of infected plants), in *rpl10* knockout lines was significantly higher than in Col-0 and *rpl18* knockout lines (Fig. 3D). Therefore, loss of rpL10 function recapitulated the enhanced susceptibility phenotype to geminivirus infection of *nik* null alleles (Fontes et al., 2004), indicating that *rpL10* may be genetically associated with the NIK1-mediated signal transduction pathway. Although the $DPI^{50\%}$ exhibited by Col-O and *rpl18* mutant lines were similar, as indicated by *t*-test analysis ($P>0.05$), there was a tendency for the mutant line to show a slightly enhanced susceptibility to geminivirus infection that might be a result from pleiotropic effects caused by inactivation of a ribosomal gene.

Discussion

NIK has been described as a transmembrane signaling receptor that mediates an antiviral defense response based on the *in vitro* biochemical properties of the kinase, its inhibition by the geminivirus NSP and the enhanced susceptibility phenotype to geminivirus infection of knockout lines (Fontes et al., 2004). We report here the identification of the ribosomal protein rpL10 as an interacting protein and a substrate of receptor NIKs. We provide three lines of evidence that rpL10 functions as a downstream component of the NIK-mediated antiviral signaling. Firstly, NIK receptors from *Arabidopsis* are capable to bind and phosphorylate rpL10 *in vitro*. Secondly, the *in vitro* NIK phosphorylation is specific for rpL10 because, although rpL18 was also isolated by its capacity to interact with the kinase domain of NIK1, it is not phosphorylated by NIK1, NIK2 or NIK3 *in vitro*. Finally, inactivation of *rpL10* gene enhances geminivirus susceptibility, a phenotype resembling that of *nik1* knockout lines. However, as a ribosomal protein, the enhanced susceptibility to virus infection displayed by *rpl10* knockout lines may be due to pleiotropic effects from inactivation of a general translation-controlling ribosomal gene rather than to an inhibition of a specific antiviral response. The finding that inactivation of *rpL18* gene did not enhance geminivirus susceptibility to the same extent as in *rpl10* null alleles argues against this hypothesis and further substantiates the notion that *rpL10* is linked to the NIK-mediated antiviral signaling.

In our infectivity assay, we have expressed infectivity efficiency as time (dpi), instead of minimal dosage of inoculum, to get 50% infected plants. Using biolistic inoculation of *Arabidopsis* at seven-leaf stage, we have observed that the lines do not respond to different doses of viral DNA in a reproducible way because dilution of our inoculum did

not always correlate statistically with a decrease in infectivity in all lines tested (Santos et al., 2008). Very likely, by DNA bombardment, as opposed to agroinoculation (Sunter et al., 2001), we cannot control the amount of virus DNA that effectively infects each leaf, as the viral DNA is randomly dispersed in several sites of inoculation.

Our results indicate that rpL10 plays an important role in plant antiviral response as the downstream effector of NIK1 signaling. The ribosomal protein L10 gene (At1g14320) is structurally related to QM (accession P27635, 69% identity) originally identified from the Wilms' tumor cell line as a candidate tumor-suppressor gene (Dowdy et al., 1991) and has been shown to regulate the proto-oncogene c-Yes (Oh et al., 2002). In plants and animals, rpL10/QM-like protein plays a role in development (Eisinger et al., 1993; Marty et al., 1993) and may act as a tumor suppressor (Monteclaro and Vogt, 1993). The chicken QM homolog, designated Jif-1 (Jun interactor factor-1), has been identified by its capacity to interact with the transcriptional factor Jun and to inhibit the formation of Jun–Jun homodimer (Monteclaro and Vogt, 1993). As a ribosomal protein, QM/Jif-1 is located to the cytoplasm but translocates to the nucleus to influence cjun-mediated transcription and apoptosis (Imafuku et al., 1999). Yeast QM homologous genes, such as GRC5 and QSR1, participate in translational control of gene expression (Karl et al., 1999) and an *Entamoeba histolytica* QM homolog exhibits extraribosomal functions associated with suppression of cell proliferation (Chavez-Rios et al., 2003). Recently, a QM-like protein from tomato has been shown to suppress Bax-induced cell death in yeast through the inhibition of ROS generation (Chen et al., 2006). These putative rpL10 functions, transcriptional and translational control as well as apoptosis and cell proliferation suppression, may serve as potential host defense strategies against virus. The determination of the subcellular localization of rpL10 in *Arabidopsis* and whether NIK-mediated phosphorylation of the ribosomal protein would regulate its intracellular trafficking and/or function will shed light into the underlying controlling mechanism of this antiviral signaling that has the potential to interfere with normal developmental and cell proliferation events.

Material and methods

Yeast two-hybrid screening

The NIK1 C-terminal kinase domain (KD, encoding amino acids 298–638) was amplified by PCR from U19571 cDNA and cloned into SalI and SstI sites of pBDLeu (Invitrogen Life Technologies, Inc.), yielding pBD-KDNIK1. An *Arabidopsis thaliana* cDNA library was prepared from mRNA isolated from aerial tissues and fused to the GAL4 activation domain in the Leu-pEXAD502 vector (Invitrogen Life Technologies, Inc; Florentino et al., 2006.). The yeast reporter strain MaV203 (Trp-Leu-Ura-) was transformed sequentially with pBD-KDNIK1 and 25 μg of pEXAD502 cDNA libraries, along with 3 mg of salmon sperm carrier DNA, using the lithium acetate/polyethylene glycol method. Transformants were plated on synthetic dropout medium lacking Trp, Leu, Ura, and His but supplemented with 25 mM 3-aminotriazole and cultured for 3 to 5 days at 30 °C. Approximately 5×10^6 transformants were obtained, as estimated based on the number of transformants growing on the SD-Trp-Leu plate. The interactions were further confirmed by measuring β-galactosidase activities from yeast extracts with *o*-nitrophenyl β-D-galactopyranoside, (ONPG; Sigma/Aldrich), as described by Uhrig et al. (1999). All tests were repeated four times. Negative controls with untransformed yeast and transformed MaV203cells with the DNA constructs alone and a standard positive control containing yeast cells transformed with pGAL4 (that expresses the complete GAL4 protein upon yeast transformation) were included in the assays. Plasmid DNA was recovered from yeast and transformed into *Escherichia coli* strain XL-1 Blue (Stratagene) by electroporation.

Plasmid constructions

The clones pGST-KDNIK1, pGST-KDNIK2, pGST-KDNIK3 containing the kinase domain-encoding DNA of *NIK1, NIK2* or *NIK3* fused to *GST* have been previously described (Fontes et al., 2004). The plasmids pAD-L10 and pAD-L18 harboring the *rpL10* cDNA and *rpL18* cDNA, respectively, were isolated by two-hybrid screening; their coding sequences were amplified by PCR with appropriate extensions and then introduced by recombination into the entry vector pDONR201. GST-fused to rpL10 or rpL18 sequences were generated by transferring the appropriate DNA fragment from pDONR201 to the bacterial expression vector pDEST15 to yield the clones pGST-L10 and pGST-L18.

Purification of GST-fusion proteins

The expression plasmids pGST-L10, pGST-L18, pGST-KDNIK1,pGST-KDNIK2 and pGST-KDNIK3 were transformed into *E. coli* strain BL21, and the synthesis of the recombinant protein was induced by 0.4 mM isopropyl-β-D-thiogalactopyranoside for 16 h at 22 °C. The GST fusions were affinity-purified using GST-Sepharose beads (GE Healthcare), according to manufacturer's instructions. Protein concentration was determined according to Bradford (1976).

Protein kinase assay

Purified GST-KDNIK1, GST-KDNIK2 or GST-KDNIK3 (500 ng) were incubated alone or with GST-L10, or GST-L18 for 45 min at 25 °C in 30 μL of kinase buffer containing 18 mM HEPES, pH 7.4, 10 mM MgCl2, 10 mM MnS04, 1 mM DTT, 10 μM ATP, and 5 μCi [γ-^{32}P]ATP. Phosphoproteins were resolved by SDS-PAGE. The gel was stained with Coomassie brilliant blue to verify protein loading, dried, and subjected to autoradiography. Incorporated radioactivity in protein bands was quantified by phosphoimaging and equal protein loading was further confirmed by densitometry using the Multi Gauge V3.0 software (Fujifilm).

Plant material, growth conditions, and genotyping

The Columbia (Col-0) ecotype of *A. thaliana* was used as the wild-type. The *rpl10* (SALK_010170) and *rpl18* (SALK_014456) mutants were from the SALK Institute. Seeds were surface sterilized and cold treated at 4 °C for 2 days in the dark and then exposed to white light. Seedlings were grown at 22 °C on plates containing Murashige-Skoog medium for 3 weeks and then transferred to soil. Plants were grown in a growth chamber at 2 °C under long-day conditions (16 h light/8 h dark). The genotyping of SALK_010170 and SALK_014456 seeds was performed by PCR.

Reverse transcription (RT)-PCR

Total RNA was extracted from the plant tissues using TRIzol (Invitrogen). Reverse transcription (RT)-PCR assays were performed with rpL18- or rpL10-specific primers as described previously (Cascardo et al., 2000). The presence of contaminating DNA was assessed in control reactions conducted without reverse transcriptase. PCR was also carried out with actin-specific primers to assess the quantity and quality of the cDNA. The PCR comprised 40 cycles of 45 s at 94 °C, 1 min and 30 s at 50 °C, and 1.min at 72 °C.

CaLCuV inoculation and analysis of infected plants

A. thaliana plants at the seven-leaf stage were inoculated with plasmids containing partial tandem repeats of CaLCuV DNA-A and DNA-B by biolistic delivery (Santos et al., 2008) and the course of infection was monitored as described previously (Fontes et al., 2004; Florentino et al., 2006; Carvalho et al., 2008). We used an attenuated form of the virus, in

which the coat protein ORF in CaLCuV DNA-A was interrupted by introducing a stop codon at amino acid position 47. Total nucleic acid was extracted from systemically infected leaves, and viral DNA was detected by PCR with DNA-A or DNA-B specific primers. In each experiment, 20 plants of each line (Col-0, *rpl18* and *rpl10* null alleles) were inoculated with 2 μg of tandemly repeated DNA-A plus DNA-B per plant. The course of infection was registered with data from three independent experiments. $DP1^{50\%}$ (days post-inoculation to reach 50% of infected plants) was obtained with data from four independent experiments.

Acknowledgments

We thank Dr Joanne Chory in whose laboratory this work was initiated and ABRC for the T-DNA insertion mutants. This research was supported by the Brazilian Government Agencies CNPq grant 470878/2006-1, FAPEMIG grant EDT 523/07 and FINEP grant 01.07.0610.00 (to E.P.B.F.). C.S.R. was supported by a graduate fellowship from CAPES and J.P.B.M. by an undergraduate scholarship from CNPq. A.A.S. is a CNPq postdoctoral fellow.

References

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Carvalho, C.M., Fontenelle, M.R., Florentino, L.H., Santos, A.A., Zerbini, F.M., Fontes, E.P.B., 2008. A novel nucleocytoplasmic traffic GTPase identified as a functional target of the bipartite geminivirus nuclear shuttle protein. Plant J. 55, 869–880.

Cascardo, J.C.M., Almeida, R.S., Buzeli, R.A.A., Carolino, S.M.B., Otoni, W.C., Fontes, E.P.B., 2000. The phosphorylation state and expression of soybean BiP isoforms are differentially regulated following abiotic stresses. J. Biol. Chem. 275, 14494–14500.

Chavez-Rios, R., Arias-Romero, L.E., Almaraz-Barrera, M. de J., Hernandez-Rivas, R., Guillen, N., Vargas, M., 2003. L10 ribosomal protein from Entamoeba histolytica shares structural and functional homologies with QM/Jif-1: proteins with extraribosomal functions. Mol. Biochem. Parasitol.127 151–160.

Chen, C., Wanduragala, S., Becker, D.F., Dickman, M.B., 2006. Tomato QM-like protein protects Saccharomyces cerevisiae cells against oxidative stress by regulating intracellular proline levels. Appl. Environ. Microbiol. 72, 4001–4006.

Dowdy, S.F., Lai, K.-M., Weissman, B.E., Matsui, Y., Hogan, B.L.M., Stanbridge, E.J., 1991. The isolation and characterization of a novel cDNA demonstrating na altered mRNA level in nontumorigenic Wilms' microcell hybrid cells. Nucleic. Acids Res. 19, 5763–5769.

Eisinger, D.P., Jiang, H.P., Serrero, G., 1993. A novel mouse gene highly conserved throughout evolution: regulation in adipocyte differentiation and in tumorigenic cell lines. Biochem. Biophys. Res. Commun. 196, 1227–1232.

Florentino, L.H., Santos, A.A., Fontenelle, M.R., Pinheiro, G.L., Zerbini, F.M., Baracat-Pereira, M.C., Fontes, E.P.B., 2006. A PERK-like receptor kinase interacts with the geminivirus nuclear shuttle protein and potentiates viral infection. J. Virol. 80, 6648–6656.

Fontes, E.P.B., Santos, A.A., Luz, D.F., Waclawovsky, A.J., Chory, J., 2004. The geminivirus NSP acts as virulence factor to suppress an innate transmembrane receptor kinase-mediated defense signaling. Genes Dev. 18, 2545–2556.

Gafni, Y., Epel, B.L., 2002. The role of host and viral proteins in intra- and inter-cellular trafficking of geminiviruses. Physiol. Mol. Plant Pathol. 60, 231–241.

Imafuku, I., Masaki, T., Waragai, M., Takeuchi, S., Kawabata, M., Hirai, S., Ohno, S., Nee, L.E., Lippa, C.F., Kanazawa, I., Imagawa, M., Okazawa, H., 1999. Presenilin 1 suppresses the function of c-Jun homodimers via interaction with QM/Jif-1. J. Cell Biol. 147, 121–133.

Karl, T., Önder, K., Kodzius, R., Pichová, A., Wimmer, H., Thür, A., Hundsberger, H., Löffler, M., Klade, T., Beyer, A., Breitenbach, M., Koller, L., 1999. GRC5and NMD3function in translational control of gene expression and interact genetically. Curr. Genet. 34, 419–429.

Lazarowitz, S.G., Beachy, R.N., 1999. Viral movement proteins as probes for intracellular and intercellular trafficking in plants. Plant Cell 11, 535–548.

Mariano, A.C., Andrade, M.O., Santos, A.A., Carolino, S.M.B., Oliveira, M.L., Baracat-Pereira, M.C., Bromoshenkel, S.H., Fontes, E.P.B., 2004. Identification of a novel receptor-like protein kinase that interacts with a geminivirus nuclear shuttle protein. Virology 318, 24–31.

Marty, I., Brugidou, C., Chartier, Y., Meyer, Y., 1993. Growth-related gene expression in Nicotiana tabacum mesophyll protoplasts. Plant J. 4, 265–278.

Monteclaro, F.S., Vogt, P.K., 1993. A Jun-binding protein related to a putative tumor suppressor. Proc. Natl. Acad. Sci. U.S.A. 90, 6726–6730.

Oh, H.S., Kwon, H., Sun, S.K., Yang, C.-H., 2002. QM, a putative tumor suppressor, regulates proto-oncogene c-yes. J. Biol. Chem. 277, 36489–36498.

Rojas, M.R., Hagen, C., Lucas, W.J., Gilbertson, R.L., 2005. Exploiting chinks in the plant's armor: evolution and emergence of geminiviruses. Annu. Rev. Phytopathol. 43, 361–394.

Santos, A.A., Florentino, L.H., Pires, A.B.L., Fontes, E.P.B., 2008. Geminivirus: biolistic inoculation and molecular diagnosis. Methods Mol. Biol. 451, 563–579.

Shiu, S.H., Bleecker, A.B., 2001. Receptor-like kinases from *Arabidopsis* form a monophyletic gene family related to animal receptor kinases. Proc. Natl. Acad. Sci. 98, 10763–10768.

Sunter, G., Sunter, J.L., Bisaro, D.M., 2001. Plants expressing tomato golden mosaic virus AL2 or beet curly top virus L2 transgenes show enhanced susceptibility to infection by DNA and RNA viruses. Virology 285, 59–70.

Uhrig, J.F., Soellick, T.-R., Minke, C.J., Philipp, C., Kellmann, J.-W., Schreier, P.H., 1999. Homotypic interaction and multimerization of nucleocapsid protein of tomato spotted wilt tospovirus: identification and characterization of two interacting domains. Proc. Natl. Acad. Sci. U.S.A. 96, 55–60.

Vanitharani, R., Chellappan, P., Fauquet, C.M., 2005. Geminiviruses and RNA silencing. Trends Plant Sci. 10, 144–151.

Wang, H., Buckley, K.J., Yang, X., Buchmann, R.C., Bisaro, D.M., 2005. Adenosine kinase inhibition and suppression of RNA silencing by geminivirus AL2 and L2 proteins. J. Virol. 79, 7410–7418.

Zhang, X.S., Choi, J.H., Heinz, J., Chetty, C.S., 2006. Domain-specific positive selection contributes to the evolution of *Arabidopsis* leucine-rich repeat receptor-like kinase (LRR RLK) genes. J. Mol. Evol. 63, 612–621.