The discovery of each new viral-protein-interacting host factor in infection by subverting defense responses (13, 19, 28, 48, 49) or for modulation of virus interactions, which are required either for basic compatibility shown to depend extensively on virus-host intramolecular infection. Accordingly, the geminivirus infection cycle has been cellular machinery to perform their basic replication and intercellular trafficking of viral DNA (14, 26). which act in a cooperative manner to mediate the intra- and

component encodes two proteins, the nuclear shuttle protein (NSP, or BV1) and the movement protein (MP, or BC1), AC3, and AC4), which are involved in replication of the viral genome (AC1, or Rep, and AC3, or REn), transactivation of viral genes (AC2, or TrAP), and encapsidation of the viral genome (AV1, or CP) (9, 10, 11, 23, 44, 45). The DNA-B component encodes two proteins, the nuclear shuttle protein (NSP, or BV1) and the movement protein (MP, or BC1), which act in a cooperative manner to mediate the intra- and intercellular trafficking of viral DNA (14, 26).

The family Geminiviridae represents a group of plant DNA viruses characterized by a single-stranded circular genome and unique twinned icosahedral particles (17, 35). Species of this family are taxonomically grouped into four genera according to the insect vector, host range, and genomic organization, which can be in either single or double configuration. In the bipartite geminiviruses, the virus-encoded replication, encapsidation, and movement functions are segregated into two genomic components, designated DNA-A and DNA-B. DNA-A has the potential to code for five gene products (AV1, AC1, AC2, AC3, and AC4), which are involved in replication of the viral genome (AC1, or Rep, and AC3, or REn), transactivation of viral genes (AC2, or TrAP), and encapsidation of the viral genome (AV1, or CP) (9, 10, 11, 23, 44, 45). The DNA-B component encodes two proteins, the nuclear shuttle protein (NSP, or BV1) and the movement protein (MP, or BC1), which act in a cooperative manner to mediate the intra- and intercellular trafficking of viral DNA (14, 26).

Like any other plant virus, geminiviruses rely on the host cellular machinery to perform their basic replication and movement functions toward the establishment of a productive infection. Accordingly, the geminivirus infection cycle has been shown to depend extensively on virus-host intermolecular interactions, which are required either for basic compatibility (1, 3, 4, 6, 24, 25, 27, 29, 40, 41) or for modulation of virus infection by subverting defense responses (13, 19, 28, 48, 49).

The discovery of each new viral-protein-interacting host factor has provided insights into the molecular bases of pathogenicity (51). In the case of geminiviruses, their potential as a model system to elucidate basic cellular components of the plant replication and transport machinery has become increasingly apparent (18, 26, 35). Particularly interesting is the unique aspect of the geminivirus infection cycle that requires a movement function to facilitate the transport of viral DNA from the nucleus, where viral replication takes place, to the cytoplasm (14, 26).

Our understanding of geminivirus movement in infected plants has advanced with the genetic and biochemical characterization of the movement proteins from Squash leaf curl virus (SLCV) and Bean dwarf mosaic virus. From these investigations, it has been conceptually established that NSP shuttles viral DNA between the nucleus and the cytoplasm, whereas MP is predominantly involved in mediating the cell-to-cell movement of viral DNA across the wall (14). However, two distinct mechanisms for cell-to-cell trafficking of viral DNA, which basically differ in the nature of the viral DNA complex transported into the adjacent uninfected cells, are supported by the relevant data (30, 37). In the first model, NSP facilitates the intracellular movement of the viral genome from the nucleus to the cytoplasm, whereas MP is involved in transporting the viral DNA to adjacent cells via plasmodesmata (30, 34). The second model, as in the case of the phloem-limited SLCV, holds that MP facilitates NSP-mediated intracellular transport of viral DNA from the nucleus to the cytoplasm and then mediates the transport of the NSP-DNA complex to adjacent cells via endoplasmic-reticulum-derived tubules induced by the viral infection (20, 36, 37, 38, 50). Regardless of the mechanism of geminivirus cell-to-cell trafficking, the fundamental role of NSP in virus movement pre-
predicts that this viral protein may interact with host factors in different subcellular compartments. Accordingly, NSP has been shown to interact with an Arabidopsis thaliana nuclear acetylase, designated nuclear shuttle protein interactor (AtNSI), as well as with plasma membrane receptor kinases, designated NIKs (NSP-interacting kinases), from tomato, soybean, and Arabidopsis (28, 29). In Arabidopsis, NSP interacts with three members of the LRR-receptor-like kinase (RLK) family, NIK1, NIK2, and NIK3, which have been shown to be authentic serine/threonine kinases with biochemical properties consistent with a receptor-signaling function (13).

Despite the interaction of NSP with acetylase and receptor-like kinases, NSP does not function as a substrate for either of these host enzymes. In fact, AtNSI does not acetylate NSP, but rather, it acetylates CP (29). During rolling-circle replication of viral DNA, CP is thought to mediate a single-stranded DNA replication pool and making them available for NSP binding (29). Consistent with this model, overexpression of AtNSI enhances the susceptibility of Nicotiana tabacum cv. Samsun to Tomato crinkle leaf yellows virus (TCrLYV) (accession number AY090556) also interact with the NsAK protein. In NSP inhibits its kinase activity to suppress a NIK-mediated infection (4, 29). In the case of the receptor-like kinase NIK, with this model, overexpression of AtNSI enhances the susceptibility of Nicotiana tabacum cv. Samsun to Tomato crinkle leaf curl virus (CaLCuV) infection, and disruption of the NSP capacity to bind AtNSI generates a mutant virus defective for infection (4, 29). In the case of the receptor-like kinase NIK, NSP inhibits its kinase activity to suppress a NIK-mediated antiviral response (13). Accordingly, inactivation of NIK genes increases the susceptibility of Arabidopsis mutants to virus infection (13). Here, we extend the characterization of the putative basic network of NSP-interacting host proteins and provide evidence for the biological significance of complex formation. We show that NSP also interacts with a proline-rich extensin-like receptor kinase (PERK)-like protein from Arabidopsis that belongs to the receptor-like serine/threonine kinase superfamily and is here referred to as NsAK (for NSP-associated kinase). NSP-NsAK complex formation is not virus specific, because NSPs from Tomato golden mosaic virus (TGMV) and Tomato crinkle leaf yellows virus (TCrLYV) (accession number AY090556) also interact with the NsAK protein. In contrast to the receptor-like kinase NIK, the NsAK protein seems to potentiate infectivity and may be a positive regulator of NSP function, as disruption of NsAK expression attenuates virus infection.

**Plasmid DNA was recovered from yeast and transformed into E. coli strain XL-1 Blue (Stratagene) by electrotransformation.**

**MATERIALS AND METHODS**

**Yeast two-hybrid screen.** 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.). The pBD-NSPCLCV clone, which contains the GAL4 DNA-binding domain fused to NSP sequences from CalLCuV, has been previously described (13). The yeast reporter strain Ma203 (MATa leu2-3,112 trp1-901 his3-200 ade2-101 gal4 gal80 spal10::UAS GAL1::lacZ his3/his3 UAS GAL1::HIS3-LYS2 can1R cyh2R) is deficient in producing tryptophan, leucine, and uracil (Trp– Leu– Ura–). Ma203 cells were transformed sequentially with pBD-NSPCLCV and 25 μg of pExAD502 cDNA libraries, along with 3 μg 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-amino triazole and cultured for 5 days at 30°C. The interactions were further confirmed by measuring β-galactosidase activities from yeast extracts with o-nitrophenyl β-D-galactopyranoside, as described previously (46). Approximately 5 × 10⁶ transformants were obtained, as estimated based on the number of transformants growing on the synthetic-dropout–Trp–Leu– plate.
22°C under long-day conditions (16 h light/8 h dark). The genotyping of the SALK_008504 seeds was performed by PCR. Wild-type NsAK alleles were identified with the NsAK-Fwd (5'-H11032-CAGTTCCTCAACTCGATCTAGTCCC-3'//H11032) and NsAK-Rvs (5'-H11032-CCTCCAATGGCGATTCCTACC-3'//H11032) primers and, for detection of the nsak alleles, the NsAK-Fwd primer was used in combination with the SALK LBa1 (5'-H11032-TGGTTCACGTAGTGGGCCATCG-3'//H11032) primer.

CaLCuV inoculation and analysis of infected plants. Arabidopsis 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 as described previously (39). Total nucleic acid was extracted from systemically infected leaves, and viral DNA was detected by PCR with DNA-A- or DNA-B-specific primers (33).

RESULTS
Identification of a PERK-like kinase domain that interacts with NSP. To identify host proteins that interact with NSP, we

FIG. 1. Alignment of the deduced protein sequence of NsAK to those of PERK-like proteins. The sequences of NsAK (At5g24550), the Brassina napus BnPERK1, and the Orysa sativa OsPERK1 were retrieved from databases. The dots represent identity to NsAK, and dashes are gaps introduced by CLUSTALW to maximize identity. The proline-rich regions are shown in boldface, and a putative transmembrane domain is boxed. The 11 subdomains typical of the eukaryotic protein kinase family are underlined and identified by Roman numerals.

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performed yeast two-hybrid screens and identified several NSP-interacting proteins, including the NIKs already described (13, 28). We describe here the characterization of NSAK, a PERK-like kinase with a transmembrane receptor configuration (Fig. 1). From a total of 5 × 10^6 independent double transformants that were assayed for Ura and His prototrophy and β-galactosidase activity, three positive clones were found that encoded a truncated kinase domain from the Arabidopsis gene At3g24550, designated NSAK (NSP-associated kinase). The deduced NSAK protein contains 652 amino acid residues and a C-terminal serine/threonine kinase catalytic domain with all 11 subdomains typical of the eukaryotic kinase family (Fig. 1). Based on the proline-rich modular organization of its N-terminal domain, NSAK falls into the PERK-like subfamily of the RLK family (42). Among the members of the PERK-like RLK subfamily from Arabidopsis, NSAK is most closely related to the At3g24400-encoded product (63% identity), followed by At3g24540 (62%) and At1g52290 (54%), whose functions are unknown. Extending the analysis to general plant proteins, NSAK was found to be closely related to the viral protein with either the intact NSAK protein (lane 2) or its potentially active kinase domain (lane 5) was rarely detected by our in vitro assay. This scenario for NSP-NSAK complex formation resembles that of enzyme-substrate interaction under a high catalytic efficiency and may indicate that NSAK phosphorylates the viral protein. In this case, inability of the kinase domain to perform catalysis would trap the enzyme-substrate complex in a nonproductive interaction, increasing the stability of the complex formation, as in the case of the KDNsAK truncated protein. Consistent with this notion, truncated NSAK polypeptides, which were also produced by the in vitro-transcribed and -translated reaction mixtures of KDNsAK and KDNsAK, bound to GST-NSP (lanes 5 and 8), but not to GST alone, indicating that NSP specifically and stably interacted with defective versions of truncated NSAK, but not with the potentially active NSAK kinase domain.

To directly assess the capacity of NSAK to phosphorylate NSP, we attempted to express the NSAK kinase domain in E. coli using several bacterial expression systems. We also tried to overexpress NSAK in Arabidopsis both as intact and as GFP- and TAP-tagged fusion proteins. Except for the KD-NSAK inactive truncated kinase domain, which was produced in E. coli, all the potentially active recombinant kinases failed to be expressed either in E. coli or in plants. Given that both full-length NSAK and its kinase domain were efficiently transcribed and translated in vitro (Fig. 2), the transcription/translation system was then adjusted to perform in vitro phosphorylation assays (Fig. 3A). Several endogenous reticulocyte proteins were phosphorylated by the reticulocyte kinases (lane 1), and so was the GST-NSP fusion protein (lane 4), which displayed a threefold-increased level of 32P labeling compared to the GST-NSP-comigrating 52-kDa-band background (Fig. 3B, compare bars 1 and 4). Nevertheless, inclusion of in vitro-transcribed and -translated NSAK in the phosphorylation reaction promoted a threefold increase in the 32P labeling of GST-NSP signal (bar 2) compared to the reticulocyte kinase-phosphorylated GST-NSP signal (bars 4 and 5) and 14-fold higher than the corresponding 52-kDa-band background (bar 1). The incorporation

| Bait         | pAD-ΔKD-NsAK | Activity in prey indicated* | pAD-SBP |
|--------------|--------------|----------------------------|---------|
| pBD-NSPCLCV  | +            | +                          | U + H   |
| pBD-NSPGTMV  | +            | +                          | U + H   |
| PBD-NSPGTLYV | +            | +                          | U + H   |
| pBD-Leu      | +            | +                          | U + H   |

*The bait proteins were expressed as GAL4 DNA-binding domain fusions, and the prey proteins were expressed as GAL4 activation domain fusions in yeast. ΔKD corresponds to the truncated C-terminal kinase domain of NSAK. SBP is an unrelated sucrose-binding protein from soybean used as a control in the two-hybrid assays.

3AT, 25 mM 3-aminotriazole; β-Gal, β-galactosidase activity; +, presence; −, absence. Values for activity are the mean ± standard deviation from four replications.
of radioactive phosphate occurred in the NSP portion, as GST by itself was not phosphorylated by either NsAK or KDNsAK (bars 2 and 5). Together, these results demonstrate that NsAK possesses a functional kinase domain at the carboxyl terminus and that NSP is phosphorylated by NsAK in vitro. Isolation of

FIG. 2. In vitro interaction of NSP with NsAK. (A) Schematic representation of in vitro-translated NsAK-truncated proteins. Different truncations of NsAK cDNA were cloned into a T7 RNA polymerase-dependent transcription vector, pDEST14. P indicates the proline-rich repeated domains. TM denotes the transmembrane domain. NBS corresponds to the nucleotide binding site that encompasses subdomains I and II, as described for kinases. The position of the subdomain VIb that corresponds to the kinase active site is also indicated. (B) In vitro pull-down protein-protein interaction. In vitro-transcribed and -translated 35S-labeled proteins, as indicated, were allowed to interact with bacterially expressed GST or GST-NSP linked to glutathione-Sepharose beads. After extensive washing of the beads, the retained proteins were separated by SDS-PAGE and visualized through fluorography. The input contained samples (10% reaction) of the respective transcription and translation reaction mixtures.

FIG. 3. Phosphorylation assay. (A) NsAK and KDNsAK were transcribed and translated in vitro, and the reaction mixtures were incubated with [γ-32P]ATP and with either purified E. coli-produced GST-NSP or recombinant GST. After separation on 10% SDS-PAGE, the phosphoproteins were visualized by autoradiography. In lane 1, the reticulocyte lysate mixture was incubated with [γ-32P]ATP in the absence of in vitro transcription DNA constructs. (B) GST-NSP phosphoprotein (bars 2, 4, and 5) was quantified by phosphorimaging and normalized to the highest-molecular-weight phosphoprotein band signal (internal control), indicated by an arrowhead in panel A. Bars 1 and 6 show the radioactive signal incorporated into the 52-kDa background band, expressed as a percentage of the internal control signal. Bar 3 corresponds to the relative phosphorylation level of GST-comigrating bands. (C) NsAK and NIK were transcribed and translated in vitro, and the reaction mixtures were incubated with [γ-32P]ATP and purified GST-NSP. After the incubation period, the GST-NSP-phosphorylated protein was isolated on glutathione-Sepharose beads, separated by SDS-PAGE, and visualized by autoradiography.
the GST-NSP-phosphorylated protein from the phosphorylation reaction mixture further confirmed these results (Fig. 3C).

The NsAK transcripts are expressed ubiquitously in Arabidopsis. To determine the expression pattern of Ns4K mRNA, we performed semiquantitative RT-PCR with total RNA from various Arabidopsis tissues (Fig. 4A). The Ns4K mRNA was detected in all tissues investigated, and its steady-state level showed only minor variations among the different tissues. When normalized to the control band, it appeared that NsAK mRNA accumulation predominated in flowers. All other tissues showed intermediate mRNA levels.

Recently, it has been demonstrated that the geminivirus protein REn induces expression of its partner, a NAC protein (40). It was of interest to determine the effect that NSP expression might have on Ns4K transcript accumulation. Total RNA from leaves of NSP-expressing Arabidopsis was analyzed by semiquantitative RT-PCR (Fig. 4B). Expression of NSP from CalLCuV (lanes 2 and 3) or TCrLYV (lanes 4, 5, and 6) did not induce Ns4K transcript accumulation in transgenic leaves. Likewise, geminivirus infection had no apparent effect on Ns4K expression in Arabidopsis leaves and roots (Fig. 4C).

Inactivation of the Ns4K gene results in attenuation of symptoms and reduces the efficiency of viral infection. To examine directly the biological significance of NAK-NSP interactions in vivo, we identified a transfer DNA (T-DNA) insertional mutation in the At3g24540 (NAK) gene (Fig. 5A). RT-PCR was performed on leaf RNA samples from wild-type (Col-0) and nsak knockout (KO) lines. With the gene-specific primers, we detected no accumulation of the corresponding transcript in the respective homozygous T-DNA insertion mutant, confirming that it was a null allele (Fig. 5B).

Wild-type Col-0 and nsak mutant plants were inoculated with CalLCuV DNA-A and DNA-B. Both Col-0 and nsak KO lines developed typical CalLCuV symptoms with distinct intensities. In fact, disease symptoms varied in severity from extreme stunting with severe chlorosis in Col-0 lines to mild stunting with epinasty and moderate chlorosis in nsak lines (Fig. 6A). The accumulation of viral DNA was detected in all symptomatic plants (Fig. 6B). The attenuated infection in nsak KO lines was reflected during the course of infection. As judged by symptom appearance, inactivation of Ns4K alleles reduced the efficiency of virus infection in comparison with Col-0 plants that displayed a higher infection rate (Fig. 6C). In other experiments, the infectivity data, expressed as the number of days postinoculation required to get 50% infected plants, further confirmed the results (Fig. 6D).

**DISCUSSION**

Begomovirus NSP shuttles the viral DNA between the nucleus and the cytoplasm and cooperates with MP to move the viral DNA cell to cell across the wall. This facilitated trafficking
of NSP indicates that the viral protein interacts extensively with host factors to usurp the endogenous host transport machinery. We report here the identification of NsAK, a novel cellular interaction partner for NSP. Interaction of an inactive kinase domain of NsAK was identified in the yeast two-hybrid system and further substantiated in an in vitro interaction assay. The latter observation argues for a direct interaction between the two proteins. However, we were unable to detect in vitro a stable interaction between NSP and an intact NsAK protein. The simplest explanation for the failure to detect interaction between the intact proteins is that the in vitro-synthesized active NsAK folded in a closed conformation that prevented NSP binding. Although we were unable to completely rule out this possibility, we provided two lines of evidence that argue against this hypothesis. First, disruption of NsAK expression indicated that NsAK is required for maximal viral infectivity. This observation suggests that any conformational constraint on NsAK function would be alleviated upon infection. Furthermore, the in vitro-transcribed and -translated NsAK was able to phosphorylate NSP, confirming that binding between the proteins might have occurred. More likely, a high catalytic efficiency ($K_{cat}/K_{m}$) of NsAK with NSP as a substrate prevented the formation of a sufficiently stable enzyme-substrate complex to be detected in our assay. Among many other possible reasons, this might also explain why we were not able to demonstrate an interaction in vivo by a classical coimmunoprecipitation assay.

NSP has also been shown to bind specifically to three members of the RLLII-RLK family, designated NIK, through their serine/threonine kinase domains. However, it binds to both active and defective kinases, and it is not phosphorylated by the proteins, but rather, it inhibits the kinase activity (13). Loss

FIG. 6. An nsak knockout line exhibits enhanced tolerance of geminivirus infection. (A) Symptoms associated with CaLCuV infection in the knockout line. Tandemly repeated viral DNA-A and DNA-B were introduced into plants by biolistic inoculation. Shown are Col-0 (bottom right) and nsak (bottom left) plants infected with CaLCuV at 15 days postinoculation (DPI). On the top, Col-0 (right) and nsak (left) are mock-inoculated plants. (B) Viral-DNA accumulation in infected lines. Total DNA was isolated from greenhouse-grown Col-0 and nsak plants at 7 DPI and diagnosed by PCR with gene-specific primers. IN refers to viral-DNA-inoculated plants, and UN refers to mock-inoculated plants. (C) The onset of symptoms is delayed in an nsak knockout line. Ecotype Col-0 and nsak lines were infected with CaLCuV DNA by the biolistic method. The values represent the percentages of systemically infected plants at different numbers of DPI and are given as mean ± standard deviation (SD) of three determinations from independent experiments. The asterisks indicate significant differences at a P value of ≤0.05. (D) Infection rates in nsak null alleles. The infection rate was expressed as number of DPI required to get 50% infected plants (DPI 50%). The data are the mean ± SD of three independent experiments.
of NIK gene function enhances susceptibility to geminivirus infection, suggesting that NSP acts as a virulence factor to suppress NIK-mediated antiviral defenses. Our results suggest that binding of NSP to the NsAK kinase domain results in a productive enzyme-substrate interaction that, in contrast to the NSP-NIK interaction, may regulate NSP function. Consistent with this hypothesis, NSP from SLCV has been shown to be posttranslationally modified by phosphorylation (31). In addition, we provided evidence for an in vivo functional link between NSP and NsAK, as loss of NsAK function led to attenuation of viral infection. This nsk mutant phenotype implicates NsAK as a positive contributor to geminivirus infection, which most likely acts through regulation of NSP function. Cell-to-cell trafficking of plant viruses has been demonstrated to be regulated by the phosphorylation state of viral MPs that are phosphorylated by plasmodesma-associated protein kinases (7, 47). In the case of NSP, however, as a facilitator of both intracellular and MP-mediated intercellular transport of viral DNA, a phosphorylation event appears equally likely to occur in the nucleus, nuclear envelope, cytoplasm, or plasma membrane. Thus, the determination of the precise localization of NsAK will provide valuable information about the functional significance of the interaction between NsAK and NSP.

Protein sequence analysis revealed that NsAK belongs to the PERK-like RLK subfamily and, as such, is structurally organized into an N-terminal proline-rich domain and a serine/threonine C-terminal domain. We provided evidence that NsAK is an authentic serine/threonine kinase, as the in vitro-translated protein or its carboxyl domain enhances the phosphorylation level of NSP in an in vitro translation mixture background (Fig. 3). This is consistent with the structural-motif prediction for NsAK, as its C-terminal region contains all 11 of the conserved subdomains of protein kinases, in addition to specific signatures of serine/threonine kinases (8, 16). These include the putative active site HrdKssNxLLD in subdomain VIIb; the DFG motif in subdomain VII, which may chelate Mg$^{2+}$ ions; and the highly conserved APE motif in subdomain VIII, which is assumed to be involved in the recognition of substrates and in autophosphorylation. As a member of the PERK-like RLK subfamily, the NsAK N-terminal portion is rich in proline and shares sequence similarity with the extensin family of cell wall proteins (42). The Brassica napus PERK-like RLR homolog was localized to the plasma membrane and has been shown to be rapidly induced by mechanical stresses (43).

As a wound-induced and plasma membrane-localized member of the RLK family that has been fundamentally implicated in a wide range of signal transduction pathways, it has been proposed that the Brassica napus homolog is involved in transducing the wounding signal (42, 43). Various wounding stimuli are often generated during the process of plant pathogen infection. This is particularly true in the case of the insect-transmitted geminiviruses. We found here, however, that the Ns4K transcript level was not altered during the onset of geminivirus infection, although the biolistic method of inoculation generates a wound stimulus by mechanically injuring the leaves. The rather constitutive and ubiquitous expression of NsAK argues for a housekeeping function of this functional serine/threonine kinase.

Even though a complete knockout of nsk T-DNA insertion mutation resulted in attenuation of geminivirus infection, it did not totally block the infection, as would be expected if NsAK had an essential role in regulating NSP function. However, this result was not surprising, because the Arabidopsis genome encodes 14 NsAK homologs from the PERK-like RLK subfamily (42, 43), and some of them might partially replace NsAK function during geminivirus infection. If such a strict NSP dependence on a specific member of a gene family in fact exists, it is reasonable to assume that NSP evolved to accommodate a certain degree of promiscuity to recruit the pivotal kinase activity to its function.

The specific interactions of NSP with members of the RLLI-II RLK subfamily as a suppressor of kinase activity and antiviral defenses (13), and with a member of the PERK-like–RLK subfamily as a kinase substrate (this work), may indicate that the binding of NSP to serine/threonine kinase domains is an inherent property of the viral protein to cover both virulence and basic compatibility functions. Functionally, the binding of NSP to NIK1 (RLLI-II RLK) antagonizes its binding to NsAK (PERK-like), as NSP inhibits the NIK kinase activity but acts as a NsAK substrate. The site of NSP binding to NIK1 was mapped to an 80-amino-acid-residue stretch that encompasses the putative active site for serine/threonine kinases (subdomain VIIb, HrdKssNxLLD) and the activation loop (subdomain VII, DFGAk/rx, plus subdomain VIII, GtxGyaPEY) (13). As a potential substrate for NsAK, one may predict that NSP interacts with NsAK through its substrate binding site, which conceptually overlaps the corresponding NSP-interacting region on NIK (16, 22). Nevertheless, the presence of the conserved subdomains VIIb, VII, and VIII per se does not fulfill the requirement for NSP-specific binding to kinases, because NSP does not interact stably with other receptor-like serine/threonine kinases that contain these conserved subdomains, such as BRLI (for brassinosteroid insensitive 1) and SERK (for somatic embryogenesis receptor-like kinase) (13). More likely, discrete contacts with divergent residues of the catalytic domains of RLKs confer the required specificity for binding. While the identification of NsAK-NSP interaction sites would allow the construction of mutants with impaired binding capacities, the predicted functional redundancy of the PERK-like RLK gene family members and the antagonistic effect of NSP binding to NIK and to NsAK complicate the use of these mutant proteins to evaluate the significance of NsAK-NSP interaction in the geminivirus infection cycle. In contrast, the identification and subsequent targeting of the NSP phosphorylation sites in a mutagenesis-based approach will allow us to handle the NsAK-NSP interaction separately and hence to elucidate the role of phosphorylation in NSP function.

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