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REVIEW PAPER

NSP-interacting kinase, NIK: a transducer of plant defence signalling

Anésia A. Santos[1,2], Kênia V. G. Lopes[1], Jorge A. C. Apfata[3] and Elizabeth P. B. Fontes[1,2,*]

[1] Departamento de Bioquímica e Biologia Molecular, BIOAGRO, Universidade Federal de Viçosa, 36571.000, Viçosa, MG, Brazil
[2] Instituto Nacional de Ciência e Tecnologia em Interações Planta-Praga, CNPq/FAPEMIG, Universidade Federal de Viçosa, 36571.000, Viçosa, MG, Brazil
[3] Departamento de Biologia Vegetal, Universidade Federal de Viçosa, 36571.000, Viçosa, MG, Brazil

* To whom correspondence should be addressed. E-mail: bbfontes@ufv.br

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Abstract

The NSP-interacting kinase, NIK, belongs to the five leucine-rich repeats-containing receptor-like serine/threonine kinase subfamily that includes members involved in plant development and defence. NIK was first identified by its capacity to interact with the geminivirus nuclear shuttle protein (NSP) and has been strongly associated with plant defence against geminivirus. Recent studies corroborate its function in transducing a defence signal against virus infection and describe components of the NIK-mediated antiviral signalling pathway. This mini-review describes the role of NIK as a transducer of a novel layer of plant innate defence, presents new data on NIK function, and discusses its possible involvement in plant development.

Key words: Defence signalling, NIK1, NSP-interacting kinases, nuclear shuttle protein, receptor-like kinases, geminivirus.

NIKs (NSP-interacting kinases): a functionally redundant branch of the LRR-RLKII subfamily in counteracting virus infection

NIK receptors belong to the plant defence group of the leucine-rich repeat (LRR) receptor-like kinase (RLK) subfamily, designated LRR-RLKII (Shiu and Bleecker, 2001; Dievart and Clark, 2004). This subfamily of RLKs is constituted by 14 proteins harbouring four complete LRRs (with 24 residues) and a fifth incomplete LRR (with 16 residues) arranged in a single continuous block within the extracellular domain (Zhang *et al.*, 2006). Based on sequence conservation and structural features, the members of the LRR-RLKII subfamily are clustered into three distinct branches: (i) antiviral defence proteins; (ii) developmental and defence proteins, such as the somatic embryogenesis receptor-like kinases (SERK-like) including SERK1 (Hecht *et al.*, 2002; Santos and Aragão, 2009) and SERK3/BAK1 [brassinosteroid-insensitive receptor 1 (BRI1)-associated kinase 1; Li *et al.*, 2002; Nam and Li, 2002; Chinchilla *et al.*, 2007; Heese *et al.*, 2007]; and (iii) functionally unassigned proteins. The *Arabidopsis* NSP-interacting kinase 1, NIK1 (At5g16000), NIK2 (At3g25560), and NIK3 (At1g60800) are in the defence group I of the LRR-RLKII subfamily and are virulence targets of the bipartite geminivirus nuclear shuttle protein, NSP (Fontes *et al.*, 2004). NSP from CaLCuV (cabbage leaf curl virus; Hill *et al.*, 1998) interacts with all three NIKs from *Arabidopsis* to suppress their kinase activity (Fontes *et al.*, 2004).

The NSP–NIK interaction is also conserved among geminivirus NSPs and NIK homologues from different hosts (Mariano *et al.*, 2004). Tomato and soybean NIK homologues also interact stably with NSP from CaLCuV (Fontes *et al.*, 2004) and with NSPs from the tomato-infecting geminiviruses TGMV (tomato golden mosaic virus; Fontes *et al.*, 1994; Mariano *et al.*, 2004), TCrLYV (tomato crinkle leaf yellows virus; Galvão *et al.*, 2003; Mariano *et al.* 2004), and ToYSV (tomato yellow spot virus; Andrade *et al.*, 2006; Carvalho *et al.*, 2008). Several

lines of evidence indicate that NIK functions in defence. NSP from CaLCuV acts as a virulence factor to suppress the kinase activity of transmembrane receptor NIKs (Fontes *et al.*, 2004). Secondly, loss of NIK1, NIK2, or NIK3 function in *Arabidopsis* is also linked to an enhanced susceptibility phenotype to CaLCuV infection (Fontes *et al.*, 2004; Rocha *et al.*, 2008; Santos *et al.*, 2009). In addition, overexpression of NIK1 from *Arabidopsis* in tomato plants attenuates symptom development and delays ToYSV infection (Carvalho *et al.*, 2008).

Recent progress towards elucidating the NIK-mediated antiviral signalling includes the identification of the ribosomal protein L10 (rpL10) as the immediate downstream effector in the pathway (Carvalho *et al.*, 2008; Rocha *et al.*, 2008). Phosphorylation of rpL10 by NIK promotes translocation of the ribosomal protein to the nucleus where it may function to mount a defence response that negatively impacts virus infection. The bipartite geminivirus NSP suppresses NIK activity through specific binding to the kinase domain and enhances geminivirus pathogenicity. Upon geminivirus infection, rpL10 is trapped within the cytoplasm to prevent the establishment of a host environment that disfavours virus proliferation and/or spread.

Components of the NIK-mediated signalling pathway

Recent research has provided new insights into the components and regulatory mechanisms of the NIK-mediated signalling pathway. The ribosomal proteins L10 (rpL10) and L18 (rpL18) were both isolated through two-hybrid screening by their capacity to bind to the kinase domain of NIK1 (Rocha *et al.*, 2008). However, only rpL10 has been shown to be biochemically and genetically linked to the NIK signalling pathway. The ribosomal protein L10 is phosphorylated by NIK1, NIK2, and NIK3 *in vitro* and by NIK1 *in vivo*, whereas rpL18 is not an NIK substrate (Carvalho *et al.*, 2008; Rocha *et al.*, 2008). Phosphorylation of rpL10 by NIK1 redirects the ribosomal protein to the nucleus, while rpL18 remains in the cytoplasm when co-expressed with NIK1 in tobacco leaves (Carvalho *et al.*, 2008). Furthermore, loss of rpL10 function, but not rpL18 function, increases susceptibility to geminivírus infection, recapitulating the *nik1* null allele phenotype (Rocha *et al.*, 2008).

Despite the observation that rpL18 does not serve as an immediate effector of NIK signalling, rpL18 binds to the kinase domain of NIK1 with high affinity. Different rounds of two-hybrid screenings of distinct *Arabidopsis* cDNA libraries for NIK1-interacting partners resulted in the independent isolation of rpL18 genes with high frequency [four rpL18 cDNA clones from screening 1×10^5 clones from a leaf cDNA library (Rocha *et al.*, 2008) and seven rpL18 cDNA clones from screening 6×10^5 clones from a seedling cDNA library (unpublished)]. Binding of rpL18 to NIK does not affect its kinase activity, but competes efficiently with rpL10 binding. These results raise the question as to whether rpL18 functions as a negative regulator of the NIK-mediated signalling pathway or whether NIK prevents rpL18 from functioning as a positive contributor in viral infection. The latter hypothesis cannot be ruled out simply by the observation that inactivation of the *rpL18aB* gene (T-DNA insertion in At2g34480) does not alter geminivirus infection (Rocha *et al.*, 2008) because the *Arabidopsis* genome encodes three rpL18 homologues (*rpL18aA*, *rpL18aB*, and *rpL18aC*) which might function redundantly in virus infection (Barakat *et al.*, 2001).

NIK as a kinase receptor

The C-terminal kinase domain of NIKs contains all of the 11 conserved subdomains of protein kinases, in addition to specific signatures of serine/threonine kinases in subdomains V1b and VIII (Hanks *et al.* 1988). NIK exhibits trans-autophosphorylation activity *in vitro* and substrate phosphorylation activity *in vitro* and *in vivo* (Fontes *et al.*, 2004; Carvalho *et al.*, 2008; Rocha *et al.*, 2008). Mutations in the activation loop (A-loop) of NIK that block autophosphorylation activity also impair the capacity of NIK to elicit a defence response against geminivirus (Santos *et al.*, 2009), establishing that it is an authentic defence signal transducer. Likewise, a perfect correlation has been found between the kinase activity of NIK and the efficiency of NIK-driven nuclear relocation of rpL10. A constitutively active mutant of NIK is more effective at redirecting rpL10 to the nucleus, and inactive mutants of NIK fail to change the cytosolic localization of rpL10. In summary, mutations in the A-loop similarly affect the capacity of NIK to elicit an antiviral response and to mediate a phosphorylation-dependent nuclear relocalization of the rpL10 downstream component. This positive correlation is consistent with the notion that the regulated nucleocytoplasmic shuttling of rpL10 links the antiviral response to receptor activation.

Possible mechanisms of NIK activation

As a single-pass transmembrane receptor kinase, NIK is expected to dimerize or multimerize with itself and/or co-receptors to promote transphosphorylation and subsequent activation of the kinase. In mammalian and plant cells, the oligomerization of single-pass transmembrane receptor kinases has been proposed to be either induced or stabilized by ligands as the critical early event that triggers signalling and transduction from the receptor (Schlessinger 2000; Vert *et al.*, 2005; Hubbard and Miller, 2007; Kim and Wang, 2010). However, there is a complete lack of information with respect to the nature and identity of possible ligands, stimuli, or mechanisms that trigger or stabilize NIK dimerization or multimerization with a co-receptor. Because NIK functions in geminivírus infection (Carvalho *et al.*, 2008; Rocha *et al.*, 2008), the virus itself could be interpreted as a stimulus that activates the NIK signalling pathway, although the molecular basis for viral infection-induced receptor activation is currently unknown.

BAK1/SERK3, the best characterized member of the LRRII subfamily of RLKs, functions as a co-receptor of BRI1 in brassinosteroid signalling and of FLS2 in the plant innate immunity response (for a review, see Chinchilla *et al.*, 2009). This scenario of BAK1 functioning as a co-receptor of LRR-RLK receptors resembles the activation mechanism of epidermal growth factor (EGF) receptors (Schlessinger, 2002). EGF receptor type I, ERBB1, exists as pre-formed dimers but depends on ligand-induced hetero-oligomerization with ERBB2 for full activation of the EGF signalling response. SERK1 also dimerizes with BRI1 *in vitro* and *in vivo*, and SERK4, also designated BAK7, forms a complex with BAK1 or BRI1 in transducing developmental signals (Karlova *et al.*, 2006, He *et al.*, 2007; Albrecht *et al.*, 2008; Jeong *et al.*, 2010). From these recent studies with SERK-like co-receptors, a common theme is that the five LRRs-containing RLKs function as co-receptors for ligand-binding LRR-RLKs in a stimulus-dependent fashion. As members of the LRR-RLKII subfamily, NIKs are likely to target an LRR-RLK-mediated signalling pathway as well, although an LRR-RLK partner of NIKs has yet to be identified.

Activation of many kinases requires phosphorylation of the activation segment that is defined by the region delimited by two conserved tripeptide motifs, DFG and APE (Nolen *et al.*, 2004). This region is highly conserved among members of the LRR-RLKII subfamily and other members of the extended LRR-RLK family, such as BRI1, which belongs to the LRR-RLK23 subfamily. For some of these receptors, such as NIK1, BAK1, and BRI1, the phosphorylation status of the activation segment has been shown to dictate their kinase activity (Carvalho *et al.*, 2008; Wang *et al.*, 2008; Santos *et al.*, 2009; Yun *et al.*, 2009). NIK1 is phosphorylated *in vitro* at the conserved positions Thr474 and Thr469 (Santos *et al.*, 2009). NIK1 Thr474 aligns at the same position as SERK1 Thr468 and SERK3/BAK1 Thr455, which may be functionally analogous phosphorylation sites. SERK1 Thr468 is absolutely essential for *in vitro* kinase activity (Shah *et al.*, 2001), and BAK1 Thr455 plays a critical role in BAK1 signalling (Wang *et al.*, 2008; Yun *et al.*, 2009). In addition to being phosphorylated *in vitro*, replacement of Thr474 with alanine impairs the capacity of NIK1 to elicit a defence response and to redirect rpL10 to the nucleus *in vivo* (Carvalho *et al.*, 2008; Santos *et al.*, 2009). In contrast, replacement of Thr474 with a phosphomimetic aspartate residue increases autophosphorylation activity and NIK-mediated relocalization of rpL10 to the nucleus. Taken together, these results support the notion that phosphorylation at the essential Thr474 residue within the A-loop constitutes a key regulatory mechanism for NIK activation. Thus, the NIK1 residue Thr474 is functionally equivalent to the corresponding BAK1 Thr455 and BRI1 Thr1049, which align at the same position in their respective A-loop and have been shown to be required for kinase function and signalling *in planta* (Wang *et al.*, 2005, 2008; Santos *et al.*, 2009).

Further support for the notion that autophosphorylation at Thr474 is a key event that promotes kinase activation arose from studies of the inhibitory effect of geminivírus NSP on NIK mutant proteins (Santos *et al.*, 2009). The NSP-binding site corresponds to an 80 amino acid stretch (positions 422–502) of NIK that encompasses the putative active site for Ser/Thr kinases (subdomain VIb–HrDvKssNxLLD) and the A-loop (subdomain VII–DFGAk/rx, plus subdomain VIII–GtxGyiaPEY; Fontes *et al.*, 2004). Binding of NSP to NIK inhibits 50% of its kinase activity and promotes a further 50% inhibition of the residual activity of A-loop alanine substitution mutants but not the phosphomimetic T474D mutant (Santos *et al.*, 2009). In this case, replacement of the essential Thr474 residue with an aspartate residue does not impair NSP binding but bypasses its inhibitory effect on kinase activity. These results suggest that the NSP inhibitor acts upstream of the phosphorylation at position 474.

While phosphorylation at Thr474 is linked to an A-loop-dependent mechanism for NIK function, phosphorylation of Thr469 appears to have an autoinhibitory role (Santos *et al.*, 2009). Replacing Thr469 with alanine relieves repression and enhances substrate phosphorylation. Furthermore, mutation at Thr469 does not inhibit autophosphorylation activity or impair the capacity of the mutant protein to elicit a defence response and to redirect rpL10 to the nucleus. This phosphorylation scheme for kinase regulation opens up an unprecedented view for negative regulation of kinase activity through phosphorylation of conserved sites within the A-loop of plant RLKs. Although phosphorylation of threonine residues within the A-loop has been demonstrated for SERK1 *in vitro* and for BRI1 and BAK1 *in vivo*, the functionally relevant phosphorylated residues on these LRR-RLKs positively regulate kinase activity (Shah *et al.*, 2001; Wang *et al.*, 2005, 2008). In the case of NIK, it has been proposed that autophosphorylation of Thr469 within the NIK1 A-loop negatively controls rpL10 phosphorylation and hence allows the kinase to control the extent of the response in a sustained signalling pathway more efficiently. Whether this inhibitory mechanism is specific for the rpL10 substrate, providing NIK1 with the capacity to phosphorylate pathway components differentially, remains to be determined. In the case of BAK1, the A-loop residue Thr450, which is equivalent to NIK1 Thr469 (Fig. 1), plays independent and separate roles in brassinosteroid signalling and flagellin signalling (Wang *et al.*, 2008).

NIK-mediated antiviral signalling pathway

Based on current data and common features of the LRR-RLKII family, a mechanistic model is proposed for a NIK-mediated defence signalling pathway and its interaction with the geminivirus NSP (Fig. 2). In this model, upon unidentified stimuli, the LRR extracelullar domain undergoes oligomerization, allowing the intracellular kinase domains to transphosphorylate and to activate one another. Alternatively or additionally, NIK may serve as a co-receptor for a defence signalling cascade and interacts with an unidentified

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                                          A-Loop
                                        465 468 469  474
AT1G71830_SERK1        EEFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
AT4G33430_SERK3/BAK1   EEFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
AT1G34210_SERK2        EEFEAVVGDFGLARLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
AT2G13790_SERK4        EEFEAVVGDFGLAKLMNYNDSHVTTAVRGTIGHIAPEYLSTGKSSEKTD
AT2G13800_SERK5        EEFEAVVGDFGLAKLMNYNDSHVTTAVRGTIGHIAPEYLSTGKSSEKTD
AT5G16000_NIK1         DYCEAVVGDFGLAKLLDHQDSHVTTAVRGTVGHIAPEYLSTGQSSEKTD
AT3G25560_NIK2         DYFEAVVGDFGLAKLLDHEESHVTTAVRGTVGHIAPEYLSTGQSSEKTD
AT1G60800_NIK3         EDFEAVVGDFGLAKLLDHRDSHVTTAVRGTVGHIAPEYLSTGQSSEKTD
AT2G23950              EYFEAVVGDFGLAKLLNHEDSHVTTAVRGTVGHIAPEYLSTGQSSEKTD
AT4G30520              ECFEAVVGDFGLAKLLNHADSHVTTAVRGTVGHIAPEYLSTGQSSEKTD
AT5G45780              ESFEAIVGDFGLAKLLDQRDSHVTTAVRGTIGHIAPEYLSTGQSSEKTD
AT5G10290              EDFEAVVGDFGLAKLVDVRRTNVTTQVRGTMGHIAPEYLSTGKSSERTD
AT5G65240              EDFEAVVGDFGLAKLVDVRRTNVTTQVRGTMGHIAPECISTGKSSEKTD
AT5G63710              NNFEPVLGDFGLAKLVDTSLTHVTTQVRGTMGHIAPEYLCTGKSSEKTD
                       :  *.::******:*::   ::*** ****:****** :.**:***:**
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Fig. 1. Sequence alignment of the activation segment (boxed) among NIKs and other members of the LRR-RLKII subfamily. The activation segment of NIK1 was compared with the indicated members of the *Arabidopsis* LRR-RLKII subfamily. Conserved residues as potential phosphorylation sites are shown in bold and the numbering scheme refers to the NIK primary structure.

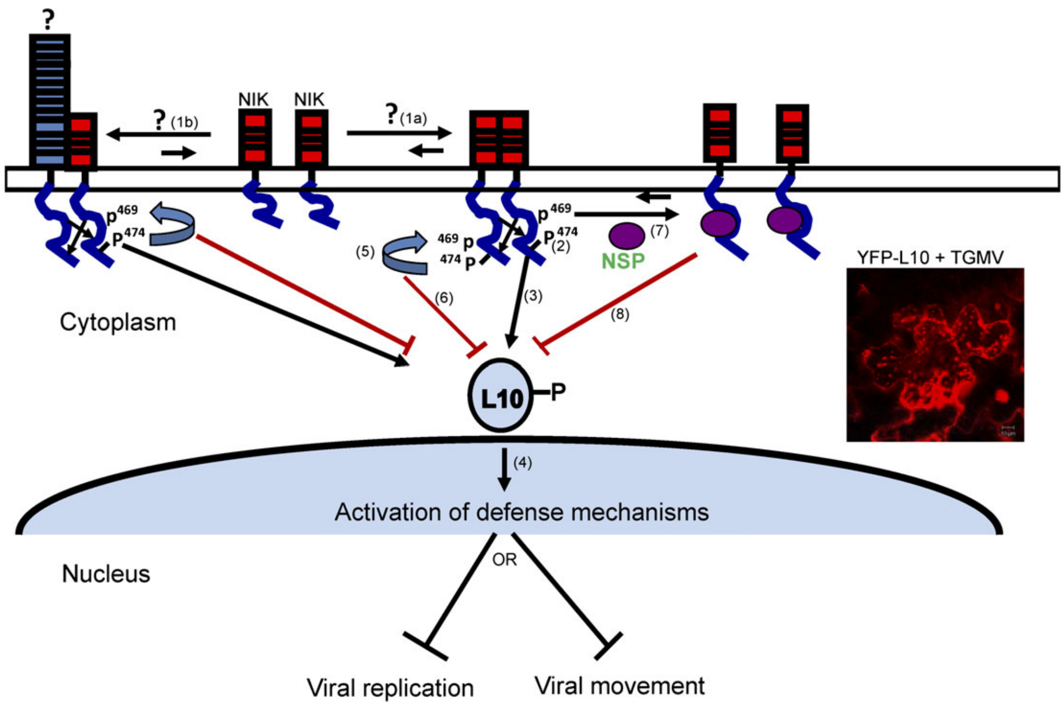

Fig. 2. Mechanistic model for the NIK-mediated defence signalling pathway. Stress-induced oligomerization of the extracellular domain of NIK (1a) brings the intracellular kinase domains into proximity and allows them to transphosphorylate Thr474 and activate one another (2). Alternatively, NIK interacts with an unknown ligand-binding LRR-RLK in a stimulus-dependent manner (1b). Upon activation, NIK phosphorylates rpL10 (3) promoting its translocation to the nucleus (4), where it may mount a defence strategy that prevents virus proliferation and/or movement. Autophosphorylation of Thr474 also leads to phosphorylation of Thr469 (5), which then inhibits substrate phosphorylation (6). This two-component phosphorylation scheme provides a mechanism by which NIK can control the extent of substrate phosphorylation during sustained NIK-mediated signalling. Conversely, binding of NSP to the NIK kinase domain (A-loop) inhibits autophosphorylation at Thr474 (7) and prevents receptor kinase activation and rpL10 phosphorylation (8), trapping rpL10 in the cytoplasm of infected cells (see the punctuate bodies in the insert).

ligand-dependent LRR-RLK receptor in response to virus infection. In this scenario, the active kinase then recruits and phosphorylates the downstream component rpL10 to propagate the defence signalling cascade that impairs virus replication and/or movement. Regulation of kinase activity may be dictated by two components: a conserved Ser/Thr kinase activation component that results from autophosphorylation of Thr474 and an inhibitory component at a distinct residue (Thr469) within the A-loop that down-regulates substrate phosphorylation. Phosphorylation at Thr474 activates the kinase and may precede phosphorylation at Thr469, providing a mechanism for NIK to control

the extent of substrate phosphorylation in a sustained signalling response. This stepwise pattern of phosphorylation within the A-loop of NIK1 is supported by the observation that tandem mass spectrometry (MS/MS) analysis of *in vitro* phosphorylated T469A-derived tryptic fragments reveals that Thr474 can be phosphorylated in this mutant protein, whereas phosphorylation at Thr469 has not been detected in the *in vitro* phosphorylated T474A mutant protein (Santos *et al.*, 2009, data not shown).

NSP counters activation of the pathway by binding to the NIK kinase domain and sterically interfering with phosphorylation of Thr474 in the A-loop. As a consequence, phosphorylation of the substrate rpL10 is impaired and the ribosomal protein is trapped in the cytoplasm during geminivirus infection (see confocal microscopy of fluorescent rpL10 in infected cells, as an insert in Fig. 2; Carvalho *et al.*, 2008). NSP inhibition of NIK1 prevents activation of the NIK-mediated signalling pathway, resulting in an intracellular environment that is more favourable to virus proliferation and spread.

In addition to questions about the mechanistic model of NIK-mediated defence response indicated in Fig. 2, it is not known how phosphorylated rpL10A delays the onset of virus infection. Yeast rpL10A is required for joining of the 40S and 60S subunits (Eisinger *et al.*, 1997) and for large subunit nuclear export through direct interaction with Nmd3p, a NES (nuclear export signal)-containing protein that is specifically associated with 60S subunits (Gadal *et al.*, 2001). By analogy with the yeast rpL10A homologue, perturbation of *Arabidopsis* rpL10A nucleocytoplasmic trafficking by NIK1 would interfere with ribosome subunit assembly and 60S subunit export from the nucleus, which would affect general translation and impair virus infection. Alternatively, putative rpL10 extraribosomal functions associated with regulation of transcriptional factors (Monteclaro *et al.*, 1993; Imafuku *et al.*, 1999; Oh *et al.*, 2002), translational control of gene expression (Karl *et al.*, 1999), and suppression of cell proliferation (Chávez-Rios *et al.*, 2003) could serve as potential host defence strategies against virus. The identification of downstream targets of rpL10A is crucial to decipher this layer of innate defence.

Are NIK-like and SERK-like receptors functionally redundant?

NIKs and SERKs belong to the same subfamily and it is likely that they functionally overlap to modulate development and defence signalling pathways. In fact, partial redundancy has been demonstrated for the more related SERK-like members (Chinchilla *et al.*, 2009; Jeong *et al.*, 2010). Several members of the SERK-like group, such as SERK1, BAK1/SERK3, and BAK7/SERK4, interact with BRI1 *in vivo* (Li *et al.*, 2002; Nam and Li, 2002; Karlova *et al.*, 2006, Jeong *et al.*, 2010) and partially substitute for one another in brassinosteroid signalling (Karlova *et al.*, 2006; Jeong *et al.*, 2010). BAK1 and BAK7 also show functional redundancy in cell death events (Jeong *et al.*, 2010). Although *in vivo* interaction between NIKs and BRI1 or SERK3/BAK1 has not been addressed, manipulation of NIK transcript levels results in developmental phenotypes that antagonize BRI1-associated phenotypes. The developmental phenotype of *nik* knockout lines resembles that of BRI1-overexpressing lines, and vice versa (see Supplementary Fig. S1 available at *JXB* online). The *nik* knockouts, *nik1*, *nik2*, and *nik3*, display longer and narrower leaves and more elongated petioles than wild-type leaves grown under short-day conditions (Supplementary Fig. S1A), resembling plants that overexpress BRI1 or its co-receptor BAK1 (Jeong *et al.*, 2010). Overexpression of NIK1 in tomato plants causes a reduction in root growth (Supplementary Fig. S1B) in contrast to BRI1-overexpressing lines that display increased root growth. These results show that an inverse correlation between BRI1 and NIK levels causes similar developmental phenotypes and, as such, NIK transcripts may impact negatively some aspect of BRI1-related growth, as opposed to the BAK function in brassinosteroid signalling.

With respect to their defence roles, it has been established that BAK1 and SERK1 cannot functionally replace NIK1 in transducing an antiviral signalling response. Loss of BAK1 and SERK1 function does not enhance susceptibility to geminivirus infection and SERK1 does not complement the enhanced susceptibility phenotype of *nik1* knockouts to geminivirus infection (Fontes *et al.*, 2004; Supplementary Fig. S2A at *JXB* online). In addition, rpL10, the downstream effector of NIK-mediated antiviral signalling, is not phosphorylated by BAK1 or SERK1 (Supplementary Fig. S2B) and is not redirected to the nucleus by these receptors (Carvalho *et al.*, 2008). Whether the receptors would function redundantly in pattern recognition receptor (PRR)-dependent signalling, which has been shown to be mediated by BAK1, remains to be determined.

Conclusion

Since the discovery of NSP-interacting kinases, several features of the NIK-mediated antiviral signalling have been elucidated (Fig. 2). It is now known that the transmembrane receptor NIK1, an authentic serine/threonine kinase transducer, must be activated to trigger a defence response against virus. Regulation of NIK kinase activity depends on a conformational change of the A-loop induced by phosphorylation of Thr474. Activated NIK regulates nucleocytoplasmic trafficking of rpL10, linking the antiviral response to receptor activation. However, major players in the defence pathway are still missing and important questions remain unanswered. What is the stimulus or molecular signal that triggers NIK activation? Does NIK serve as co-receptor for LRR-RLKs? What are the molecular events downstream of rpL10 that defend against virus infection? If the phosphomimetic T474D mutant supports a sustained NIK signalling, comparison of T474D-induced global variation in gene expression with the virus-induced transcriptome may provide insight into the signals that

trigger signalling. Alternatively, characterization of ligand-binding receptors that interact with NIK may lead to the identification of the trigger. Finally, the identification of downstream targets of rpL10A will be crucial to unravel this layer of innate defence.

Current knowledge of the transmembrane NIK receptors and their possible functional overlap with other members of the LRR-RLKII subfamily is still rudimentary. Here it is shown that SERK-like receptors cannot replace NIK1 in the antiviral signalling response. However, the observation that some developmental phenotypes of *nik* null alleles and NIK overproducers are opposite to BRI1-associated phenotypes suggests that NIK1 may be involved in the BRI1-dependent developmental pathway. The molecular basis for such cross-talk is yet to be demonstrated.

Supplementary data

Supplementary data are available at *JXB* online.

Figure S1. Developmental phenotypes associated with manipulation of NIK transcript levels.

Figure S2. SERK-like receptors cannot substitute for NIK in antiviral signalling.

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