NIK1, a host factor specialized in antiviral defense or a novel general regulator of plant immunity?

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NIK1 is a receptor-like kinase involved in plant antiviral immunity. Although NIK1 is structurally similar to the plant immune factor BAK1, which is a key regulator in plant immunity to bacterial pathogens, the NIK1-mediated defenses do not resemble BAK1 signaling cascades. The underlying mechanism for NIK1 antiviral immunity has recently been uncovered. NIK1 activation mediates the translocation of RPL10 to the nucleus, where it interacts with LIMYB to fully down-regulate translational machinery genes, resulting in translation inhibition of host and viral mRNAs and enhanced tolerance to begomovirus. Therefore, the NIK1 antiviral immunity response culminates in global translation suppression, which represents a new paradigm for plant antiviral defenses. Interestingly, transcriptomic analyses in *nik1* mutant suggest that NIK1 may suppress antibacterial immune responses, indicating a possible opposite effect of NIK1 in bacterial and viral infections.

Keywords:
begomoviruses; immune receptor; immune responses; NIK1; NSP-interacting kinase; plant antiviral immunity; translation suppression

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Abbreviations:
ETI, effector-triggered immunity; **LRR-RLK,** leucine-rich repeats receptor-like kinases; **PAMP,** pathogen-associated molecular pattern; **PRR,** pattern recognition receptor; **PTI,** PAMP-triggered immunity; **RLK,** receptor-like kinase; **RP,** ribosomal protein; **SA,** salicylic acid; **Ser,** serine; **Thr,** threonine.

Introduction

Plants depend on their immune system to detect pathogens and activate defenses against invaders. The plant innate immune system employs a perception and defense system at two levels [1]. At the first level of defense, PAMP-triggered immunity (PTI) is mediated by pattern recognition receptors (PRRs), which perceive and recognize pathogen-associated molecular patterns (PAMPs) that are presented by the pathogens [2]. So far, plant PRRs are receptor-like kinases (RLKs) or receptor-like proteins (RLPs) located at the cell surface [3]. The second level, effector-triggered immunity (ETI), involves intracellular immune receptors, designated as resistance proteins (R), which recognize – directly or indirectly – virulence effectors secreted by the pathogens into the host intracellular environment, thereby activating a defense response [4]. Plants use the second level of defense to fight virus [5, 6]. An emerging picture is the capacity of plants to trigger PTI against viruses [7, 8]. Furthermore, the pre-activation of PTI by the elicitor chitosan through interaction with chitin-binding PRRs has also been shown to be effective against viruses [9]. Two kinds of chitin elicitor-binding PRRs have been identified: a RLP, in rice, and a RLK, in Arabidopsis, which share chitin binding-extracellular lysine motifs (LysMs).

The well-characterized co-receptor of the plasma membrane-associated PRRs, designated BRASSINOSTEROID INSENSITIVE1 (BRI1)-associated kinase 1, BAK1, has been shown to be involved in antiviral immunity [7, 8]. Another immune receptor, nuclear shuttle protein (NSP)-interacting kinase 1 (NIK1), which is also located in the plasma membrane and is structurally similar to BAK1, is also involved in antiviral immunity. Nevertheless, we have shown recently that the mechanism underlying the antiviral function of NIK1 is totally different from the classical BAK1-mediated PTI. Here, we discuss the possibility that NIK1 may antagonize PTI by interacting negatively with the classical mechanism of plant anti-bacterial immunity. If this is the case, NIK1 may function as a negative co-receptor in suppressing translation and the

plant immune system through distinct branches of the NIK1-supported signaling pathways.

Like BAK1, NIK1 belongs to subfamily II of the leucine-rich repeats (LRR) receptor-like kinases (RLK)

The RLK superfamily consists of the largest group of plant receptors, comprising more than 600 members in Arabidopsis, tomato, and soybean [10–12] and more than 1,000 representatives in rice [13]. Members of the RLK superfamily are involved in development and/or defense responses against a wide range of pathogenic agents [14–16]. RLKs are structurally organized into a receptor configuration with a peptide signal, a transmembrane segment that connects a variable extracellular domain with the capacity to interact with a specific ligand to a cytosolic kinase domain that phosphorylates threonine/serine residues and, in some cases tyrosine residues, on protein substrates [15, 17, 18]. RLK-mediated signaling is frequently initiated by a ligand-dependent dimerization or oligomerization of the receptor. The immune receptor RLKs (PRRs) recognize conserved molecular signatures characteristics of a class of pathogens (PAMPs) or endogenous danger signals released by the host during a wound or pathogenic attack (DAMPs), which function as elicitors. Upon elicitor binding, the PRR undergoes dimerization/oligomerization with a co-receptor [3]. The RLK heterodimers are then auto- or trans-phosphorylated by their cytosolic kinase domains, leading to activation, recognition, and phosphorylation of the downstream components of the signaling pathway [19].

The RLK superfamily is divided into approximately 50 families, based on a phylogenetic analysis of the kinase domain, by which the RLKs with structurally similar extracellular domains were clustered together defining the families [20]. Among them, the largest family comprises RLKs, with a leucine-rich repeat (LRR) extracellular domain that is represented by more than 200 copies in the Arabidopsis genome [12, 21]. The LRR-RLK family is further subdivided into 13 subfamilies based on sequence identity, number (3–26 LRRs) and disposition of the LRR motifs in the extracellular domain. Subfamily II of LRR-RLK (LRRII-RLK) comprises 14 receptors, which possess four complete LRR motifs (with 24 amino acid residues) and a fifth incomplete LRR motif (with 16 amino acid residues), arranged in a unique contiguous block in the extracellular domain [22]. Phylogenetic analysis of this subfamily clustered the 14 components into three distinct clades: (i) antiviral defense receptors; (ii) development and defense receptors; and (iii) receptors of unknown function [22].

Subgroup II of the LRRII-RLK subfamily, involved in development and defense, is formed by the five members of the *SOMATIC EMBRYOGENESIS RECEPTOR KINASE* (*SERK1-5*) gene family, which play different functions in male sporogenesis, response to brassinosteroids (BRs), and in the control of PTI and cell death [23]. BAK1, also designated AtSERK3, is the best characterized member of this subfamily of receptors. BAK1 functions as a co-receptor of several plasma membrane-tethered ligand-dependent LRR-RLKs and thereby participates actively in distinct signaling pathways. BAK1 contributes to plant development through interactions with BRI1, an LRR-RLK that perceives the signal of the BR phytohormone [24, 25]. The BAK1-BRI1 heterodimerization initiates signaling for BR- induced developmental responses [26]. In addition to participating in BR signaling, BAK1 also plays a role in plant innate immunity against many classes of pathogens as a co-receptor through interactions with flagellin sensing 2 (FLS2) receptor, elongation factor Tu receptor (EFR) or pep one receptor 1 (PEPR1), which perceive specific PAMPs and trigger PTI [3, 27–30]. The BAK1 positive regulation in plant immunity and in BR signaling is underscored by phosphorylation reactions between the BAK1 co-receptor and the corresponding receptor.

NIK1, *NIK2*, and *NIK3* form a gene family belonging to group I of the LRRII-RLK subfamily, which is involved in antiviral defenses [22]. NIKs were first identified as virulence targets of the NSP of bipartite geminiviruses (begomoviruses) [31]. The NSP-NIK interaction is conserved among begomovirus NSPs and NIK homologs from distinct hosts. NIK homologs from Arabidopsis, tomato, and soybean interact with NSP from *Cabbage leaf curl virus* (CaLCuV) and from tomato-infecting begomoviruses, such as *Tomato golden mosaic virus* (TGMV), *Tomato crinkle leaf yellow virus* (TCrLYV), and *Tomato yellow spot virus* (ToYSV) [11, 31, 32]. These interactions inhibit the NIK kinase activity and prevent the activation of the signal transduction pathway that would trigger an antiviral defense response, creating a host environment enabling begomovirus infection [33, 34].

The NIK1/RPL10 module transduces a defense response to begomoviruses

The transmembrane receptor NIK1 was isolated through two-hybrid screening on account of its capacity to bind to the viral protein NSP, and the interaction of NIK1-NSP was further confirmed by in vitro binding assays [31, 32]. More recently, the interaction between NSP from CaLCuV and NIK1 from Arabidopsis was confirmed in planta through bimolecular fluorescence complementation (BiFC) assays, which located the NIK1-NSP complex in the cell periphery [35]. The NSP-binding site was mapped to an 80 amino acid stretch (positions 422–502) of NIK1 that encompasses the putative active site for Ser/Thr kinases (subdomain VIb–HrDvKssNxLLD) and the activation loop (subdomain VII–DFGAk/rx, plus subdomain VIII–GtxGyiaPEY) [31].

The viral NSP is encoded by the component B, DNA-B, of bipartite begomoviruses (*Geminiviridae* family) that also encodes the movement protein (MP), both viral proteins required for systemic infection [36]. A second genomic component, DNA-A, encodes all of the functions required for viral replication, transcriptional activation of viral genes, suppression of siRNA-mediated plant defenses, and encapsidation of the viral genomes as single-stranded DNA circles in twinned isometric particles. Begomoviruses replicate their genome in the nuclei of infected plants via rolling circle replication. NSP binds to the nascent viral DNA in the nucleus

and facilitates the intracellular trafficking of viral DNA between the nucleus and the cytoplasm, whereas MP potentiates its cell-to-cell movement. In addition to encoding suppressors for siRNA-mediated defenses, begomoviruses enhance their pathogenicity in susceptible hosts through the NSP-mediated suppression of the antiviral activity of the NIK1 receptor [31, 33, 35].

In addition to the virulence function of NSP as an NIK suppressor, several other lines of evidence indicate that NIK1 functions in antiviral defenses. First, loss of *NIK1*, *NIK2*, or *NIK3* function in Arabidopsis is linked to an enhanced susceptibility phenotype to CaLCuV infection [31, 33, 37]. Second, overexpression of *NIK1* from Arabidopsis in tomato plants attenuates symptom development and delays ToYSV infection [38]. Finally, as an authentic defense signal transducer, mutations in the activation loop (A-loop) of NIK1 that block autophosphorylation activity also impair the capacity of NIK1 to elicit a defense response against begomoviruses [33].

As Ser/Thr kinase receptors, NIKs contain all of the 11 conserved subdomains of protein kinases, in addition to specific signatures of serine/threonine kinases in subdomains VIb and VIII [39]. NIK1 exhibits trans-autophosphorylation activity in vitro and substrate phosphorylation activity in vitro and in vivo [31, 37, 38]. NIK1 is phosphorylated in vitro at the conserved positions Thr-474 and Thr-469, and mutations within the A-loop interfere in the NIK1 capacity of autophosphorylation [33]. Replacement of Thr-474 with alanine strongly inhibits the autophosphorylation activity, whereas replacement of Thr-474 with a phosphomimetic aspartate residue increases autophosphorylation activity and results in the constitutive activation of the NIK1 mutant receptor that it is no longer inhibited by begomovirus NSP [33]. Consistent with these findings, ectopic expression of the Arabidopsis phosphomimetic T474D mutant in tomato transgenic lines confers a higher level of tolerance to tomato-infecting begomoviruses than expression of an intact *NIK1* receptor [35]. In contrast, ectopic expression of loss-of-function Thr-474 mutants, such as T474A or the double mutant G4743V/T474A, did not reverse the enhanced susceptibility phenotype of *NIK1* knockout lines, demonstrating that Thr-474 autophosphorylation is required to transduce a defense response to begomoviruses [33]. Collectively, these results support the notion that phosphorylation at the essential Thr-474 residue within the A-loop constitutes a key regulatory mechanism for NIK1 activation.

The ribosomal protein L10 (RPL10) was isolated through two-hybrid screening by its capacity to bind to the kinase domain of NIK1 [37], and was biochemically and genetically linked to the NIK1 signaling pathway [33, 37, 38]. RPL10 is localized in the cytoplasm, but is redirected to the nucleus by co-expression with NIK1. Although RPL10 binds to NIK1 in vitro and in vivo, it is not efficiently phosphorylated by NIK1 in vitro and may not serve as a direct NIK1 substrate in vivo. Nevertheless, the nucleocytoplasmic shuttling of RPL10 is regulated by phosphorylation and is dependent on the kinase activity of NIK1. In fact, NIK1 does not relocate a phosphorylation-deficient mutant of RPL10 to the nucleus [38]. Furthermore, the gain-of-function T474D mutant is more effective at redirecting RPL10 to the nucleus, and inactive mutants of NIK1 fail to change the cytosolic localization of RPL10 [33]. Mutations in the A-loop similarly affect the NIK1 capacity to elicit an antiviral response and to mediate a phosphorylation-dependent nuclear relocalization of the RPL10 downstream component.

The mechanism of NIK1-mediated antiviral defense is underscored by suppression of host global translation: A new paradigm for antiviral defenses in plants

Since the discovery of the NIK1 receptor [32] and the downstream component RPL10 [37], progress toward deciphering this layer of plant defense has been limited for two major reasons. First, the NIK1-mediated antiviral signaling represents a resistance response evolutionarily overcome by a begomovirus virulence strategy. Therefore, in this compatible interaction, triggering the signaling pathway also elicits the side effects of virus infection, because the viral protein NSP suppresses defense to cause disease. Second, the complete lack of knowledge of the critical early events that elicit signaling and transduction from the receptor does not allow us to trigger the pathway in a controlled manner. These difficulties were recently overcome by replacing the *NIK1* gene with the constitutively activated T474D mutant in Arabidopsis and analyzing the T474D-induced changes in gene expression as the signature of a sustained NIK1 signaling pathway in the absence of virus infection [40]. Constitutive activation of NIK1 down-regulates components of the translational machinery and, thereby, causes suppression of global translation, decreasing the loading of host mRNAs in actively translating polysomes (PS). Induction of T474D expression through a dexamethasone-inducible promoter also impairs global translation, which is accompanied by a reduction in PS and monosome (NPS) fractions as well as in the RNA content associated with these fractions in the T474D lines. The transgenic lines ectopically expressing T474D were tolerant to CaLCuV, a phenotype that is associated with a symptomless infection, lower infection efficiency, reduced accumulation of viral DNA in systemic leaves, and reduced loading of coat protein viral mRNA in actively translating polysomes. Therefore, begomovirus was not capable of sustaining high levels of viral mRNA translation in the T474D-expressing lines, indicating that suppression of global protein synthesis may effectively protect plant cells against DNA viruses (see also comments by Nicaise [41]). Consistent with this argument, overexpression of T474D in tomato represses ribosomal protein genes, suppresses global protein synthesis, decreases viral mRNA association with polysome fractions, and recapitulates the enhanced resistance phenotype of the Arabidopsis T474D transgenic lines [35]. The T474D-overexpressing tomato transgenic lines were tolerant to ToYSV and *Tomato severe rugose virus* (ToSRV), which display highly divergent genomic sequences and hence are phylogenetically separated within the two major groups of begomoviruses found in Brazil [42]. These observations demonstrate the potential of a sustained NIK1-mediated defense pathway to confer broad-spectrum tolerance to begomoviruses in distinct plant species.

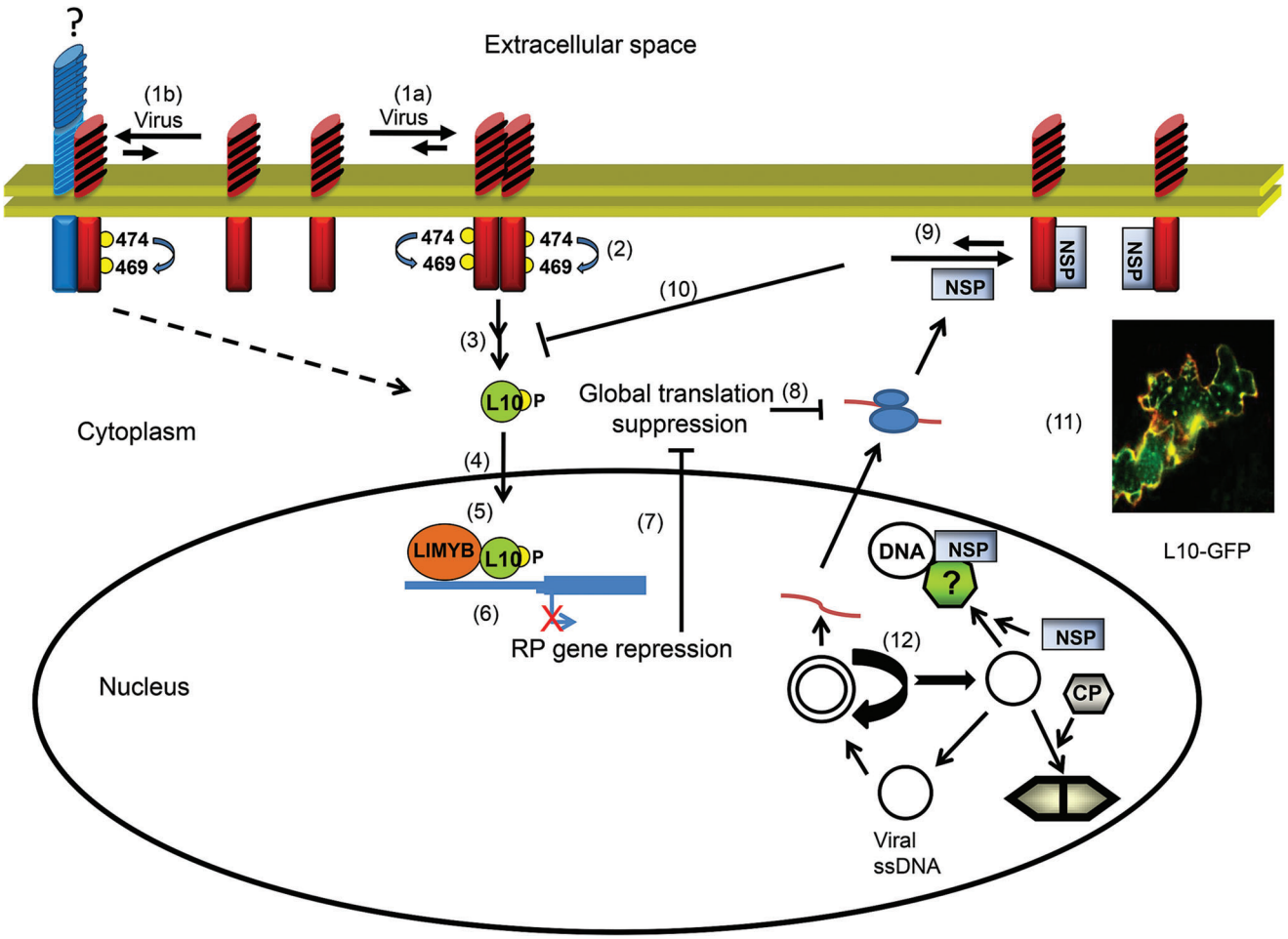

Figure 1. Mechanistic model for the NIK1-mediated antiviral signaling pathway. Virus infection-induced oligomerization of the extracellular domain of NIK1 (1a) brings the intracellular kinase domains into proximity and allows them to transphosphorylate Thr-474 and activate one another (2). Alternatively, NIK1 interacts with an unknown ligand-binding LRR-RLK in a stimulus-dependent manner (1b). Although virus infection triggers NIK1-mediated antiviral signaling, the molecular basis of such elicitation is unknown. Upon activation, NIK1 indirectly mediates the RPL10 phosphorylation (3) promoting its translocation to the nucleus (4), where it interacts with LIMYB (5) to down-regulate the expression of translation-related genes (6). Therefore, the propagation of the antiviral signal culminates with suppression of host global protein synthesis (7), which also impairs translation of viral mRNA (8). Conversely, the binding of begomovirus NSP to the NIK1 kinase domain (A-Loop) inhibits autophosphorylation at Thr-474 (9), thereby preventing receptor kinase activation and RPL10 phosphorylation (10). As a consequence, RPL10 is trapped in the cytoplasm of infected cells (11, see the punctuate bodies in the insert), creating an intracellular environment that is more favorable to begomovirus infection. The viral single-stranded DNA replicates via double-stranded DNA intermediates (12) that are transcribed in the nucleus of plant infected cell. NSP binds to nascent viral DNA and facilitates its movement to the cytoplasm in a process that may be mediated by a yet unidentified exportin-like protein (denoted as ? green).

Recent progress toward elucidation of the molecular bases for the NIK1-mediated suppression of translation includes the isolation of an RPL10-interacting MYB-domain containing transcriptional repressor LIMYB, which acts in concert with RPL10 to fully repress the expression of ribosomal protein (RP) genes, components of the translational machinery [40]. LIMYB and RPL10 interact in the nucleus of transfected cells and coordinately regulate common target promoters. T474D also down-regulates the expression of the same sub-set of LIMYB-regulated RP genes but requires the LIMYB function to repress RP gene expression. As a downstream component of the NIK1-mediated antiviral signaling and a transcriptional repressor, *LIMYB* overexpression down-regulates RP genes at the transcriptional level, suppresses translation, and enhances tolerance to begomovirus. In contrast, the loss of *LIMYB* function releases the repression of translation-related genes and increases susceptibility to virus infection. Therefore, LIMYB links NIK1 activation to global translation suppression as an antiviral immunity strategy in plants.

Based on the current data and common features of the LRRII-RLK family, we propose a mechanistic model for a NIK1-mediated defense signaling pathway and its interaction with the begomovirus NSP (Fig. 1). Upon begomovirus infection, the LRR extracellular domain undergoes oligomerization, allowing the intracellular kinase domains to transphosphorylate each other on the crucial Thr-474 residue, causing signaling transducer activation [33].

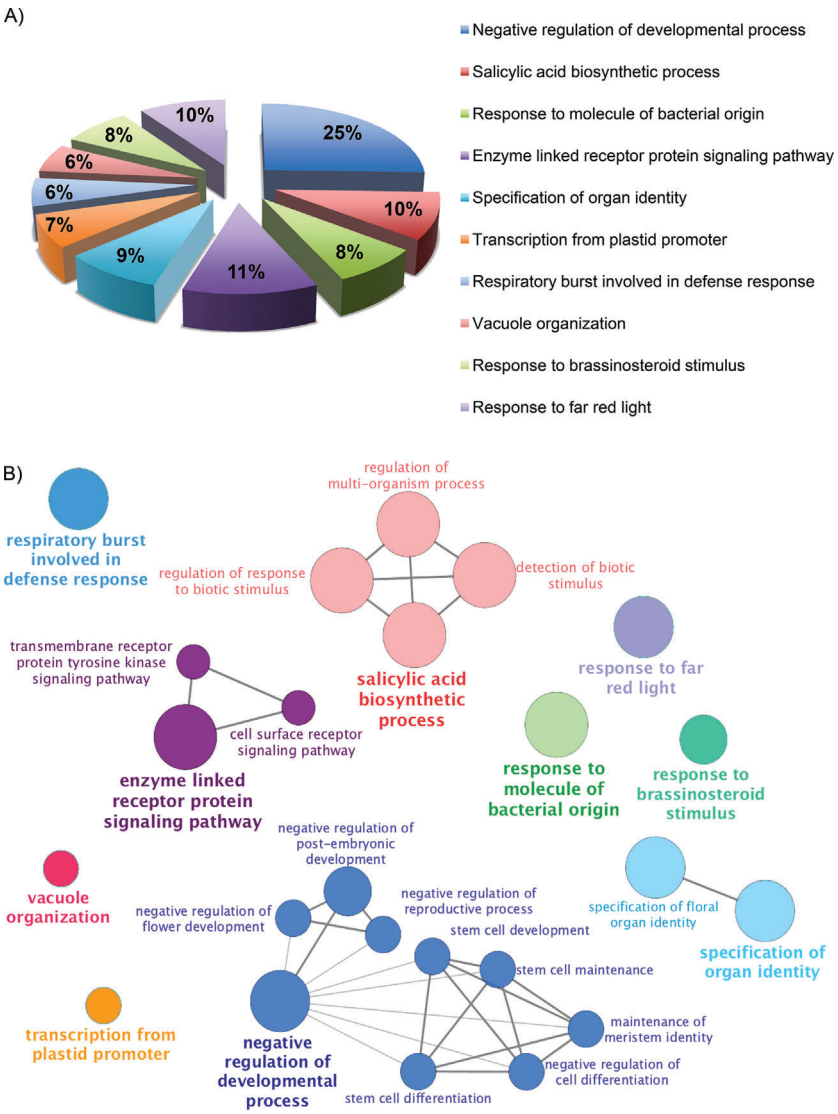

Figure 2. Selected up-regulated genes as major hubs in the plant-pathogens interactome network. **A:** Functional categorization of up-regulated hubs based on biological processes. The pie chart illustrates the distribution of up-regulated genes that represent relevant hubs across functional categories as defined by the Gene Ontology (GO) Biological process. The numbers represent the percentage of up-regulated genes in *nik1* that represent relevant hubs in each category compared to the WT control. **B:** Sub-categorization of interacting up-regulated hubs in *nik1* across the functional categorization. The figure was generated by the Cytoscape program, which connected the interacting sub-classes of a biological process. Node size reflects the gene frequency in each functional sub-category. Node color identifies the nodes associated with a given GO biological category from the list of up-regulated genes as in 2A.

Alternatively or additionally, NIK1 may serve as a co-receptor that interacts with an unidentified ligand-dependent LRR-RLK receptor in response to virus infection. In this scenario, the phosphorylation-dependent activation of NIK1 leads to the regulated relocation of RPL10 to the nucleus, where it interacts with LIMYB to fully repress translation-related genes [38, 40]. Prolonged down-regulation of translation machinery-related genes causes a suppression of global protein synthesis, reducing the association not only of host mRNAs but also of viral mRNAs with the actively translating polysomes in infected cells. Therefore, this down-regulation of cytosolic translation underlies at least partially the molecular mechanisms involved in NIK1-mediated antiviral defenses. Counteracting the NIK1 activation mechanism, NSP binds to the receptor kinase domain and sterically interferes with phosphorylation of Thr-474 in the A-loop. As a consequence, phosphorylation of the substrate RPL10 is impaired, and the ribosomal protein is trapped in the cytoplasm during begomovirus infection (see confocal microscopy of fluorescent RPL10 in infected cells, as an insert in Fig. 1 showing punctuate bodies dispersed in the cytoplasm [38]). Therefore, NSP inhibition of NIK1 prevents activation of the NIK1-mediated signaling pathway and suppresses the plant defense response.

NIK1 may be a general negative co-receptor in signaling pathways: A property that may impact negatively defense against other pathogens

Although NIK1 is structurally similar to BAK1, the mechanism of NIK1-mediated antiviral defense is distinct from the BAK1-mediated PTI. In fact, expression of the constitutively activated NIK1 mutant T474D does not induce PAMP immune response-associated marker genes but rather down-regulates translation-related genes [40]. We took advantage of the *nik1* null alleles to examine the global variation of gene expression induced by inactivation of the *NIK1* gene and examined the contrast *nik1*-Col0 from 10 day-old seedlings using the differential gene expression (DGE) method Deseq2 [43]. The differentially expressed (DE) genes were stored using SQL tables at the PostgreSQL relational database (http://inctipp.bioagro.ufv.br/arabidopsisnik0/), which listed the corresponding $\log_2$FC (fold change) and *p*-value corrected by FDR (q-value) for all DE genes.

RNA-seq data were then analyzed using the eigenvector centrality method [44] to identify up-regulated genes in *nik1* that represent relevant protein hubs in the plant-pathogens interactome network based on protein–protein and genetic interactions. By taking the fold change >1.5 as the major criterion for the eigenvector centrality metrics, the *nik1* up-regulated genes, which were retrieved from the Arabidopsis pathogens interactome network database (http://interactome.

dfci.harvard.edu/A_thaliana), were classified by gene ontology categories, and are presented in Fig. 2A. Among the differentially expressed genes that represented relevant centralization points from protein–protein and genetic interactions in the biological network, biotic stimuli responsive genes and negative regulators of development largely predominated the up-regulated list. The negative regulators of development were sub-categorized in hubs controlling stem cell differentiation, maintenance and development, flower development, cell differentiation, and post-embryonic development (Fig. 2B). This result indicated that NIK1 may also be involved in developmental control with a strong influence in stem cell development as well as floral induction and development. In this developmental category, the up-regulation of major hubs from BR signaling in the *nik1* mutant line may also implicate NIK1 as a negative regulator of the BR responses, which would be in marked contrast with the positive role of BAK1 in BRI1 pathway. The antagonistic roles of BAK1 and NIK1 may extend to include the plant immune response. In the *nik1* mutant line, the differentially expressed genes encoding important hubs in the biotic stress response are over- represented in the up-regulated list (Fig. 2A and B). These include up-regulated hubs functioning in salicylic acid (SA) signaling and in bacteria response (Fig. 2B). In the SA signaling category, relevant marker genes, such as *PR1*, *PR5*, and *NIM1-INTERACTING 1*, are up-regulated in the *nik1* mutant lines, and the hubs are represented by genes involved in perception, signaling, and SA biosynthesis. In the bacteria response category, relevant up-regulated hubs form a major antibacterial immune response group. Collectively, these results suggest that inactivation of the *NIK1* function may relieve repression of some layers of the immune response.

Conclusions and outlook

From recent studies with SERK-like co-receptors, a common theme emerges: the five LRRs-containing receptor-like kinases function as co-receptors for ligand-binding LRR-RLKs in a stimulus-dependent manner. As members of the LRRII-RLK subfamily, NIKs are likely to target LRR-RLK-mediated signaling pathways as well, although LRR-RLK partners of NIKs have yet to be identified. Recently, we have uncovered a translation control branch of the NIK1 signaling that transduces an antiviral signal to protect plants against begomoviruses, one of the largest and most successful groups of plant DNA viruses that collectively infect a variety of relevant crops in tropical and sub-tropical areas. Nevertheless, the transcriptome profiling resulting from inactivation of the *NIK1* gene suggests that NIK1 functions as a negative regulator in developmental and immune signaling pathways as co-receptors of different LRR-RLK receptors. If this is the case, the general negative function of the NIK1 co-receptor will pose a problem in targeting NIK1 for begomovirus resistance, because antibacterial immunity-related genes might be down-regulated in these virus resistant lines. The identification of new distinct signaling arms of the NIK1 transducer along with receptor partners of the NIK1 co-receptor will allow us to engineer NIK1-based defense strategies that cover antiviral immunity without compromising plant antibacterial immunity. One such strategy would be to target downstream components of the translational control branch of NIK1 signaling for genetic engineering of begomovirus resistance.

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References

1. **Dodds PN, Rathjen JP.** 2010. Plant immunity: towards an integrated view of plant-pathogen interactions. *Nat Rev Genet* **11**: 539–48.
2. **Boller T, Felix G.** 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu Rev Plant Biol* **60**: 379–406.
3. **Macho AP, Zipfel C.** 2014. Plant PRRs and the activation of innate immune signaling. *Mol Cell* **54**: 263–72.
4. **Jones JDG, Dangl JL.** 2006. The plant immune system. *Nature* **444**: 323–9.
5. **Mandadi KK, Scholthof K-BG.** 2013. Plant immune responses against viruses: how does a virus cause disease? *Plant Cell* **25**: 1489–505.
6. **Nicaise V.** 2014. Crop immunity against viruses: outcomes and future challenges. *Front Plant Sci* **5**: 660.
7. **Kørner CJ, Klauser D, Niehl A, Domínguez-Ferreras A**, et al. 2013. The immunity regulator BAK1 contributes to resistance against diverse RNA viruses. *Mol Plant Microbe Interact* **26**: 1271–80.
8. **Yang H, Gou X, He K, Xi D**, et al. 2010. BAK1 and BKK1 in *Arabidopsis thaliana* confer reduced susceptibility to *turnip crinkle virus*. *Eur J Plant Pathol* **127**: 149–56.
9. **Iriti M, Varoni EM.** 2014. Chitosan-induced antiviral activity and innate immunity in plants. *Environ Sci Pollut Res* **22**: 2935–44.
10. **Liu P, Wei W, Ouyang S, Zhang J-S**, et al. 2009. Analysis of expressed receptor-like kinases (RLKs) in soybean. *J Genet Genomics* **36**: 611–9.
11. **Sakamoto T, Deguchi M, Brustolini OJB, Santos AA**, et al. 2012. The tomato RLK superfamily: phylogeny and functional predictions about the role of the LRRII-RLK subfamily in antiviral defense. *BMC Plant Biol* **12**: 229.
12. **Shiu S-H, Bleecker AB.** 2001. Receptor-like kinases from Arabidopsis form a monophyletic gene family related to animal receptor kinases. *Proc Natl Acad Sci USA* **98**: 10763–8.
13. **Shiu S-H, Karlowski WM, Pan R, Tzeng Y-H**, et al. 2004. Comparative analysis of the receptor-like kinase family in Arabidopsis and rice. *Plant Cell* **16**: 1220–34.
14. **Afzal AJ, Wood AJ, Lightfoot DA.** 2008. Plant receptor-like serine threonine kinases: roles in signaling and plant defense. *Mol Plant Microbe Interact* **21**: 507–17.
15. **Gish LA, Clark SE.** 2011. The RLK/Pelle family of kinases. *Plant J* **66**: 117–27.
16. **Johnson KL, Ingram GC.** 2005. Sending the right signals: regulating receptor kinase activity. *Curr Opin Plant Biol* **8**: 648–56.
17. **Lin W, Li B, Lu D, Chen S**, et al. 2014. Tyrosine phosphorylation of protein kinase complex BAK1/BIK1 mediates Arabidopsis innate immunity. *Proc Natl Acad Sci USA* **111**: 3632–7.
18. **Oh M-H, Wang X, Kota U, Goshe MB**, et al. 2009. Tyrosine phosphorylation of the BRI1 receptor kinase emerges as a component of brassinosteroid signaling in Arabidopsis. *Proc Natl Acad Sci USA* **106**: 658–63.
19. **Clouse SD.** 2011. Brassinosteroid signal transduction: from receptor kinase activation to transcriptional networks regulating plant development. *Plant Cell* **23**: 1219–30.
20. **Shiu S-H, Bleecker AB.** 2003. Expansion of the receptor-like kinase/pelle gene family and receptor-like proteins in Arabidopsis. *Plant Physiol* **132**: 530–43.
21. **Lehti-Shiu MD, Zou C, Hanada K, Shiu S-H.** 2009. Evolutionary history and stress regulation of plant receptor-like kinase/pelle genes. *Plant Physiol* **150**: 12–26.

22. **Zhang XS, Choi JH, Heinz J, Chetty CS.** 2006. Domain-specific positive selection contributes to the evolution of Arabidopsis leucine-rich repeat receptor-like kinase (LRR RLK) genes. *J Mol Evol* **63**: 612–21.
23. **Albrecht C, Russinova E, Kemmerling B, Kwaaitaal M**, et al. 2008. Arabidopsis SOMATIC EMBRYOGENESIS RECEPTOR KINASE proteins serve brassinosteroid-dependent and -independent signaling pathways. *Plant Physiol* **148**: 611–9.
24. **Li J, Wen J, Lease KA, Doke JT**, et al. 2002. BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. *Cell* **110**: 213–22.
25. **Nam KH, Li J.** 2002. BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. *Cell* **110**: 203–12.
26. **Bücherl CA, van Esse GW, Kruis A, Luchtenberg J**, et al. 2013. Visualization of BRI1 and BAK1(SERK3) membrane receptor hetero-oligomers during brassinosteroid signaling. *Plant Physiol* **162**: 1911–25.
27. **Chinchilla D, Zipfel C, Robatzek S, Kemmerling B**, et al. 2007. A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature* **448**: 497–500.
28. **Heese A, Hann DR, Gimenez-Ibanez S, Jones AME**, et al. 2007. The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. *Proc Natl Acad Sci USA* **104**: 12217–22.
29. **Postel S, Küfner I, Beuter C, Mazzotta S**, et al. 2010. The multifunctional leucine-rich repeat receptor kinase BAK1 is implicated in Arabidopsis development and immunity. *Eur J Cell Biol* **89**: 169–74.
30. **Roux M, Schwessinger B, Albrecht C, Chinchilla D**, et al. 2011. The Arabidopsis leucine-rich repeat receptor-like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. *Plant Cell* **23**: 2440–55.
31. **Fontes EPB, Santos AA, Luz DF, Waclawovsky AJ**, et al. 2004. The geminivirus nuclear shuttle protein is a virulence factor that suppresses transmembrane receptor kinase activity. *Genes Dev* **18**: 2545–56.
32. **Mariano AC, Andrade MO, Santos AA, Carolino SMB**, et al. 2004. Identification of a novel receptor-like protein kinase that interacts with a geminivirus nuclear shuttle protein. *Virology* **318**: 24–31.
33. **Santos AA, Carvalho CM, Florentino LH, Ramos HJO**, et al. 2009. Conserved threonine residues within the A-loop of the receptor NIK differentially regulate the kinase function required for antiviral signaling. *PLoS ONE* **4**: e5781.
34. **Santos AA, Lopes KVG, Apfata JAC, Fontes EPB.** 2010. NSP-interacting kinase, NIK: a transducer of plant defence signalling. *J Exp Bot* **61**: 3839–45.
35. **Brustolini OJB, Machado JPB, Condori-Apfata JA, Coco D**, et al. 2015. Sustained NIK-mediated antiviral signalling confers broad-spectrum tolerance to begomoviruses in cultivated plants. *Plant Biotechnol J* (in press). doi: http://dx.doi.org/10.1111/pbi.12349
36. **Hanley-Bowdoin L, Bejarano ER, Robertson D, Mansoor S.** 2013. Geminiviruses: masters at redirecting and reprogramming plant processes. *Nat Rev Microbiol* **11**: 777–88.
37. **Rocha CS, Santos AA, Machado JPB, Fontes EPB.** 2008. The ribosomal protein L10/QM-like protein is a component of the NIK-mediated antiviral signaling. *Virology* **380**: 165–9.
38. **Carvalho CM, Santos AA, Pires SR, Rocha CS**, et al. 2008. Regulated nuclear trafficking of rpL10A mediated by NIK1 represents a defense strategy of plant cells against virus. *PLoS Pathog* **4**: e1000247.
39. **Hanks SK, Quinn AM, Hunter T.** 1988. The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. *Science* **241**: 42–52.
40. **Zorzatto C, Machado JPB, Lopes KVG, Nascimento KJT**, et al. 2015. NIK1-mediated translation suppression functions as a plant antiviral immunity mechanism. *Nature* **520**: 679–82.
41. **Nicaise V.** 2015. Lost in translation: an antiviral plant defense mechanism revealed. *Cell Host Microbe* **17**: 417–9.
42. **Albuquerque LC, Varsani A, Fernandes FR, Pinheiro B**, et al. 2012. Further characterization of tomato-infecting begomoviruses in Brazil. *Arch Virol* **157**: 747–52.
43. **Love MI, Huber W, Anders S.** 2014. Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2. *Genome Biol* **15**: 550.
44. **Bonacich P.** 1987. Power and centrality: a family of measures. *Am J Sociol* **92**: 1170–82.