doi:10.1038/nature14171

SUPPLEMENTARY DISCUSSION

Supplementary Discussion 1: The mechanistic model of NIK1-mediated antiviral signaling[1].

Stress-induced oligomerization of the extracellular domain of NIK1 brings the intracellular kinase domains into proximity and allows them to transphophorylate Thr-474 and activate one another[1]. Alternatively, NIK1 interacts with an unknown ligand-binding LRR-RLK in a stimulus-dependent manner. Activated NIK1 regulates the nucleocytoplasmic trafficking of the ribosomal protein RPL10 in turn, linking the antiviral response to receptor activation. NSP counters the activation of the pathway by binding to the NIK1 kinase domain and sterically interfering with the phosphorylation of Thr-474 in the A-loop. Apart from the identification of RPL10 as a downstream effector in NIK1-mediated antiviral immunity, mechanistic knowledge of the signaling pathway is lacking, and the molecular nature of the defense response remains unclear. The limited progress toward deciphering this layer of plant defense is likely due to our complete lack of knowledge of the molecular bases of the critical early events that trigger signaling and transduction from the receptor. This lack of knowledge makes it impossible to elicit the signaling pathway in a controlled manner without also eliciting the side effects of virus infection, as the viral protein NSP suppresses the signaling pathway to cause disease. To overcome this limitation, we generated a NIK1 phosphomimetic mutant by replacing the key threonine residue at position 474 with an aspartate residue (T474D)[2]. The T474D gain-of-function mutant receptor displayed a 1.5-fold increase in phosphorylation activity and an enhanced capacity to relocate RPL10 to the nucleus. In this study, we replaced the normal NIK1 receptor with the gain-of-function mutant in transgenic *Arabidopsis* lines to understand the molecular bases of the NIK1-mediated defense mechanism.

Supplementary Discussion 2: Ectopic expression of T474D did not activate typical viral defenses, such as SA signaling or virus-induced gene silencing

Compelling evidence in the literature has revealed a fundamental role for members of the Arabidopsis LRRII-RLK subfamily as co-receptors for transducing defense and development signals[3]. As a member of the LRR-RLKII subfamily, NIK1 may serve as a receptor for the co-receptor BAK1, which has also been implicated in viral immunity[4]. However, the constitutive activation of NIK1 and LIMYB overexpression did not induce typical BAK1-mediated defense responses, such as PTI or SA signaling marker genes, but rather repressed translation-related genes and impaired translation. In fact, our data indicate that virus infection is the trigger of the NIK1-mediated antiviral signaling and the output of transducing the defense signal consists in suppression of translation. Therefore, we reported here the identification of a new downstream component of the NIK1-mediated antiviral immunity, LIMYB, which is linked to a translational control branch of the antiviral signaling pathway.

Supplementary Discussion 3: Differential polysome loading of specific mRNAs by T474D expression

The association of the host mRNAs (*RBCS*, *AtWWP1*, *S13* and *S39* genes) in actively translating polysome (PS) fractions was significantly reduced in T474D-overexpressing lines compared to the wild-type line, although to a different extent; *S39* and viral mRNA were more affected. The differential reduction on polysome loading of specific mRNAs mediated by T474D may be linked to their intrinsic structural properties (size, uORFs, 5' and 3' UTR cis-regulatory elements), which can affect their specific association with ribosomal components[5-7]. Therefore, the activation of NIK1 reduces the global levels of translation, but the effect may not be the same

for all mRNAs. This down-regulation of cytosolic translation might at least partially underlie the molecular mechanisms involved in NIK1-mediated antiviral defenses. Although the half-life of plant ribosomes is not known, the ribosomes in plants are expected to have a relatively slow turnover time. Accordingly, the fluctuations of RP transcript levels during undisturbed diurnal cycles have little impact on ribosome abundance[8]. Despite the high stability of plant ribosome complexes, we found a 12% reduction in the ribosome content after 8h of T474D expression, as an indicative that activation of NIK1 may have also affected ribosome turnover in addition to mediating a down-regulation of RP gene expression. The NIK1-mediated phosphorylation and subsequent relocation of RPL10 to the nucleus may disrupt ribosome unit and affect ribosome turnover. Yeast RPL10 is positioned close to the peptidyl-transferase site on the large subunit; it is required for joining of the 40S and 60S subunits 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[9]. Disruption of RPL10-60S association may impact the correct assembly of the 60S subunit; thereby increasing the ribosome turnover rate. Plant RPL10 may also play fundamental roles in ribosome function, as it has been shown to be essential for development and to function as a translational control factor under UV stress[10].

Supplementary Discussion 4: Conclusion

In conclusion, the constitutive activation of NIK1 in the T474D lines impaired translation; this activation might constitute an excellent strategy for fighting begomovirus infection in host cells. Consistent with this finding, the overexpression of the downstream component LIMYB repressed RP gene expression, suppressed translation and enhanced tolerance to begomovirus. Nevertheless, antiviral defense systems mediated by suppression of global host translation have not been previously identified in plants. However, as begomoviruses

rely completely on the plant translational machinery and cannot circumvent host translational regulation, the global repression of translation is expected to significantly affect virus infection, as was observed in the T474D- and *LIMYB*-overexpressing lines. The demonstration that immune receptor-mediated defense signaling controls translation in plant cells represents a new paradigm for antiviral defenses in plants.

References

1. Santos AA, Lopes KVG, Apfta JAC, Fontes EPB. NSP-interacting kinase, NIK: a transducer of plant defence signalling. J. Exp. Bot. 2010; 61:3839-3845.
2. Santos AA, Carvalho CM, Florentino LH, Ramos JJO, Fontes EPB. Conserved threonine residues within the A-loop of the receptor NIK differentially regulate the kinase function required for antiviral signaling. PLoS ONE 2009; 4:e5781.
3. Shiu SH, Bleecker AB. Receptor-like kinases from Arabidopsis form a monophyletic gene family related to animal receptor kinases. Proc. Natl. Acad. Sci. USA 2001; 98:10763–10768
4. Kørner CJ, et al. The immunity regulator BAK1 contributes to resistance against diverse RNA viruses. *Mol. Plant Microbe Interact*. 2013; **26**:1271-1280.
5. Rosado A, Li R, van de Ven W, Hsu E, Raikhel NV. Arabidopsis ribosomal proteins control developmental programs through translational regulation of auxin response factors. *Proc Natl Acad Sci USA*. 2012; **109**: 19537-19544.
6. Schepetilnikov M, Dimitrova M, Mancera-Martínez E, Geldreich A, Keller M, Ryabova LA. TOR and S6K1 promote translation reinitiation of uORF-containing mRNAs via phosphorylation of eIF3h. *EMBO J*. 2013; **32**: 1087-1102.

7. Sorenson R, Bailey-Serres J. Selective mRNA sequestration by OLIGOURIDYLATE-BINDING PROTEIN 1 contributes to translational control during hypoxia in Arabidopsis. *Proc Natl Acad Sci USA*. 2014; **111**: 2373-8.

8. Pal SK, Liput M, Piques M, Ishihara H, Obata T, Martins MC, Sulpice R, van Dongen JT, Fernie AR, Yadav UP, Lunn JE, Usadel B, Stitt M. Diurnal changes of polysome loading track sucrose content in the rosette of wild-type arabidopsis and the starchless pgm mutant. Plant Physiol. 2013; 162:1246-1265.

9. Johnson AW, Lund E, DahlbergJ. Nuclear export of ribosomal subunits. Trends Biochem Sci. 2002; 27: 580-585.

10. Ferreyra MLF, Pezza A, Biarc J, Burlingame AL, Casati P. Plant L10 Ribosomal Proteins Have Different Roles during Development and Translation under Ultraviolet-B Stres. Plant Physiol. 2010; 153: 1878–1894.