A nucleoporin is required for induction of Ca\(^{2+}\) spiking in legume nodule development and essential for rhizobial and fungal symbiosis


Edited by Sharon R. Long, Stanford University, Stanford, CA, and approved November 14, 2005 (received for review October 11, 2005)

Nuclear-cytoplasmic partitioning and traffic between cytoplasmic and nuclear compartments are fundamental processes in eukaryotic cells. Nuclear pore complexes mediate transport of proteins, RNAs and ribonucleoprotein particles in and out of the nucleus. Here we present positional cloning of a plant nucleoporin gene, Nup133, essential for a symbiotic signal transduction pathway shared by *Rhizobium* bacteria and mycorrhizal fungi. Mutation of Nup133 results in a temperature sensitive nodulation deficient phenotype and absence of mycorrhizal colonization. Root nodules developing with reduced frequency at permissive temperatures are ineffective and electron microscopy show that *Rhizobium* bacteria are not released from infection threads. Measurement of ion fluxes using a calcium-sensitive dye show that Nup133 is required for the Ca\(^{2+}\) spiking normally detectable within minutes after application of purified rhizobial Nod-factor signal molecules to root hairs. Localization of NUP133 in the nuclear envelope of root cells and root hair cells shown with enhanced yellow fluorescent protein fusion proteins suggests a novel role for NUP133 nucleoporins in a rapid nuclear–cytoplasmic communication after host–plant recognition of symbiotic microbes. Our results identify a component of an intriguing signal process requiring interaction at the cell plasma membrane and at intracellular nuclear and plastid organelle-membranes to induce a second messenger.

Materials and Methods

Plant Material. Isolation of *nup133*-1, *nup133*-2 mutants previously called *sym3*-1 and *sym3*-2 were described in ref. 16, and *nup133*-3 and *nup133*-4 (previously called *sym3*-3 and *sym4*) were also isolated in the ecotype Gifu B-129 background. Plants were grown with or without *M. loti* strain NZP2235, TONO, and R7A. The root hair curling procedure has been described (6).

Electrophysiology. Seedlings of *L. japonicus* were germinated, mounted, and microinjected with Oregon Green-488 BAPTA-1 (Molecular Probes) essentially as described (9). Fluorescence was imaged by using a Nikon TE2000 inverted microscope coupled to a Hamamatsu Photonics digital charge-coupled device (CCD) camera. The excitation wavelength of 488 nm with an 11-nm bandpass was selected by using an Optoscan Monochromator (Cairn, Faversham, Kent, U.K.), and an emission filter of 545 (±15) nm was used. Images covering the protruding part of the root hair, including the entire nuclear region, were collected every 5 s with a 200-ms exposure using METAFLUOR software, and derivative traces were generated by using Microsoft EXCEL. The data presented were transformed to first derivative traces (in arbitrary units) as described (15).

Conflict of interest statement: No conflicts declared.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviation: eYFP, enhanced yellow fluorescent protein.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AJ890251, AJ890252, and AP008949–AP008952).

**To whom correspondence should be addressed. E-mail: stougaard@mb.au.dk.

© 2006 by The National Academy of Sciences of the USA

www.pnas.org/cgi/doi/10.1073/pnas.0508883103

PNAS | January 10, 2006 | vol. 103 | no. 2 | 359–364
scribed by Wais et al. (17) using the formula $Y = X_{n+1} - X_n$, where $Y$ is the change in fluorescence and $X_n$ and $X_{n+1}$ are the fluorescence intensity measurements at time points $n$ and $n+1$ separated by $5$-s intervals. After microinjection, root hairs were left at least 20 min before Nod-factor addition, and only cells showing active cytoplasmic streaming were used for analysis; at least 95% of such cells in wild-type plants induce calcium spiking in response to Nod-factor. Nod factors, isolated from a reverse phase C18 column, were added directly to the incubation chamber to give an estimated final concentration of $10^{-8}$ M. In the experiments done with the mutants, seven, four, six, and three cells were analyzed all with separate seedlings of the nup133-1, nup133-2, nup133-3, and nup133-4 mutants, respectively. An additional 10 cells were assayed by using three seedlings of the nup133-3 mutant grown at 10°C and assayed at 15°C.

**Map-Based Cloning and cDNA Isolation.** An F2 mapping population was established by crossing a nup133-1 mutant and a wild-type L. japonicus ecotype “MG-20.” F2 plants homozygous for the nup133-1 mutant allele were identified after screening for the nonnodulation mutant phenotype. In total, 822 homozygous F2 mutant plants were analyzed. Microsatellite markers and single nucleotide polymorphism developed from BAC and TAC clones anchored to the general genetic map of the region were used for fine mapping and for building the physical TAC/BAC contig. Five TAC/BAC clones from MG-20 and two BAC clones from Gifu were assembled to cover the Nup133 region between the two flanking markers 1F24R and T47D06. On the basis of sequence differences between the two parents, additional PCR markers were developed inside this contig, and the region was narrowed down to 22 kb between markers 1F24-7 and T45D07-3. Finally, clone LjT44M23 from MG-20 containing the entire Nup133 region was sequenced as a part of the Nup133 from Gifu. A full-length cDNA clone of nup133 was obtained from cDNA libraries prepared from total RNA of wild-type and human nup133 sequences was done by using CLUSTALX (www-igbmc.u-strasbg.fr/BioInfo/ClustalX/Top.html). The full-length cDNA sequence is GenBank accession no. AJ890252, and nup133 mRNA is GenBank accession no. AJ890251.

**Localisation of Nup133 Protein.** For localization of NUP133 protein, a full length Nup133 cDNA was fused to enhanced yellow fluorescent protein (eYFP) coding region to create an N-terminal in frame fusion. The recombinant gene was inserted between the 35S promoter and pANOS 3′ terminal, and cloned into the pIV10 plasmid. Transgenic hairy roots were observed by using confocal laser microscopy (LSM510 META, Zeiss).

**Results**

**nup133 Mutants Are Nodulation Deficient.** We have phenotypically characterized Lotus nup133 mutants and cloned the Nup133 gene belonging to the common symbiotic pathway. Four monogenic recessive mutant alleles, nup133-1, nup133-2, nup133-3, and nup133-4 (Table 2, which is published as supporting information on the PNAS web site), were originally found in a screen for symbiotic mutants and assigned to the same locus by diallelic crosses. The nup133-1 allele has the strongest effect on nodulation, and nup133-1 mutants do not nodulate when inoculated at 22°C with the Lotus microsymbiont, Mesorhizobium loti strain NZP2235 (Table 1). Two other M. loti strains, TONO and R7A, trigger formation of a few small ineffective nodules (Fig. 1 A and B). On average nup133-1 mutants developed one nodule on four of 25 plants and six of 28 plants inoculated with TONO and R7A, respectively. Compared to wild-type plants, this is a 20-30 fold reduction in nodulation frequency (Table 1). The nup133-2, nup133-3, and nup133-4 mutants are nodulation deficient and developed small ineffective nodules with a frequency similar to the controls inoculated at 5° to 30-fold after inoculation with one of the three M. loti strains (Table 1). When inoculated at 26°C, nup133-2, nup133-3, and nup133-4 were nonnodulating, revealing a temperature-sensitive nodulation deficiency (Table 1).

**Nod-Factor Perception and Calcium Oscillations.** The earliest visible cellular change in wild-type plants after inoculation is root hair deformation and root hair curling (Fig. 1 C). In the nup133-1 mutants inoculated with NZP2235, root hair swelling and in nup133-4 also root hair branching were observed (Fig. 1 D), whereas root hair curling was not observed. These observations indicate that Nod-factor perception did occur in the mutants. This conclusion was supported by microelectrode measurements of Nod-factor induced alkalization in the root hair space of nup133-1, nup133-2 and nup133-3 mutants (data not shown). In wild type, this response is associated with rapid calcium influx (6, 15). Nodules that develop...
_promoted on the *nup133*-1 mutants with TONO were further characterized by microscopy. Sections of mutant nodules show that they are developmentally arrested, either empty nodules or nodules with only few cells containing bacteria (Fig. 1 F and G). Electron microscopy of mutant nodule sections shows infection threads and bacteria inside plant cells contained in structures resembling enlarged infection droplets (Fig. 1 H–J). Symbiosomes containing endocytosed bacteria were not observed in these sections. To investigate whether infection of these ineffective nodules occurred through root hairs, we examined *nup133* mutant root hairs in the infection zone for presence of infection threads. After inoculation with strain NZP2235 or TONO constitutively expressing LacZ, root hair infection threads of *nup133*-1, *nup133*-2, *nup133*-3, and *nup133*-4 mutants were visualized by X-Gal staining. With this technique, up to 20 infection threads are visible in the infection zone of wild-type plants. At the permissive temperature, one infection thread was found on 14 *nup133*-1 mutant plants; in *nup133*-2, no infection threads were found in 14 plants; in *nup133*-3, no infection threads were found in 12 plants; in *nup133*-4, one infection thread was found in four plants. These rare infection threads are most likely associated with the occasional formation of ineffective root nodules observed on *nup133* mutants.

Measurements of ion fluxes in wild type root hairs using the calcium-sensitive dye Oregon Green-488 BAPTA-1 show Ca\(^{2+}\) spiking ∼10 min after the addition of Nod-factor (Fig. 2A). In contrast, calcium spiking was not detected in the four allelic *nup133* mutants (Fig. 2A). Sixteen individual root hairs from nine seedlings of the *nup133*-3 mutant were tested and no Ca\(^{2+}\) spiking was detected, even though 10 of these cells were from plants grown and assayed at the lower temperature that permits low level nodulation. An additional 14 root hairs (each from a separate seedling) from the *nup133*-1, *nup133*-2, and *nup133*-4 mutants were also tested, and none induced Ca\(^{2+}\) spiking after Nod factor addition. Because our success rate for observing Ca\(^{2+}\) spiking in wild type is 94% of those root hairs chosen for assay after microinjection, it is absolutely clear that mutations in the *nup133* gene block Nod-factor induced Ca\(^{2+}\) spiking in young root hairs, because 30 individual root hairs from four independent mutants all lacked this response. Microinjection of *L. japonicus* root hairs is laborious and technically demanding, so this technique does not lend itself to identification of rare cells that might induce Ca\(^{2+}\) spiking. Furthermore, we are unable to microinject older root hairs. Therefore, the occasional infection and nodulation events observed with these mutants could be due to either a lack of requirement for Ca\(^{2+}\) spiking or that Ca\(^{2+}\) spiking is induced occasionally in some cells, possibly even among cell types where root-hair calcium measurements are not possible using the microinjection approach. Nevertheless, it is very clear that *nup133* mutations can block Ca\(^{2+}\) spiking in those young root-hair

### Table 1. Nodulation phenotype of *nup133* alleles inoculated with three different *M. loti* strains at two temperatures

<table>
<thead>
<tr>
<th>Plant</th>
<th>Rhizobium strain</th>
<th>22°C</th>
<th>26°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nodulation ratio</td>
<td>No. of nodules per plant</td>
<td>Nodulation ratio</td>
</tr>
<tr>
<td>Wild type</td>
<td>NZP2235</td>
<td>24/24</td>
<td>6.4</td>
</tr>
<tr>
<td>Nup133-1</td>
<td>NZP2235</td>
<td>33/33</td>
<td>4.68</td>
</tr>
<tr>
<td>Nup133-2</td>
<td>NZP2235</td>
<td>0/29</td>
<td>0</td>
</tr>
<tr>
<td>Nup133-3</td>
<td>NZP2235</td>
<td>11/34</td>
<td>0.68</td>
</tr>
<tr>
<td>Nup133-4</td>
<td>NZP2235</td>
<td>13/37</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Promoted on the *nup133*-1 mutants with TONO were further characterized by microscopy. Sections of mutant nodules show that they are developmentally arrested, either empty nodules or nodules with only few cells containing bacteria (Fig. 1 F and G). Electron microscopy of mutant nodule sections shows infection threads and bacteria inside plant cells contained in structures resembling enlarged infection droplets (Fig. 1 H–J). Symbiosomes containing endocytosed bacteria were not observed in these sections. To investigate whether infection of these ineffective nodules occurred through root hairs, we examined *nup133* mutant root hairs in the infection zone for presence of infection threads. After inoculation with strain NZP2235 or TONO constitutively expressing LacZ, root hair infection threads of *nup133*-1, *nup133*-2, *nup133*-3, and *nup133*-4 mutants were visualized by X-Gal staining. With this technique, up to 20 infection threads are visible in the infection zone of wild-type plants. At the permissive temperature, one infection thread was found on 14 *nup133*-1 mutant plants; in *nup133*-2, no infection threads were found in 14 plants; in *nup133*-3, no infection threads were found in 12 plants; in *nup133*-4, one infection thread was found in four plants. These rare infection threads are most likely associated with the occasional formation of ineffective root nodules observed on *nup133* mutants.

Measurements of ion fluxes in wild type root hairs using the calcium-sensitive dye Oregon Green-488 BAPTA-1 show Ca\(^{2+}\) spiking ∼10 min after the addition of Nod-factor (Fig. 2A). In contrast, calcium spiking was not detected in the four allelic *nup133* mutants (Fig. 2A). Sixteen individual root hairs from nine seedlings of the *nup133*-3 mutant were tested and no Ca\(^{2+}\) spiking was detected, even though 10 of these cells were from plants grown and assayed at the lower temperature that permits low level nodulation. An additional 14 root hairs (each from a separate seedling) from the *nup133*-1, *nup133*-2, and *nup133*-4 mutants were also tested, and none induced Ca\(^{2+}\) spiking after Nod factor addition. Because our success rate for observing Ca\(^{2+}\) spiking in wild type is 94% of those root hairs chosen for assay after microinjection, it is absolutely clear that mutations in the *nup133* gene block Nod-factor induced Ca\(^{2+}\) spiking in young root hairs, because 30 individual root hairs from four independent mutants all lacked this response. Microinjection of *L. japonicus* root hairs is laborious and technically demanding, so this technique does not lend itself to identification of rare cells that might induce Ca\(^{2+}\) spiking. Furthermore, we are unable to microinject older root hairs. Therefore, the occasional infection and nodulation events observed with these mutants could be due to either a lack of requirement for Ca\(^{2+}\) spiking or that Ca\(^{2+}\) spiking is induced occasionally in some cells, possibly even among cell types where root-hair calcium measurements are not possible using the microinjection approach. Nevertheless, it is very clear that *nup133* mutations can block Ca\(^{2+}\) spiking in those young root-hair

### Table 1. Nodulation phenotype of *nup133* alleles inoculated with three different *M. loti* strains at two temperatures

<table>
<thead>
<tr>
<th>Plant</th>
<th>Rhizobium strain</th>
<th>22°C</th>
<th>26°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nodulation ratio</td>
<td>No. of nodules per plant</td>
<td>Nodulation ratio</td>
</tr>
<tr>
<td>Wild type</td>
<td>NZP2235</td>
<td>24/24</td>
<td>6.4</td>
</tr>
<tr>
<td>Nup133-1</td>
<td>NZP2235</td>
<td>33/33</td>
<td>4.68</td>
</tr>
<tr>
<td>Nup133-2</td>
<td>NZP2235</td>
<td>0/29</td>
<td>0</td>
</tr>
<tr>
<td>Nup133-3</td>
<td>NZP2235</td>
<td>11/34</td>
<td>0.68</td>
</tr>
<tr>
<td>Nup133-4</td>
<td>NZP2235</td>
<td>13/37</td>
<td>0.49</td>
</tr>
</tbody>
</table>

**Fig. 1.** Phenotype of *nup133* mutants and genetic complementation. (A) Wild-type root nodules. (B) Ineffective nodule on *nup133*-1 inoculated with the *M. loti* TONO strain. (C) Root hair curling on a wild-type seedling inoculated with *M. loti*. (D) Root hair swelling on a wild-type seedling inoculated with *M. loti*. (E) Nodules on transgenic root showing complementation of *nup133*-2. (F) Sections of mature wild-type root nodule. (G) Section of *nup133*-2 nodule. (H–J) Electron micrographs of an ineffective nodule showing infection threads (arrow) and *M. loti* bacteria within enlarged infection droplet structures (arrow). (K and L) Electron micrographs of a wild-type root nodule showing a branched infection thread releasing an *M. loti* containing infection droplet (white arrow) and bacteroids (black arrow) surrounded by peribacteroid membrane (arrowhead). The symbiosome consists of bacteroids enclosed within a peribacteroid membrane. (Scale bars: 1 mm in A, B, and G and 50 μm in C–F and H.)
cells normally assayed by us and others (8, 9, 15, 17) for this phenotype.

Taken together, the phenotypic characterization of a temperature-dependent nonnodulation phenotype, the impaired mycorrhizal colonization (4, 5), the lack of normal Nod-factor induced alkalization in the root hair space, and the lack of Nod-factor induced calcium spiking in developing root hairs, lead us to conclude that NUP133 is part of the common pathway required to mount a response to signals from rhizobial and mycorrhizal symbionts.

**Positional Cloning of Nup133.** To characterize the gene molecularly, Nup133 was isolated by using a positional cloning approach. On the genetic map of Lotus, the Nup133 locus is located on the short arm of chromosome 1 (20). Subsequent fine mapping in an F2 population and genotyping of 822 mutant plants identified markers delimiting Nup133 to a 0.06 cM region of 22 kb (Fig. 5, which is published as supporting information on the PNAS web site). Located within this region are four genes predicted to encode a binding protein, a Ca\(^{2+}\) transporting ATPase, and a homologue of Arabidopsis expressed protein, respectively (Fig. 5). Considering the suggested role for calcium as second messenger, the search for mutant alleles was initially focused on sequencing the Ca\(^{2+}\) transporting ATPase gene. Contrary to our expectations, this gene was not mutated. Subsequent sequencing of gene regions corresponding to the homologue of Arabidopsis expressed protein in nup133-1, nup133-2, nup133-3, and nup133-4 identified the Nup133 gene.

Short deletions shifting the reading frame, leading to premature stop codons, were found in nup133-1, nup133-2, and nup133-4 (Table 2). A retrotransposon is inserted in nup133-3. All four mutant alleles were complemented by the cloned Nup133 using Agrobacterium rhizogenes to generate transgenic roots (19). The wild-type gene, including a 1.8-kb promoter region and a 3.6-kb 3' region was introduced into nup133-1, nup133-2, nup133-3, and nup133-4 mutant plants via A. rhizogenes and the nodulation phenotype scored after inoculation with strain NZP2255 of M. loti. All mutants were complemented with high efficiency for nodulation (Fig. 1E and Table 3, which is published as supporting information on the PNAS web site). Complementation for mycorrhizal colonization was tested and obtained in the nup133-1 mutant. Complementation was scored as normal colonization and abundant arbuscule formation in transgenic roots.

Sequencing of full-length cDNAs isolated from a Lotus leaf library determined the transcription start site at least 62 bp upstream of the start codon and a 3’ untranslated region of 210 nucleotides (Fig. 5). Alignment of genomic and cDNA sequences defined eight exons in Nup133 (Fig. 5). The Lotus Nup133 cDNA encodes a conceptual protein of 1,309 aa, corresponding to 146.5 kDa. Southern hybridization indicates that NUP133 is encoded by a single gene in both the small genome of Lotus japonicus and the large genome of pea (data not shown). In the fully sequenced Arabidopsis and rice genomes, Nup133 homologs are single functionally uncharacterized genes in each species. The Arabidopsis predicted protein At2g05120 is 54% identical, and the predicted rice protein (AAN52748) is 47% identical to NUP133. Less similar, with 20% global identity, are yeast and human Nup133 nucleoporins (Fig. 6, which is published as supporting information on the PNAS web site). Three-way alignment of human, yeast, and Lotus proteins identifies a set of 59 conserved amino acids positioned along the length of the proteins suggesting common origin of corresponding genes. This finding is supported by conservation of the two first intron positions among 25 introns of the human gene and eight introns of plant Nup133 genes. The Lotus NUP133 protein was identified as a Nup133 nucleoporin by PSI-BLAST. After two iterations, the Lotus NUP133 amino acid sequence aligned to the entire length of Nup133 proteins from mouse and human with E values of 0 and overall identities of ~20%. The N-terminal domain of human Nup133 consists of an α/β domain with a seven-bladed β-propeller fold, and the C-terminal domain was predicted to be all α-helical (21). Secondary structure prediction and fold recognition on Lotus NUP133 suggest a similar overall structure and recognize the β-propeller structure of human Nup133 within the first 600 N-terminal residues of Lotus NUP133. From secondary structure predictions and an alignment with human Nup133, the N-terminal α/β domain and C-terminal α helical domain of Lotus NUP133 were estimated to localize between residues 50–535 and 555–1309, respectively. The positions of β-propeller blades suggested by secondary structure prediction are shown on the alignment of N-terminal domains of Lotus NUP133 and human Nup133 proteins in Fig. 3.

**Expression of Nup133 in Different Plant Organs.** The symbiotic mutant phenotype suggests a function for Nup133 in root tissues. To test this prediction, expression of Nup133 in different plant organs was determined by Northern and quantitative RT-PCR analysis. Results of both analyses demonstrate that Nup133 transcripts are present in all organs tested (Fig. 2B and C). No significant induction was detected in roots several days after inoculation with M. loti (data not shown). The observed expression in all organs tested and the similarity to a nucleoporin would predict a general function for Nup133 and a mutant phenotype affecting overall plant development. For example, mutation of the PAUSED gene (encoding an homolog of nuclear export receptor for tRNA) in Arabidopsis affected shoot and apical meristem growth, leaf development, and lateral root formation (22). No such general effect was observed.
sequencing of mutant alleles, and successful complementation of yeast and mammalian cells. In control experiments, an eYFP–NUP133 N-terminal fusion protein expressed into a temperature-sensitive yeast strain deleted for the Nup133p gene. Complementation was not achieved, and the GFP fusion protein was not localized to the nuclear rim as expected for a nuclear pore protein. However, the punctuate appearance of eYFP–NUP133 fluorescence (Fig. 4H, I, and K) is comparable to the punctuate localization characteristic for proteins residing in nuclear pore complexes of yeast and mammalian cells. In control experiments, expression of eYFP alone resulted in an even distribution of fluorescent protein within nuclei (Fig. 4A–C).

**Discussion**

The collective evidence obtained by genetic and physical mapping, sequencing of mutant alleles, and successful complementation unequivocally identified the nucleoporin Nup133 gene. The presence of only one gene copy in Lotus, pea, and the fully sequenced Arabidopsis and rice genomes, together with conservation of two intron positions, suggest that plant Nup133 genes are closest ho-

![Fig. 3](image)

![Fig. 4](image)
molinos of yeast and mammalian Nup133 genes. Further evidence from protein sequence conservation in Lotus NUP133, comparable to the general level of conservation in the NUP133 protein family and the overall α/β/α domain structure together with the predicted N-terminal seven-bladed β-propeller domain support the identification of Lotus NUP133 as a member of the Nup133 nucleoporin family. Localization of eYFP fusion proteins at the nuclear rim makes it most likely that NUP133 and its homologs are components of the nuclear pore complex in plants. The punctuate localization of eYFP–NUP133 fluorescence, characteristic for nuclear pore proteins, supports this interpretation (Fig. 4 H, I, and K).

In yeast, Nup133p is a constituent of the Nup84p complex and in mammals the corresponding protein is a component of the Nup107–160 complex residing in the nuclear basket structure of the nuclear pore (24–27). Studies in yeast and HeLa cells suggest a function in export of mRNA, nuclear pore assembly and distribution (18, 28). Such a general function for the Lotus NUP133 nucleoporin is difficult to reconcile with the predominantly symbiotic phenotype observed. Interestingly, Drosophila has one example of cell-specific developmental effects in a nucleoporin mutant that is also affected in the response to fungal and bacterial pathogens. Mutation of members only, the gene encoding a nucleoporin homologous to mammalian Nup133, leads to the death of Drosophila larvae (29).

In Arabidopsis, recently published results show that a putative nucleoporin 96 protein is required for both basal resistance against bacterial pathogens and for R-gene-mediated pathogen resistance (30). These parallels are tantalizing and might have implications for bacterial pathogens and for R-gene-mediated pathogen resistance (30). These parallels are tantalizing and might have implications for the emerging comparative studies of IL-1β and IFN-α in plants (30). These parallels are tantalizing and might have implications for bacterial pathogens and for R-gene-mediated pathogen resistance (30).

In Arabidopsis, recently published results show that a putative nucleoporin 96 protein is required for both basal resistance against bacterial pathogens and for R-gene-mediated pathogen resistance (30). These parallels are tantalizing and might have implications for the emerging comparative studies of IL-1β and IFN-α in plants (30). These parallels are tantalizing and might have implications for bacterial pathogens and for R-gene-mediated pathogen resistance (30). These parallels are tantalizing and might have implications for the emerging comparative studies of IL-1β and IFN-α in plants (30). These parallels are tantalizing and might have implications for bacterial pathogens and for R-gene-mediated pathogen resistance (30). These parallels are tantalizing and might have implications for bacterial pathogens and for R-gene-mediated pathogen resistance (30).