

The *Nicotiana attenuata* LECTIN RECEPTOR KINASE 1 is involved in the perception of insect feeding

Gustavo Bonaventure

Max Planck Institute of Chemical Ecology; Department of Molecular Ecology; Jena, Germany

The *Nicotiana attenuata* LECTIN RECEPTOR KINASE 1 (LecRK1) has been recently identified as a component of the mechanism used by plants to suppress the *Manduca sexta*-triggered accumulation of salicylic acid (SA). The suppression of the SA burst by LecRK1 allows for the unfettered induction of jasmonic acid (JA)-mediated defense responses against *M. sexta* herbivory. LecRK1 contains a multi-domain extracellular region composed of a G-type Lectin domain and a PAN-AP domain separated by a variable sequence with low similarity to an EGF domain. The LecRK1 intracellular region is composed of a single domain structure with predicted Ser/Thr protein kinase activity. The multi-domain structure of the extracellular region of LecRK1 adds a level of complexity in terms of the potential ligands that this receptor protein could recognize.

Insect-associated Elicitors and Identification of Na-LecRK1

The recognition of phytophagous insects by plants induces a set of very specific responses aimed to deter tissue consumption and to reprogram the metabolism and development of the plant to tolerate the herbivore. The recognition of insects by plants requires the plant's ability to perceive chemical cues generated by the insects and to distinguish a particular pattern of tissue disruption.¹ Relatively little is known about the molecular basis of insect perception by plants and the signaling mechanisms directly associated to this perception. During insect feeding,

components of the oral secretions (OS) or saliva of insects become into contact with plant cells and elicitors present in these insect-derived fluids are perceived by plant cells to initiate the activation of specific signaling cascades.^{1,2} Importantly, several examples have also shown that OS components can interfere with or even suppress the activation of defense responses in plants. Thus, although some OS components are perceived by plants as a signal of herbivore attack, others interfere with the induction of defense responses allowing the insect to feed in a "stealthy" manner. For example, mandibular glands of *Helicoverpa zea* secrete salivary glucose oxidase (GOX), an enzyme that functions as an effector to suppress the induced defenses of the host plant.³ Experimental evidence suggests that GOX contributes to the initial oxidative burst of H₂O₂ observed in leaves damaged by herbivores⁴⁻⁶ and the enhanced oxidative burst suppresses the induction of plant defense responses. GOX activity in *Spodoptera exigua* OS produce high levels of H₂O₂ that induce a strong salicylic acid (SA) burst in *N. attenuata* leaves.⁷ These high levels of SA attenuate the induction of JA-mediated defense responses.

The insect-associated elicitors that act during folivory by chewing insects are diverse in structure. They can be enzymes (e.g., glucose oxidase, β -glucosidase),^{4,8} fatty acid-amino acid conjugates (FACs),^{2,9} sulfur-containing fatty acids (caeliferins),¹⁰ fragments of cell walls (e.g., pectins and oligogalacturonides),¹¹ or peptides released from digested plant proteins (e.g., inceptins; proteolytic fragments of the chloroplastic ATP synthase γ -subunit).¹²

Keywords: plant-insect interactions, insect elicitor, jasmonic acid, lectin receptor kinase, salicylic acid, defense responses

Submitted: 09/30/11

Accepted: 10/06/11

DOI: 10.4161/psb.6.12.18324

Correspondence to: Gustavo Bonaventure;
Email: gbonaventure@ice.mpg.de

Addendum to: Gilardoni PA, Hettenhausen C, Baldwin IT, Bonaventure G. The *Nicotiana attenuata* NaLecRK1 gene suppresses the insect-mediated inhibition of induced defense responses during *Manduca sexta* herbivory. *Plant Cell* 2011; 23(9):3512-32; PMID:21926334; DOI:10.1105/tpc.111.088229.

Table 1. Examples of chewing insect-associated elicitors that induce specific responses in plants during insect folivory

Elicitors	Insect species	Plant species
Glucose Oxidase (GOX)	<i>Helicoverpa zea</i>	<i>Nicotiana tabacum</i> (tobacco)
	<i>Spodoptera exigua</i>	<i>Nicotiana attenuata</i> (coyote tobacco)
β-glucosidase	<i>Helicoverpa armigera</i>	<i>Medicago truncatula</i>
	Other Lepidoptera and Hymenoptera	<i>Solanum lycopersicum</i> (tomato)
N-acyl-amino acids (FACs)	<i>Pieris brassicae</i>	<i>Phaseolus lunatus</i> (lima beans)
		<i>Zea mays</i> (maize)
Caeliferins	<i>Spodoptera exigua</i>	<i>Brassica oleracea</i> (cabbage)
	<i>Manduca sexta</i>	<i>Zea mays</i>
Inceptin	<i>Teleogryllus taiwanemma</i>	<i>Glycine max</i> (soybean)
	<i>Drosophila melanogaster</i>	<i>Solanum melongena</i> (eggplant)
Oligouronides	Several Lepidoptera	<i>Nicotiana attenuata</i>
		<i>Solanum nigrum</i>
Protease inhibitors (PIs)	<i>Schistocerca americana</i>	<i>Zea mays</i>
		<i>Arabidopsis thaliana</i>
Produced by degradation of the plant ATP synthase γ-subunit during folivory by <i>Spodoptera frugiperda</i>		<i>Vigna unguiculata</i> (cowpea)
	Produced by degradation of plant cell walls during insect folivory	<i>Solanum lycopersicum</i>

Importantly, most of these insect-associated elicitors are not general elicitors of responses against chewing insects in all plant species but are usually restricted to particular plant-insect associations (Table 1). This selectivity probably reflects the evolutionary history of both plants and their interacting insects and, hence, it is crucial to understand the mechanisms of plant-insect interactions in the evolutionary context of the interaction.²

N. attenuata is an annual fire-chasing plant native to the Great Basin desert of the southwestern USA which has evolved a large number of specific induced responses against generalist and specialist herbivore species that co-exist with the plant in the same environment. For example, *N. attenuata* responds very specifically to the attack by *M. sexta* larvae. Among the responses induced by *M. sexta* folivory are changes in the expression of more than 500 genes, 90 proteins, 170 metabolites and the differential production of leaf volatile organic compounds (VOCs), jasmonic acid (JA), ethylene (ET) and salicylic acid (SA).¹³⁻¹⁷ Critical defense responses against *M. sexta* folivory are the accumulation of the defense molecules 17-hydroxygeranylinalool diterpene glycosides (HGL-DTGs), nicotine, phenylpropanoid-polyamine conjugates

and protease inhibitors (PIs).¹⁸⁻²¹ The accumulation of these defense molecules depend on de novo biosynthesis of JA and jasmonyl-Isoleucine (JA-Ile). In addition to these jasmonates, ET and SA also play critical roles in the modulation of induced JA-mediated defense and tolerance responses.^{7,22,23}

As mentioned above, relatively little is known about the molecular basis of insect perception by plants and the signaling mechanisms directly associated to this perception. With the aim of identifying signal transduction components of the pathways operating early during the response to *M. sexta* larval attack, a SuperSAGE (serial analysis of gene expression) approach combined with next generation sequencing (NGS) was recently used to quantify the early transcriptional changes elicited by the FAC *N*-linolenoyl-glutamic acid (18:3-Glu) in *N. attenuata* plants.¹⁷ The analysis targeted mRNAs encoding regulatory components: rare transcripts with very rapid FAC-elicited kinetics. Among the 547 differentially expressed transcripts, more than 25% corresponded to putative regulatory components, including 22 protein kinases.¹⁷ Among these protein kinases was LECTIN RECEPTOR KINASE 1 (LecRK1).

LecRK1 is Indispensable during *M. sexta* Herbivory to Suppress the Insect-Mediated Inhibition of Defense Responses

Gene function analysis performed by reducing *LecRK1* expression in *N. attenuata* plants by both virus induced gene silencing (VIGS) and inverted repeated RNA interference (ir-RNAi) revealed that LecRK1 is essential to mount a full defense response against *M. sexta* folivory; larvae growing on plants with reduced expression of LecRK1 were 40–100% larger than larvae growing on wild type plants.²⁴ The increased rates of larval growth were correlated with reduced levels of induction of several critical defense molecules, namely nicotine, HGL-DTGs and trypsin protease inhibitors (TPIs). The expression of threonine deaminase (*TD*) was also several fold reduced in plants with reduced expression of LecRK1 compared with wild type plants. The reduced induction of these defense molecules was independent of the capacity of the plants to produce JA; the accumulation of JA and JA-Ile during *M. sexta* herbivory was similar in wild type plants and plants reduced in the expression of LecRK1. However, the accumulation of SA was increased by 2-fold in the latter.²⁴ The ectopic expression of the *nabG* gene prevented the increased accumulation of SA during *M. sexta* herbivory and fully restored the defense response against this herbivore in plants with reduced expression of LecRK1. The results indicated that *N. attenuata* LecRK1 is indispensable to suppress the SA-mediated inhibition of defense responses and thereby to stimulate the unfettered JA-mediated induction of defense metabolites (Fig. 1).

Analysis of *LecRK1* mRNA expression in plants deficient in JA biosynthesis or perception showed that jasmonates inhibit the induction of the *LecRK1* gene (Fig. 1). Moreover, plants with reduced expression of SIPK (SA-inducible protein kinase) and WIPK (Wound-inducible protein kinase) showed that these two regulatory components have a positive effect on the expression of *LecRK1*, consistent with their central role in the activation of defense responses against *M. sexta* herbivory²⁵ (Fig. 1). These results revealed that the induction of *LecRK1* expression is under

tight control; it is induced by OS elicitation but the levels of induction are checked by jasmonates (in a COI1-dependent manner). Thus, in this case, jasmonate levels would tune *LecRK1* expression and thereby the accumulation of SA levels during insect herbivory (Fig. 1).

The amino acid sequence of *LecRK1* contains a predicted N-terminal (Nt) extracellular region, a single transmembrane spanning α -helix and a C-terminal (Ct) cytoplasmic region (Fig. 2). The extracellular Nt region contains a predicted 22 amino acid signal sequence to the secretory pathway, a G-type Lectin domain, an Epidermal Growth Factor-like domain (EGF) domain and a PAN-AP (plasminogen/apple/nematode) domain. The Ct cytoplasmic region contains a predicted functional Ser/Thr kinase domain. The closest homologs to *LecRK1* in other plant species that have been extensively studied are the brassica self-incompatibility determinant S receptor kinase (SRK) acting in self-recognition of pollen. SRK binds a cycteine-rich protein at a hypervariable region in the Nt extracellular region.²⁶ *LecRK1* has also close homology to the *N. glutinosa* RLK1 (Receptor-like Kinase 1) that interacts with elicitor (a conserved protein of ~98 amino acids) from *Phytophthora capsici*.²⁷ The Lectin domains in the *LecRK* family of proteins usually do not carry all the conserved residues found in soluble lectins and which are responsible for carbohydrate binding. Moreover, the presence of the PAN-AP domain which has been shown to bind proteins and carbohydrates and the variable region between the lectin and the PAN-AP domains add a level of complexity in terms of the potential ligands that *LecRK1* could recognize. The identification of these ligands will provide critical information about the mechanisms used by plants to perceive lepidopteran herbivory.

Acknowledgments

The DFG (BO3260/3-1) and the MPG are acknowledged for funding.

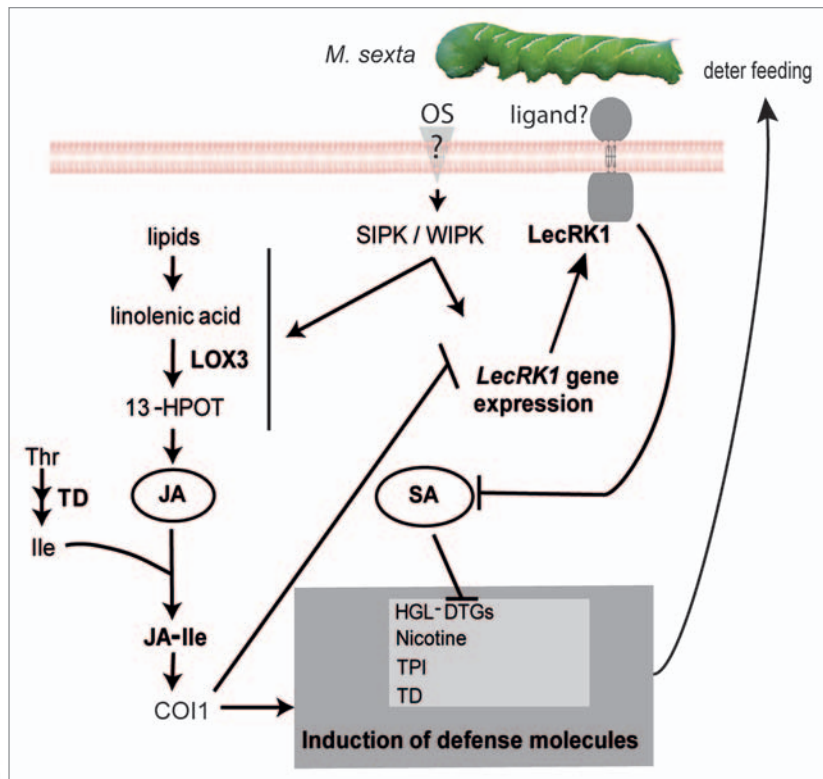


Figure 1. Proposed model for the role of *LecRK1* in the *N. attenuata* defense response against *M. sexta*. During herbivory by *M. sexta* larvae, the larval OS induce the expression of the *LecRK1* gene, however, *LecRK1* is also constitutively expressed. The induction of *LecRK1* depends on the activity of SIPK and WIPK, which in parallel activate the biosynthesis of JA.¹⁶ JA is conjugated to Ile to form JA-Ile, and TD supplies Ile for this biosynthetic process.²⁸ JA-Ile induces the accumulation of defense metabolites or proteins (including nicotine, TPIs, HGL-DTGs and TD) via COI1-dependent mechanisms^{29,30} and inhibits the expression of *LecRK1*. *LecRK1* suppresses the accumulation of SA induced by *M. sexta* herbivory which in turn allows for an unfettered induction of the defense metabolites nicotine, TPIs, HGL-DTGs and TD. LOX3, lipoxygenase 3; 13-HPOT, 13S-hydroperoxy-octadecatrienoic acid; SIPK, SA-induced protein kinase; WIPK, Wound-induced protein kinase; TD, threonine deaminase.

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