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シロイヌナズナの PAMP 受容体 RLP23 が関与する植物免疫機構に関する研究

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CHAPTER I. Plant immune responses are quantitatively different between nlp24-induced immunity and flg22-induced immunity

Plants recognize conserved microbial molecules, referred to as pathogen-associated molecular patterns (PAMPs), and activate immunity. In *Arabidopsis thaliana*, immune responses are induced by two known PAMPs, nlp24 derived from secreted proteins termed necrosis- and ethylene-inducing-like proteins (NLPs), conserved in a broad range of fungi, bacteria and oomycetes, and flg22 derived from bacterial flagellin. Here, I found that there were quantitative differences between nlp24- and flg22-induced immune responses. The expression of *PAD3*, a gene required for camalexin synthesis, and *ERF1*, a gene responsive to ethylene, were strongly induced by nlp24 treatment in comparison to flg22. Moreover, the timing of gene expression for these genes were also distinct between nlp24- and flg22-induced immunity in *A. thaliana*. Conversely, the reactive oxygen species (ROS) production and MAPK activation were more induced by flg22 than nlp24. The flg22 peptide is recognized by the leucine-rich repeat receptor kinase (LRR-RK) type receptor FLAGELLIN-SENSITIVE 2 (FLS2), while the nlp24 peptide is recognized by the leucine-rich repeat receptor protein (LRR-RP) type receptor RLP23 that lacks a kinase domain in the intracellular region. Complemented *Arabidopsis rlp23* mutant with chimeric receptors having the intracellular kinase domain of FLS2 and the extracellular LRR domain of RLP23 exhibited the ROS production after nlp24 treatment. However, the amount of ROS production observed in these transgenic plants treated with

nlp24 was not comparable to that in wild-type *Arabidopsis* treated with flg22, and was even lower than that in transgenic plants harboring full-length RLP23. These results suggest that the structural differences in the pattern-recognition receptors are insufficient to fully explain the molecular mechanisms underlying the differences between nlp24-induced immunity and flg22-induced immunity.

CHAPTER II. *Arabidopsis* BAK1 plays distinct roles between nlp24-induced immunity and flg22-induced immunity

During PAMP-induced immunity, pattern recognition receptors engage with their corresponding co-receptors to mediate downstream signaling. FLS2 interacts with its co-receptor BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) immediately following flg22 treatment. On the other hand, RLP23 interacts with BAK1 upon nlp24 treatment and also constitutively interacts with another co-receptor SUPPRESSOR OF BIR1-1 (SOBIR1). I revealed that nlp24-induced immunity involves both BAK1-dependent and BAK1-non-essential pathways, whereas flg22-induced immunity largely relies on BAK1. All tested immune responses, including ROS production, expression of *WRKY33* and *PAD3* expression, and MAPK activation, were diminished in the double mutants of *BAK1* and its homolog *BAK1-LIKE 1* (*BKK1*) following flg22 treatment. However, the nlp24 treatment induced MAPK activation and the expression of *WRKY33* and *PAD3* in the *bak1-5 bkk1* mutant, although the immune responses were not intact. The ROS production was not induced in *bak1-5 bkk1* after nlp24 treatment. In contrast, the *sobir1-12* mutant failed to activate all the tested immune responses after nlp24 treatment, while it retained the induction of the immune responses

following flg22 treatment to the same extent as wild-type Col-0. Moreover, the pretreatment with nlp24 resulted in a reduction of the growth of a bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 in the *bak1-5 bkk1* mutant. This reduction was not observed in *pad3* mutant pretreated with nlp24. These results indicate the presence of a BAK1-non-essential pathway in addition to the BAK1-dependent pathway in nlp24-induced immunity, and suggest that BAK1 is involved in the differences between nlp24-induced immunity and flg22-induced immunity.

CHAPTER III. RLP23 is required for *Arabidopsis* immunity against the grey mould pathogen *Botrytis cinerea*

The flg22 receptor FLS2 has been reported to be essential for the resistance to bacterial pathogen *P. syringae* pv. *tomato* DC3000. However, the actual contribution of nlp24 receptor RLP23 to plant immunity against pathogens is still unclear. Here, I revealed that RLP23 was required for *Arabidopsis* immunity against the necrotrophic fungal pathogen *Botrytis cinerea.* In contrast, RLP23 was dispensable for the immunity against the necrotrophic fungus *Alternaria brassicicola* and the hemi-biotrophic fungus *Colletotrichum higginsianum*. Interestingly, microscopic observation revealed that the invasion ratio of *B. cinerea* was increased in the *rlp23* mutants compared with the wild-type plants. *B. cinerea* possesses two *NLP* genes, named *BcNEP1* and *BcNEP2*. *BcNEP1* was preferentially expressed before/during invasion, in contrast to the *BcNEP2* that was expressed at the late infection phase. The nlp sequences derived from BcNEP1 as well as BcNEP2 were recognized by *Arabidopsis* RLP23, although the nlp sequence of BcNEP1 contained three additional amino acids that were absent in BcNEP2

and other NLPs, such as the NLPs of *C. higginsianum*, *Phytophthora parasitica* (oomycete) and *Bacillus subtilis* (bacterium). I found that *A. brassicicola* expressed its *NLP* gene (*AbNLP1*) preferentially at the late infection phase, rather than before/during pathogen invasion. I also found that the co-inoculation of *A. brassicicola* with the synthetic nlp peptide from BcNEP1 strengthened the immunity against *A. brassicicola*. Collectively, these results revealed that RLP23 contributes to the *Arabidopsis* pre-invasive resistance to *B. cinerea* via the recognition of the NLP proteins, mainly BcNEP1, which are secreted at the early infection phase.