Title of Project： Rice NLR genes, their function and evolution

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【Purpose and Background of the Research】

Rice crop feeds more than 50% of the world population. Blast disease caused by a fungus *Magnaporthe oryzae is* the most devastating disease of rice. To control blast disease, deploying rice resistance genes is the most effective mean. Plant pathogens manipulate host by secreting and injecting effector molecules to host cells. A subset of pathogen effectors are recognized by plant resistance proteins (R-proteins) coded by resistance gene (*R*-genes) and trigger strong host resistance. Such effectors recognized by the hosts are named avirulence (AVR) effectors. The majority of R-proteins are Nucleotide-binding Leucine-rich repeat Receptors (NLRs). We have isolated three *Magnaporthe oryzae AVR* genes (*AVR-Pia*, A*VR-Pii* and A*VR-Pik*) and are studying their interactions with cognate rice *R*-genes, *Pia*, *Pii* and *Pik*, respectively. Each of the three *R*-genes comprises a pair of NLR genes coding for "Sensor NLR" and "Helper NLR": *Pik=Pik-1+Pik-2*; *Pia=RGA5+RGA4*; *Pii=Pii-2+Pii-1*). Sensor NLRs contain extra integrated domains (IDs) in addition to the canonical CC, NB and LRR domains, and these IDs have similarities to domains of host proteins (Fig.1).
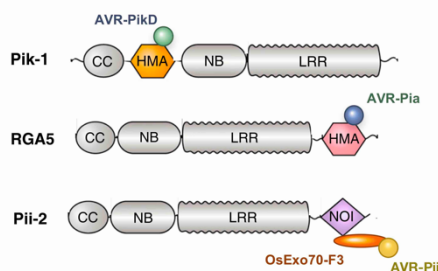

Fig.1 Recognition of *Magnaporthe oryzae* AVRs by rice "Sensor NLRs" is mediated by Integrated Domains (IDs). AVR-PikD and AVR-Pia bind HMA IDs of Pik-1 and RGA5, whereas AVR-Pii binds host OsExo70-F3 protein which in turn binds NOI ID of Pii-2.

The host proteins with ID-like domains are hypothesized to be the targets of pathogen effectors. These domains were most likely incorporated to NLR as IDs during evolution to detect pathogen effectors. Based on these findings, we set out to further understand the molecular function of rice NLRs and to engineer durable resistance against blast disease.

【Research Methods】

(1) Engineering of rice NLRs to broaden the recognition specificities: Rice *Pik-1* locus has multiple alleles with different HMA domain sequences. They show recognition specificities to variable *AVR-Pik* alleles due to differential binding of AVR-Pik variants to Pik-1 HMA variants. However, all AVR-Pik variants strongly bind to their host target proteins; small HMA proteins (sHMAs). In this project, we engineer broad spectrum Pik-1 by replacing Pik-1 HMA ID with HMA domain from sHMA. We also found that *Pia/Pias* locus has highly divergent alleles with different IDs. For instance, Pia Sensor NLR RGA5 has HMA domain, whereas Pias Sensor NLR Pias-1 has DUF761 domain in an identical position. We engineer ID of *Pia/Pias* locus by inserting domains of host proteins that are common target of pathogen effectors. Such NLR engineering is expected to confer broad spectrum resistance to rice.

(2) Functional analysis of paired NLRs: In Pia, Helper NLR (RGA4) is known to trigger hyper-sensitive (HR)-like cell death whereas Sensor NLR (RGA4) suppresses RGA4 as well as senses AVR. In Pik and Pii, Helper NLR and Sensor NLR do not seem to be involved in negative regulation and they cooperate to transduce resistance signal. In this project, we elucidate molecular mechanisms of Pia negative regulation and Pii/Pik cooperation. Additionally, we employ systematic knockout of NLRs to understand NLR regulation networks.

(3) Understanding molecular function of AVR-host target interactions: We have isolated AVRs and many effectors from *M. oryzae*. We will study their host interactors and understand molecular functions of these interactions.

【Expected Research Achievements and
  Scientific Significance】

Better understanding of rice NLR molecular function will allow us to engineer NLRs that may confer rice with broad spectrum resistance against blast and other diseases.

【Publications Relevant to the Project】

· Structural basis of pathogen recognition by an integrated HMA domain in a plant NLR immune receptor. Maqbool A., Saitoh H. et al. (2015) *eLife* doi:10.7554/eLife. 08709.002.

【Term of Project】 FY2020-2024

【Budget Allocation】 118,900 Thousand Yen

【Homepage Address and Other Contact Information】

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