Ligand induced cleavage and nuclear localization of the rice XA21 immune receptor

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Abstract

The rice XA21 receptor confers immunity to the Gram-negative bacterial pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) upon recognition of the conserved microbial signature $AxY^{S}22$. Here, we demonstrate that the intracellular kinase domain of XA21 translocates to the nucleus upon $AxY^{S}22$-mediated perception and that this translocation event is required for XA21-mediated immunity.

Plants and animals perceive conserved microbial signatures via plasma membrane and cytoplasmically localized receptors[1]. Such immune receptors, often called pattern recognition receptors, include animal TLRs (Toll-like receptors) and plant receptor kinases. These receptors require serine-threonine kinases of the non-arginine aspartate (non-RD) subclass to transduce the immune response[2]. Whereas RD kinases are regulated by autophosphorylation of the activation segment, a centrally located loop that sits close to the catalytic center, very little is known about non-RD kinase activation.

In plants, well-studied immune receptors include rice XA21 (Xanthomonas resistance 21), Arabidopsis FLS2 (flagellin sensitive 2) and EFR (elongation factor Tu receptor). In animals, studies have focused on TLR1, 3, 5, 6, 7, 8, and 9, which signal through non-RD interleukin-1 receptor-associated kinases 1 (IRAK1), and TLR3 and TLR4, which signal through non-RD receptor interacting protein 1 (RIP1) kinases[1-3]. A general theme that has emerged from these studies is that non-RD kinase activity is at least partially dispensable for the innate immune response in both plants and animals[1] and that the kinases function partly as phosphorylation-mediated scaffold proteins that recruit different signaling components[3]. Furthermore, for XA21, the inactive state is promoted by autophosphorylation of specific residues in the juxtamembrane domain by an associated ATPase[4]. These results suggest that non-RD kinases are activated in a manner distinctly different from the well-characterized RD kinases.

Similar to the plant immune receptors, all members of the epidermal growth factor receptor (EGFR) family have an extracellular ligand binding domain, a transmembrane domain, and a cytoplasmic kinase domain. Many of these receptors require a nuclear translocation step for their signal transductions. For example, in response to binding their corresponding ligands, the intact protein or the intracellular domain of EGFR, ErbB-2 (v-erb-a erythroblastic leukemia viral oncogene homolog 2), ErbB-3, and ErbB-4 are translocated to the nucleus[5,6]. ErbB-2 and ErbB-4 carry proline-rich carboxyl termini that contain intrinsic transcription activity and function as transcriptional regulators in the nucleus[7-9]. EFGR interacts with the transcription factors STAT3 (signal transducer and activator of transcription 3), STAT5, and E2F1 (E2F transcription factor-1). Each of these transcription factors is then regulates expression of target genes [10-12]. Such nuclear translocation events have not been reported for receptor kinases governing the innate immune response.

Rice *XA21* confers immunity to the Gram-negative bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*)[13]. XA21 binds a conserved sulfated peptide, called $AxY^{S}22$ peptide, derived from the *Xoo* Ax21 (activator of XA21-mediated immunity) protein[14]. We previously reported that upon binding to $AxY^{S}22$, XA21 undergoes a proteolytic cleavage, producing a 110 kDa N-terminal cleavage product in transgenic rice plants expressing a N-terminal Myc-tagged XA21 (Myc-XA21)[14-16]. Here we show that a 70 kDa C-terminal cleavage product, corresponding to the kinase domain fused to cyan fluorescent protein (CFP), is also released after infection of transgenic rice plants carrying a C-terminal CFP-tagged XA21 (XA21-CFP) [17] (data not shown).

To localize the XA21 intracellular domain *in vivo*, we investigated the cellular distribution of C-terminal green fluorescent protein (GFP) tagged XA21 (*XA21-GFP*) in rice protoplasts. Consistent with our previous results[15], XA21-GFP is primarily localized to the plasma membrane in the absence of $AxY^{S}22$ (Fig. 1a). GFP alone localizes to both the nucleus and cytoplasm (data not shown). In contrast, treatment with $AxY^{S}22$ (Fig. 1a) or with supernatants prepared from *Xoo*[14] (data not shown), triggers significant accumulation of the XA21-GFP inside the nucleus (24.1% ± 3.8 of the protoplasts accumulated XA21-GFP protein in the nucleus, data not shown). Nuclear localization was not observed in protoplasts treated with water or the biologically inactive peptide, AxY22[14] (1.2% ± 0.1 and 1.8% ± 0.7, respectively) (Fig. 1a). These results indicate that the XA21 intracellular domain translocates to the nucleus in protoplasts after treatment with biological active $AxY^{S}22$.

To investigate if the XA21 intracellular domain translocates to the nucleus in mature plants, we generated stable transgenic rice plants expressing biologically active XA21-GFP (*Ubi*-XA21-GFP) (data not shown). After *Xoo* infection or $AxY^{S}22$ treatment, we observed a significant increase of the XA21 intracellular domain in the nuclei-enriched fraction (Fig. 1b and c). In contrast, the intracellular domain was not detected in nuclei-enriched fractions after mock infection and was not detected in nuclei-depleted fractions. These results indicate that $AxY^{S}22$ recognition triggers nuclear translocation of the XA21 intracellular domain *in planta*.

Because XA21 does not carry a proline-rich domain indicative of intrinsic transcription activity, we hypothesized that the nuclear-localized XA21 interacts with transcription factors to control transcriptional reprogramming. To test hypothesis, we assessed if the rice transcription factor OsWRKY62, which negatively regulates XA21-mediated immunity[18], interacts with the

XA21 intracellular domain in plant cells. Indeed, using bimolecular fluorescence complementation (BiFC) assays, we showed that the XA21 intracellular domain and OsWRKY62 interact exclusively in the nucleus of rice protoplasts (data not shown).

We next assessed whether the nuclear localization of the XA21 intracellular domain is critical for XA21-mediated immunity. For this purpose, we generated transgenic rice plants expressing XA21 fused to a C-terminal nuclear export signal (NES)[19] (*Ubi*-XA21-GFP-NES*)* and inoculated with *Xoo* (data not shown). Out of twelve independently transformed rice plants expressing *Ubi-XA21-GFP-NES*, all displayed significantly enhanced susceptibility to *Xoo* compared with the control transgenic rice plants expressing *Ubi-XA21-GFP*, lacking the NES. The NES did not affect the expression or stability of XA21-GFP (data not shown). The enhanced susceptibility in *Ubi*-XA21-GFP-NES was quantified by lesion length and growth curve analysis (Fig. 2 and data not shown). Rice plants expressing XA21-GFP-NES under the control of its native promoter (*Native*-XA21-GFP-NES) were also susceptible compared to resistant *Native*-XA21-GFP, lacking NES (data not shown). These results demonstrate that nuclear translocation of the XA21 intracellular domain is required for XA21-mediated immunity.

The observation that the non-RD kinase domain of a plant immune receptor is cleaved and localized to the nucleus where it interacts with a transcriptional regulator has not previously been reported. However, studies of animal TLRs and their associated non-RD kinases have demonstrated a similar nuclear localization requirement for activation of TLR-mediated immune responses. For example, upon challenge with lipopolysaccharide or interleukin-1, IRAK1 localizes to the nucleus and binds to NF-κB-responsive elements located within the *IκB*-α promoter to regulate *IκB*-α transcription[20-23]. Nuclear-localized IRAK1 also binds promoters of STAT3 and interleukin-10 upon lipopolysaccharide challenge and subsequently regulates interleukin-10 gene expression[22,24].

Together these results suggest a model where non-RD kinases translocate to the nucleus following recognition of conserved microbial signatures where they directly interact with transcription factors to regulate the immune response.

Figure legends

Figure 1. Nuclear translocation of XA21-GFP after treatment with AxY^S22 peptide or *Xoo*.

(a) XA21-GFP was expressed in rice protoplast cells using polyethylene glycol (PEG)-mediated transformation. AxY^S22 or AxY22 peptides were applied 16 hours after transformation. Expression of the introduced genes was observed one hour after peptide treatment. *NLS-DsRed* (nuclear localization signal-*Discosoma* sp. red fluorescent protein) was co-transformed as a nucleus marker. Images were collected with a Leica True Confocal Scanner SPE confocal microscope and coded in green and red for GFP and DsRed, respectively. Arrowheads mark the nuclei. Scale bar, 10 μm.

(b) Western blot analysis of XA21-GFP proteins extracted from transgenic rice plants expressing *XA21-GFP* under the control of the *Ubi* promoter (*Ubi*-XA21-GFP, homozygous 5-5-4). Equal amount of total proteins (75 μg) were extracted from leaf discs of Kitaake and *Ubi*-XA21-GFP plants three hours after Mock, *Xoo*, or AxY^S22 treatment. XA21-GFP and C-terminal cleavage product, detected with an anti-GFP antibody, displayed bands of approximately 170 and 70 kDa, respectively. Equal loading of total proteins was confirmed by Coomassie blue staining of proteins (lower panel).

(c) Western blot analysis of XA21-GFP extracted from nuclei-depleted and nuclei-enriched fractions from *Ubi*-XA21-GFP line (homozygous 5-5-4) detected by the anti-GFP antibody. Nuclei-enriched (100 μg) and nuclei-depleted (100 μg) fractions were prepared from total proteins in (b). XA21-GFP and C-terminal cleavage product-GFP displayed bands at approximately 170 and 70 kDa, respectively. Cytolsolic phosphoenolpyruvate carboxylase (PEPC) and nuclear histone H3 protein were used as cytosolic and nuclear markers, respectively.

Figure 2. Nuclear export of XA21 disrupts XA21-mediated immunity.

Transgenic plants expressing XA21 carrying a nuclear export signal (*Ubi*-XA21-GFP-NES) developed long lesion lengths after *Xoo* inoculation. Rice leaves were photographed twelve days after inoculation (DAI) with *Xoo*. From left to right: Kitaake, *Ubi*-XA21-GFP (homozygous 5-5-4), *Ubi*-XA21-GFP-NES (7-22-1 and 8-21-1), and *Native*-XA21-GFP (9-7-2) rice plants.

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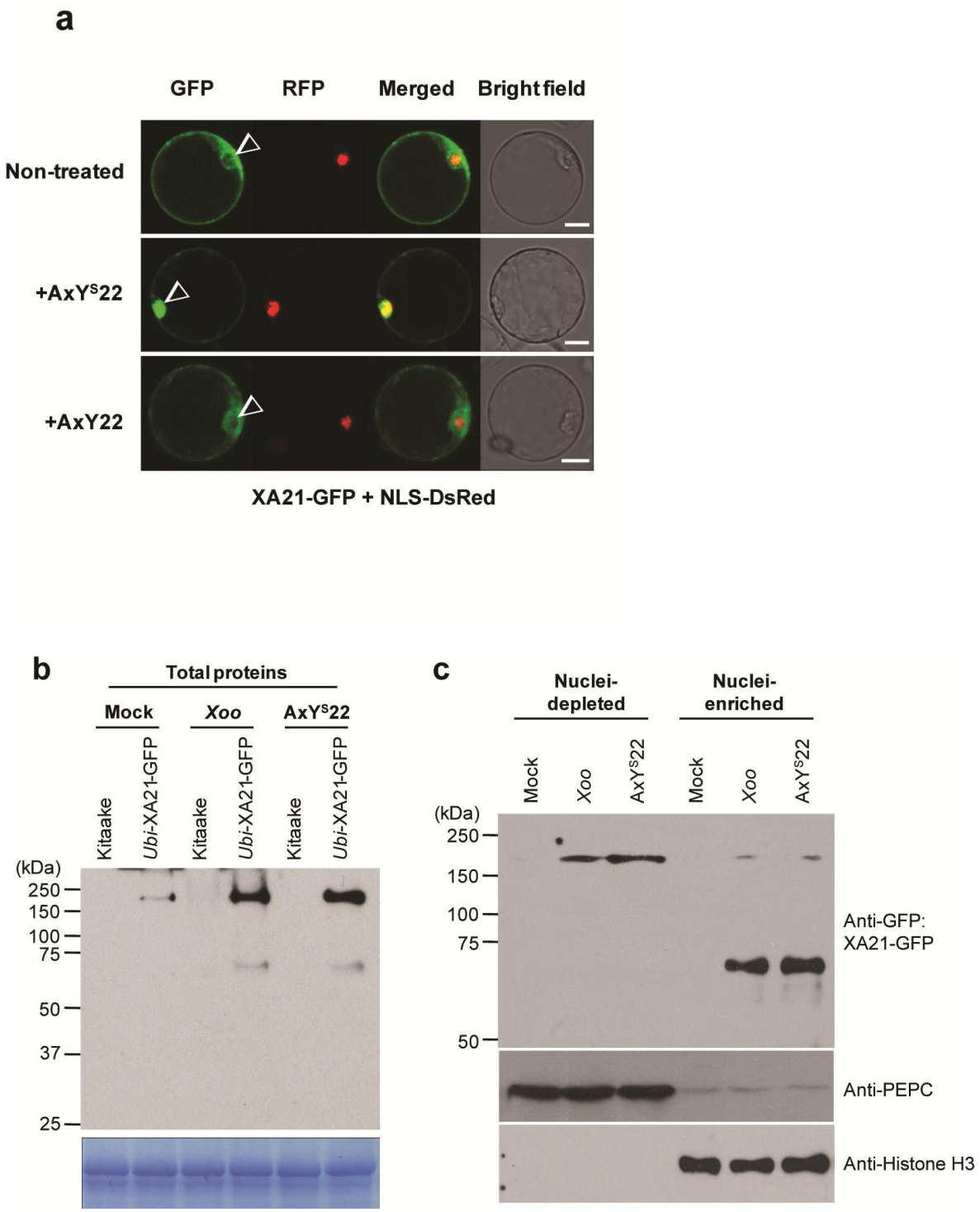
Authorship

C.J.P and P.C.R. designed the project. C.J.P. performed experiments. C. J. P. and P.C.R. wrote the paper.

Conflict of Interest

The authors declare no competing financial interests.



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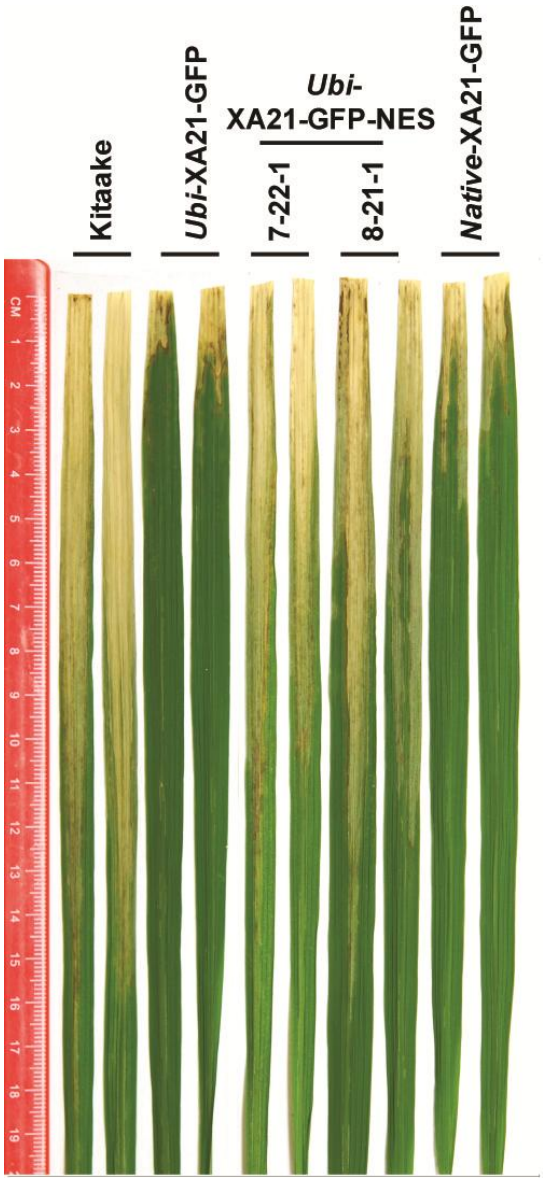

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