Biochemical and Biophysical Research Communications xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Rice BiP3 regulates immunity mediated by the PRRs XA3 and XA21 but not immunity mediated by the NB-LRR protein, Pi5

Chang-Jin Park [a,c], Min-Young Song [b], Chi-Yeol Kim [b], Jong-Seong Jeon [b], Pamela C. Ronald [a,*]

[a] Department of Plant Pathology and the Genome Center, University of California Davis, Davis, CA 95616, USA
[b] Graduate School of Biotechnology & Crop Biotech Institute, Kyung Hee University, Yongin 446-701, Republic of Korea
[c] Department of Bioresources Engineering, Sejong University, Seoul 143-747, Republic of Korea

ARTICLE INFO

Article history:
Received 9 April 2014
Available online xxxx

Keywords:
BiP
Magnaporthe oryzae
NB-LRR
Oryzae sative
PRR
Xanthomonas oryzae pv. *oryzae* (*Xoo*)

ABSTRACT

Plant innate immunity is mediated by pattern recognition receptors (PRRs) and intracellular NB-LRR (nucleotide-binding domain and leucine-rich repeat) proteins. Overexpression of the endoplasmic reticulum (ER) chaperone, luminal-binding protein 3 (BiP3) compromises resistance to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) mediated by the rice PRR XA21 [12]. Here we show that BiP3 overexpression also compromises resistance mediated by rice XA3, a PRR that provides broad-spectrum resistance to *Xoo*. In contrast, BiP3 overexpression has no effect on resistance mediated by rice Pi5, an NB-LRR protein that confers resistance to the fungal pathogen *Magnaporthe oryzae* (*M. oryzae*). Our results suggest that rice BiP3 regulates membrane-resident PRR-mediated immunity.

1. Introduction

Plants recognize conserved microbial signatures via pattern recognition receptors (PRRs) [1]. Well-characterized PRRs include the Arabidopsis flagellin sensitive 2 (FLS2) receptor, the Arabidopsis elongation factor Tu receptor (EFR), and the rice XA21 (Xanthomonas resistance 21) receptor [2–4]. These proteins share a similar structure: an extracellular leucine-rich repeat (LRR) domain, a transmembrane (TM) domain, and an intracellular non-arginine-aspartate (non-RD) kinase domain. The non-RD kinase motif is a hallmark of kinases involved in the initial stages of PRR-mediated immunity. In contrast, RD kinases are associated with a function in developmental processes [5]. Rice XA3 (also known as XA26) also shares this LRR–TM–non-RD kinase domain structure [6,7]. XA3 also confers another important trait characteristic of PRRs: broad-spectrum resistance. For these reasons, XA3 is predicted to be a PRR [1,8,9].

Membrane-bound PRRs are synthesized in the endoplasmic reticulum (ER) where they are subject to ER-quality control [10–13]. The ER-quality control is a conserved process in eukaryotic cells responsible for monitoring correct folding and processing of membrane and secretory proteins [14]. Many ER proteins, including HSP70 luminal-binding protein (BiP), HSP40 ERdj3B, stromal-derived factor 2 (SDF2), calreticulin3 (CRT3), UDP-glucose glycoprotein glucosyl transferase (UGGT), and ER retention defective 2B (ERD2B), are involved in ER-quality control [10,11,15]. Recent research suggests that the ER-resident chaperone BiP, an integral protein of the ER quality control system, is one of the main chaperones regulating biogenesis and degradation of membrane-resident PRRs. For example, in BiP3-overexpressing rice plants, XA21-mediated immunity is compromised and accumulation/processing of XA21 after *Xoo* inoculation is significantly inhibited [12]. These results indicate that BiP3 regulates XA21 protein stability and processing and that this regulation is critical for resistance to *Xoo*. In Arabidopsis, a large ER chaperone complex BiP/ERdj3B/SDF2 is required for the proper accumulation of EFR, further supporting a role for ER-quality control in membrane-resident PRR-mediated function [11].

In contrast to PRRs that recognize conserved microbial signatures, intracellular NB-LRR proteins, containing nucleotide-binding and leucine-rich repeat domains, perceive highly variable pathogen-derived effectors in the cytoplasm directly or indirectly. Many NB-LRR proteins, including Arabidopsis RPM1, RPS2, and RPS5,

Abbreviations: ER, endoplasmic reticulum; BiP3, luminal-binding protein 3; BRI1, brassinosteroid-insensitive 1; EFR, elongation factor Tu receptor; FLS2, flagellin sensitive 2; HSP70, heat shock protein 70; *M. oryzae*, *Magnaporthe oryzae*; NB-LRR, nucleotide-binding domain and leucine-rich repeat; non-RD, non-arginine-aspartate; PRR, pattern recognition receptor; XA21, Xanthomonas resistance 21 receptor; *Xoo*, *Xanthomonas oryzae* pv. *oryzae*.

* Corresponding author. Fax: +1 530 752 6088.
E-mail address: pcronald@ucdavis.edu (P.C. Ronald).

http://dx.doi.org/10.1016/j.bbrc.2014.04.093
0006-291X/

tobacco N and rice Pi-ta and Pi5, have been characterized [16–20]. In tobacco, a number of ER resident chaperones, including BiP, are up-regulated during the earlier stages of N immune receptor-mediated defense against *Tobacco mosaic virus* [21]. In Arabidopsis, BiP2 is also involved in folding and secretion of pathogenesis-related (PR) proteins [22]. In a *bip2* mutant, increased PR protein synthesis after benzothiadiazole S-methylester (BTH, salicylic acid analog) treatment is not accompanied by a concomitant increase in BiP protein accumulation, resulting in impaired BTH-induced resistance. These results suggest that components of ER-quality control may also play a role in NB-LRR-mediated immunity [23].

A differential requirement for gene governing ER-quality control has been observed in structurally-related receptors. For example, Arabidopsis FLS2-mediated responses are not impaired in the mutants, *sdf2*, *crt3*, *uggt*, and *erd2b* [10,11]. In contrast, these genes which encode ER proteins, are all required for EFR-mediated signal transduction. Similarly, overexpression of BiP3 in rice does not affect rice brassinosteroid-insensitive 1 (OsBRI1)-mediated signaling, even though OsBRI1 shows an overall structural similarity with XA21. In contrast to XA21, OsBRI1 carries the RD class of kinases and regulates growth and developmental responses. As observed for rice, Arabidopsis BiPs fail to interact with wild-type BRI1 [24]. In animals, processing of Toll-like receptors (TLRs), which are key determinants of the innate immune response, also require specific ER chaperones. For example, despite of its role as a general housekeeping chaperone, mouse ER gp96 is specific for processing TLR2, 4, 5, 7, and 9 in macrophages [25–27]. Although gp96-deficient macrophages fail to respond to flagellin, the ligand for TLR5, mutant macrophages display normal development and activation by interferon-c, tumor necrosis factor-a, and interleukin-1b [27].

Based on these results, we hypothesize that ER chaperones are specific to their substrates, differentially regulating plant responses. To test this hypothesis, we chose two different types of receptors, the membrane-resident PRR XA3 and the intracellular NB-LRR protein Pi5, and assessed if BiP3 overexpression affects these immune responses. We found that overexpression of BiP3 compromises XA3-mediated immunity to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) but not Pi5-mediated immunity to *Magnaporthe oryzae* (*M. oryzae*). These results indicate that BiP3 regulates PRRs and that BiP3 overexpression does not lead to a general ER stress response.

2. Materials and methods

2.1. Plant materials and growth conditions

The endoplasmic reticulum (ER) chaperone *BiP3* overexpressing (BiP3ox) transgenic line 1A-6 [12] and its background cultivar Kitaake, IRBB3 monogenic line carrying *Xa3* [7], and IRBL5-M monogenic line carrying *Pi5* [28] were used in this study. The IRBB3 and IRBL5-M lines were crossed with BiP3ox 1A-6 to generate BiP3ox/XA3 and BiP3ox/Pi5 plants, respectively. Self-pollinated seeds (F_2) were collected and were used in pathogen inoculations. Rice plants were grown in a greenhouse at 30 °C during the day and at 20 °C at night in a light/dark cycle of 14/10 h.

2.2. Xanthomonas oryzae pv. oryzae inoculation and lesion length measurements

Rice plants were grown in the greenhouse normally until they were six-week-old and transferred to the growth chamber. Growth chambers were set on 14 h light:10 h dark photoperiod, 28/26 °C temperature cycle, and 85/90% humidity. The *Xoo* strains PXO61 and PXO79 were used to inoculate rice by the scissors-dip method [3,29]. *Xoo* strains PXO61 and PXO79 were grown on PSA plate (Peptone Sucrose Agar, 10 g/L peptone, 10 g/L sucrose, 1 g/L glutamic acid, 16 g/L agar, and pH 7.0) containing cephalexin (100 μg/L) for three days and suspended with water at OD = 0.5 (600 nm) for inoculation. Only the top two to three expanded leaves of each tiller were inoculated.

2.3. Magnaporthe oryzae inoculation and disease evaluation

M. oryzae R01-1, which is incompatible with the *Pi5*-carrying line [30] and compatible with the cultivar Kitaake, has been commonly used to evaluate disease resistance. *M. oryzae* R01-1 was grown on rice flour medium containing 20 g/L rice flour powder, 10 g/L dextrose, and 12 g/L agar in the dark for 2 weeks at 22 °C [30]. Conidia were induced for 5 days by scratching the plate surface with a sterilized loop. Agar blocks covered with spores were placed on the injured spot of 2.0 mm diameter according to the leaf punch inoculation [31]. The second fully expanded leaves from the top of five-week-old plants were used for *M. oryzae* inoculation. The inoculated plants were placed in sealed containers to maintain humidity in the dark for 24 h at 24 °C and then incubated at the same relative humidity under a 14/10 h (light/dark) photoperiod. For disease evaluation, blast lesion lengths were measured 10 days after inoculation.

2.4. Genomic DNA isolation and PCR analysis

Genomic DNAs were extracted from leaves of individual plants following the protocol described previously [32]. The gene-specific primers for PCR were as follows: for *BiP3*, 5′-GCTGCTGCTATTGCGT ACGGTTTGGACA-3′ and 5′-AATCATCGCAAGACCGGCAACAGG-3′; for *Pi5-1*, 5′-TTATGAGATTAGGAGTGTAT-3′ and 5′-ATGTAAA GGCAAAAGCTGAT-3′; and for *Pi5-2*, 5′-CTCTTGGTGATCTTTGTTAC-3′ and 5′-GGATGATGTGATCTGCAGAG-3′; and for *Xa3*, 5′-CACCC CACGCAAGCCTCTCA-3′ and 5′-CTCCGTCATCAGCCATACACTCAC-3′. We carried out PCR experiments. PCR conditions were pre-denaturation for 3 min at 94 °C, followed by 35 cycles of polymerization, each consisting of 30 s of denaturation at 94 °C, annealing for 30 s at 58 °C, and an extension step of 30 s at 72 °C. The PCR product was separated on a 1.5% agarose gel and stained with ethidium bromide to detect the amplicons.

3. Results

3.1. Overexpression of BiP3 compromises membrane-resident PRR XA3-mediated immunity

As is typical of PRRs, XA3 confers broad-spectrum resistance to *Xoo* strains including PXO61 and PXO79 [33]. To explore the biological role of BiP3 in XA3-mediated immunity, we first investigated the presence of a functional *Xa3* gene in the genome of rice cultivar Kitaake. Using the scissors-dip-method, we inoculated Kitaake plants with *Xoo* strains PXO61 and PXO79. Fig. 1A shows typical leaves from Kitaake wild type and *Xa3*-possessing IRBB3 plants 14 days after inoculation with *Xoo* strains. While IRBB3 was highly resistant, showing short lesions (approximately 4–5 cm), the inoculated leaves of Kitaake plants developed long water-soaked lesions (approximately 17–20 cm) typical of bacterial blight disease (Fig. 1B). These results indicate that Kitaake plants do not possess a functional *Xa3* gene.

A segregation ratio of 17:6 (approximately 3:1), reflecting *BiP3ox* insertion at a single locus, was observed in the progeny of a transgenic Kitaake line overexpressing *BiP3* (BiP3ox 1A) (data not shown). To generate XA3 plants overexpressing *BiP3*, we first crossed IRBB3 (XA3, pollen donor) with transgenic Kit-

Please cite this article in press as: C.-J. Park et al., Rice BiP3 regulates immunity mediated by the PRRs XA3 and XA21 but not immunity mediated by the NB-LRR protein, Pi5, Biochem. Biophys. Res. Commun. (2014), http://dx.doi.org/10.1016/j.bbrc.2014.04.093