Plant Science 210 (2013) 53–60

Contents lists available at SciVerse ScienceDirect

Plant Science

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

The endoplasmic reticulum-quality control component SDF2 is essential for *XA21*-mediated immunity in rice

Chang-Jin Park[a,b,1], Rita Sharma[a,c,1], Benoit Lefebvre[a,2], Patrick E. Canlas[a], Pamela C. Ronald[a,c,*]

[a] Department of Plant Pathology and The Genome Center, University of California Davis, Davis, CA, United States
[b] Department of Bioresources Engineering, Sejong University, Seoul, South Korea
[c] Joint BioEnergy Institute, Emeryville, CA, United States

ARTICLE INFO

Article history:
Received 17 December 2012
Received in revised form 17 April 2013
Accepted 7 May 2013
Available online 14 May 2013

Keywords:
ER-QC
Receptor-like kinase
SDF2
Xanthomonas oryzae pv. *oryzae*
XA21

ABSTRACT

Plant genomes contain large number of plasma membrane (PM)-localized immune receptors, also called pattern recognition receptors (PRRs). PRRs are synthesized in the endoplasmic reticulum (ER) and then translocated to the PM, where they recognize conserved pathogen-associated molecular patterns (PAMPs) and activate innate immune response. The rice XA21 immune receptor confers resistance to the Gram-negative bacterial pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). To identify components that mediate *XA21*-mediated signaling, we performed co-purification experiments using C-terminal GFP tagged XA21 protein. Several endoplasmic reticulum-quality control (ER-QC) proteins including stromal-derived factor 2 (SDF2) co-purified with XA21. Silencing of the *SDF2* genes in the *XA21* rice genetic background compromises resistance to *Xoo* but does not affect plant growth and development.

1. Introduction

Plants perceive conserved microbial signatures *via* plasma membrane (PM)-localized immune receptors, often called pattern recognition receptors (PRRs) [1]. Well-studied PRRs include rice XA21 [Xanthomonas resistance 21; [2]], *Arabidopsis* FLS2 [flagellin sensitive 2; [3]] and *Arabidopsis* EFR [elongation factor Tu receptor; [4]]. These PRRs typically carry or associate with the non-RD (non-Arg-Asp) kinase motif to regulate innate immunity [5].

PRR proteins, as all PM proteins, must enter the secretory pathway for proper folding and assembly in the endoplasmic reticulum (ER) before translocation to their final destination in the PM. To ensure transportation of only correctly folded proteins, the ER utilizes a complex quality control (QC) system composed of many molecular chaperones, carbohydrate-binding lectins and glycan-modifying enzymes [6,7]. One of the ER-QC systems relies on the direct binding of the heat shock protein (HSP) 40-like chaperone, ERdj3B, to unfolded/misfolded proteins. ERdj3B further recruits HSP70 family luminal binding proteins, BiPs, for their retention in the ER in an ATP-dependent manner. The second system is characterized by N-linked glycosylation followed by glucosidase-mediated trimming of glucose residues producing monoglycosylated client proteins. These proteins are then recognized and folded by the lectin chaperones, calnexin (CNX) and calreticulin (CRT). The incorrectly folded proteins are re-glucosylated by UDP-Glc glycoprotein-glucosyltransferase (UGGT) and again subjected to CNX/CRT cycle. In parallel, formation of disulphide bonds between free thiol groups in the client proteins is mediated by protein disulphide isomerases (PDIs) and thiol oxidoreductases [8].

Although several proteins that participate in receptor-mediated signaling have been intensively studied, very few components involved in receptor biogenesis and translocation have yet been characterized. Recent genetic evidence indicates that the molecular chaperones and co-chaperones play critical role in biogenesis, maturation, and stabilization of PRRs through ER-QC pathway [8–14]. For example, a genetic screen for elf18-insensitive (*elfin*) mutants in *Arabidopsis* revealed that *crt3* fails to accumulate EFR proteins, suggesting that EFR is a substrate for CNX/CRT3 [9]. An ER complex comprising the stromal-derived factor-2 (SDF2), the HSP40 ERdj3B, and the HSP70 BiP proteins is required for proper accumulation of EFR in *Arabidopsis* [11]. Similarly, the rice Hop/Sti1–HSP90 chaperone complex is required for maturation of chitin elicitor receptor kinase 1 (OsCERK1) in ER before transport to the PM [15].

Abbreviations: ER-QC, endoplasmic reticulum-quality control; SDF2, stromal-derived factor 2; LRRs, leucine-rich repeats; PM, plasma membrane; PR, pathogenesis-related; PRR, pattern recognition receptor; XA21, Xanthomonas resistance 21; *Xoo*, *Xanthomonas oryzae* pv. *oryzae*.

* Corresponding author at: Department of Plant Pathology and The Genome Center, University of California Davis, Davis, CA, United States. Tel.: +1 530 752 1654; fax: +1 530 752 6088.
E-mail address: pcronald@ucdavis.edu (P.C. Ronald).

[1] These authors contributed equally.

[2] Present address: INRA UMR441-CNRS UMR2594, Laboratory of Plant Microbe Interactions, Castanet-Tolosan, France.

0168-9452/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.plantsci.2013.05.003

We have also shown previously that the ER chaperone BiP3 in rice interacts with XA21 *in vivo* and its overexpression compromises *XA21*-mediated immunity [16,17].

Although the ER-QC machinery is well conserved among eukaryotic cells, a differential genetic requirement for ER-QC dependency has been observed in structurally related receptor-like kinases. For example, *Arabidopsis FLS2*-mediated responses are not impaired in the mutants, *sdf2*, *crt3*, *uggt*, and *erd2b* [9,11]. In contrast, all of these ER proteins are required for *EFR*-mediated signal transduction. Similarly, overexpression of *BiP3* in rice compromises *XA21*-mediated immunity but does not affect rice *Brassinosteroid-insensitive 1* (*OsBRI1*)-mediated signaling, even though OsBRI1 shows an overall structural similarity with XA21. As observed for rice, *Arabidopsis* BiPs fail to interact with wild-type BRI1 [18]. These results suggest that ER-QC components are specific to their substrates resulting in differential regulation of plant responses.

To identify proteins involved in *XA21*-mediated signaling, we purified an *in vivo* XA21 complex from transgenic rice plants expressing a C-terminal green fluorescent protein (GFP)-tagged XA21. Several ER-QC related proteins co-purified with XA21 suggesting a role for these proteins in biogenesis and functionality of the XA21 receptor. One of the co-purified proteins is orthologous to *Arabidopsis SDF2* , which is required for regulation of *EFR* function and the unfolded protein response [11,19]. *Arabidopsis SDF2* is a single copy gene encoding a protein with an N-terminal signal peptide and three repeats of the MIR (mannosyltransferase, inositol-3-phosphate receptor, and ryanodine receptor) domain [11]. AtSDF2 localizes to the ER in a complex with HSP40 ERdj3B and the HSP70 BiP [11]. Here, we investigate the role of rice SDF2 in regulating *XA21*-mediated immunity.

2. Materials and methods

2.1. Plant material and growth conditions

Rice (*Oryza sativa* L.) plants (cultivar Kitaake) were grown for about six weeks in the green house and transferred to growth chamber for *Xoo* inoculations. Growth chambers were set on a 14 h light and 10 h dark photoperiod with 28/26 °C temperature cycle and 85–90% humidity. Healthy and well-expanded leaves from six-week-old rice plants were inoculated with *Xoo* strain Philippine race 6 (designated as *Xoo* in text). The plant tissues, both prior and after inoculation, were collected for nucleic acid and protein extractions.

2.2. In vivo purification of XA21

Total proteins were extracted from 40 g of six-week-old rice leaves in 80 ml of ice-cold Extraction Buffer II [0.15 M NaCl, 0.01 M Na-phosphate pH 7.2, 2 mM EDTA, 1% Triton X-100, 10 mM β-mercaptoethanol, 20 mM NaF, 1% Plant protease inhibitor cocktail (Sigma), 2 mg/ml antipain, and 2 mg/ml aprotinin]. After filtration through a Nylon mesh followed by centrifugation at 48,000 × *g* for 60 min at 4 °C, the supernatant was mixed with 80 μl of magnetic bead-conjugated GFP-Trap (chromotek) and incubated overnight at 4 °C. The beads were then washed twice in 0.5 ml of Extraction Buffer II without proteinase inhibitors. The proteins were eluted with Laemmli sample buffer (Bio-Rad). Proteins were separated on precast 7.5% tris/glycine gels (Bio-Rad) and stained with Bio-Safe Coomassie Stain (Bio-Rad). Western blot was performed using mouse anti-GFP antibodies (Santa Cruz).

2.3. Protein identification

Proteins were identified in three independent purification experiments carried out using LC–MS/MS analysis at the Genome Center Proteomics Core Facility, University of California, Davis. Proteins were prepared using standard *in gel* reduction, alkylation and trypsin digestion procedures. Digested peptides were extracted from the gel pieces, mixed as specified and analyzed by an LTQ XL (Thermo Scientific) mass spectrometer. Peptides were identified by searching the MS/MS spectra against a UniProt rice database using X! Tandem was searched with a fragment ion mass tolerance of 0.40 Da and a parent ion tolerance of 1.8 Da. Iodoacetamide derivative of cysteine was used as fixed modification. Deamidation of asparagine and glutamine, oxidation of methionine and tryptophan, sulphone of methionine and acetylation of the N-terminus were used as variable modifications. Sample comparison and spectrum analysis were performed using the Scaffold Viewer. The Protein Prophet algorithm was used to assign protein probabilities (Table 1). Proteins co-purified with XA21, in at least two independent experiments and represented by at least two unique peptides with a scaffold probability higher than 95% and absent from the Kitaake wild type control, were defined as interacting partners of XA21.

2.4. Plasmid construction for SDF2 overexpression and silencing in rice

The rice genome contains two *SDF2* genes, designated here as *OsSDF2-1* (Os08g17680) and *OsSDF2-2* (Os08g34190). 654 nucleotide cDNA fragments encoding full-length OsSDF2-1 and OsSDF2-2 proteins were amplified from rice cDNA using primers, 5′-CACCATGGCCGCCGCGTCGTT-3′/5′-ATATTTTTGCTGGTTCACCGGGAG-3′ (for *OsSDF2-1*) and 5′-CACCATGGCCGCGTCCGTTCTCGCGCT-3′/5′-TTACTTGCTTTTGTTAACGGGAAGG-3′ (for *OsSDF2-2*). The PCR products were cloned into pENTR/D-TOPO vector (Invitrogen) to create OsSDF2-1/pENTR/D-TOPO and OsSDF2-2/pENTR/D-TOPO according to the instructions provided by the manufacturer and the insert was confirmed by sequencing. For silencing both *OsSDF2-1* and *OsSDF2-2* in rice, 300 bp fragment of *OsSDF2-2* was excised from OsSDF2-2/pENTR/D-TOPO using *Hinc*II/*Sph*I and then ligated into 295 bp fragment of OsSDF2-1/pENTR/D-TOPO, predigested with *Nae*I and *Sph*I, to create a chimeric *SDF2* construct (*chSDF2*) in pENTR/D-TOPO (Fig. S3). The chSDF2/pENTR/D-TOPO was recombined into the pANDA silencing vector [20] using gateway LR Clonase (Invitrogen). To construct the *Ubi OsSDF2-1* and *Ubi OsSDF2-2* overexpression constructs, OsSDF2-1/pENTR/D-TOPO and OsSDF2-2/pENTR/D-TOPO were recombined into the gateway-compatible Ubi-CAMBIA-1300 [21].

2.5. Rice transformations

Rice transformations were performed as described previously [21]. *Agrobacterium* strain EHA105 was used to infect rice callus for transformation. Transformants of Kitaake and Nat-XA21 plants carrying the plasmids were selected using hygromycin selection marker.

2.6. Expression analysis

For reverse transcriptase-polymerase chain reaction (RT-PCR) and quantitative real time (qRT)-PCR analysis, total RNAs were extracted from leaves using TRIzol® reagent (Invitrogen). The RT reaction was performed following the manual for QuantumRNA 18S Internal Standards (Ambion). PCR analyses were performed with primers pairs, 5′-ATAGTGAGACCGCAACCAGATACT-3′/5′-GACTTCCCACTGCCCTCAATCTC-3′ (for *OsSDF2-1*) and 5′-GCCGTCGCGTTCCTCCTC-3′/5′-CTGCCCACCACCAAGCCTGTT-3′ (for *OsSDF2-2*). After 28 cycles, the amplified products were resolved by gel electrophoresis. For qRT-PCR analysis, RNA samples were