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Cell wall reorganization during infection in fungal plant pathogens☆

Marie Nishimura

National Institute of Agrobiological Sciences, 2-1-2, Kan-non dai, Tsukuba, Ibaraki 305-8602, Japan

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ABSTRACT

The fungal cell wall, which is mainly composed of polysaccharides, is a major source of pathogen-associated molecular patterns (PAMPs). Because PAMPs recognition activates immunity in plants, successful pathogens have developed immune-evasion strategies. Studies of various fungal rice pathogens indicated that masking the cell wall surface with α-1,3-glucan, a polysaccharides that is not degradable by plants, is a fungal PTI evasion strategy. Interestingly, accumulation of α-1,3-glucan at the surface was specifically observed in presence of plants or plant factor(s). Since the surface α-1,3-glucan protected the fungal cell wall from digestive enzymes and interfered with PAMPs generation by host enzymes, fungal α-1,3-glucan is a potential target for plant protection strategies.

1. Introduction

Cell wall is the outermost layer of fungi that is exposed to the surrounding environment. The cell wall is essential for fungi not only for maintaining structure but also for protecting the cells from environmental stresses. The fungal cell wall is mainly composed of polysaccharides; β-1,3-glucan, β-1,6-glucan, α-1,3-glucan, chitin/chitosan and mannan and/or galactomannan. In ascomycete filamentous fungi, a skeletal core is formed by branched β-1,3-glucan and chitin; this core structure is embedded in amorphous polysaccharides such as α-1,3-glucan. Studies on medically important fungal pathogens indicated that the organization and composition of the amorphous polysaccharides varies with fungal classes or genera and according to environmental conditions [1,2]. Environmental factors which trigger reorganization of fungal cell wall components and fungal mechanisms that recognize these factors have not been sufficiently studied.

In plant-fungus interactions, conserved and specific cell wall polysaccharides of various fungi, such as chitin, are recognized as pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors (PRRs) in plants. Recognition of PAMPs triggers immunity (PTI) in plants; various cellular defense responses in plants are associated with PTI, including generation of reactive oxygen species, production of antimicrobial enzymes/compounds, reinforcement of the plant cell wall and changes in gene expression. These responses together block invasion of a broad range of fungi [3,4]. Nevertheless, fungal pathogens invade host plants. This fact led to the idea that successful fungal pathogens have mechanisms to evade the host PTI. Intensive studies have clarified several PTI evasion strategies of fungal pathogens, most of which are effector-mediated. Fungal effectors are generally small secreted proteins with no homology to proteins of known function. Fungal pathogens secrete effectors that suppress host immune responses in various ways, e.g. modulating host metabolisms and defense pathway, sequestering PAMPs from the host PRRs and interfering with antifungal enzymes of hosts [3–6]. In contrast, known effector-independent strategy in plant fungal pathogens are less diverse; they are limited to reorganizing cell wall polysaccharides [4–6]. Here, I spotlight a fungal 'stealth infection' strategy which does not rely on secretion of effectors [7,11]. In this strategy, fungal plant pathogens utilize α-1,3-glucan, a nondegradable polysaccharide for most plants, to protect the fungal cell walls from plant immune responses.

2. Recognition of a plant factor triggered reorganization of cell wall polysaccharides in *Magnaporthe oryzae*

M. oryzae (*Pyricularia oryzae*) is a model plant pathogen for the study of plant-microbe interactions. At the same time, this fungus has caused the most serious economic damage to rice production in the world. Under natural conditions, infection by this fungus starts with attachment of the conidia to host surfaces. *M. oryzae* forms an

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E-mail address: marie@affrc.go.jp.

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infection-specific structure called an appressorium at the tip of the germ tube (germling) extended from the conidium on plant surfaces. A penetration peg developed from the appressorium perforates the plant epidermal layer and then expands to form a primary hypha, which differentiates into a secondary infectious hypha in plant cells. Appressoria are also induced on artificial substrates such as plastic or glass cover slips. The response of the fungus to artificial and natural plant surfaces appeared to be similar in terms of formation of appressoria. However, histochemical staining of major cell wall polysaccharides using specific fluorophore-labeled antibodies or lectins revealed that the fungal responses to plant hosts and artificial substrates are distinct. When fungal conidia were incubated on glass coverslips, the core polysaccharides, β-1,3-glucan and chitin, were clearly detected on appressoria and germ tubes but α-1,3-glucan was detected only on appressoria (Fig. 1A–C, 'None'). However, on plant surfaces, chitin and α-1,3-glucan, but not β-1,3-glucan, were clearly detected on appressoria and germ tubes (Fig. 2A) [7]. This result indicated that the presence of a plant factor(s) promoted reorganization of cell wall polysaccharides in *M. oryzae*. Indeed, in the presence of 1,16-hexadecanediol, the cutin monomer known as an inducer of appressorium formation, α-1,3-glucan became detectable on glass coverslips (Fig. 1A–C, '+ 1,16-hexadecanediol') [7,8].

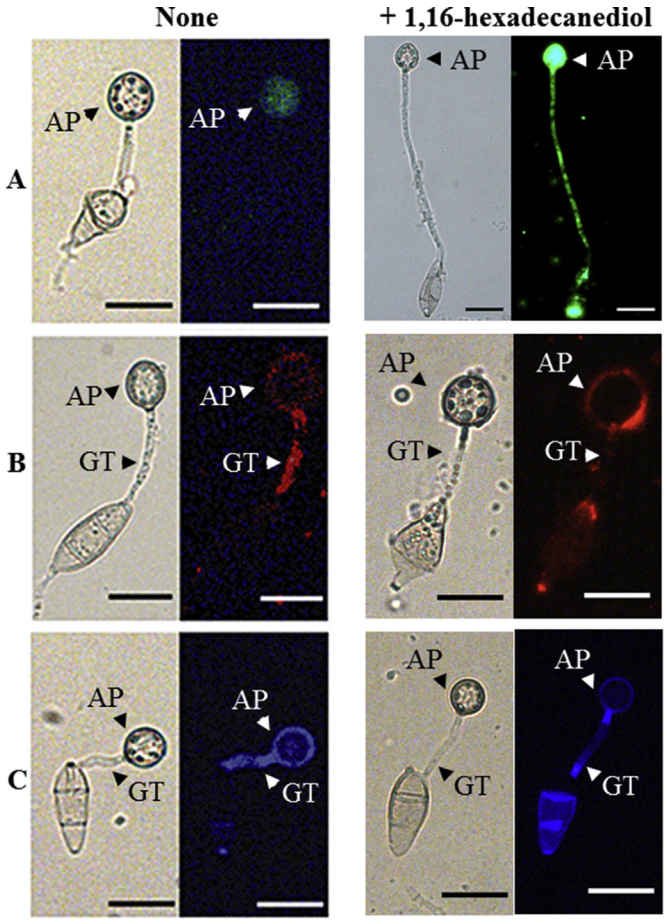

Fig. 1. Plant cutin monomer induced α-1,3-glucan accumulation on the cell wall on glass coverslips. Detection of cell wall polysaccharides on appressorium-forming conidia on glass coverslips in the absence/presence of plant cutin monomer, 1,16-hexadecanediol at 24 h after incubation (hai). α- and β-1,3-glucan were detected by fluorophore-labeled antibodies, chitin by fluorophore-labeled lectin. Bright-field optics (left panels of each pair) and epifluorescent optics (right panels of each pair). **(A)** α-1,3-glucan, **(B)** β-1,3-glucan **(C)** chitin. AP, appressoria; GT, germ tubes. Scale bar = 10 μm. Figure modified from Fujikawa et al. (2009) [7].

The Mps1 MAP kinase of *M. oryzae* is an orthologue of the cell wall integrity (CWI) MAP kinase of *Saccharomyces cerevisiae* [9]. Biochemical and immunohistological studies using mutants showed that α-1,3-glucan synthesis fully depended on Mps1 activity and that 1,16-hexadecanediol activated Mps1 signaling in *M. oryzae* [7]. Considering that sensors recognize environmental signals and activate CWI MAP kinase pathways in other fungi [1,10], it was very likely that sensor(s) for the CWI pathway recognized the plant factor(s) in some way. The fact that *M. oryzae* reorganizes cell wall polysaccharides upon recognition of the plant surface factor(s) suggested that the fungus recognizes whether or not it is on host plant surfaces before invasion.

3. Major fungal rice pathogens masked cell wall surfaces of infectious hyphae with α-1,3-glucan

M. oryzae, *Cochlioborus miyabeanus* (*Bipolaris oryzae*) and *Rhizoctonia solani* cause blast, brown spot and sheath blight, respectively, which are the top-three fungal diseases causing serious damage to world rice production. These pathogens are phylogenetically distant; *M. oryzae* and *C. myabeanus* belong to different classes within Ascomycota, while *R. solani* is a basidiomycete. In addition, these pathogens have different infection style; *M. oryzae* is hemibiotrophic whereas *C. miyabeanus* and *R. solani* are necrotrophic. Although these fungi are very different as pathogens, histochemical staining of major cell wall polysaccharides using fluorophore-labeled antibodies or lectins revealed that these pathogens specifically accumulated α-1,3-glucan at accessible surfaces of cell walls in infectious hyphae [11]. The following subsections summarize the patterns of histochemical staining for each fungus. Because antibodies and lectins were used for the staining, polysaccharides located at accessible surfaces of cell walls might be detected.

3.1. Cell wall polysaccharide localization of infectious hyphae in M. oryzae

Histochemical staining clearly detected α-1,3-glucan but β-1,3-glucan and chitin were hardly observable (Fig. 2B). Thus, detectable polysaccharides on cell wall of infectious hyphae were different from those on germ tubes. However, β-1,3-glucan and chitin became detectable after enzymatic removal of α-1,3-glucan by a bacterial α-1,3-glucanase treatment (Fig. 2C). In addition, immunoelectron microscopy revealed that α-1,3-glucan tended to locate outside the β-1,3-glucan in the cell walls of infectious hyphae [7]. Together, these results show that α-1,3-glucan accumulated at the surface and masked β-1,3-glucan and chitin in the cell walls of infectious hyphae.

3.2. Localization of cell wall polysaccharides in C. miyabeanus

Histochemical staining was performed with vegetative and infectious hyphae of *C. miyabeanus* by the same method used for the *M. oryzae* cell wall staining. On the vegetative hyphae, chitin and β-1,3-glucan, but not α-1,3-glucan, were clearly detected (Fig. 3A). In contrast to this, α-1,3-glucan became observable but chitin was undetectable on the infectious hyphae (Fig. 3B). After the enzymatic digestion of α-1,3-glucan, chitin became detectable and β-1,3-glucan became more apparent (Fig. 3C). Thus, α-1,3-glucan specifically accumulated on cell wall of infectious hyphae, masking chitin and partly masking β-1,3-glucan [11].

3.3. Localization of cell wall polysaccharides in R. solani

Similar to *M. oryzae* and *C. miyabeanus*, histochemical staining