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α -1,3-glucan functions as camouflage during infection in the rice blast fungus *Magnaporthe oryzae*

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The fungal cell wall, the outermost layer of the cell, is a good target for recognition of fungal invaders by the host cells. Fungal cell walls are mainly composed of polysaccharides, e.g., β -glucans, α -glucans, chitin/chitosan, and mannans. In general, β -1,3-glucan and chitin form the skeletal core, while non-skeletal polysaccharides differ between fungal species and with growth conditions. Innate immune systems found in vertebrates, invertebrates and plants are activated upon receptor recognition of conserved structural patterns of cell-wall components of the fungal invaders and enables an immediate defence of the host cells against a broad range of fungi. As a result of activation of the innate immune systems, various defence responses are induced in the host cells. In plants, fungal cell wall chitin and β -glucans have been reported to evoke innate immune responses such as secretion of anti-fungal enzymes (β -glucanases and chitinases), production of anti-fungal agents and reinforcement of the plant cell wall. As such, recognition of the fungal cell-wall polysaccharide is very important in plant-microbe interactions. It was assumed that pathogens have mechanisms to protect themselves from host immune attacks during infection; however, the protection mechanisms in plant pathogens were poorly understood.

To better understand fungal strategies circumventing the host plant immune responses, we used fluorescent labels to investigate localization of major cell wall polysaccharides of the rice blast fungus, *Magnaporthe oryzae*, during rice infection. Our cytological studies showed that α -1,3-glucan and chitosan were the major polysaccharides at accessible surface of infectious hyphae. However, β -1,3-glucan and chitin also became detectable after enzymatic digestion of α

-1,3-glucan (Fig. 1). Immunoelectron microscopic observation of infectious hyphae revealed that α -1,3-glucan was localized in the outer portion of the cell wall compared to β -1,3-glucan, supporting our microscopic observation that surface α -1,3-glucan masks β -1,3-glucan and chitin in the cell wall. Moreover, although no accumulation of α -1,3-glucan on germ tubes was observed on cover glass, it was clearly detected in the presence of exogenous plant wax or on plant surfaces (Fig. 2). These results provide evidence that α -1,3-glucan accumulation is induced by a plant cue (wax). Using a genetic mutant related to signal transduction pathways in *M. oryzae*, we have shown that Mps1 MAP kinase, an ortholog of MAP kinase involved in cell-wall integrity in *Saccharomyces cerevisiae*, is required for the accumulation of α -1,3-glucan and is activated in the presence of the plant wax. We further demonstrated that the accumulation of α -1,3-glucan on the cell wall was correlated with resistance of the cell wall against chitinase digestion. Taken together, these results show that the fungus accumulates α -1,3-glucan on the surface of the fungal cell wall in response to plant wax in an Mps1 MAP-kinase-dependent manner to physically and functionally mask the cell wall.

It is important to note that genes encoding α -1,3-glucanase are not found in the rice genome. Our results, together with the rice genome information, strongly suggest that the function of the surface α -1,3-glucan is to not only protect the fungal cell wall from digestive enzymes produced by plant cells but also to camouflage chitin and β -1,3-glucan in the cell wall from being recognized during infection by the innate immune system of rice (Fig. 3).

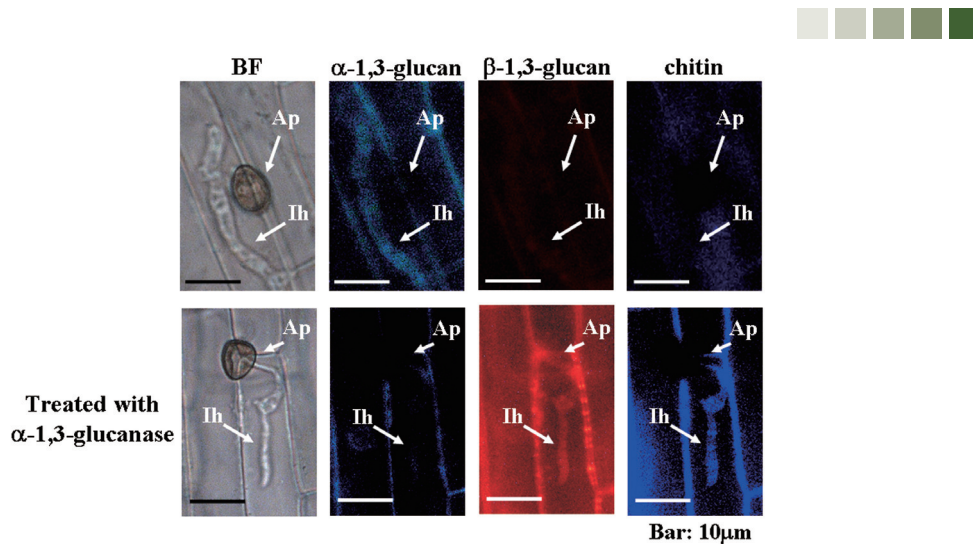


Fig. 1 Detection of cell wall polysaccharides on infectious hyphae developed in rice sheath cells. After enzymatic digestion of α -1,3-glucan, β -1,3-glucan and chitin became detectable, indicating that α -1,3-glucan masks β -1,3-glucan and chitin in the fungal cell wall. Images were taken at 24h post inoculation. Ap: appressorium, Ih: infectious hypha.

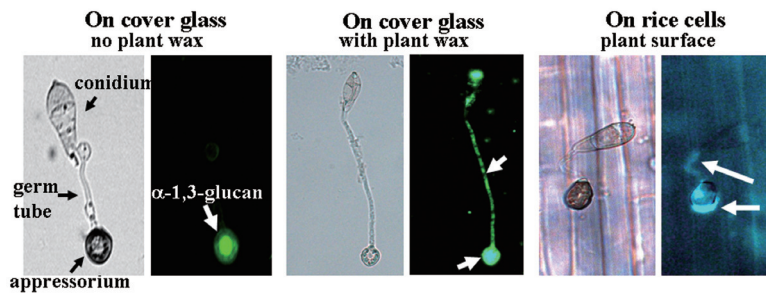


Fig. 2 Induction of α -1,3-glucan accumulation on the fungal cell wall by plant cue (wax). α -1,3-glucan was only detected on appressorium developed on a cover glass (left panel). In contrast, α -1,3-glucan was detected in entire cells in the presence of plant wax (center panel) and on the surface of rice cells (right panel).

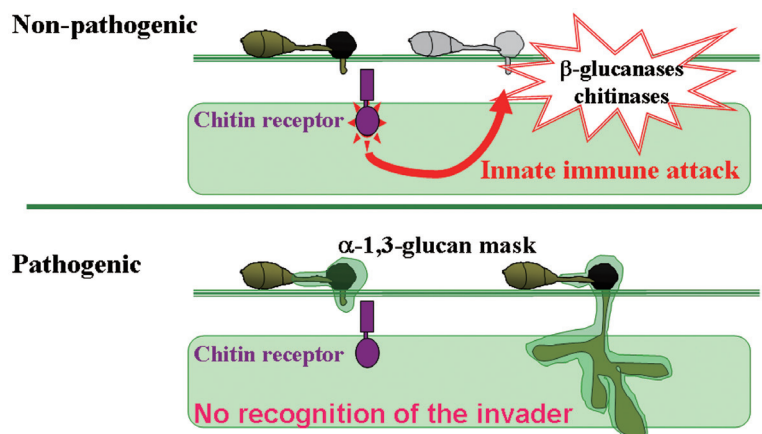


Fig. 3 Stealth technology of the rice blast fungus. Rice innate immune system attacks fungal invaders by secretion of anti-fungal enzymes/agents upon recognition of chitin exposed on the fungal cell wall. However, rice failed to detect *M. oryzae* camouflaged with α -1,3-glucan mask that allows the fungus to successfully invade into rice cells.

Reference

Fujikawa T, Kuga Y, Yano S, Yoshimi A, Tachiki T, Abe K, Nishimura M (2009) Dynamics of

cell wall components of *Magnaporthe grisea* during infectious structure development. *Molecular Microbiology*, 73: 553-570.