Calcium signaling for appressorium formation of *Magnaporthe grisea*

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Rice blast, caused by ascomycete fungus *Magnaporthe grisea* (anamorph, *Pyricularia grisea*), is the most threatening disease in rice production throughout the world. Rice blast has been considered as a model system to study plant-pathogen interactions not only due to the economic significance of this disease but also classical and molecular tractability of both pathogen and host. Moreover, rice blast is the only pathosystem in which both host and pathogen genome sequences are available.

The rice blast fungus, *M. grisea*, forms a specialized infection structure, an appressorium, to penetrate its host. During the last decade, much has been learned about environmental cues and signal transduction pathways involved in appressorium development. Several genes involved in signaling pathways including G-proteins, adenylate cyclase, cAMP-dependent-protein kinase A and mitogen-activating protein kinases have been cloned and functionally characterized to be implicated in appressorium development, maturation, and subsequent infection. Calcium-related signaling pathway was also suggested to play a major role in appressorium formation of *M. grisea,* but molecular mechanism is largely unknown.

Intracellular Ca^{2+} plays a crucial role in cellular signaling and can regulate a wide range of physiological functions and cell development in diverse organisms. Intracellular Ca^{2+} signaling is regulated by phosphoinositide-specific phospholipase C (PI-PLC). Generally, PI-PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce two second messengers, inositol-1, 4, 5-triphosphate (IP_3) and diacylglycerol (DAG). These messengers then promote the release of the Ca^{2+} from intranacellular stores and protein kinase C (PKC) activation, respectively. To understand fungal pathogenesis, many scientists have focused on Ca^{2+} signaling. An intracellular Ca^{2+}gradient can be directly acted on the regulation of cytosolic Ca2+ by intracellular store under physiological and pathological responses in filamentous fungi (Dean 1997). In filamentous fungi, Ca^{2+} /calmodulin-dependent signalings have been implicated in a variety of cellular processes including conidial germination of *Metarhizium anisopliae*, hyphal tip growth of *Saprolegnia ferax*, and appressorium differentiation of *Colletotrichum* species and *M anisopliae*. In *M. grisea*, Ca^{2+} and DAG signalings were suggested to be involved in

appressorium formation. However, most studies relied on inhibitor experiments to investigate a role of calcium signaling in filamentous fungi, and how calcium signaling plays in infection-related fungal morphogenesis is still largely unknown.

To understand the molecular function of PI-PLC, a gene (*MPLC1*) encoding a PI-PLC was cloned and characterized in *M. grisea*. Mutant (Δ*mplc1*) lacking a MPLC1 showed significant reduction in appressorium formation and failed to penetrate and proliferate within rice plant tissues. Δ*mplc1* mutant also showed impaired ability to maintain intracellular calcium homeostasis during appressorium formation. MPLC1 was also essential for both asexual and sexual reproduction in this fungus. Introduction of an intact copy of MPLC1 rescued all defective phenotypes in the mplc1 mutant. Moreover, a mouse PLC-1 (*mPLC-1*) gene also complemented defective phenotypes of the Δ*mplc1* mutant, including appressorium formation and pathogenicity on rice plants.

These results demonstrate that MPLC1-mediated Ca^{2+} signaling plays important roles in infection-related morphogenesis and host colonization of *M. grisea*. Our finding would not only provide insights into clear understanding of signal transduction pathway required for pathogenicity of *M. grisea* but also lead decipher kingdom-wide understanding of signal transduction in fungal and mammalian systems.