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AWARD

Morphogenesis and adaptive strategies for infection in plant pathogenic fungi

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Introduction

To successfully infect host plants, plant pathogenic fungi must accomplish critical initial steps: attachment, lysis, and mechanical breach of the host surface. To overcome the barriers posed by the host plants, fungi have evolved a range of infection strategies, including the formation of specialized infection structures and the secretion of cell-wall-degrading enzymes, toxins, and effectors. One such specialized structure is the appressorium, which fungi use to penetrate the plant surface. Appressoria are often melanized and generate high turgor pressure to facilitate this penetration. Following successful penetration, fungi proliferate and subsequently transition to the sporogenesis phase during the later stages of infection. Although the molecular mechanisms of spore formation are not fully understood in many plant pathogenic fungi, fungal cells often form hyphal aggregates. These aggregates are embedded in a mucilaginous matrix, where subsequent cell wall remodeling and spore maturation occur. This review summarizes recent findings on the molecular mechanisms underlying appressorium development and the initiation of spore formation, based on studies of two model plant pathogenic fungi: *Colletotrichum orbiculare*, which causes cucumber anthracnose, and *Ustilago maydis*, which causes corn smut.

The unique role of cell cycle regulators in *Colletotrichum orbiculare*

Colletotrichum orbiculare is the causal agent of cucumber anthracnose disease. The infection process is initiated through the recognition of specific surface cues by aseptate conidia. Following germination of the conidia, the emerging germ tubes differentiate into dome-shaped appressoria, which subsequently undergo nuclear division (Kubo and Takano 2013; Takano et al. 2001). From random gene-insertional mutagenesis in *C. orbiculare*, the *CoBUB2* gene was identified as responsible for the pathogenicity-deficient phenotype on cucumber (Fukada and Kubo 2015). CoBub2 harbours a Rab GTPase-activating protein (GAP) domain, with highly conserved homologous sequences observed across yeast and filamentous fungi. In *Saccharomyces cerevisiae*, Bub2 forms a two-component GAP complex with Bfa1, which targets the GTPase Tem1. The Bub2-Bfa1 GAP complex in *S. cerevisiae* plays a critical role in monitoring cell cycle checkpoints, ensuring accurate distribution of replicated genomic material and preventing premature mitotic exit (Hu and Elledge 2002). By analysing the timing of entry into the DNA replication phase (S phase), we found that the *C. orbiculare cobub2* and *cobfa1* mutants displayed deficiencies in monitoring the G1 to S phase transition during conidial germination and appressorium formation. Thus, we conclude that the CoBub2-CoBfa1 GAP complex plays a distinct role in regulating G1/S phase progression in *C. orbiculare*, differing from its function in homologs in yeasts. To assess whether the regulation of G1/S progression by CoBub2/CoBfa1 is conserved across other plant pathogenic fungi, we investigated the role of Bub2 in G1/S progression in *C. higginsianum* and *Magnaporthe oryzae*, other representative species that penetrate host plants via appressoria. Our findings demonstrate that *BUB2* is a crucial negative regulator of G1/S progression and initiates septum formation in these fungi (Fukada et al. 2019).

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Proper cell cycle progression is essential for appressorium-mediated penetration

What explains the differing role of Bub2 in the cell cycle compared to its yeast homologs? We hypothesized that downstream components of CoTem1 GTPase differ among species. To test this hypothesis, we performed a yeast two-hybrid analysis using CoTem1 as bait, identifying CoNpc2 in *C. orbiculare* (Kodama et al. 2022). CoNpc2 and its homolog CoNpc1 are sterol-binding proteins crucial for sterol export from lysosomes in humans and yeasts (Ikonen 2008). Although *conpc2* and *conpc1* mutants showed no cell cycle defects, deletions of *CoBUB2*, *CoTEM1*, *CoNPC1*, and *CoNPC2* resulted in similar defects in appressorial vacuolar morphogenesis. Consistent with observation that *cobub2* mutants failed to form a penetration peg due to impaired septin and actin assembly at the appressorium pore (Fukada and Kubo 2015), *CoNPC2* and *CoNPC1* are critical for membrane integrity, actin assembly, penetration peg formation, and appressorial cone development by facilitating sterol transport and distribution (Kodama et al. 2022). Therefore, we conclude that the CoBub2-CoBfa1 and CoTem1 cascade that controls cell cycle machinery is required for appressorium penetration and works in conjunction with proper intracellular sterol transport mediated by CoNpc2 and CoNpc1.

Successful spore formation in *Ustilago maydis* is facilitated by secreted hydrophobic proteins

Even after successfully penetrating host plants, plant pathogenic fungi must continue to evade plant defense mechanisms and produce spores to facilitate secondary infections during the later stages of infection. The biotrophic basidiomycete fungus *Ustilago maydis*, which causes smut disease in maize, exemplifies this process. Following penetration of maize tissue, *U. maydis* dikaryotic cells invade and proliferate, forming large tumors. In the later stages, sporogenesis is initiated by the formation of large fungal aggregates embedded in a mucilaginous matrix. The tumors eventually rupture, releasing diploid spores into the environment (Tollot et al. 2016). To promote colonization, *U. maydis* produces 467 putative secreted effector proteins, which are expressed in discrete waves (Lanver et al. 2018). Although most functionally characterized effector proteins are associated with biotrophic development (Lanver et al. 2017; Ludwig et al. 2021), none of the effectors expressed during tumor and spore formation had been characterized.

Among the most upregulated effectors during tumor formation, we identified a novel core effector, Lep1 (Late Effector Protein 1), in *U. maydis* (Fukada et al. 2021). In tumors, *lep1* mutants exhibited reduced hyphal aggregation, failed to undergo massive late proliferation, and produced only a few spores. Constitutive expression of *lep1* induced cell aggregation and enhanced the hydrophobicity of the filamentous colony surface. Lep1 bound to the cell wall of biotrophic hyphae and associated with the repellent protein Rep1 when constitutively expressed in hyphae. We conclude that Lep1 acts as a novel type of cell adhesin that functions together with other surface-active proteins to facilitate the proliferation of diploid hyphae and the morphological changes associated with spore formation.

Conclusion

In experiments with *C. orbiculare*, we identified the *BUB2* gene, which regulates G1/S phase progression of the cell cycle through a reverse genetics approach. This finding led to the identification of CoBfa1, which forms a GAP complex; CoTem1, a downstream GTPase; and CoNpc2, which is involved in sterol transport and interacts with CoTem1. The molecular mechanism by which *BUB2* gene disruption accelerates G1/S phase progression in the three tested plant pathogenic fungi, differing from homologs in yeasts and non-pathogenic fungi, remains unclear. However, it is more evident that reduced pathogenicity resulting from *BUB2* gene disruption is likely due to impaired appressorium core development, attributed to disrupted sterol transport by CoNpc1 and CoNpc2.

In the study of *U. maydis*, we identified Lep1 as a crucial factor for the formation of hyphal aggregates observed prior to spore formation. Lep1 is highly conserved among smut fungi, suggesting its role in fungal morphology rather than interaction with host plant-specific proteins. Indeed, Lep1 binds directly to the cell wall of *U. maydis*, rather than migrating to the host plant cells. It acts as a novel kind of adhesin, promoting hyphal aggregation. This finding demonstrates that *U. maydis* secretes Lep1 to orchestrate the formation of hyphal aggregates, thereby enhancing spore production. Determining whether hyphal aggregation in tumor tissue depends solely on Lep1 or also involves quorum sensing and extracellular matrix biosynthesis remains a significant challenge, as these processes occur late in fungal development.

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Declarations

Conflict of interest The author declares no conflict of interest.

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