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Article Addendum

Cutinase and hydrophobin interplay

A herald for pathogenesis?

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Surface-penetrating phytopathogenic fungi frequently form appressoria. These are specialised infection structures pivotal to fungal ingress into the host. Recently, we demonstrated that one member of a family of cutinases in *Magnaporthe grisea* is involved in surface sensing, mediating appressorium differentiation and penetration peg formation and hence facilitates host penetration. Cutinase2 serves as an upstream activator of cAMP/PKA and DAG/PKC signalling cascades and is essential for full virulence. Here, we speculate on the role of rice blast hydrophobins as surface interactors facilitating fungal cutinase activity.

Introduction

The cuticle overlays the plant cell wall and cloaks all aerial plant parts in a mantle of wax and cutin. Saprophytic leaf-litter fungi decompose this cuticle enzymatically, but surface-penetrating phytopathogenic fungi appear to breach this barrier by mechanical force. Certain undisputed roles have been ascribed to some fungal cutinases and their products, such as in spore attachment to the host,[1] in the perception of host-derived signals[2-4] and in carbon procurement.[5] However, a definitive role for cutinases in the enzymatic degradation of the plant cuticle by phytopathogenic fungi has courted much controversy over the past 20 years.[6,7] Originally, investigations into the pea pathogen *Fusarium solani* f. sp. *pisi* led Kolattakudy and coworkers[8,9] to propose that upon landing on its host, the low basal cutinase activity of virulent strains enzymatically releases plant cutin monomers. These are sensed by the pathogen and trigger a rise in fungal cutinase activity, which dissolves the plant cuticle, so effecting fungal ingress. However, it was later demonstrated that *F. solani* f. sp. *pisi* cutinase null mutants do not differ in pathogenicity from the wild-type strain,[10,11] so kindling the controversy.[6] Subsequent research has described cutinase knock-out mutants in a range of plant pathogenic fungi[12-15] but has failed to resolve unequivocally the dispute.

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A Family of Cutinase Genes in *M. grisea*

M. grisea asexual spores alight on a host plant, germinate and develop a dome-shaped appressorium at the end of the germ tube. The developmental cues for appressorium formation include surface hardness, hydrophobicity and a lack of exogenous nutrients.[16-18] The turgor pressure generated within this melanin-caged infection structure can reach 8 MPa;[19] it forces a fine penetration peg through the plant cuticle to initiate plant infection.

The identification of sixteen putative cutinase sequences within the *M. grisea* genome[20,21] therefore seemed intriguing. Such a large number could reflect functional redundancy or varying specificity of these enzymes. Previously, Sweigard et al[22,23] demonstrated that *M. grisea* cutinase1 is dispensable for pathogenicity but did not rule out a role for cutinases in cuticular penetration.

Cutinase2 is a Virulence Determinant

We identified a specific *M. grisea* cutinase, *CUT2*, which shows a dramatic uplift in transcription during appressorium maturation and penetration.[20,24] A *cut2* mutant shows reduced extracellular serine esterase and plant cutin-degrading activity in vitro and attenuated pathogenicity on rice and barley. On hydrophobic, appressorium-inducing plastic the *cut2* mutant forms multiple elongate germ tubes and misshapen appressoria, and a small subset of wild-type like appressoria. We demonstrated that Cut2 plays no part in spore or appressorium adhesion or in appressorial turgor generation, but mediates the formation of the penetration peg. Exogenous application of synthetic cutin monomers, cAMP, and diacylglycerol (DAG) restores the morphological and pathogenicity defects of the *cut2* mutant to wild-type levels. The partial rescue of the mutant by addition of the β-adrenoreceptor antagonist propranolol hints at some interplay between Cut2 and one of the many G-protein coupled receptors (GPCRs) in *M. grisea*.[25] We proposed that Cut2 is an upstream activator of the cAMP/PKA and DAG/PKC signalling pathways that direct appressorium formation and infectious growth in *M. grisea*. In essence, Cut2 is required for surface sensing leading to correct germling differentiation, penetration and full virulence in this model fungus.[20]

Cutinase and Surface Proteins—More than a Superficial Relationship?

The compromised appressorium differentiation of the *cut2* mutant seen on artificial surfaces, in the apparent absence of cutin, is intriguing. This finding implies that *cut2* is compromised in its ability to perceive the hydrophobicity signal. Furthermore, addition of synthetic cutin monomers (1,16-hexadecanediol) is sufficient to restore appressorium formation by the wild type strain on hydrophilic glass.[2,3] However, it only partially restores appressorium formation in *cut2*.[20] Collectively, these data suggest that in the wild-type strain the plant-induced signalling cascade triggered by Cut2 is interlinked with that triggered by hydrophobicity. Such interdependency could be due to the requirement of both signals to reach a specific threshold, sufficient to activate the downstream signalling pathways. The experimental data thus far support the existence of an interplay between cutinase and hydrophobic surface recognition.

This integration of physical and chemical surface cues is not without precedent. Recently, the filamentous saprophyte *Aspergillus oryzae* was shown to break down the biodegradable plastic polybutylene succinate-coadipate (PBSA) via the concerted action of the cutinase CutL1 and the hydrophobin RolA.[26,27] RolA is adsorbed onto the hydrophobic PBSA surface and following a change in protein conformation specifically recruits cutinase CutL1, restricting the lateral movement of RoIA across the surface.[26] Subsequently, CutL1 condenses onto the surface, initiating CutL1-dependent PBSA hydrolysis. Similar findings have been described with the novel hydrophobic surface binding protein, HsbA.[28]

Perspective

Could the degradation of PBSA be an accurate mimic of the natural processes occurring in the establishment of disease by plant penetrating fungi? Several pieces of evidence support this notion. Firstly, PBSA appears structurally similar to natural wax polymers found in plant cuticles[29] and hence *A. oryzae* appears to recognize PBSA polymers as a cuticle analogue.[26,28] Secondly, carbon-starvation, coupled with the presence of PBSA polymer, induces transcription of RolA.[27] Of the three hydrophobins residing in the *M. grisea* genome (*MPG1*, *MHP1*[30] and MGG09134.5), the class 1 Mpg1 is most similar to RolA. Mpg1 is considered to be involved more with surface interaction and host recognition than with appressorium formation itself.[31] Despite this, *MPG1* is highly expressed during appressorium formation in *M. grisea,* when nutrients are scarce;[32,33] however it is not yet known whether cutin monomers boost *MPG1* transcription.

So could one or more *M. grisea* hydrophobins, or indeed other unidentified surface active protein(s) be adsorbed onto the leaf surface, and subsequently recruit one of the multiple cutinases to facilitate cutin degradation? Is an Mpg1-like protein acting in concert with Cut2 alone? Could this be facilitated by one or more cutinases produced at low levels prior or coincident with spore and/or appressorium attachment? These hypotheses are testable, but complicated by the residency in the *M. grisea* genome of 3 hydrophobins, 16 cutinases (Cut1 is most homologous to the *A. oryzae* CutL),[22,26,28] and at least one surface binding protein (homologous to the *A. oryzae* HsbA[28]). Interplay of surface active proteins and their role in host recognition and pathogenesis remains a compelling topic.

Downstream of such initial complex interactions, the cutin monomer ligand released by Cut2 is likely to be perceived by one of the 61 GPCRs identified in *M. grisea*.[25] The cuticle-derived signal is then transduced to trigger the signalling pathways that ensure correct morphogenesis in the rice blast fungus.[20] Identification of a specific receptor, possibly Pth11-like,[3] and verification of such an interaction would open up another avenue of research. Basic understanding of the strategies employed by a fungus to sense its host represents the first step towards sustainable plant protection.

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