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Short Commentary

Cell Wall-Derived Acetic Acid may Serve as an Important Messenger for Metabolic Switching-Mediated Infection-Specific Cell Differentiation in the Rice Blast Fungus

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COMMENTARY

The hemibiotrophic fungus *Pyricularia (Magnaporthe) oryzae* causes rice blast, which is the most devastating and widespread disease of rice [1]. Recently, wheat blast caused by *P. oryzae* has also become a serious threat to global wheat production [2,3]. *P. oryzae* senses extracellular conditions and differentiates a specialized infection cell, called an appressorium, to penetrate the plant cuticle and cell wall; therefore, this fungus is not only considered as an important crop pathogen but also as a model organism to study infection-specific cell differentiation and molecular mechanisms of plant-pathogen interactions.

The infection process of this fungus is initiated by the attachment of a conidium to the plant leaf surface. The germinated conidium elongates a germ tube, and then elaborates the appressorium at the tip of the polarized germ tube. The progression of appressorium development is accompanied by single cell division and differentiation. The matured appressorium generates an enormous turgor of up to 8.0 MPa and produces a penetration hypha to breach the cuticle of the leaf [4].

Appressorium development requires a switch from germ tube elongation to appressorium formation via the recognition of extracellular physical and chemical cues, for example, surface hydrophobicity, hardness and host plant-derived chemicals [5]. The received cues are transmitted to pivotal signal transduction pathways for appressorium formation, the cyclic AMP (cAMP)-protein kinase A (PKA) pathway and mitogen-activated protein (MAP) kinase cascade [6]. However, not all cues are required for infection-specific cell differentiation, as appressorium formation can be induced solely by the hydrophobicity and hardness of an artificial hydrophobic surface in the absence of plant components.

In our recent article, we found that acetic acid generated by chitin-binding protein 1 (Cbp1), encoding chitin deacetylase (CDA), may be an important messenger for appressorium formation on hydrophobic surfaces in *P. oryzae* [7]. Chitin is one of the common polysaccharide components of the fungal cell wall and is detected by plant pattern recognition receptors as a microbe-associated molecular pattern. CDA chemically converts chitin into chitosan by removing acetyl groups from N-acetylglucosamine residues of chitin; therefore, it was hypothesized that Cbp1 contributes to cell wall modification for changing cell wall components and evading plant recognition (a stealth mechanism).

*CBP1*has been cloned from a cDNA library representing an early stage of germ tube development [8]. *CBP1* is a putative mucin gene with a signal peptide and a Ser/Thr cluster but lacks a distinct transmembrane domain and cytoplasmic tail region [9]. Actually, Cbp1 was specifically expressed during appressorium formation and localized at the cell surface.

Mutants which lost the CDA activity (Cbp1-D161A) of Cbp1 (including null mutants) show significantly delayed appressorium formation on hydrophobic surfaces, but this phenotype is restored by 3-isobutyl-1-methylxanthine (a cAMP-PKA pathway activator) [8,9]. In addition, chitosan accumulation at the tip of the germ tube is lost in the *Δcbp1* mutant. These results indicated that the spatiotemporal CDA activity of Cbp1 was involved upstream of signal transduction for appressorium formation.

We hypothesized that acetic acid, which is another product of the Cbp1-catalyzed conversion of chitin into chitosan, is a candidate for as an upstream messenger of the signal transduction for appressorium formation [7]. We first investigated whether Cbp1 CDA activity affects the pH at the tip of the germ tube using the pHusion system [10], because low molecular weight acetic acid is thought to diffuse immediately after it is generated. As expected, acidification could be detected at the tip of the germ tube in the wild-type strain, but not in the *Δcbp1* mutant [7]. The detected time point (4 h post inoculation) corresponded to that of the observed chitosan accumulation [9].

Next, we added exogenous acetic acid to evaluate the direct involvement in appressorium formation. Surprisingly, extremely low concentrations of acetic acid (1 fM) restored appressorium

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formation in the *Δcbp1* mutant, although 1 mM acetic acid severely inhibited conidial germination and appressorium formation. The appressroium formation rate obtained by exogenous 1 fM acetic acid in the *Δcbp1* mutant (ca. 50%) was lower than that of the wild-type strain (ca. 80%). Other tested acids, propionic acid and sorbic acid (at fM concentrations), also restored appressorium formation in the *Δcbp1* mutant. However, these fatty acid solutions did not change the pH value of the conidial suspension [7]. From these results, we concluded that the extremely low concentrations of fatty acids partially stimulate Cbp1-mediated appressorium formation.

Many pathogenic microbes including *P. oryzae* use the glyoxylate cycle, which is an anaplerotic pathway of the citric acid cycle, to assimilate fatty acids for gluconeogenesis under starvation conditions, such as infection processes [11]. We generated unique glyoxylate cycle gene (isocitratelyase: ICL1) mutants (*Δicl1*) and double mutants (*Δicl1*/*Δcbp1*) to evaluate the relationship between acetic acid and this metabolic pathway. In these mutants, appressorium formation rates were decreased relative to those of the wild-type strain, and acetic acid could not restore the phenotype [7]. These results suggested that acetic acid generated by the Cbp1 activity stimulates the glyoxylate cycle and induces appressorium formation.

During the infection process, lipid bodies of geminating conidia move to the tip of the germ tube and are degraded for appressorium turgor generation [12]. This lipolysis process leads to β-oxidation of fatty acids, which are finally metabolized in the glyoxylate cycle [13,14]. Recently, it was reported that the methylcitrate cycle, which metabolizes propionyl-CoA (propionate), is also required for appressorium formation and virulence in *P. oryzae* [15].

From these findings and our data, it seemed that exogenous acetic acid, propionic acid, and sorbic acid were directly used as a carbon source in the glyoxylate cycle and/or methylcitrate cycle; however, the amounts of these acids would not be sufficient for gluconeogenesis of the infection-related cell remodeling because the number of acid molecules at the fM order was only approx. 100 per one conidium. This suggested the existence of (a) mediator (s) and/or amplifier (s) to trigger the activation of the glyoxylate cycle and appressorium formation.

Meanwhile, it is likely that Cbp1 produced sufficient amounts of acetic acid as a carbon source of the glyoxylate cycle and as a messenger of appressorium formation; because, the acidification at the tip of the germ tube was detected only in the wild-type strain, and extremely low concentrations of acetic acid could not fully restore the phenotype of the *Δcbp1* mutant. The pH shift at the tip of the germ tube may also be a direct trigger for appressorium formation. Further, intracellular acetate synthesis may coordinately produce acetic acid for gluconeogenesis, similar to the plant drought-response [16].

One of the candidates for an upstream sensor interacting with Cbp1 is Msb2 signaling mucin. Msb2 can perceive the plant wax and surface hardness, and then can directly activate the MAP kinase cascade [17,18]. Although the *Δmsb2* single mutant retained some pathogenicity, the *Δmsb2*/*Δcbp1* double mutant failed to activate the MAP kinase Pmk1 and to form appressorium formation, which resulted in non-pathogenic [18]. Therefore, Msb2 may also interact with Cbp1 on hydrophobic surfaces.

In addition, Geoghegan and Gurr reported that exogenous chitosan fully restored appressorium formation in the CDA gene-deleted mutants [19]. These authors mentioned that chitosan does not have a role as a stealth molecule, but instead mediates the adhesion of germlings to surfaces, thereby allowing the perception of the physical stimuli necessary to promote appressorium formation.

Taken together, we demonstrated that the cell wall-derived acetic acid and extremely low concentration of acetic acid may directly or indirectly stimulate metabolic switching and cell differentiation in *P. oryzae*. For pathogenic microbes, the use of acetic acid derived from the cell wall as an alternative carbon source for gluconeogenesis and as a signaling messenger for an infection specific cell remodeling is a reasonable strategy to survive under starvation conditions (infection processes). Moreover, with the exception of some animal hormone signals, biological activity at this low concentration has no precedent in cell biology.

However, we have yet to obtain direct evidence that acetic acid is generated by Cbp1 activity and that cell wall-derived acetic acid acts as a signaling messenger for metabolic switching and cell differentiation. Further analyses are required to identify the mediator and/or amplifier of acetic acid, and integrate the complicated signaling pathways influenced by acetic acid in *P. oryzae*.

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