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Infection biology: Molecular recognition of fungal spores stimulates host hygiene

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Hygienic behaviors that remove pathogens can be crucial in preventing disease. But how are such behaviors stimulated? A new study shows that *Drosophila* recognize proteins on the surface of *Metarhizium* spores as a cue to initiate grooming and spore removal.

Entomopathogenic fungi are widespread pathogens of insects. In between hosts, entomopathogenic fungi exist in the environment as hardy, but dormant, spores. When these spores come into contact with a potential host, they adhere to the insect's cuticle, awake from dormancy, and grow hyphae into the body to establish an internal infection[1,2]. The fungus consumes the insect from the inside, then bursts back out of the insect cadaver and showers spores into the environment to set the stage for subsequent infection of a new host. The victim insect's best chance of avoiding this disastrous fate is to rapidly remove the spores while they are still dormant, before the hyphae penetrate the body. But how can an insect know when it has fungal spores on its body, and how can it remove them in time? A new study by Shang *et al.*[3] published in this issue of *Current Biology* addresses these questions, demonstrating that an insect host can molecularly surveil for spore surface proteins and that detection of spores stimulates a robust grooming response. Intriguingly, the interaction between host and fungal proteins is highly specific, involving single genes from multigene families on both sides, potentially allowing for both tight coevolution and a wide breadth of recognition.

Proteins containing common in fungal extracellular membrane (CFEM) domains are, as the name indicates, found frequently in the extracellular membranes of fungi. CFEM-containing proteins are diverse in structure and sequence and perform a variety of roles, sometimes being implicated in virulence[4–7]. Shang *et al.*[3] scanned the sequenced genomes of nine species of the entomopathogenic fungus *Metarhizium* and found that generalist species tend to have a more diverse array of CFEM-containing proteins than species that specialize on a narrow host range. In particular, the genome of the generalist *M. robertsii* encodes 18 CFEM-containing proteins, divided into six subfamilies. Nearly all of these contain either predicted transmembrane domains or putative glycophosphatidylinositol anchors, suggesting that the proteins could be integrated into the fungal cell membrane or wall[8]. Shang *et al.*[3] systematically deleted the genes encoding each of these 18 proteins and tested the ability of each mutant to infect a model insect host, *Drosophila melanogaster*. Individual deletion of three genes significantly increased time-to-death of inoculated flies, indicating that the *presence* of the fungal gene is *protective* to the host (the authors refer to these as "negative virulence" factors). However, the increased rate of mortality is observed only when mutant fungal spores are dusted onto the surface of the fly; these mutant spores show no difference in lethality compared with wild-type spores when injected into the *D. melanogaster* body cavity to bypass the cuticle.

There is a natural hypothesis that could explain how the presence of CFEM-containing proteins on the fungus might protect the insect host from infection. The insect may have the capacity to recognize these proteins as belonging to a fungus and interpret them as a cue to activate a defensive response. This would be analogous to stimulation of the immune system via pattern recognition receptors (PRRs), which recognize conserved microbial molecular structures (often called microbe-associated or pathogen-associated molecular patterns, MAMPs or PAMPs)[9–11]. However, in this case, because CFEM-containing proteins exert an effect only when they are outside the fly, the host response does not appear to be conventionally immunological.

Shang *et al.*[3] used a series of genetic manipulations to test this hypothesis, focusing specifically on one CFEM-containing protein, Mcdc9. By incorporating in-frame green fluorescent protein (GFP), they confirmed that Mcdc9 is localized to the surface of *M. robertsii* spores. They then used Mcdc9 as bait in a yeast-two-hybrid screen against a library of *D. melanogaster* cDNAs to search for a potential host receptor. They report a single major hit from this screen, a chemosensory protein called CheA75a. Heterologous expression of Mcdc9 and CheA75a in *Escherichia coli* confirmed that they can physically interact. Furthermore, *cheA75a* is highly expressed in *D. melanogaster* legs and wings, suggesting a localization that is appropriate for defensive surveillance against fungal spores. Topical exposure to fungal spores activates a robust grooming response in *D. melanogaster*, wherein the flies use their forelegs and mouthparts to remove the contaminating contagion[12]. Shang *et al.*[3] used electrophysiology to demonstrate that the *M. robertsii* Mcdc9 activates a neurological response in the *Drosophila* foreleg, which presumably stimulates grooming behavior. They also demonstrated that spore clearance through grooming was far less effective in flies that have *cheA75a* knocked down by RNAi, and that *cheA75a* knockdown flies succumbed more rapidly to topical exposure (though not injection) with *M. robertsii* spores. Reciprocally,

spore clearance was less effective, internal fungal burden was increased, and time-to-death was accelerated in wild-type flies that were topically exposed to *M. robertsii* spores lacking functional Mcdc9. Altogether, the data strongly support a model by which *D. melanogaster* use CheA75a to recognize *M. robertsii* spores by virtue of their display of Mcdc9 protein, and recognition stimulates grooming behavior to remove the spores and prevent internal infection (Figure 1).

The molecular interaction between Mcdc9 and CheA75a appears to be specific and evolutionarily derived. CheA75a is a fairly divergent member of the CheA family of chemosensory proteins and is apparently an innovation of the Schizophora group of true flies. In yeast two-hybrid assays, Shang *et al.*[3] showed that *M. roberstii* Mcdc9 also physically interacts with the CheA75a ortholog from *D. suzukii*, though not with a much more distantly related homolog from the mosquito *Anopheles gambiae*, nor with other seven CheA family members from *D. melanogaster*. The Mcdc9 ortholog from the related generalist *M. anisopliae* physically interacts with *D. melanogaster* CheA75a, and *cheA75a* knockdown flies are similarly susceptible to *M. anisopliae* infection. Of the two other CFEM-containing fungal proteins that result in increased virulence toward *D. melanogaster* when deleted, neither interact with CheA75a in the yeast two-hybrid assay.

Why would a pathogenic fungus continue to display a protein that triggers a host defensive response? The answer may lie in an intriguing pattern of host specificity in protein effects. Shang *et al.*[3] tested the ability of their 18 *M. robertsii* mutants that are deficient for individual CFEM-containing proteins to topically infect *Galleria mellonella* (waxmoth) larvae. They again identified three genes that, when removed, increased virulence toward *G. mellonella*. However, these were not the same three genes that caused increased virulence toward *D. melanogaster* when deleted. Moreover, one of the genes actually showed opposite effects on these two host species: deletion decreased virulence toward *D. melanogaster* but increased virulence toward *G. mellonella*. Three additional deletions reduced virulence toward *G. mellonella* and one additional deletion reduced virulence toward *D. melanogaster*, illustrating that CFEM-containing proteins can behave as conventional virulence-promoting factors in some contexts. Thus, the generalist entomopathogenic *Metarhizium* species may express a diversity of CFEM-containing proteins because they enable infectivity in many contexts, even if some hosts have evolutionarily learned to recognize a subset of those proteins as a signature of threat. It is tempting to speculate that the specialist *Metarhizium* species express fewer CFEM-containing proteins because they have lost those that are recognized by their hosts.

The interactions between pathogen CFEM-containing proteins and host recognition proteins present a ripe arena for future research. The proteins are encoded as multigene families in both host and pathogen. The data from Shang *et al.*[3] are reminiscent of gene-for-gene models wherein infection success depends on whether the host expresses proteins that are capable of recognizing particular pathogen proteins[13,14]. These models, which have been most fully developed in plant systems, raise specific predictions about breadth of host range as a function of which proteins are displayed by both pathogen and prospective host. Future studies could examine the evolutionary dynamics of the CFEM-containing proteins and their receptors in the host, including testing rates of gene family turnover and identifying potentially adaptive sequence divergence. Much remains to be understood mechanistically as well. How does recognition of *M. robertsii* Mcdc9 by *D. melanogaster* CheA75a trigger the behavioral grooming response? What are the neurological circuits involved and how are these engaged? Grooming in *Drosophila* is a prescribed, genetically encoded behavior[15,16] so deciphering the neurobiology may be tractable. Thinking

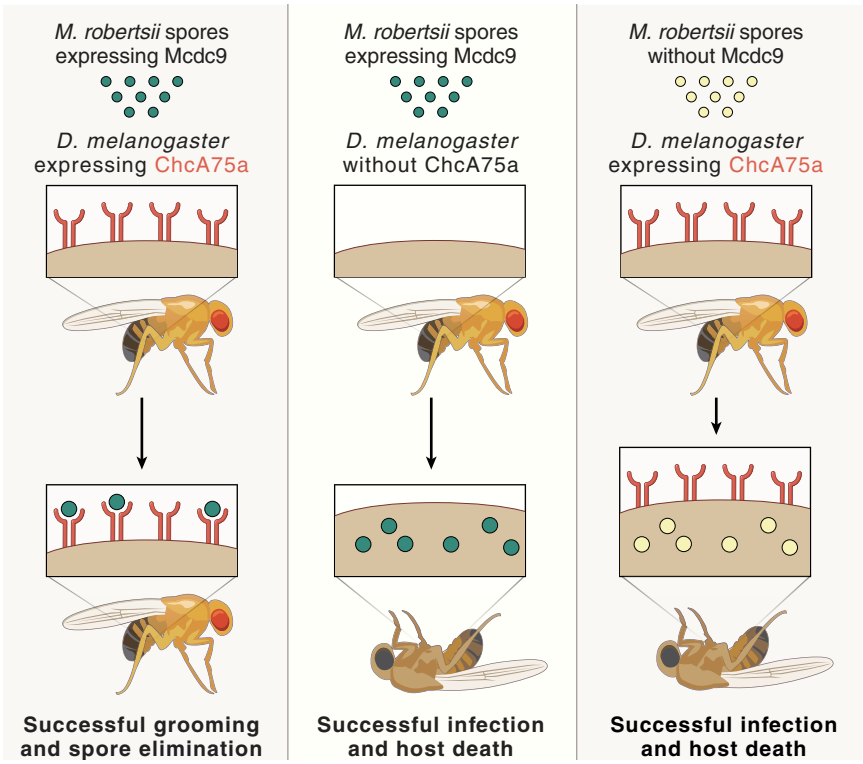

Figure 1. Schematic illustration of how spore recognition protects the host from infection.
Mcdc9 is a protein expressed on the surface of spores of the fungus *M. robertsii*. The *D. melanogaster* receptor ChcA75a recognizes Mcdc9, and recognition stimulates the host to remove spores through grooming. In the absence of either Mcdc9 or ChcA75a, grooming is induced at a lower level and host death is accelerated.

more broadly, are the stimulated responses to different CFEM-containing proteins similar across insect hosts, and, if so, do they depend on the same downstream signaling mechanisms? Or is recognition a universal trigger that stimulates diverse responses? Social insect species often exhibit allogrooming to remove pathogens from colony mates[17,18]. Does allogrooming rely on similar molecular cues? This new work by Shang *et al.*[3] uses elegant genetics and varied experimental approaches to uncover a novel mechanistic trigger for a behavioral defense response, and in doing so raises a plethora of exciting new research questions that can be pursued in this and other systems.

DECLARATION OF INTERESTS

The author declares no competing interests.

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Developmental neuroscience: Boosting inhibition boosts learning

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Compared to adults, children learn differently and more efficiently. A new study shows that in children a rapid boost of inhibition evoked during learning leads to better stabilization of learned items due to reduced retrograde interference.

Learning is hard. Acquiring a foreign language as an adult reminds you of the ease with which you learned as a kid within a few years languages, skills in math and physics, and playing an instrument. This does not mean that as a child learning was not demanding, but compared to adulthood it appeared almost effortless. Why is that? Why do children apparently learn differently and more effectively? A new study from Sebastian Frank and colleagues