Molecular toolkit unlocks life cycle of the panzootic amphibian pathogen *Batrachochytrium dendrobatidis*

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Amphibians are facing an extinction crisis that threatens up to 50% of all species (1, 2). Uniquely, a pathogenic fungus, *Batrachochytrium dendrobatidis* (*Bd*), is now recognized as a proximate driver of these declines (3). The pathogen's widespread global host range in >400 species of amphibian on 5 continents gives unfortunate candidacy for this being the most destructive emergence of infectious disease ever witnessed. *Bd* is a basal fungal lineage in the Chytridiomycota. These fungi are characteristically aquatic and flagellate, and the lack of any chytrid pathogens of vertebrates has led to the group remaining poorly characterized relative to other fungi, both at the taxonomic and molecular level. As a consequence, the mechanisms utilized by *Bd* to infect and cause disease in amphibians remain shrouded in mystery. The recent sequencing of two *Bd* genomes has created an opportunity to leverage comparative genomics and molecular biology to unlock the life cycle of this secretive fungus. The report by Rosenblum *et al.* (4) in a recent issue of PNAS represents the first use of this genomic information by using whole-genome arrays to compare patterns of global gene expression for two stages of *Bd*, the sessile sporangium and the infectious flagellate zoospore. This new study has shown that >55% of the ≈9,000 genes in the *Bd* genome are undergoing differential expression between these two stages. Mining the predicted function of these genes by identifying similarities with genes of known function in other species (gene ontology) has provided the first clues into the higher mechanisms that orchestrate *Bd* growth, infection, and pathogenicity in amphibians.

The acquisition of >160 fungal genomes (5) is increasingly allowing the evolutionary origin and diversification of infection-associated innovations to be mapped across the fungal pathosphere. The ecological niche that a fungus occupies is to a large extent defined by the products that it secretes, because fungi are osmotrophs and live by secreting enzymes into the environment to degrade specific polymers for nutrition. Therefore, comparing the transcriptomes of divergent lineages allows the identification of common "motifs" that are associated with specific ecological niches, which in the case of pathogens can translate into genes with an infection-specific role. The identification of fungalysin metallopeptidases as a gene family that has expanded to >25 members in *Bd* is a clue to the important function of these secreted peptides (4). Metallopeptidases have been shown to be highly up-regulated in fungi that infect human and animal skin, the Dermatophytes, accounting for up to 36% of total secreted protein extracts (6). In the dermatophytes, these proteins are associated with survival in keratin-rich tissues; skin, nails, and hair. It is well known that *Bd* is highly keratinophilic, surviving in the keratin-rich mouthparts of tadpoles and then infecting the keratinized stratum corneum as it is formed during metamorphosis. Therefore, that Rosenblum *et al.* (4) find overexpression of metalloproteases in *Bd* sporangia is evidence that this gene family is a key pathogenicity factor. Interestingly, only a single fungalysin was found to be more abundant in zoospores compared with sporangia. Currently, nothing is known about the mechanism that *Bd* zoospores use to colonize amphibian skin and to gain entrance to host cells; therefore, this gene may provide the key to unlocking this process.

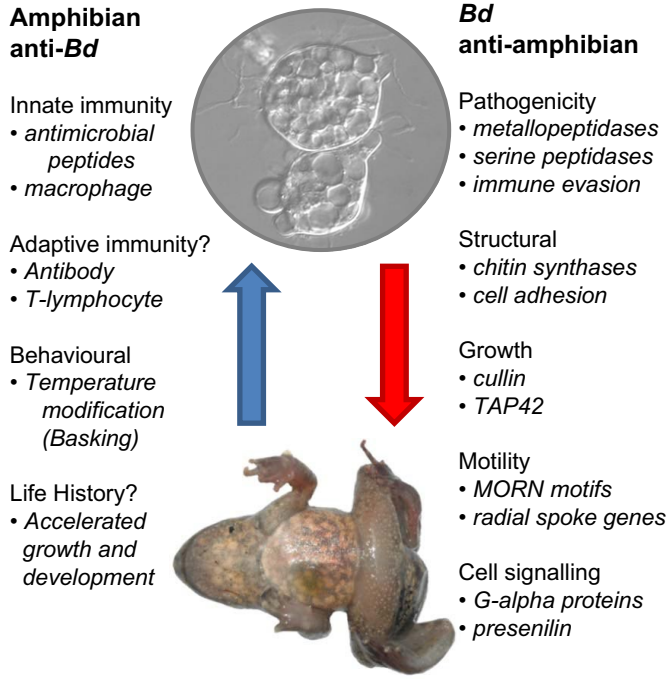

Fig. 1. Potential interactions governing the outcome of the amphibian–*Bd* interaction. On the left-hand side are listed known and suspected host mechanisms for combating infection. On the right-hand side are listed *Bd* genes discovered by Rosenblum *et al.* that are putatively important in infection and pathogenicity. The photomicrograph of *Bd* sporangia is courtesy of Louise Walker and Neil Gow. The amphibian is a recently metamorphosed *Alytes obstetricans* juvenile that died of chytridiomycosis; photograph by Jaime Bosch.

Is the *Bd* Secretome the Key to Overwhelming Host Antimicrobial Defense Systems?

One of the most striking features of the emergence of *Bd* has been the finding that, although many hundreds of species can become infected, only a percentage of these go on to manifest disease and subsequent mortality. Research has

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shown that a key host-defense mechanism is the production of secreted skin peptides with antimicrobial properties; research has found that amphibian species that produce peptides with anti-*Bd* activity show increased survival (7). The current study shows that serine-type peptidases constitute a large gene family in the *Bd* genome and show differential expression between the two *Bd* life stages, with a bias toward expression in the sporangia. A clear hypothesis to be tested is whether these peptidases are secreted by *Bd* to overcome amphibian antimicrobial defense molecules. The recent report of an amphibian-secreted peptide (OGTI) in *Odorrana grahami* that inhibits serine protease activity suggests that amphibians may have evolved specific defenses against fungal proteases. In this case, amphibian innate immunity may hinge on their ability to degrade the fungal secretome to prevent infection and pathogenicity (Fig. 1) (8).

However, combating *Bd* by using chemical warfare may not be enough to prevent invasion of the host tissue by *Bd*. Research on plant pathogens has shown that infection requires the secretion of protein effectors that suppress host defenses and bias cellular metabolism in favor of the pathogen (9). One such effector is the RXLR-motif that oomycetes use for host-cell targeting and transport of effector proteins into host cells (10). Although such effector motifs have not yet been identified in fungi, the largely unannotated nature of basal fungal genomes means that they could well occur without yet being discovered. However, Rosenblum *et al.* (4) do find differential expression of several genes that are related to vertebrate clathrin, ITAM and Interleukin 1 genes. It is known that the human pathogenic fungus *Candida albicans* manipulates host defenses by suppressing macrophage production of reactive nitrogen species while stimulating Interleukin 1 (11). The occurrence and expression of these genes by *Bd* raises the possibility that they may have evolved to mimic vertebrate host proteins as a means of either sheltering *Bd* from the immune system or for gaining entry to the host cell. The important next step is to reproduce the current study in vivo, rather than by using in vitro culture. Recent work by Bignell *et al.* (12) uses transcript profiling of *Aspergillus fumigatus* during mouse infection to show, by using minute quantities of samples, that subtelomeric expression of genes is important in early infection. Using such in vitro expression techniques will provide key insights into the amphibian–*Bd* interaction (Fig. 1).

Amphibians may have evolved specific defenses against fungal proteases.

Although this work raises more questions that answers, it has unlocked a barrage of information that can be leveraged to understanding the thus-far cryptic interaction between *Bd* and its host amphibian species. Excitingly, the study formulates hypotheses that can be rapidly tested in model host–*Bd* experimental systems, and used to understand contemporaneous patterns of amphibian decline. For instance, the apparently stable nature of infected *Alytes muletensis* populations in Mallorca (13) may be due to attenuated virulence of the introduced strain of *Bd* on the island (M.C.F., unpublished data). Comparing expression profiles between these virulent and attenuated lineages of *Bd* could well provide the key to our further understanding of the evolution of pathogenicity in this most destructive of organisms.

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