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Editorial overview: Host-microbe interactions: fungi

Chad A Rappleye and Duncan Wilson

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Chad A Rappleye

Ohio State University, Department of Microbiology, Columbus, OH 43210, USA
e-mail: rappleye.1@osu.edu

Chad Rappleye started in research under the mentorship of John Roth working on *Salmonella typhimurium* gene expression at the University of Utah where Dr. Rappleye received his B.S. degree. Chad Rappleye received his PhD from the University of California, San Diego where he studied the biology of cell polarization in the laboratory of Raffi Aroian. He transitioned to the field of fungal pathogenesis for his postdoctoral studies at Washington University. There, as a Damon Runyon Cancer Research Foundation Postodoctoral Fellow he worked with William Goldman on the virulence determinants of the fungus *Histoplasma capsulatum*. Chad Rappleye is currently an Associate Professor and Chair of the Graduate Studies Committee in the Department of Microbiology at Ohio State University. His group uses molecular genetics to identify and characterize the factors that enable pathogenic fungi to survive host defense mechanisms, with a particular focus on the primary pathogen *Histoplasma capsulatum*.

Duncan Wilson

Medical Research Council, Centre for Medical Mycology at the University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen, UK
e-mail: Duncan.Wilson@abdn.ac.uk

Fungal pathogens of humans continue to plague human health. In developed nations, heightened risk for fungal infection is linked to many modern medical advancements such as organ and stem cell transplantation (and the ensuing immunosuppression), chemotherapy, inflammatory cytokine modulation, and even anti-bacterial therapeutics. In developing nations, immunocompromisation (e.g., due to HIV) is a major driver of morbidity and mortality due to fungal disease. In addition, the endemic systemic fungal pathogens cause disease even in immunocompetent individuals. Thus, fungal disease remains a significant burden for populations around the world.

The virulence of fungi is largely a function of the degree to which the immune response can control or eliminate fungal cells, which is countered by mechanisms expressed by fungi to defeat such immune defenses. The opportunistic fungal pathogens of humans include the environmentally-acquired saprophyte *Aspergillus fumigatus*, which is typically controlled by host phagocytes, often in the absence of any inflammation. In contrast, *Candida* species can maintain a commensal relationship with humans, being effectively controlled by immune defenses, but not eliminated. Primary fungal pathogens such as *Cryptococcus* species, and the dimorphic fungal pathogens (including the genera *Coccidioides*, *Histoplasma*, *Blastomyces*, *Paracoccidioides*, and *Emergomyces*) are effective in combating host defenses of the innate immune response. This spectrum of host-control and how fungal pathogens can influence the outcome of the host response are the subject of the reviews in this issue on host-pathogen interactions of human fungal pathogens.

In this issue, several reviews explore how *C. albicans* establishes itself in different host environments, balancing commensalism with pathogenesis. Many aspects of *Candida* biology impact its ability to colonize diverse tissues, including surface components, micronutrient acquisition and fungal morphology. Indeed, some postulate the plasticity of *Candida* is key to its success as both commensal and pathogen. While much of the past research on *Candida* has focused on how *Candida* causes mucosal and systemic infections, these situations are a rather small minority of the state of *Candida* within the host. The vast majority of humans harbor *Candida* as a commensal, yet only relatively recently have studies addressed this aspect. In this issue, Perez reviews recent studies identifying features of *Candida* that enable colonization of the gastrointestinal tract, including fungal morphologies and Candida interactions with non-fungal microflora.

C. albicans readily forms biofilms on biotic and abiotic surfaces, and these biofilms are a significant source of fungal cells that cause disease in hosts with reduced immune defenses. Wall *et al.*, describe key studies that show biofilm formation is a highly regulated process to which a significant portion of the *Candida* genome is involved, further underscoring the important role of biofilms in *Candida* biology and pathogenesis. While considerable attention has been paid to regulators and structural features necessary for biofilm formation, dispersal of cells from biofilms has only recently been investigated. In this issue, Wall *et al.* highlight key studies that show dispersal is a regulated process, and that the yeast-like dispersal cells have higher virulence than broth-grown yeast cells.

Early investigations of *Candida* morphology indicated hyphal cells were necessary for invasive disease as well as biofilm formation, and consequently hyphae were generally regarded as the virulent form of *Candida*. However, the new findings regarding the importance of yeast-like dispersal cells in *Candida* pathogenesis, as well as the importance of yeast cells in gastrointestinal tract colonization, cautions against assumptions that yeast-like forms are avirulent simply because filamentous forms are required for virulence. The emerging picture is that the polymorphic nature of this fungal pathogen facilitates *Candida* pathogenesis and thus regulation of morphology and the corresponding morphology-specific gene expression remains a central question.

The signaling cascades and the *Candida* transcription factors controlling *Candida* morphology have been reviewed previously and in this issue, Kadosh provides a refreshing perspective by highlighting understudied aspects of gene regulation such as chromatin structure and translational regulation. This review places the operation of specific transcription factors in the context of the chromatin structure surrounding filamentation and virulence genes as well as the availability of general transcriptional machinery subunits. In addition, Kadosh discusses the role of translational control of central factors in hyphal differentiation, specifically the 5’UTRs which result in fine-tuning (both increasing and decreasing) the degree of hyphal gene expression. This multitude of control levels highlights the regulatory complexity underlying the decision to produce hyphae, but also suggests novel targets for inhibition of *Candida* filamentation by antifungal therapeutics.

C. albicans hyphae secrete candidalysin, a peptide toxin that can both facilitate pathogenesis or activate host defenses. Naglik *et al.*, provide a historical perspective on the discovery and elucidation of the role of candidalysin. The toxin mediates damage of host cell membranes that initiate danger-type or damage-type responses from the host, including indirect activation of EGFR signaling, the activation of Th17 responses and subsequent neutrophil recruitment for control of *Candida*. However, in the absence of host responses to candidalysin-induced danger/damage signals, cellular lysis can contribute to Candida pathogenesis. As both a virulence and ‘avirulence’ factor, the evolution and function of candidalysin is an intriguing question.

The fungal cell wall is the primary site of fungal interaction with the host immune system. In this issue, two reviews discuss *A. fumigatus* cell wall components. Zacharias and Sheppard present an inventory of the cell surface polysaccharides of *A. fumigatus* and discuss how each influences the host response to *Aspergillus* infection. Mackrel and Steele focus on host side of the host-pathogen interaction and discuss the role of the lung epithelium integrity in keeping *Aspergillus* cells within the airways and the host receptors that recognize *Aspergillus* components. One of the interesting features is how exposure of different polysaccharides enables the host to tailor the response to the appropriate threat level. Conidia are readily cleared without necessitating an inflammatory response whereas recognition of cell wall beta-glucans as the conidia germinate trigger stronger pro-inflammatory responses. This calculated response is essential to preserve lung function in light of the constant inhalation of *Aspergillus* conidia. Zacharias and Sheppard discuss how other cell wall polysaccharides such as alpha-glucan and galactosaminogalactan (GAG) can decrease immune responses. Zacharias and Sheppard use the discussion of host recognition of *Aspergillus* chitin as a reminder that the context of host-recognition of fungal polysaccharides matters; the size of chitin polysaccharides or oligosaccharides triggers pro-inflammatory or non-host protective Th2 responses, respectively and the presence of other saccharides can further alter host responses. Thus, caution is advised when interpreting studies with isolated polysaccharides as the results may not accurately reflect the interaction of the host with the intact fungal cell. Invasive aspergillosis is largely due to lack of host immune defenses, and Mackrel and Steele provide the new perspective that immunosuppression in at-risk patients is not equivalent. Consequently, different therapeutic strategies to combat invasive aspergillosis are required for the different immunosuppressive regimens patients receive.

Nutritional immunity encompasses the competition between host and pathogen for essential nutrients. The battle for iron is well known. Both elemental iron and heme iron in the host can serve as iron sources for fungal pathogens as long as the fungus expresses mechanisms to acquire it in the face of host sequestration. In this issue, Horianopoulos and Kronstad review the multiple ways through which *Cryptococcus neoformans* captures iron from the host including high affinity transport, xenosiderophores, and heme uptake. Their review also discusses how iron uptake is not sufficient, but the iron must also be incorporated into Fe-S clusters in the mitochondria. These findings are intriguing as the enhanced proliferation of *Cryptococcus gattii* has been correlated with increased mitochondria and/or mitochondria function. In this issue, Roy and Kornitzer detail the molecular machinery of *Candida* that enables this fungus to utilize host heme as a source for iron. This process involves a number of surface CFEM-family proteins as well as endocytic factors which may extend to other ascomycete fungi to overcome iron nutritional immunity in mammalian hosts.

Iron is not the only micronutrient sequestered from fungal pathogens. In this issue, Wilson and Deepe discuss the struggle to withhold zinc from fungal pathogens and the fungal countermeasures to maintain zinc homeostasis in a zinc-limited environment. Host mechanisms to limit zinc availability depend on whether the pathogen is extracellular (e.g., *Candida*, *Aspergillus*, and *Cryptococcus*) or intracellular (*Histoplasma*). For limitation of extracellular zinc, various phagocytic cells release zinc-sequestering S100A proteins, which are an essential fungal-inhibiting component of neutrophil extracellular traps (NETs). Restriction of zinc from intracellular pathogens is accomplished by production of metallothioneins. Intriguingly, Wilson and Deepe discuss how zinc limitation also potentiates production of reactive oxygen against fungi implicating host limitation of zinc as a two-fold immune. However, fungal pathogens are not powerless in this struggle for zinc, and Wilson and Deepe balance the discussion by highlighting fungal molecules that assist fungal pathogens in acquiring limited zinc, including the *Candida* zincophore Pra1. While Pra1 has been lost from some human fungal pathogens, nearly all have homologs of Zrt-type zinc transporters which enable fungi to obtain zinc. The requirement for these transporters for fungal virulence highlights the importance of zinc-limitation or acquisition and provides another example of nutritional immunity in the host-pathogen balance.

Many fungal pathogens have been shown to release extracellular vesicles, but their biological importance is only beginning to be elucidated. Bielska and May review studies that characterize the biogenesis and release of fungal extracellular vesicles. These vesicles contain a variety of nucleic acids and proteins, including fungal virulence factors. Release of these vesicles may be an alternative way for fungal secretion of these virulence factors, although studies defining how cellular proteins are selected for inclusion in extracellular vesicles are in their infancy. Identification of the mechanisms and cellular pathways for extracellular vesicle production discussed by Bielska and May are facilitating functional studies on the importance of extracellular vesicles. Notably, extracellular vesicles have been shown to induce both pro-inflammatory and anti-inflammatory responses from host cells indicating they are previously unknown modulators of the host-fungus interaction.

The dimorphic fungal pathogens comprise a group of related ascomycete fungi. The central feature of the dimorphic fungal pathogens is the thermally regulated transition of the environmental mycelial form to the virulent spherule or yeast form that causes disease in mammals. In contrast to polymorphic fungi (e.g., *C. albicans*), the dimorphic fungi have non-filamentous morphologies within the host. Expression of many of the known factors that promote virulence is specific for the virulent form illustrating division not only of morphology but of lifestyle. In this issue Dr Sil provides a review of the molecular circuitry that regulates the dimorphic transitions of *Histoplasma*, explaining how two circuits comprise opposing regulatory pathways, the antagonism of which nicely accounts for the switch to exclusively yeasts cells at 37°C or the mycelial form at lower temperatures. How temperature is sensed and transduced into signals that push these antagonistic circuits to one or the other outputs remains an intriguing question for future studies.

Recent molecular phylogenetic studies, aided by advancements in genome sequencing technologies, continue to identify distinct populations and subdivisions leading to new phylogenetic species designations. In this issue, Van Dyke *et al.* summarize the current taxonomic relationships among the Onygenales. In their review, they note how phylogenetic studies have moved some species into different genera and even specified a new genus, *Emergomyces*, that is becoming one of the most common endemic mycoses in South America. Taxonomic refinements hold value especially when phylogenetic divisions are associated with unique factors or mechanisms underlying fungal pathogen virulence. In this regard, Van Dyke *et al.* highlight studies that show established virulence mechanisms differ between dimorphic fungal genera (e.g., *Histoplasma* versus *Paracoccidioides*) and non-pathogenic species, as well as between species within a genus (e.g.,c *Histoplasma*). While dimorphism appears to be largely conserved among pathogenic and non-pathogenic dimorphic fungi, human dimorphic fungal pathogens have lost some genes encoding plant degradation enzymes reflecting a mammal-associated lifestyle. With the ever expanding species definitions, Van Dyke *et al.*, advise caution, particularly in designating species with names based on geography as the distribution of many of these new species is not specific to the implied endemicity.

Within a given species, generation of genetic variation may facilitate infection of different niches within the host in commensal or pathogenic situations. The mechanisms by which the genome of *C. albicans* changes and evolves in response to stress and colonization pressures is intriguing and links the two fields of evolution and fungal pathogenesis. In this issue, Ene *et al.* review the mechanisms at both the nucleotide and chromosomal level that drive variation in the *Candida* genome. The largely diploid and heterozygous nature of *C. albicans* permits a high frequency of nucleotide changes on which selection can operate. Loss of this heterozygosity, whether by individual gene conversion or chromosome-level changes, has occurred in isolates as a consequence of host colonization and antifungal selective pressures. Ene *et al.* highlight studies that demonstrate passage of *C. albicans* through the GI tract induces changes in virulence determinants that enhance commensal colonization of the GI tract at the expense of systemic virulence. In addition, aneuploidy has emerged as a mechanism selected by antifungal treatment to increase gene dosage for increased resistance to azole drugs in *C. albicans*. The high degree of genome variation may be one of *Candida*'s secrets for successful colonization of human hosts and persistance as a commensal microorganism.

The threat of fungal resistance to front-line antifungal compounds reached a new level of concern with the recent identification of *Candida auris*. While some fungi are naturally resistant to certain antifungals (e.g., *Cryptococcus* and *Histoplasma* to the echinocandins and *A. fumigatus* to fluconazole), *C. auris* is recognized as a muti-drug resistant fungal pathogen. In this issue, Rhodes and Fisher review the genomic analyses that have defined the major *C. auris* geographic clades and discuss some of the polymorphisms and transcriptional variations that correlate with the enhanced resistance of *C. auris* to azole- and echinocandin-class antifungals. Interestingly, not all of *C. auris*'s drug resistance is associated with polymorphisms in the known targets of these antifungals. Fortunately, *C. auris* does not have increased virulence above other *Candida* species. Thus, the major concern surrounding *C. auris* is treatment of individuals who contract infections with this drug-resistant fungus, a situation which highlights the need for continued pursuit of new therapeutics to combat pathogenic fungi.