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Host-microbe interactions: The response of fungal and oomycete pathogens to the host environment

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The interactions of fungi and oomycetes with plant and animal hosts involve specific adaptations that allow the pathogens to adhere to and penetrate tissue, to remodel metabolic capabilities to utilize host nutrients, and to deal with stresses generated by host defense mechanisms. These fascinating adjustments require major reprogramming events as the pathogens move from the environment (with its own challenges) to host tissue. This year's collection of six reviews on the interactions between fungi or oomycetes and their hosts reflect several emerging themes in our developing view of pathogen response to the host environment. These themes include the demonstrated utility of genomic resources for the discovery and characterization of pathogen traits with key roles in adaptation to the host environment. The benefits of the long-awaited appearance of these genomic resources for fungal and oomycete pathogens are clearly apparent from the seamless inclusion of genomic approaches in each of the reviews. In particular, transcriptome analyses show great promise for defining the broad features of pathogen gene expression during infection. A second theme expands on the long-standing appreciation of the importance of secreted proteins for fungal pathogens of both animals and plants. Striking observations in recent years elevate secretion to a new level of sophistication with the identification of proteins from plant pathogens that are translocated into host cells. This work raises the possibility that these pathogens not only modify the extracellular host environment, but also manipulate the intracellular compartment in ways that may contribute to virulence or suppress host defense. These observations may ultimately reach the level of detail available for the effector proteins that are delivered into host cells by bacterial pathogens of plants and animals. Additional themes concern the importance of metabolic remodeling in the host environment for nutrient acquisition and utilization, and the interconnections between metabolism, morphogenesis, and the response to stress. Mechanistic details are appearing from genetic and genomic approaches, and provide notice for what promises to be an explosion in the application of similar approaches in a wide range of fungal and oomycete species.

As described in the first review, *Candida albicans* has emerged as perhaps the best characterized fungal pathogen of humans in terms of the analysis of gene expression during infection. Earlier detailed studies characterized specific factors that play roles during infection including secreted hydrolases and adhesins that are expressed in a niche-specific fashion in the host. In addition, much effort has gone into characterizing the relevance of the yeast-hyphal transition and associated changes in gene expression as a component of the infection process. Brown, Odds and Gow present an overview of approaches to evaluate the expression of *C. albicans* genes during infection and describe the body of evidence that leads to the question of whether environmental adaptation is the main key to pathogenicity. The expression of specific sets of virulence factors during infection has been studied in detail including the analysis of

secreted aspartyl proteases, secreted lipases, phospholipase B and adhesions of the Als family and Hwp1. These studies highlight the importance of secreted products for nutrient acquisition and colonization of host tissue. Brown, Odds and Gow focus part of their discussion on the challenges of “going global” in the performance of *in vivo* gene expression experiments. These challenges include collecting sufficient fungal cells from infected tissue, minimizing the impact of contaminating mammalian RNA, dealing with heterogeneity in the fungal population, controlling parameters during infection, and dealing with host variability. This valuable discussion, much of it referencing the pioneering work of Bernhard Hube’s group, is essential reading for anyone contemplating similar experiments. To date, global experiments have examined the transcriptional response of *C. albicans* to host cells (e.g., macrophages, neutrophils), fluids (e.g., blood), and tissues (e.g., reconstituted epithelium, perfused liver), as well as to actual infections (kidney, peritoneum). These studies highlight the metabolic and stress adaptations of the fungus to allow nutrient acquisition and protection from host defenses.

Klein and Tebbets review the latest findings for the systemic dimorphic fungal pathogens that include *Blastomyces dermatitidis*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, and *Penicillium marneffii*. These fungi share the ability to undergo a temperature-dependent morphological transition reflected in their growth as molds in the soil and conversion to a yeast-like or spherule form upon inhalation into the lungs of mammalian hosts. As with *C. albicans*, more global approaches with microarrays are now augmenting the analysis of small sets of genes in these fungi. In the case of the dimorphic fungi, single yeast-phase specific factors have been characterized with regard to their importance during infection, and many of these factors function at the cell surface. For example, α -(1,3)-glucan in the cell wall is known to correlate with virulence in *B. dermatitidis*, *H. capsulatum* and *P. brasiliensis*, and interference with the function of the *AGS1* gene for α -(1,3)-glucan synthase impairs virulence in *H. capsulatum*. Other cell-surface localized virulence functions include the adhesin BAD1 that binds to chitin in the cell wall of *B. dermatitidis* and the exported calcium binding protein CBP1 that is important for pathogenicity and growth in macrophages. Although sequencing projects have been completed or are underway for most of the dimorphic pathogens, global gene expression approaches have so far only been applied to *H. capsulatum* and *P. brasiliensis*. These studies focused on the identification of phase-specific genes. For example, microarray experiments with *H. capsulatum* identified genes that were differentially expressed in the mold and yeast forms. Along with the first insights into global changes in gene expression, efforts have been made to begin identifying the master regulators of the dimorphic transition with the important goal of understanding how these pathogens sense temperature changes. Klein and Tebbets highlight a recent breakthrough in this area with the identification of a hybrid histidine kinase (DRK1) as global regulator of dimorphism and virulence. The discovery of this kinase emphasizes a growing appreciation for the role of two-component signaling systems involving histidine kinases in fungal pathogens. For example, similar systems that involve the Hog1 MAP kinase in mediating the response to stress have been characterized in *C. albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans*.

In their review on *Cryptococcus neoformans*, Brown, Campbell and Lodge summarize recent work on the mechanisms that this basidiomycete fungal pathogen employs to deal with a variety of stresses encountered during infection. These stresses include host temperature, hypoxia, nutrient deprivation, and oxidative and nitrosative components of host defense. As with other fungal pathogens, single gene approaches have yielded insights into mechanisms of stress adaptation. Thus, detailed studies of stress-related genes identified roles for trehalose and mitochondrial superoxide dismutase (SOD) in the response to elevated temperature, as well as roles for SOD (but not catalase), the glutathione system, laccase and peroxiredoxin (Tsa1) in resistance to oxidative stress. Tsa1 and flavohemoglobin also participate in resistance to nitric oxide. The framework of signaling pathways and regulatory factors that mediate the response

to stress is starting to emerge. Thus, the histidine kinase/Hog1 stress pathway has been characterized along with a sterol-response element binding protein that mediates adaptation to hypoxia. Additionally, the calcineurin pathway that is critical for growth at 37°C has been characterized in great detail and several components of the protein kinase C pathway have been found to function in the maintenance of cell wall integrity. Transcription factors for the oxidative and nitrosative stress responses (Yap4 and Atf1) and iron sensing (Cir1) have also been characterized recently. The genome sequence of *C. neoformans* has enabled both transcriptome and proteome studies to explore stress- and infection-related gene expression. These studies have focused on the fungal response to low oxygen, elevated temperature, reactive oxygen and nitrogen species, phagocytosis and growth in central nervous system. In light of the findings regarding nutrient acquisition and host adaptation in other fungi such as *C. albicans*, an intriguing area of new investigation for *C. neoformans* is exploring the link between metabolic homeostasis and stress revealed by connections between the pentose phosphate pathway, nitrosative stress and fungal gene expression during experimental meningitis.

Turning to the plant side of fungal pathogenesis, Ellis, Dodds and Lawrence summarize elegant recent work on the identification of secreted proteins that are produced by biotrophic fungi and that trigger or suppress host defense responses. The biotrophic fungal pathogens in the rust and powdery mildew groups are economically important in terms of crop loss, but they are challenging pathogens to investigate because they are difficult to grow in axenic culture and they cannot easily be transformed. Avirulence genes have been identified genetically in the basidiomycete flax rust fungus *Melampsora lini*, and the products of these genes trigger a defense response in host plants carrying the appropriate resistance gene. A map based cloning approach led to the isolation of the AvrL567 locus that appears to encode a secreted avirulence protein expressed in fungal haustoria, the feeding structures that establish an intimate connection (exchange center) with the host by growing inside plant cells. Expression analyses identified other haustoria-expressed and secreted proteins and several of these represent additional avirulence factors. Remarkably, expression of the avirulent form of AvrL567 in a resistant transgenic flax plant led to a defense response indicating that the plant detects the fungal avirulence product intracellularly. Similarly, two avirulence proteins from the barley powdery mildew pathogen are also recognized in the cytoplasm of resistant plant cells although, surprisingly, these proteins do not have N-terminal secretion signals. The overall implication is that these proteins are secreted by the fungi and taken up by the host cell during infection, although the mechanism(s) by which the proteins enter plant cells is not yet clear. Ellis, Dodds and Lawrence describe tantalizing information for the flax rust avirulence protein AvrM and the ToxA toxin of *Pyrenophora tritici-repentis* that indicates the existence of host uptake machinery. Finally, the analysis of the genome of another biotrophic pathogen, *Ustilago maydis*, identified clusters of genes for secreted proteins. Although avirulence functions have not been found for *U. maydis*, gene disruption experiments can be performed in this fungus. This approach revealed that five of the gene clusters encoding predicted secreted proteins play a role in disease on the maize host. Specifically, deletions for four of the clusters reduced virulence and deletion of the fifth cluster actually increased virulence. Taken together, these studies with biotrophic fungal pathogens of plants illustrate the importance of secreted proteins in the interaction with the host.

The theme of secreted proteins is continued and reinforced in the review by Morgan and Kamoun. In this case, the secreted proteins are avirulence effectors that mediate interactions between oomycete pathogens of the genera *Phytophthora* and *Hyaloperonospora* and host plants. Although these pathogens are not fungi, they have similar morphologies and cause devastating plant diseases such as late blight of potato. Morgan and Kamoun focus on the fascinating discovery that four oomycete avirulence proteins all have signal peptides for secretion and share a conserved N-terminal motif RXLR that appears to function in

translocation of the proteins into plant cells. These proteins are recognized by resistance proteins located in the host cell cytoplasm. As described by Morgan and Kamoun, a remarkable finding was that the RXLR motif is similar to a targeting signal for proteins of the malaria parasite that translocate into host red blood cells. Experimental evidence indicates that sequence conservation reflects functional conservation for this signal. As with the avirulence proteins in the rust and powdery mildew fungi, a key mystery for the RXLR proteins in oomycetes has to do with the translocation machinery. Morgan and Kamoun present a clear model that focuses attention on key steps in the process including secretion of the proteins outside the pathogen cell and translocation into the plant cell.

Among the fungal pathogens of plants, the rice blast fungus *Magnaporthe grisea* is clearly the best characterized in terms of genomic, genetic and cell biology approaches to understand the infection process. Caracuel-Rios and Talbot review the latest findings for *M. grisea* including highlights from the annotation of the recently published genome sequence. Key observations from the sequence include the identification of genes for an estimated 784 secreted proteins and for a large set of putative G-protein coupled receptors, as well as the remarkable discovery of phylogenetic evidence for multiple horizontal gene transfers. In terms of functional studies, *M. grisea* is an important model for developmental aspects of plant infection including the formation of the specialized infection structure, the appressorium, and ramification of hyphae in host tissue. Caracuel-Rios and Talbot describe a number of elegant recent results that link the cell cycle, autophagy, lipid metabolism and MAP kinase signaling pathways to appressorium formation and infection. Details of the growth of *M. grisea* hyphae inside rice plant cells have also appeared recently with the fascinating discovery that the fungus can move from cell to cell via plasmadesmata. The close association of fungal and host cells in this case is particularly interesting in light of the potential delivery of pathogen proteins, as described in the reviews for the rust and oomycete pathogens. As with these other pathogens, *M. grisea* is known to produce avirulence proteins that trigger a defense response when recognized by intracellular host resistance proteins. The secretion and delivery of these and other proteins appears to depend on MgApt2, a golgi-localized protein that is required for exocytosis during infection. Finally, Caracuel-Rios and Talbot describe large-scale insertional mutagenesis approaches that promise to facilitate functional analysis of the ~10,000 genes in the genome. These studies further highlight the utility of the genome sequence.

In summary, the recent developments presented in these reviews nicely reflect the marriage of single gene approaches with transcriptome, proteome and insertional mutagenesis studies that are now possible due to the rapidly accumulating genome sequences. These global approaches are effectively ramping up the discovery of candidate genes for functional analysis, and prompting an even greater emphasis on the study of signaling pathways and master regulators to understand adaptation. As a package, the reviews also highlight the value of monitoring parallel developments in pathogens of both plants and animals. This is elegantly demonstrated by the conserved function of translocation sequences between the oomycete pathogens and Plasmodium. In general, secreted proteins and the pathogen cell surface stand out as key features of all of the pathogen interactions. These studies validate current projects to define the secretome for several pathogens and emphasize the importance of detailed genome annotation to recognize secreted proteins of potential importance. In light of the delivery of effector proteins into host cells by plant pathogens, it will be interesting to follow parallel developments for the animal pathogens. Given recent studies, further investigations with *H. capsulatum* and *C. neoformans* may provide insights into the roles of pathogen proteins after phagocytosis. For example, *H. capsulatum* is an intracellular pathogen of macrophages and survives by inhibiting or reversing phagolysosomal acidification [1]. Furthermore, intracellular growth of *C. neoformans* in macrophages results in damage to the phagolysosomal membrane and the accumulation of vesicles filled with polysaccharide capsule from the fungus [2]. *C.*

neoformans also has the remarkable ability to escape from phagocytic cells, a process that may involve an influence of the pathogen on actin polymerization [3,4].

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Biography

Jim Kronstad is a professor in the Michael Smith Laboratories, an interdisciplinary research unit founded by the Nobel Prize-winning chemist Dr. Michael Smith. Jim's research focuses on investigating the interactions of basidiomycetous fungal pathogens with animal and plant hosts, with an emphasis on the characterization of host signals that influence pathogen gene expression.