HHS Public Access

Author manuscript

Curr Opin Microbiol. Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

Curr Opin Microbiol. 2015 August ; 26: 10–16. doi:10.1016/j.mib.2015.03.006.

Microsporidia-Host Interactions

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Abstract

Microsporidia comprise one of the largest groups of obligate intracellular pathogens and can infect virtually all animals, but host response to these fungal-related microbes has been poorly understood. Several new studies of the host transcriptional response to microsporidia infection have found infection-induced regulation of genes involved in innate immunity, ubiquitylation, metabolism, and hormonal signaling. In addition, microsporidia have recently been shown to exploit host recycling endocytosis for exit from intestinal cells, and to interact with host degradation pathways. Microsporidia infection has also been shown to profoundly affect behavior in insect hosts. Altogether, these and other recent findings are providing much-needed insight into the underlying mechanisms of microsporidia interaction with host animals.

Introduction

Microsporidia comprise a phylum of fungal-related, obligate intracellular parasites. This phylum contains species that parasitize almost all types of animals, including humans, fish, bees, and other insects. Over 1400 species of microsporidia have been described thus far and new species are being discovered each year [1-3]. Some species of microsporidia have a very narrow host range, while others have a relatively broad host range, including vertebrates and invertebrates. Transmissible microsporidia spores are often described as ubiquitous and have been detected in diverse environments ranging from deep sea vents [1] to intercontinental dust [4]. Microsporidia spores invade hosts with a polar tube to inject themselves directly into the host cell, where they undergo their entire replicative life cycle, and then ultimately differentiate back into spores to return to the environment. These microbes are widespread, but poorly understood, despite their importance to human health and agriculture.

The medical relevance of microsporidia was appreciated when they were found to be responsible for lethal diarrhea in AIDS patients, and death in transplant and immunocompromised patients. Microsporidia can infect any organ system, but predominantly infect the intestine in humans. There is a lack of drugs that are both safe and

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effective for treating microsporidiosis. For example, fumagillin is one of the few compounds that are effective in killing some species of microsporidia but unfortunately it is toxic to humans [5]. Some groups report that the prevalence of microsporidia infections in humans increasing, with many individuals carrying latent and asymptomatic infections [6-8]. For further details on the clinical relevance of microsporidia, we refer readers to a recent review of this topic [9].

Microsporidia also affect agriculturally relevant animals, predominantly through infections of fish and insects. Microsporidia have been responsible for the collapse of fisheries, and they have also been implicated in honey bee colony collapse disorder, a disease that is decimating the honey bees that pollinate many essential crops. Recently, progress has been made in developing vaccines and cell lines for study of fish infections by microsporidia [10-14]. Due to space limitations, we direct the reader to existing reviews of microsporidia infections and treatments in fish [15,16], and in honey bees [17].

Here we focus primarily on progress made in the basic research of microsporidia-host interactions. We review findings from genomic, transcriptional, cell biological, immunological, and behavioral studies published in the last two years that provide new insight into how hosts respond to these ubiquitous intracellular pathogens.

Analysis of microsporidian genomes and host-interacting proteins

There has been a rapid increase in the number of microsporidian genome sequences available, which has helped address questions of phylogeny, evolution and pathogenesis of the microsporidia. Microsporidia were originally classified as protists, but are now generally accepted to be a sister taxa to the fungi based on phylogenomic analysis [18,19]. Because of the challenges in manipulating microsporidia in the lab, it has been difficult to use this newly acquired microsporidian genome information to perform functional analysis. However, new findings have emerged that are providing insight into the microsporidian obligate intracellular lifestyle. A particular focus has been on the proteins secreted by microsporidia into the host cell, since these factors likely hold the key to microsporidia survival within the host cell. Importantly, two recent reports have experimentally verified secretion of some of these proteins from pathogen cells. The first example relates to the finding that many microsporidia genomes encode a secretion signal sequence on the enzyme hexokinase, which catalyzes the first step in glycolysis and the pentose phosphate pathway [19]. This secretion sequence was shown to be functional in a heterologous yeast expression system, where it could direct traffic through the yeast secretory system, supporting a model where microsporidia hexokinase could be directed into the host cell and perhaps promote anabolic metabolism *in vivo* [19]. Hexokinase secretion was recently verified experimentally using antibodies directed against hexokinase of *Antonospora locustae*, a species of microsporidia that infects locusts [20]. Interestingly, hexokinase localized to the nucleus in these studies, suggesting that it could alter host gene expression. This study also provided experimental confirmation for other pathogen proteins previously predicted to be secreted into host cells. A second set of microsporidia proteins that recently were experimentally verified as secreted came from genomic and proteomic analysis of *Spraguea lophii*, which infects *Lophius* monkfish [21]. The authors identified proteins released into the extracellular

media from spores that were germinated *in vitro*, and found several microsporidia-specific proteins, as well as RICIN-B lectin-like proteins. The RICIN-B lectin-like proteins are also encoded in the genomes of other microsporidian species, and could possibly interact with carbohydrates found on host proteins. It will be exciting to functionally connect some of these secreted proteins with phenotypes long known to be caused by microsporidia infection, such as the dramatic 'xenoma' growths induced by many microsporidia infections in fish [22]. The past few years have seen many newly published microsporidia genomes, and these are covered in a recent review to which we direct readers for more information relating to progress in deciphering microsporidian biology using genomics [23].

Using this newly acquired genome information, several studies have focused on proteins that are unique to microsporidia, to learn more about the biology that characterizes these parasites and how they interact with their hosts. In particular, microsporidia-specific proteins such as spore wall proteins and polar tube proteins have received attention [24-29]. Some of these studies suggest a role for these unique proteins in promoting host cell entry. For example, it is thought that spore wall proteins may aid in adherence of spores to the host cell and thereby contribute to spore infectivity. The Zhang group recently reported that blocking either spore wall protein 16 (SWP16) or spore wall protein SWP11 using *in vitro* antibody treatments caused a 20% decrease in the adherence of *Nosema bombycis* spores to host cells in each case [24,26]. Further studies of proteins unique to microsporidia may provide insight into what underlies the unique properties of these parasites.

Host transcriptional response to microsporidia infection

Despite microsporidia being ubiquitous and significant parasites, very little was known about how host animals alter their gene expression in response to infection until just recently. This gap in our understanding has now been filled through analysis of the microsporidia-induced host response for several species, including insect hosts that have been studied for decades, as well as the nematode *C. elegans*, which has only recently been studied as a host for microsporidia infection. See Table 1 for a summary of pathways subjected to transcriptional regulation upon microsporidia infection in the host species that are discussed below.

One of the first animals described as a host for microsporidia was the silkworm *Bombyx mori*, which can be infected by the microsporidia *Nosema bombycis*. Indeed, Louis Pasteur was one of the first scientists to describe microsporidia infection in silkworm, which causes a disease called pébrine. This disease is characterized by small larval size, delayed development, molting problems, and 'prickly ash spots'. To understand more about the silkworm response to microsporidia infection, the Zhou group recently analyzed changes in host transcription. They used a genome-wide (23K) microarray chip for *B. mori* and examined host transcriptional response to *N. bombycis* at 2, 4, 6, and 8 days post infection [30]. Then more recently, these transcriptional studies were extended by examining additional early timepoints with a more modern Digital Gene Expression (DGE) analysis method [31]. In both studies, the authors highlight the differential expression of many genes active in the synthesis and metabolism of a key regulator of silkworm development, juvenile hormone. These changes in gene expression are likely responsible for increases in juvenile hormone

during infection [30], which in turn is likely responsible for the small body size and delayed development that are symptoms of silkworm pébrine disease [30,31]. Interestingly, juvenile hormone also accumulates upon infection in *Nosema ceranae*-infected honey bees. However, as opposed to causing stunted growth, in honey bees extra juvenile hormone may cause precocious foraging behaviors that are associated with microsporidia infection [32], although the cause of precocious foraging is still disputed [33]. The link between juvenile hormone regulation and symptoms of pébrine in silkworms is intriguing as a possible connection between changes in host gene expression and complex symptoms of disease. Additionally, the microarray study compared *N. bombycis*-induced transcriptional changes to changes resulting from infection by 4 non-microsporidian pathogens and found that 34/70 differentially regulated *B. mori* immune genes were uniquely regulated during infection by *N. bombycis*. Genes in the Toll and JAK/STAT pathways were found to be upregulated in expression, as well as several classes of anti-microbial peptides [30]. These findings were largely confirmed in the study using DGE [31].

In addition to the innate immune signaling pathways described above, many insects also use a melanization pathway to defend against microbes. The microarray study found genes of the serine protease cascade of the melanization pathway to be down-regulated upon infection with microsporidia. The authors postulate that secretion of serpins by the pathogen could be responsible for this down-regulation of host defense, and go on to show that hemolymph from *N. bombycis*-infected silkworms has slower rates of *in vitro* melanization than does uninfected silkworm hemolymph [30]. Interestingly, the serine protease cascade was also found to be downregulated in an RNA-seq study of *Nosema ceranae*-infected honey bees [34]. The DGE study of silkworms on the other hand found that lysozyme and lectins, key players in the melanization defense pathway were upregulated upon silkworm infection with *N. bombycis* [31]. However, lysozyme was downregulated in the honey bee system [34]. Taken together, these findings suggest that the melanization pathway may be a battleground for the ongoing arms race between host and pathogen, with each seeking to alter this important defense pathway to its own advantage.

A recently developed model host for studying microsporidia infection is the nematode *C. elegans*, which provides a tractable host with many genetic and molecular tools available for study. *Nematocida parisii* is a microsporidian species shown to naturally infect the intestines of *C. elegans* nematodes from around the world [35,36]. The transcriptional response of *C. elegans* to *N. parisii* microsporidia infection was measured using RNA-seq at 5 timepoints during infection and compared to transcriptional responses to other pathogens of *C. elegans*. Genes upregulated by *N. parisii* infection were largely distinct from those upregulated by infection with the extracellular pathogens *Pseudomonas aeruginosa* or *Staphylococcus aureus*, although there was extensive overlap in the set of genes downregulated by these distinct infections [37]. This finding is similar to the results of the *B. mori* microarray study described above, which found a high proportion of microsporidia-specific changes in gene induction compared to infection with other pathogens [30]. Interestingly, there was a striking similarity in the *C. elegans* host genes upregulated during *N. parisii* infection as compared to genes upregulated by viral infection, indicating a common host response to these very distinct intracellular pathogens. Many of the commonly upregulated genes

contain F-box, FTH, and MATH domains that are associated with ubiquitin-mediated degradation [37]. The authors provide several additional lines of evidence to show that ubiquitin-mediated pathways are involved in the host response to microsporidia infection. In particular, they show that two downstream outputs of ubiquitin, the proteasome and autophagy, provide defense against infection. RNAi knock-down of proteasome subunits, as well as autophagy factors LGG-1 (Atg8/LC3 homolog) or ATG-18 led to increased pathogen load. Furthermore, they showed that ubiquitin as well as autophagy markers are targeted to parasite cells, and that the parasite may suppress that targeting [37]. Interestingly, in the silkworm model of microsporidia infection, DGE analysis of differentially expressed genes found that autophagy genes were regulated during infection by *N. bombycis*, particularly early in infection (6 hpi) [31]. Although autophagy genes were not induced by *N. parisii* infection, they did appear to play an important role in defense [37]. Thus, autophagy and other ubiquitin-mediated processes may be a common host response to intracellular infection by microsporidia.

A growing theme in host defense in *C. elegans*, as well as in other hosts, is that immune defense genes are induced when core host processes commonly targeted by pathogens are perturbed [38]. In keeping with this theme, *C. elegans* appears to induce intracellular defense genes in response to perturbation of proteasome function. In particular, E3 ubiquitin ligase components, which are induced by RNAi knock-down of proteasome subunits, as well as by pharmacological inhibitors of the proteasome, are also induced by microsporidia or viral infections [37]. Thus, microsporidia infection may be detected through the increased demand placed on the proteasome, although there are likely to be other cues as well. A recent study using a proteomics technique also supports the hypothesis that microsporidia counteracts host degradation pathways [39]. Proteomic analysis of infected and uninfected honey bee midguts identified 14 differentially expressed proteins, one of which was a proteasome subunit that was about half as abundant upon *Nosema ceranae* infection. Perhaps challenging the host proteasome is a common mechanism of pathogenesis employed by different species of microsporidia.

Microsporidia use host intracellular trafficking pathways for exit and remodel host cytoskeleton

A critical stage in the life cycle of any intracellular pathogen is to exit from the host cell and be transmitted to a new host, which requires the pathogen to navigate and interact with host pathways. Very little was known about microsporidia exit from host cells until recently. Previous analysis of microsporidia life cycles had assumed that microsporidia lyse host cells in order to exit. Indeed, several other intracellular pathogens such as *Chlamydia* have been shown to use such a strategy [40]. However, recent discoveries in *C. elegans* have found microsporidia can use a very well-orchestrated, multi-step exit strategy that does not lyse cells, but rather enables the host to live for a surprising length of time during prolific pathogen production, although microsporidia infection does eventually kill this host [35,41].

Earlier work in the *C. elegans* host system showed that host animals were alive while contagious, indicating that the *C. elegans*-infecting species of microsporidia, *Nematocida parisii* is able to exit from host intestinal cells and be excreted from the animal without

causing death [35]. Additional studies of this host cell exit process indicated it to be non-lytic, because intestinal cells continued to exclude a small dye that enters perforated cells, even at timepoints when animals were actively excreting *N. parisii* spores [41]. More recently, it was shown by electron microscopy that intracellular spores are contained in a separate membrane compartment that can fuse with the host plasma membrane [42]. Importantly, *N. parisii* spores exit exclusively from the apical side of polarized intestinal cells, which allows them access to the lumen of the intestinal tract and therefore a route to excretion [41]. The recycling endosome regulator RAB-11 was identified by an RNAi screen to be instrumental in orchestrating the fusion of microsporidia-containing compartments with the host apical membrane. RNAi against RAB-11 decreased spore exit and reduced the transmission of infection from infected animals to their neighbors [42]. The authors propose that the action of this polarized smGTPase, RAB-11, may be responsible for the apical-only direction of parasite exit, an example of elegant repurposing of host trafficking machinery in response to infection.

Another interesting finding from studies of microsporidia in the *C. elegans* model system, is the degree to which the host cytoskeletal system is remodeled during infection. It was first noted that exit of *N. parisii* from host cells requires an intestinal-specific isoform of *C. elegans* actin, ACT-5. This protein is also mislocalized early during infection [41]. Interestingly, differential levels of host actin upon infection were identified in a proteomic study of the mosquito, *Aedes aegypti*, when it was co-infected with two species of microsporidia [43]. Although there are many possible roles actin could play during intracellular infection by parasites, perhaps use of actin during host cell exit is a common strategy employed by many different species of microsporidia. This finding also suggests that researchers should be cautious about using actin gene levels to normalize expression in transcriptional studies, since actin genes may not be have consistent levels of expression during infection [43].

Natural variation in host resistance and clearance of microsporidia

One recent study of microsporidia infection demonstrates how genetic variation in host responses to parasitic infections can affect host fitness across generations. In a study of the resistance of different strains of the roundworm *C. elegans* to its naturally occurring microsporidian parasite, *N. parisii*, the authors found that a strain of *C. elegans* from Hawaii had about 30-fold increased resistance to infection compared to the laboratory strain from England, as assessed by pathogen load. Furthermore, Hawaiian worms had more progeny than British worms after infection, indicating that the increased resistance could lead to a selective advantage. The enhanced resistance and fecundity of this strain when challenged with *N. parisii* appears to be due to the surprising ability of this strain to clear intracellular infection, but only during early larval stages of development [44]. Clearance of *N. parisii* from the intestinal epithelial cells of *C. elegans* in vivo is a striking finding, given that *C. elegans* does not have known professional immune cells. It would be interesting to analyze the mechanism of clearance in other hosts, to determine whether this epithelial clearance can also be used in hosts that have a professional immune system.

Changes in host behavior resulting from microsporidia infection

In addition to modulating host activities on a cellular level, microsporidia such as the honey bee-infecting species, *Nosema ceranae*, can also alter host behavior [45-48]. European honey bee populations have been decimated recently due to a phenomenon called 'colony collapse disorder' that may be at least partially due to infection by *Nosema ceranae.* A recent report suggests that "homing success", a measure of the bees' ability to return to the hive after kidnapping and being released from a far-away location, was significantly reduced in *N. ceranae*-infected bees compared to control animals [49]. This difference was largely due to decreased flight times and increased rest intervals of infected bees, rather than differences in navigation or other flight characteristics. The authors note that although this inability to return home can reduce colony size, it also can mitigate spread of infection throughout the colony, highlighting the complexity of factors at play in host response to microsporidia infections.

In another fascinating example of the complex behavioral changes that can occur upon microsporidia infection, Shi et al present data for a mechanism by which microsporidia infection can prevent locust swarming [50]. In their paper, the authors propose that the locust-infecting microsporidia, *Antonospora* (*Nosema) locustae*, acidifies the hindgut of the host locust during infection, which reduces growth of a particular commensal bacterial species that is responsible for producing pheromones that promote swarming behavior. Volatiles from the feces of infected locusts were less attractive to healthy locusts than volatiles from the feces of uninfected animals. In addition to reducing the onset of aggregating behaviors, RNAseq data shows that microsporidia infection suppresses synthesis of dopamine, a neurotransmitter that helps maintain swarming [50]. This study again illustrates how far-reaching the impact of microsporidia infection can be in a host, with changes in the host microbiome due to microsporidia adversely affecting important behaviors like locust swarming.

Conclusions

In conclusion, exciting progress has been made recently in investigating host responses to microsporidia infection. Many of the studies described above highlight the struggle between host and parasite for control of host defense pathways including innate and cellular immune pathways, cellular clearance and autophagy, and the proteasome. Interactions between host and pathogen manifest across many levels of host biology, ranging from transcriptional changes in the genome, to cytoskeletal and trafficking modifications within cells, and even to alterations in host behavior (Figure 1). Studying these interactions should help us understand infectious disease caused by microsporidia, and more generally the needs of both hosts and parasites.

Acknowledgements

We thank Michael Botts and Lianne Cohen for helpful comments on the manuscript. This work was supported by NIH predoctoral training grant T32 GM008666 and a NSF Predoctoral Fellowship to S.C.S.; and NIAID R01 AI087528, the Searle Scholars Program, Packard Foundation and Burroughs Wellcome Fund fellowships to E.R.T.

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Highlights

- Hexokinase and lectin-like proteins are candidate effectors secreted by microsporidia
- Microsporidia infection induces robust transcriptional changes in many host animals
- Host ubiquitin and autophagy machinery target microsporidia cells in nematodes
- Microsporidia exploit endocytic recycling of host nematode for directional exocytosis
- Microsporidia infection regulates honey bee homing and locust swarming behavior

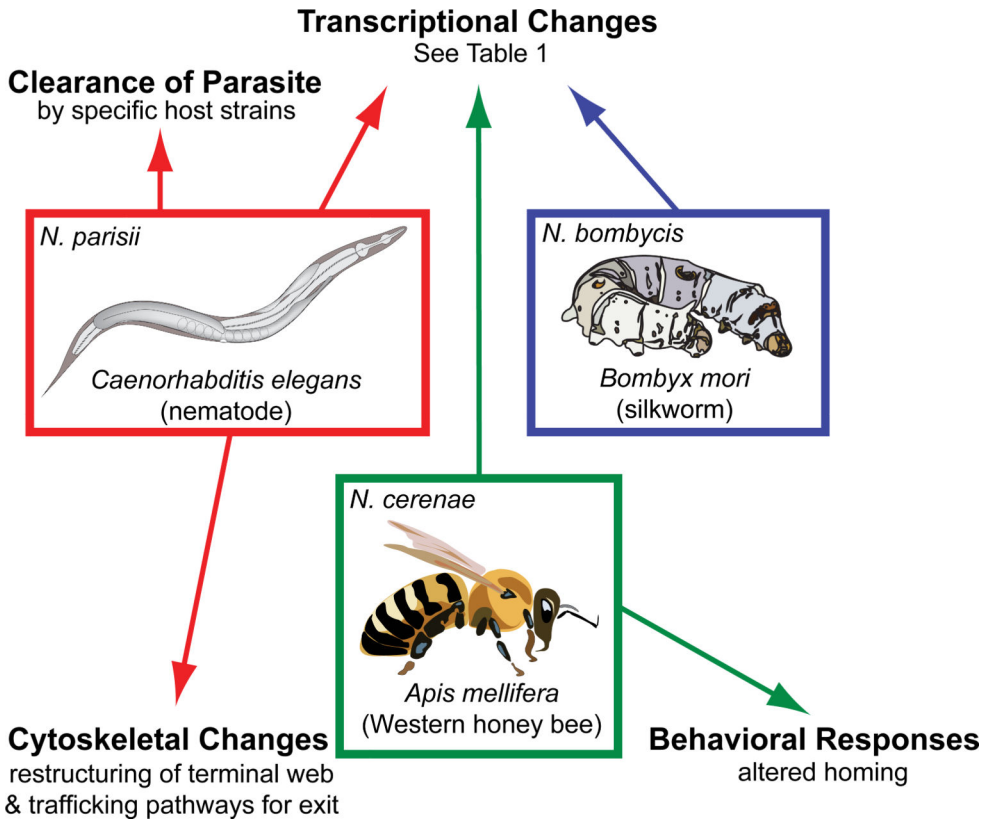

Figure 1. Microsporidia-host interactions

Recent studies conducted in nematodes, silkworms, and honeybees have enhanced our understanding of the basic biology of host-parasite interactions by examining how hosts respond to microsporidia infection. Examples of host responses studied in each host-parasite pair discussed in detail in this review are summarized above.

Table 1

Differentially regulated host pathways upon microsporidia infection.

	Host / microsporidia		
Differentially regulated pathways	***C. elegans / N. parisii***	***B. mori / N. bombycis***	***A. mellifera / N. ceranae***
Autophagy		X	
Ubiquitin proteasome system	X	X	X
Melanization		X	X
Innate Immunity- Toll		X	
Innate Immunity- IMD			
Innate Immunity- JAK/STAT		X	
C type lectins	X	X	
Antimicrobial peptide production		X	
Metabolism	X	X	X
References	[37]	[30,31]	[34,39]