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Proteome of *Aedes aegypti* larvae in response to infection by the intracellular parasite *Vavraia culicis*

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Abstract

We report on the modification of the *Aedes aegypti* larval proteome following infection by the microsporidian parasite *Vavraia culicis*. Mosquito larvae were sampled at 5 and 15 days of age to compare the effects of infection when the parasite was in two different developmental stages. Modifications of the host proteome due to the stress of infection were distinguished from those of a more general nature by treatments involving hypoxia. We found that the major reaction to stress was the suppression of particular protein spots. Older (15 days) larvae reacted more strongly to infection by *V. culicis* (46% of the total number of spots affected; 17% for 5 days larvae), while the strongest reaction of younger (5 days) larvae was to hypoxia for pH range 5–8 and to combined effects of infection and hypoxia for pH range 3–6. MALDI-TOF results indicate that proteins induced or suppressed by infection are involved directly or indirectly in defense against microorganisms. Finally, our MALDI-TOF results suggest that *A. aegypti* larvae try to control or clear *V. culicis* infection and also that *V. culicis* probably impairs the immune defense of this host via arginases-NOS competition.

Keywords: Proteomics; Proteome; *Aedes aegypti*; Microsporidia; *Vavraia culicis*; Nitric oxide synthase

1. Introduction

Insects must resist attack from various pathogens of a microbial or eukaryotic origin. Their defences start with physical barriers to prevent infectious agents from gaining entry into the body cavity. These include the thick waxy cuticles of exoskeleton and the peritrophic matrix lining the digestive tract. Invaders managing to breach these defenses often arrive in the host's hemocoel where they are exposed to both humoral and cellular components of the host's innate immune system. These include a wealth of molecules involved in recognizing and signaling the presence of foreign or non-self material. These responses set in motion cellular defense mechanisms, including phagocytosis and encaspulation, as well as causing cells, such as those in the fat body, to rapidly produce and release a battery of anti-microbial and anti-fungal peptides into the hemocoel. Additional responses include those to protect the host, for example, by repairing damaged tissues or buffering against free radicals produced during intensive bouts of metabolic activity.

Much of our knowledge on the immune system of invertebrates has been derived from studies involving the genetics of *Drosophila melanogaster*. This has been through its comparison with other organisms or by experimental approaches making use of molecular and genetic tools developed using this species as a model organism. However, data from other organisms are making an increasing contribution to this field. Mosquitoes in particular feature in this diversification following the sequencing of the *Anopheles gambiae* genome (Holt et al., 2002). For example, diversity in the recognition and effector molecules of the *Drosophila* and *Anopheles* immune systems is indicative of adaptive evolutionary change associated with their divergent ecologies and contrasting exposure to

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pathogens (Zdobnov et al., 2002). Furthermore, direct experimental evidence of how mosquito genes function in response to pathogens is also becoming available with the application of techniques such as double-stranded RNA knockdown (Blandin et al., 2004).

Despite these advances in the genetic domain relatively less is known about the mechanisms initiating these responses, the proteins or molecules directly affecting parasite development, or how parasites evade or try to manipulate them. Protein-based approaches promise to make a useful contribution in this area (Engström et al., 2004; Biron et al., 2005a). Recent improvements in the techniques of 2-DE (two-dimensional gel electrophoresis) and mass spectrometry, in combination with accumulating database resources, have made it possible to characterize the entire protein complement (or proteome) expressed by individual cells, tissues, or whole organisms. These techniques have already been applied to *D. melanogaster* in characterizing how the profile of hemolymph proteins changes within the minutes or hours following wounding, challenge by immune-stimulatory molecules, or infection by microorganisms (e.g. Levy et al., 2004; Vierstraete et al., 2004a,b). In these studies, researchers have been able to identify increases or decreases in the presence of particular proteins and in many cases have been able to assign them a functional role, e.g. as antimicrobials or responding to oxidative stress.

In this study we investigated the proteome of the yellow fever mosquito *Aedes aegypti* (L.) in response to infection by the microsporidian parasite *Vavraia culicis* Weiser. The microsporidia are amitochondrial eukaryotes currently thought to be closely related to the fungi, if not directly descended from them (Keeling et al., 2000). Their host range spans most of the animal kingdom, including man, but they have mainly been described from invertebrate hosts (Becnel and Andreadis, 1999). In particular, they are among the most common pathogens infecting natural populations of mosquitoes (Castillo, 1980). *Vavraia culicis* itself has been reported from natural populations of *Aedes*, *Anopheles* and *Culex* mosquitoes (Weiser, 1980), and has been subject to investigation in a variety of laboratory studies (Bano, 1958; Reynolds, 1970; Kelly et al., 1981; Agnew et al., 1999, 2004; Bedhomme et al., 2004). Host larvae become infected when they ingest the parasites' spores from their aquatic environment. Germination causes a tube coiled within the spore to be everted with force. It is thought this tube crosses the peritrophic matrix and penetrates epithelial cells lining the digestive tract. Contents of the spore pass down the tube and directly into the host cell's cytoplasm, from which point the parasite's development can begin. New spores are formed approximately 8–10 days p.i. They can germinate in situ and directly infect other host cells. The accumulation of spores within a cell may also cause its rupture, in which case spores can be liberated back into the intestine or into the host's hemocoel from where other cells or tissues can be infected, e.g. the fat body. Infections can either be chronic, increasing host morbidity, or cause host mortality, depending on the particular conditions involved (Agnew et al., 2004; Bedhomme et al., 2004). Little is known about the immune responses invertebrate hosts deploy against microsporidian infections. Melanized spores are occasionally reported in the hemocoel of insects with mature infections (Weiser, 1976). However, such spores must have been produced in the host concerned, thus the infection must already have been established and the parasite able to develop through to spore production.

Our study differs from the proteomic studies cited above in two important aspects. First, unlike many of the pathogens used to date, the development of the microsporidia is obligatorily intracellular; they thus pose a different type of challenge for the host immune response as compared to pathogens circulating in the hemocoel. Second, we investigated changes in the host's proteome at 5 and 15 days p.i. These intervals are much longer than those used above and correspond with different stages of infection where the parasite's development is predominantly directed towards proliferation (5 days) or spore production (15 days). Both developmental stages are likely to have different metabolic requirements and impose different stresses upon the host (Agnew et al., 2003).

We were particularly interested in changes of the host proteome that were specifically in response to *V. culicis* infection, as opposed to those in response to stress per se. To help distinguish between these possibilities, infected and uninfected larvae were subjected to hypoxia as a source of general stress by denying them access to atmospheric oxygen in the 5 h prior to being sampled. We analyzed how these treatment combinations quantitatively affected the numbers of protein spots expressed by the host proteome. We subsequently focused on those showing a qualitatively different pattern of expression, being either specifically induced or suppressed in association with infection.

2. Materials and methods

2.1. Host and parasite material

The strain of *A. aegypti* used in this study was collected by colleagues at the Pasteur Institute in Dakar, Senegal and had been maintained in the laboratory for at least 10 generations in outbred conditions (>500 breeding females) in an insect room maintained at 25 (±3)°C, >50% relative humidity and a 12 h:12 h light:dark photoperiod. The isolate of *V. culicis* used was provided by Dr J.J. Becnel of the USDA Gainesville, USA and originally isolated from *Aedes albopictus* (Fukuda et al., 1997).

2.2. Experimental protocol

Our experiment involved four treatment conditions that combined the stressful effects of infection and hypoxia.