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Research report

Differential transcriptomic responses of *Biomphalaria glabrata* (Gastropoda, Mollusca) to bacteria and metazoan parasites, *Schistosoma mansoni* and *Echinostoma paraensei* (Digenea, Platyhelminthes)

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ABSTRACT

A 70-mer-oligonucleotide-based microarray (1152 features) that emphasizes stress and immune responses factors was constructed to study transcriptomic responses of the snail *Biomphalaria glabrata* to different immune challenges. In addition to sequences with relevant putative ID and Gene Ontology (GO) annotation, the array features non-immune factors and unknown *B. glabrata* ESTs for functional gene discovery. The transcription profiles of *B. glabrata* (3 biological replicates, each a pool of 5 snails) were recorded at 12 h post-wounding, exposure to Gram negative or Gram positive bacteria (*Escherichia coli* and *Micrococcus luteus*, respectively), or infection with compatible trematode parasites (*Schistosoma mansoni* or *Echinostoma paraensei*, 20 miracidia/snail), relative to controls, using universal reference RNA. The data were subjected to Significance Analysis for Microarrays (SAM), with a false positive rate (FPR) $\leq$10%. Wounding yielded a modest differential expression profile (27 up/21 down) with affected features mostly dissimilar from other treatments. Partially overlapping, yet distinct expression profiles were recorded from snails challenged with *E. coli* (83 up/20 down) or *M. luteus* (120 up/42 down), mostly showing up-regulation of defense and stress-related features. Significantly altered expression of selected immune features indicates that *B. glabrata* detects and responds differently to compatible trematodes. *Echinostoma paraensei* infection was associated mostly with down-regulation of many (immune-) transcripts (42 up/68 down), whereas *S. mansoni* exposure yielded a preponderance of up-regulated features (140 up/23 down), with only few known immune genes affected. These observations may reflect the divergent strategies developed by trematodes during their evolution as specialized pathogens of snails to negate host defense responses. Clearly, the immune defenses of *B. glabrata* distinguish and respond differently to various immune challenges.

1. Introduction

The planorbid snail *Biomphalaria glabrata* is an important intermediate host for the larval stages of the digenetic trematode *Schistosoma mansoni*, a parasite that infects nearly 100 million people in Africa and the neotropics (Morgan et al., 2001). In addition to *S. mansoni*, *B. glabrata* can be infected by pathogens including other trematode parasites such as *Echinostoma paraensei* (Loker et al., 1987), bacteria (Bean-Knudsen et al., 1988), and likely viruses (Rondelaud and Barthe, 1992). Commonly, *B. glabrata* counters pathogens with an effective immune response that involves soluble components and cell-mediated cytotoxicity (Adema et al., 1997; Bayne, 2009; Hahn et al., 2001). In case of infection by *S. mansoni* or *E. paraensei*, however, the snail immune response may fail to clear the infection, due in part to trematode-mediated avoidance or inhibition of snail defense mechanisms (Coustau and Yoshino, 1994; Douglas et al., 1993; Lie and Heyneman, 1977; Loker et al., 1986; Loker and Hertel, 1987; Noda and Loker, 1989a,b; Roger et al., 2008).

Current understanding of the immunity of *B. glabrata* and other molluscs is incomplete (Bayne, 2009), yet a number of recent studies have identified molecules that may be relevant to their defense (Bouchut et al., 2007, 2006a,b; Guillou et al., 2007; Hanelt et al., 2008; Knight et al., 2009; Lockyer et al., 2007a,b; Mitta et

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al., 2005; Raghavan et al., 2003; Stout et al., 2009; Vergote et al., 2005). Little is known about the specific functions of many of these molecules, but we can nonetheless gain valuable information about the roles of these molecules by assessing the snail response as a whole, documenting transcriptional trends displayed in response to specific stimuli. Of particular interest is to determine the extent to which snails, as representative lophotrochozoans, can mount responses that are tailored to specific groups of pathogens, as suggested by previous comparative analysis of ESTs profiles of *B. glabrata* exposed to bacteria or parasites (Mitta et al., 2005; Hanelt et al., 2008). A comprehensive microarray-based approach can reveal whether exposure to infection elicits a "one size fits all" type of defense response, or whether snails mount different kinds of responses depending on the stimulus. This is particularly of interest when the infectious agents such as digenetic trematodes, that have an intimate evolutionary association with snails, initiate complex developmental programs that result in long-term infections that are overtly deleterious to their snail hosts.

This study employed an oligo-based microarray to survey transcriptional responses of *B. glabrata* to wounding, exposure to bacteria (Gram negative *Escherichia coli* or Gram positive *Micrococcus luteus*) and to digenetic trematodes (*S. mansoni* and *E. paraensei*). The design of the oligo-based array was targeted in the sense that it emphasizes features involved in immune or stress-related responses. The study of transcriptomic responses of snails to parasitism and other environmental stimuli is currently in its infancy, only a single other report featuring a *B. glabrata* cDNA microarray, different from the one employed here, has been published (Lockyer et al., 2008). Studies of other invertebrate host–parasite associations indicate the great potential for this approach to reveal immunological mechanisms critical to host defense or parasite survival (Abraham et al., 2004; Dimopoulos et al., 2002; Srinivasan et al., 2004; Xu et al., 2005; Baton et al., 2009). This paper aims to present and validate the oligo-based array, and to exemplify its use by comparative analysis of the responses of *B. glabrata* at a relatively early time point (12 h) following the immune challenges noted above. The results indicate that *B. glabrata* snails mount different defense responses depending on the nature of the biological stimulus.

2. Materials and methods

2.1. Live material and experimental treatments

The M line strain of the snail *B. glabrata* used in these studies serves as intermediate host for both *E. paraensei* and *S. mansoni* (PR-1 strain). Both snails and trematodes were maintained at the University of New Mexico as previously described (Stibbs et al., 1979; Loker and Hertel, 1987). Seven groups of snails were used in this study. The first group consisted of unmanipulated snails (10–12 mm shell diameter) that served as controls for snails of similar size in groups two through four. Snails in the second group were stab-wounded with a 27G hypodermic needle. Groups three and four were exposed to *E. coli* or *M. luteus,* respectively, by injection in the headfoot with 50 μL of bacterial culture in LB medium (OD_{600} of $1.0 = 8 \times 10^8$ cells/mL) using a G27 hypodermic needle (Hanelt et al., 2008). These bacteria were selected because they are common in nature, their genomes have been characterized and they are frequently used as model infectious organisms in invertebrates (Hetru and Bulet, 1997). Groups five and six consisted of snails (4–8 mm) that were exposed to *E. paraensei* or *S. mansoni,* respectively. For both trematodes, snails were exposed individually to 15–20 miracidia per snail in the wells of a 24-well plate, in artificial spring water (ASW) (Loker and Hertel, 1987) for 12 h. Size-matched snails (group seven) were sham exposed as controls. Snails from all groups were kept for 12 h in 24-well plates before RNA was extracted from whole bodies of individual snails. For each group, three biological replicates consisting of pools of 5 snails were used.

2.2. Design and generation of a B. glabrata oligonucleotide-based microarray

The *B. glabrata* 70-mer oligoarray was designed at the Center for Evolutionary and Theoretical Immunology (CETI), University of New Mexico (UNM) and contains 1152 features. Target sequences were selected from the set of 4382 unique sequences identified by cluster analysis of *B. glabrata* ESTs in GeneBank performed by Blaxter in 2006, http://www.nematodes.org/NeglectedGenomes/MOLLUSCA/wwwPartiGene.php, combined with ORESTES many of which were recorded uniquely from *B. glabrata* after bacterial challenge (Hanelt et al., 2008; Lockyer et al., 2007b). The use as selection criteria of Gene Ontology terms related to immunity, stress response, phagocytosis, encapsulation, defense, lectin, lysosome, oxidant, radical, adhesion, apoptosis, cytoskeleton, kinase, and signal transduction yielded 557 targets for features on the array. An additional 502 features on the array represent novel sequences (i.e. no similarity to entries in GenBank databases) with unknown functions were incorporated with the goal of identifying new candidate factors important to the response of *B. glabrata*. Detection of coding regions using ESTscan v2.1 (Iseli et al., 1999) was used to infer 5′–3′ directionality for unknown ESTs. The array also includes nuclear rDNA sequences and genes of the mitochondrial genome of *B. glabrata.* An additional 37 features represent mitochondrial and rDNA genes of *S. mansoni* and *E. paraensei*, as well as transcripts expressed by intramolluscan larvae of these parasites (Table 1).

Within the derived population of ESTs, unique 70-mer oligos were selected with the bioinformatics tools Yoda (Nordberg, 2005) and OligoArray v2.1 (Rouillard et al., 2003). Inclusion of 10 alien sequences (SpotReport® Alien® cDNA Array Validation System, Stratagene) facilitates normalization of relative signals for different probes. Sense 70-mer oligonucleotides were obtained from Integrated DNA Technologies (IDT) and printing and quality control testing were performed at the Hollings Marine Laboratory Genomics Core Facility (HML-GCF)/MUSC in Charleston, SC. The GenePix Array List (GAL) file and details of the features on the array (feature ID, complete cDNA sequence, oligo sequence, BLAST similarity) are provided in supplementary Tables 1 and 2. Note that many features were assigned putative identities based on BLAST results, pending validation by full experimental characterization.

For spotting on the microarray, the oligonucleotides (100 μM in deionized water) were diluted into 50% water and 50% Epoxide

Table 1
General description of features on the array.

Category	Number	Comments
Biomphalaria (with putative ID)	557	Genes, ESTs, ORESTES (clusters, singletons)
Biomphalaria (unkown, novel)	502	Genes, ESTs, ORESTES (clusters, singletons)
Biomphalaria (other)	25	rDNA genes, mitochondrial genes
Echinostoma paraensei	12	ESTs, rDNA genes, mitochondrial genes
Schistosoma mansoni	26	ESTs, rDNA genes, mitochondrial genes
SpotReport Aliens	20	10 sense, 10 antisense
Negative controls	10	Plant origin
Total	1152	