Genome-wide transcriptional analysis upon entomopathogenic nematode infection of Drosophila larvae

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Entomopathogenic nematodes (EPN) are obligate and lethal insect parasites. These EPN are symbiotically associated with entomopathogenic bacteria (EPB). Photorhabdus luminescens creating the highly pathogenic nematobacterial complex that is able to kill the host within 24 to 48 hours. H. bacteriophora with its bacterial symbionts are able to infect a broad spectrum of insect species including e.g. larvae of Drosophila melanogaster. Symbiotic bacteria help to digest host tissues and provide nutrients for themselves and developing nematodes.

For successful development within the host, EPN and their symbiotic EPB must overcome insect defences including cellular and humoral immune responses. We used the well-established tipparite model (Drosophila melanogaster, nematodes and their symbiotic bacteria), DNA chips and bioinformatic tools to compare gene expression in non-infected and infected fly larvae. We focused on the early time point of nematode infection when EPB establish themselves in the hemolymph after release from their nematode vector.

We performed a genome-wide analysis of the D. melanogaster transcriptome response to nematobacterial infection at the time point at which the nematodes reached the hemolymph. The significantly regulated transcripts after nematobacterial infection are enriched for immune genes. a. A heatmap representing the 100 most strongly regulated transcripts from the microarray (columns c1–c3 = Control: noninfected; c1–c0 = infected larvae). Each column represents an independent sample. Color key and density plot represent the level of regulation. Dark intensities indicate the most up- and down-regulated genes, respectively. The one-letter code to the right of the heatmap indicates whether the gene was previously detected in other genome-wide analysis of Drosophila larval immune response (category A–C, G, H) or is specifically regulated upon nematode infection (D); for a description of the categories compare the Venn diagram in e. b. GO classification of the 100 most strongly up-regulated genes. Immune response molecules occupy a fourth of the top 100 genes. c. Venn diagram showing differentially regulated transcripts after infection with common Gram-negative (G–) and positive (G+) bacteria, pathogenic G– bacteria, wasps and nematodes in Drosophila larvae. 381 and 104 transcripts are specifically up- or down-regulated after nematobacterial infection (comprising altogether 485 differentially regulated transcripts in category D).

We detected 642 genes whose expression was significantly influenced by nematobacterial infection; most of them (518) upregulated upon infection including highly induced genes involved in antimicrobial response and development. Based on Gene Ontology annotation we identified several factors and pathways such as Wnt, Jak-STAT or Hedgehog which could be involved in sealing and repairing of wounds caused by invading nematodes as well as a number of immune molecules that were enriched after nematode infection.

We use data from microarrays as a starting point for functional tests. D. melanogaster mutants and RNAi lines for differentially regulated genes help us to identify factors that are critical during the infection. We have already identified several key players in the immune response and further investigation is likely to characterize the infection by nematodes and their symbiotic bacteria in more detail improving our understanding of the insect immune response.


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