

# **Study of TEP3 gene in *Drosophila melanogaster* and its immune response to entomopathogenic nematodes**

Dissertação de Mestrado

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Alexandre Lemnos**

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## Abstract

Entomopathogenic nematodes (EPNs) are parasites of numerous insects that form a symbiotic relationship with Gram-negative bacteria. Upon infection, several complex interactions between EPNs and the host insect are established. *Drosophila melanogaster*, has been described as an excellent genetic tool and there is plenty of mutant lines easily available allowing the researchers to better understand the complex interactions between EPNs and the host insect upon infection.

It has been proposed that thioester-containing proteins (TEPs) are molecules with an important role in the recognition of pathogens acting as opsonins, but their role in the immune response is not fully described. Studies have shown that *Drosophila* TEP3 mutants are more susceptible to nematode infections and that higher levels of melanization and phenoloxidase as well as increased production of antimicrobial peptides in TEP4 mutants infected with *Photorhabdus luminescens* bacteria.

The aim of this study is to further evaluate the susceptibility of mutated TEP3 *D. melanogaster* upon infection with two isolates of two different species of EPNs, namely *Steinernema carpocapsae* (isolates PB and R1) and *Heterorhabditis bacteriophora* (isolates Az 148 and H221). Potential differences in response of nematode isolates were in focus, because together with known products of nematodes and their function they would help to identify immune mechanisms in which TEP3 is involved. Furthermore, experiments included the isolation of the symbiotic bacteria from the isolates used and Nanoinjecting into wildtype and TEP3 mutant larva.

Susceptibility was affected not only between two different species of nematodes used but also among different isolates. Isolate R1 proved to be the most virulent against *D. melanogaster* wildtype and TEP3 mutants while Az148 shows as the least virulent. Nanoinjection with bacteria proved pathogenicity of *Photorhabdus* and *Xenorhabdus*, but no alteration was observed in survivability of wildtype and TEP3 mutants upon bacterial nanoinjections. These results could suggest that TEP3 acts against the nematode rather than against the bacteria.

Keywords: *Drosophila*, Entomopathogenic nematodes, thioester-containing proteins, Immune system, *Photorhabdus*, *Xenorhabdus*.

## Resumo

Os nemátodos entomopatogénicos (EPNs) são parasitas de insetos capazes de formar uma relação simbiótica com as bactérias Gram-negativas. Aquando da infeção, são estabelecidas interações complexas entre EPNs e o hospedeiro (inseto). *Drosophila melanogaster*, é descrito como uma excelente ferramenta genética que compreende várias estirpes mutantes de fácil acesso, proporcionando uma melhor forma de compreensão destas interações.

Diversos estudos propuseram que as proteínas que contêm tioéster (TEPs) são moléculas associadas ao reconhecimento de agentes patogénicos, atuando como opsoninas embora o seu papel na resposta do sistema imunitário não tenha sido totalmente descrito. Vários estudos demonstraram o aumento da suscetibilidade de mutantes de *Drosophila* TEP3 a infeções com nematode bem como altos níveis de melanização e de fenoloxidase. Para o gene TEP4 foi verificado um aumento da produção de péptidos antimicrobianos aquando da infeção com a bactéria *Photorhabdus luminescens*.

Este trabalho tem como objetivo avaliar a suscetibilidade da estirpe mutada TEP3 de *D. melanogaster* aquando uma infeção com dois isolados de duas espécies diferentes de EPNs: *Steinernema carpocapsae* (isolados PB e R1) e *Heterorhabditis bacteriophora* (isolados Az 148 e H221), bem como testar a suscetibilidade aquando da infeção da bactéria isolada de cada nematode por nano injeção em larvas de *D. melanogaster* selvagens e com mutação para o gene TEP3.

Observou-se que a suscetibilidade foi afetada não só pelas duas espécies de nematodes, mas também pelos diferentes isolados. O isolado R1 evidencia uma maior virulência contra o mutante TEP3 de *D. melanogaster* enquanto o isolado Az148 apresenta-se como o menos virulento. A nano injeção com bactéria demonstrou a patogenicidade de *Photorhabdus* e *Xenorhabdus* mas não foi suficiente para alterar a sobrevivência tanto de moscas selvagens como das mutantes TEP3. Tendo em conta estes resultados, sugere-se um papel mais ativo do gene TEP3 na defesa contra o nematode e não contra a bactéria.

Palavras Chave: *Drosophila*, nemátodos entomopatogénicos, TEP, sistema imunitário, *Photorhabdus*, *Xenorhabdus*.

## Abbreviations

AMPs - Antimicrobial peptides

BGBP -  $\beta$ -glucans-binding proteins

Dopa - L-3,4-dihydroxyphenylalanine

ESPs - Excretory/secretory products

EPN – Entomopathogenic nematodes

GNBPs - Gram-negative binding proteins

IJ – Infective juveniles

LBP - LPS-binding proteins

LPS – Lipopolysaccharides

MCR - Macroglobulin complement related

PAMPs - Pathogen associated molecular pattern molecules

PGRPs - Peptidoglycan recognition proteins

PRPs - Pattern recognition proteins

PO – Phenoloxidase

proPO - prophenoloxidase

TEP – Thioester containing proteins

wt - WildType

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