The Effect of Mutations in Toll-like Receptors on *Caenorhabditis elegans* Egg-Laying signaling

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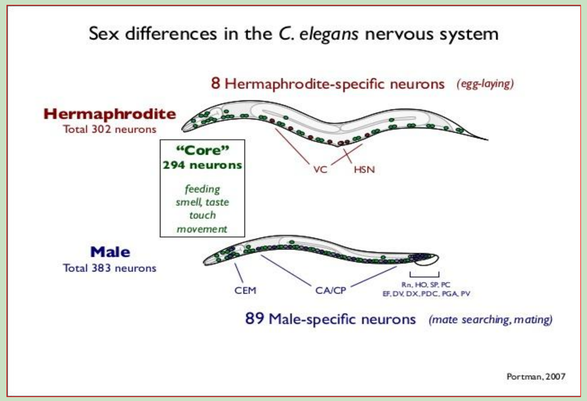
Introduction

Recent studies have shown that Tol-1 is important for the *Caenorhabditis elegans* innate immunity against gram-negative bacterium once introduced in the pharynx. This toll-like receptor (TLR) has been shown to be orthologous to mammalian TLR and subsequent downstream signaling. The biological function of TLR is to detect chemical ligands from the surrounding environment of the cell, i.e. lipopolysaccharides (LPS) released by bacterium. Once the LPS is recognized by the cell, the TLR will activate downstream protein kinases that ultimately lead to the activation of transcription factors important for the innate immune response. Additional signaling in *C. elegans* involves egg-laying that is maintained by the interaction between Hermaphrodite Specific Neurons (HSNs) and Ventral C neurons (VCs) with vulval muscles. A total of two HSNs and 6 VCs are found in *C. elegans*. These HSNs are found lateral and posterior to the vulva of the *C. elegans.* Signaling conducive to egg-laying involves first the mechanosensory neuron, PLM, that will send synaptic input to HSNs. Next, neuromodulators can be sent from HSNs to G-protein coupled receptors (GPCR) on the vulval muscles to regulate egg-laying behavior. A neuromodulator such as serotonin will increase excitability of the vulval muscles that will promote egg-laying. The role of VCs is not as significant as HSNs; however, these neurons are important for stimulation of the vulval muscles.



Objective

The objective of this experiment was to gain insight on how the Tol-1 receptor and its downstream signals impact the egg laying abilities of *C. elegans*. This was accomplished by performing three phases of experimentation: egg laying assays with TOL$^{-/-}$ mutants, egg laying assays of TOL$^{-/-}$ after exposure to the mutagenic agent ethyl methanesulfonate (EMS), and thrashing and touch assays of TOL$^{-/-}$ mutants. These egg laying, touch, and thrashing assays provide evidence to suggest that TOL-1 receptor activity contributes to the chemosensory abilities of *C. elegans* with respect to egg laying.

Materials and Methods

The strains of *C. elegans* used include the wild-type strain (N2) and the mutant *tol*-1(*nr2033*). Each were chunked on NGM plates seeded with OP50 *E. coli*. Initial screening of N2 and mutant worms included: Egg-laying assay, thrash assay, and touch assay. Additional egg-laying in ethyl methanesulfonate (EMS) occurred with mutants chosen from the initial egg-laying assay that laid the least amount of eggs in the serotonin solution. Subsequent mutants were also plated on NGM plates seeded with OP50 *E. coli*. An Olympus SZ30 Stereomicroscope was used to make observations. Solutions utilized for egg-laying were M9, serotonin dissolved in M9, and EMS

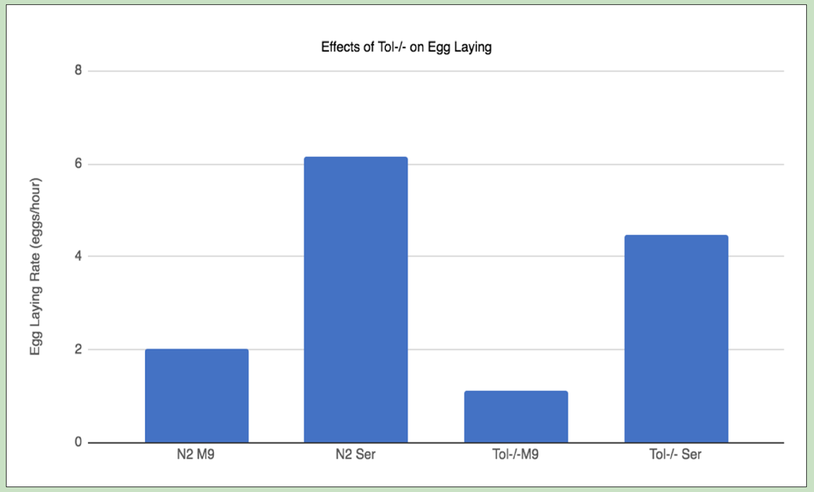


Figure 1. Bar graph comparing egg-laying rates of N2 and TOL$^{-/-}$ *C. elegans* with and without 1-hr incubation in serotonin

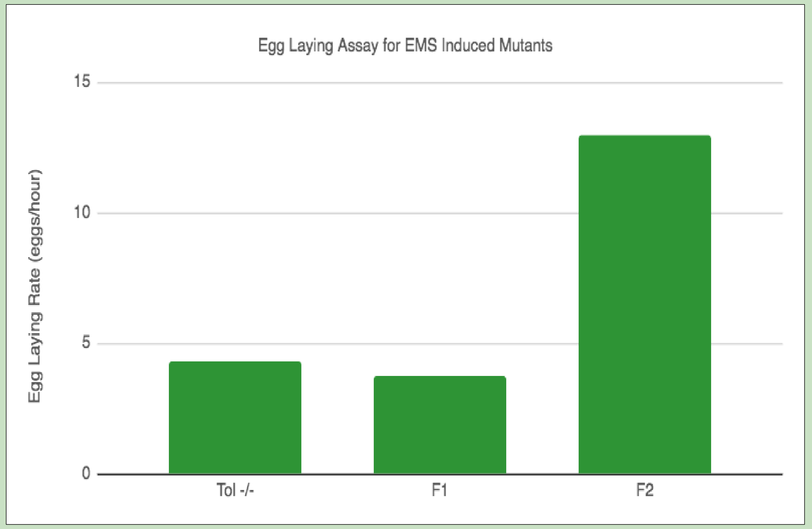


Figure 2. Bar graph comparing egg-laying rates of parent TOL$^{-/-}$(P1) *C. elegans* versus F1 and F2 progeny

Results

The TOL$^{-/-}$ mutant showed a 27% decrease in egg laying ability (figure 1), compared to the N2 wild-type, after serotonin administration via egg-laying assays. In F1, there were 215 mutants screened for egg laying and another 288 screened for F2. The F2 generation of the EMS induced TOL$^{-/-}$ mutants showed a 300% increase in egg laying rate (figure 2). Thrashing and touch assays with EMS induced TOL$^{-/-}$ mutants showed no significant changes compared to the N2 wildtype *C. elegans.*

Discussion

Data suggests the effects of mutations on the TOL-1 receptors in C. elegans result in chemosensory changes and also that TOL-1 is important in chemosensory abilities. The mechanosensory assays (touch and thrashing) result in essentially no significant changes in behavioral responses, while the egg-laying assay (using the monoamine neurotransmitter serotonin to induce egg-laying) results in quantifiable changes in egg laying behavior, suggesting TOL-1 receptors are a part of C. elegans environmental chemosensory abilities. Data implies that alterations via mutations to these TOL-1 receptors could result in a decreased ability to respond to chemical stimuli in the surrounding environment. Future studies need to be performed to conclude how these randomized (rtol-1) mutants have overcome their initial resistance to egg laying after being exposed to EMS (ethyl methanesulfonate), and whether this resistance is due to changes upstream or downstream of the TOL-1 pathway.

References

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