Immunological roles of *TmToll-2* and *TmToll-3* in response to systemic infection in *Tenebrio molitor*

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Abstract

The antimicrobial roles of Toll-like receptors have been mainly identified in mammalian models and *Drosophila*. However, its immunological function in other insects has yet to be clarified. Here, we determined the innate immune response involvement of *TmToll-2* and *TmToll-3* encountering Gram-negative, Gram-positive, and fungal infection. Our data revealed that *TmToll-2* expression could be induced by *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* infections in the fat bodies, gut, Malpighian tubules, and hemolymph of *Tenebrio molitor* young larvae. However, *TmToll-2* silencing via RNAi technology revealed that in the absence of *TmToll-2*, the final Toll signaling effector, antimicrobial peptide (AMP) genes, and relevant transcription factors were significantly downregulated mainly *E. coli* post-insult. We showed that the expression of all AMP genes was suppressed in the main immune organ of insects, namely, fat bodies, in silenced individuals, while the relevant expressions were not affected after fungal infection. Moreover, we showed that *TmToll-3* expression was induced after infection with *Staphylococcus aureus* and *Candida albicans*. Taken together, our research revealed the immunological roles of *TmToll-2* and *TmToll-3* in different organs of *T. molitor* in response to pathogenic insults.

Keywords: Toll-2, Toll-3, *Tenebrio molitor*, microbial infection, antimicrobial peptides, RNAi technology

Results

1. *TmToll-2* and *TmToll-3* developmental expression

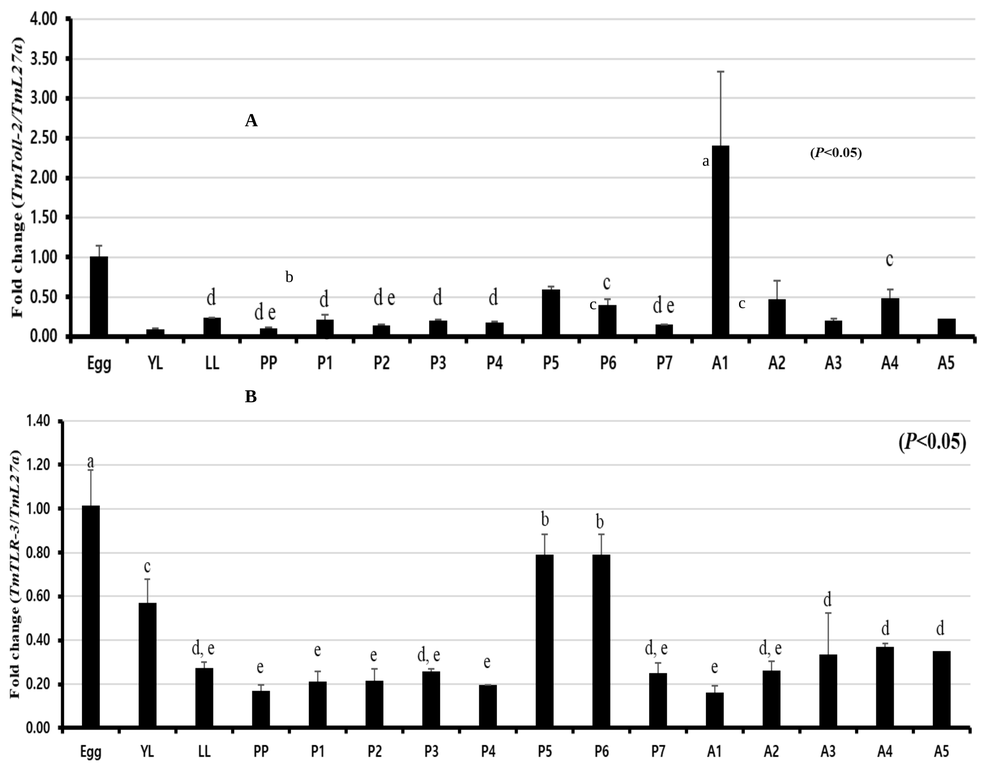

Figure 1. Relative expression levels of *TmToll-2* and *TmToll-3* mRNA in developmental stages of *T. molitor*.

Expression levels of *TmToll-2* (A) and *TmToll-3* (B) in *T. molitor* at the egg, the young larval (YL) , late larval (LL), pre-pupal (PP), 1–7-day-old pupal (P1–7), and 1–5-day-old adult (A1–5) stages.

2. Specific tissues of larvae and adults

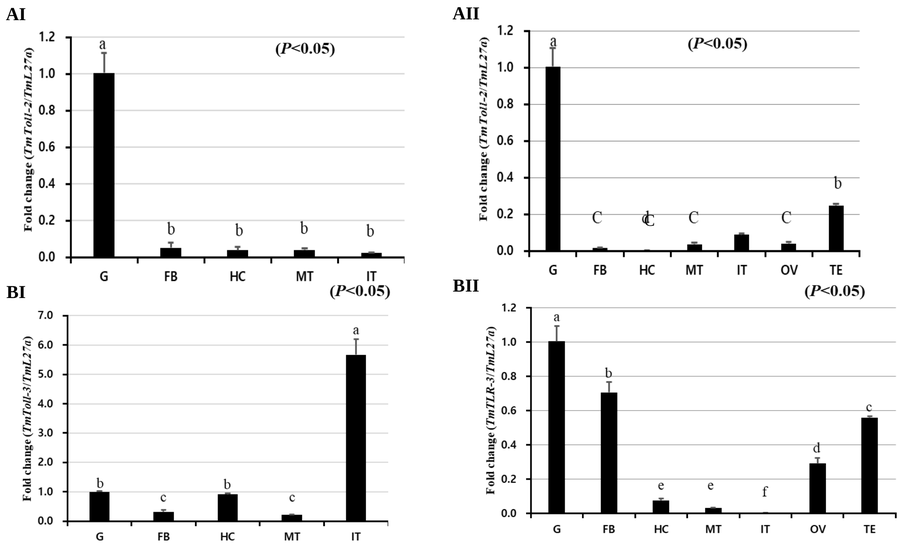

Figure 2. Relative expression levels of *TmToll-2* and *TmToll-3* mRNA in tissues of larvae and adults.

Distribution of *TmToll-2* transcripts in larval (AI) and adult tissues (AII) and *TmToll-3* transcripts in larval (BI) and adult tissues (BII). Fat body (FB), gut (GT), hemocytes (HC), integument (INT), and Malpighian tubules (MT) of late instar larvae and adults, in addition to ovaries (OV) and testes (TT) of adults, were dissected and collected from 20 early larvae and 5-day-old adults for analysis. *T. molitor* 60S ribosomal protein L27a (*TmL27a*)-encoding gene was used as an internal control.

3. *TmToll-2* and *TmToll-3* mRNA expression profiles after microbial challenge

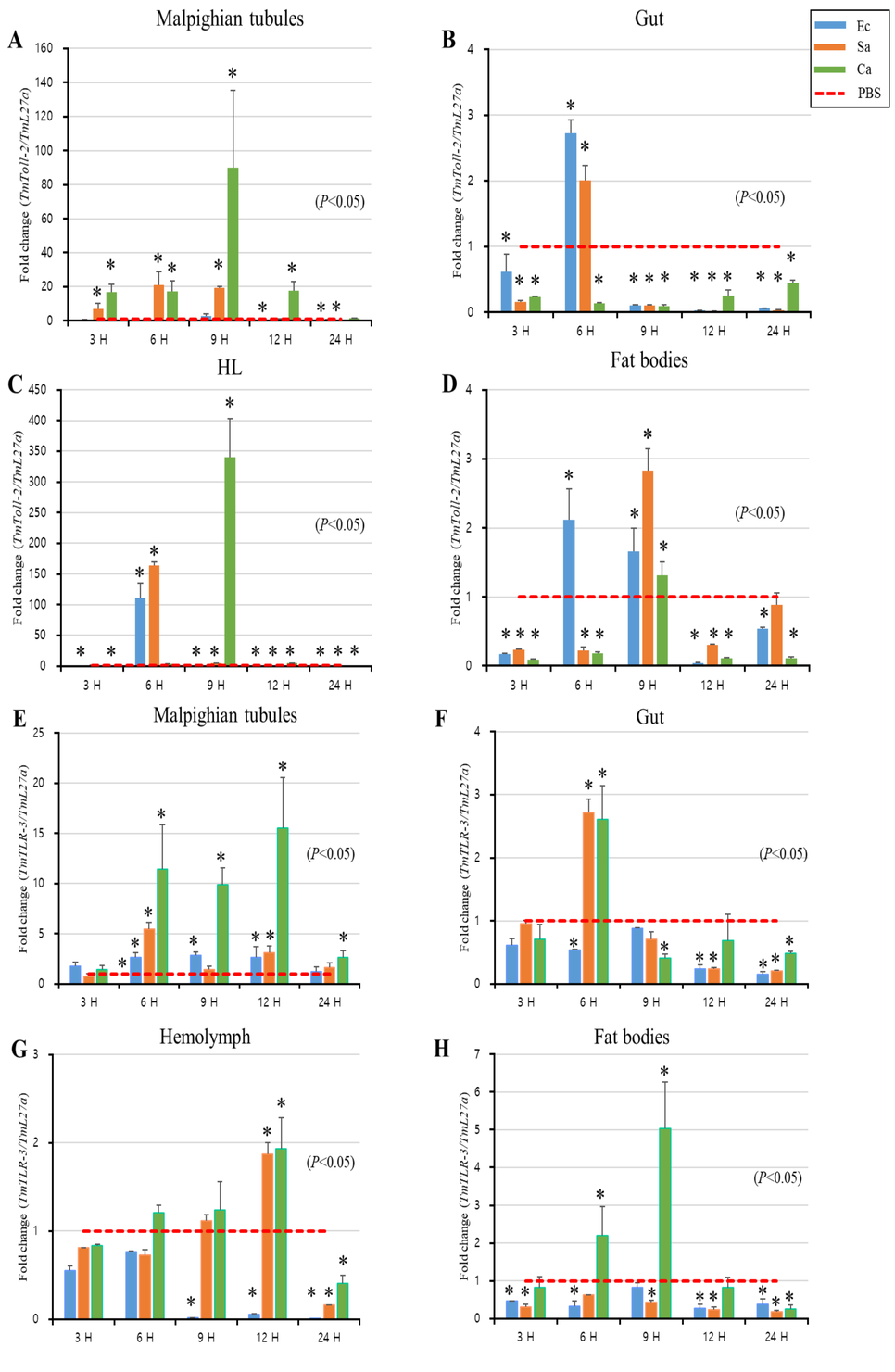

Figure 3. *TmToll-2* and *TmToll-3* mRNA expression profiles after microbial challenge.

Expression of *TmToll-2* (A-D) and *TmToll-3* (E-H) mRNA in the Malpighian tubules (A,E), gut (B,F), hemolymph (C-G), and fat bodies (D,H) of larvae infected with *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The expression was analyzed by qRT-PCR using *L27a* (*T. molitor*) as the internal control. For each time point, the expression level in the phosphate-buffered saline (PBS)-injected control (mock control) was set to 1; this is represented by a dotted line. vertical bars represent mean ± standard error from three biological replicates.

Conclusion

TmToll-2 and *TmToll-3* mRNA expression patterns were evaluated at different developmental stages and in various tissues in larvae and adults. *TmToll-2* and *TmToll-3* mRNA expression patterns were evaluated at different developmental stages and in various tissues in larvae and adults. *TmToll-2* mRNA levels was highly expressed in one-day-old adults. *TmToll3* peaked in the embryonic stage. With respect to tissue expression patterns, in larvae and adults, the highest *TmToll-2* expression levels was in the gut. In larvae, the highest *TmToll-3* expression levels was in the integument, and gut for adults. *TmToll-2* and *TmToll-3* expression in immune-challenged *T. molitor* larvae were examined after *E. coli*, *S. aureus*, and *C. albicans* injections, using PBS injection as the control. *TmTak1* and *TmTab2* expression was considerably upregulated in response to bacterial and fungal infections. For *TmToll-2*, the highest expression levels were seen in the hemolymph 9h after fungal infection. For *TmToll-3*, the highest expression levels were seen in the Malpighian tubules at 9h after fungal infection. Further comprehensive studies of possible ligands and simulators of TLRs and possible cross-talks of this signaling with other pathways should provide a clear perspective of underlying mechanisms involved in innate immunity.