Supplementary Material 1

Quantitative reverse transcription-polymerase chain reaction (RT-PCR)

The detailed conditions of the quantitative RT-PCR were described previously (Takehana A, Katsuyama T, Yano T, Oshima Y, Takada H, Aigaki T, Kurata S (2002) Overexpression of a pattern-recognition receptor, peptidoglycan-recognition protein-LE, activates imd/relish-mediated antibacterial defense and the prophenoloxidase cascade in Drosophila larvae. *Proc Natl Acad Sci U S A* **99:** 13705-13710). Primers were as follows: CG8974, 5'-CGACTATTGCCCAACGAGTT -3', 5'- CTCACTCTACGAGTGCAACA -3'; CG8509, 5'-GAGCAACAAACTGGGCTATG -3', 5'- CACGTCCTTTAACTTCGCGA -3'; CG15602, 5'-AATGAACCTGGCTCCTCCAT -3', 5'- TCGCAGACGTCTCTCAATGT -3'; CG32581, 5'-CTTAGCACGGCAAGTAGAAG-3', 5'- TGTTGCACTCGTAGAGTGAG -3'; Cecropin A, 5'- CATCTTCGTTTTCGTCGCTC -3', 5'- CGACATTGGCGGCTTGTTGA -3'; Metchnikowin, 5'- CACCGAGCTAAGATGCAACT -3', 5'- CCTCCAGTTACCTGTGTCTT -3'; Drosocin , 5'- CGGAGAAGTCATCCGATCAA -3', 5'- GCAGCCATCTGGTTATTTCG -3'; Defensin, 5'- TTGAACCCCTTGGCAATGCA -3', 5'- AGTTCTTCGTTCTCGTGGCT -3'. Each experiment is representative of at least two independent experiments.

Antibody preparation

A chimeric protein composed of glutathione S-transferase and the PGRP-domain (from Pro at amino acid position 171 to Gln at position 345) of PGRP-LE was expressed in Sf21 cells using the BacPAK Baculovirus Expression System (Clontech, Palo Alto, CA), and was purified using DEAE-Sepharose FF chromatography (Amersham Biosciences, Piscataway, NJ), followed twice by Glutathione Sepharose 4B chromatography (Amersham Biosciences). After cleaving with glutathione S-transferase-tagged excision protease, the recombinant PGRP-domain was purified by Glutathione Sepharose 4B chromatography. Immunization and affinity purification of the antiserum were performed using the previously described methods (Kurata S, Saito H, Natori S (1992) The 29-kDa hemocyte proteinase dissociates fat body at metamorphosis of Sarcophaga. *Dev Biol* **153:** 115-121). For immunostaining, affinity purified antibody was absorbed with fixed *PGRP-LE*^{112} larval tissues three times. Western blotting was performed with rabbit anti-PGRP-LE at 1:15 and rabbit anti-Hrp48 at 1:2000. The hemocyte fractions were prepared from larval hemolymph by centrifugation (130 *g*) and resulting supernatants were used the hemolymph (plasma) fraction.

Immunohistochemistry

The third instar larvae were dissected in cold PBS and fixed in PEM (100 mM PIPES pH 6.9, 2 mM $MgSO_4$, 1 mM EGTA, 4 % formaldehyde) for 20 min on ice. After washing with PBT (PBS containing 0.3 % Triton X-100), blocking was performed in PBTB (PBS containing 0.3 % Triton X-100 and 5% BSA) for 1 h at 4 °C. Antibody staining was performed by using the primary antibodies mouse anti-β-galactosidase (Promega, Madison, WI) at 1:5000 and anti-PGRP-LE at 1:4 overnight at 4°C. Immunofluorescent detection was performed using Cy3-conjugated anti-IgGs (Jackson ImmunoResearch, West Grove, PA). Nuclear staining was performed with 0.1 μg/ml DAPI. After washing with PBTB, the fat body and the trachea were dissected in PBS, and mounted in Vectashield (Vector Laboratories, Burlingame, CA). The preparations were analyzed on a Zeiss Axioplan fluorescent microscope and Leica confocal microscope.