Supplemental Data

A Serpin that Regulates Immune Melanization in the Respiratory System of *Drosophila*

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**Figure S1. Identification of a Mutation in the *spn77Ba* Gene**

(A) Genomic DNA from *l(3)77ABi[W7]/TM6C* flies was used as a template to amplify the region covering the *spn77Ba* gene by PCR, and the PCR products were sequenced. At position 247 in the shown sequence, a peak representing an adenine was present in addition to a peak representing the wild-type guanine, indicating that the *l(3)77ABi[W7]* allele is a G-to-A mutation at that position.

(B) The G-to-A mutation in *l(3)77ABi[W7]* is at the first nucleotide of the second intron of 57 bp in the *spn77Ba* gene. The change of this absolutely conserved nucleotide is predicted to block splicing of the intron. In the unspliced transcript, a stop codon toward the 5' end of the intron is predicted to result in a truncated polypeptide only about a third of the full-length Spn77Ba protein. 5' (▼) and 3' (▲) primers were designed to amplify the cDNA region covering the mutation. The PCR product is predicted to be 311 bp for wild type and 368 bp for the mutant. Exon sequences are shown in uppercase letters and intron sequences in lowercase letters. Amino acids are shown in three-letter codes.

(C) RT-PCR analysis showed PCR products corresponding to both spliced and unspliced transcripts predicted in *l(3)77ABi[W7]/TM6C* flies (RT PCR product). No-RT control did not yield any detectable PCR product, thus excluding the possibility of genomic DNA contamination. Sequence analysis (not shown) confirmed that the apparently larger RT-PCR product corresponds to *spn77Ba* RNA containing the un-spliced intron.
Figure S2. Spn77Ba Mutants are Early Lethal

(A) About 31% of embryos homozygous for the l(3)77ABi[W7] mutation did not hatch, and 55% and 12% of the surviving l(3)77ABi[W7] larvae died at the first instar or second instar stages, respectively.

(B) Embryos homozygous for the l(3)77ABi[W7] mutation appeared to be fully differentiated. Dead embryos showed melanization, visible as discrete spots (arrows) throughout the body but not in the trachea.

(C) Dead l(3)77ABi[W7] first and second instar larvae had melanized spots (arrows) in the body cavity but not visibly in the trachea system.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Fly Stocks

The w1118 line was used as the wild-type Drosophila strain unless otherwise indicated. The following lines were described earlier: UAS-MP1-IR, UAS-MP1, UAS-MP2-IR, UAS-MP2 (Tang et al., 2006); UAS-Spn27A (Ligoxygakis et al., 2002b); spezm7 (Lemaitre et al., 1996); pshi, UAS-psh (Ligoxygakis et al., 2002a); seml (Michel et al., 2001); vkg-GFP (Morin et al., 2001); Drs-GFP (Ferrandon et al., 1998); tubP-Gal80ts (McGuire et al., 2003); UAS-PGRP-SA, UAS-GNBP1 (Gobert et al., 2003). RNAi lines for PO genes were obtained from the Vienna Stock Center and National Institute of Genetics, Mishima, Japan. All other lines were obtained from the Bloomington Stock Center, including the Df(3L)rdgC-co2/TM6C, Sb stock carrying the deficiency that uncovers the spn77Ba gene as well as the stocks bearing 8 different lethal mutations, l(3)77ABA, c, d, f-j, uncovered by the same deficiency (Lukinova et al., 1999).

Identification of a Mutation in the spn77Ba Gene

Standard crosses were used to generate flies with the genotype UAS-Spn77Ba; da-Gal4, Df(3L)rdgC-co2/TM6C, Sb, which were then crossed to flies carrying each of the 8 l(3)77AB lethal mutations over TM6C, Sb. Only the cross with the l(3)77ABi[W7] mutation resulted in non-Sb progeny, suggesting that Spn77Ba expression rescued the lethality caused by this mutation and that therefore this mutation is in the spn77Ba gene.

RNA Analysis

Total RNA was isolated from whole larvae, pupae, or 1-2 day old adult males using the TRIzol™ Reagent (Invitrogen) according to manufacturer’s instructions. SuperScript™ II reverse transcriptase (Invitrogen) was used to make cDNAs. Quantitative RT-PCR was performed essentially as described earlier (Pili-Floury et al., 2004). All values were averages of numbers derived from at least three independently prepared cDNA samples.
SUPPLEMENTAL REFERENCES


