able conditions, circulate and cause cases of poliomyelitis has important implications for current and future strategies of the World Health Organization (WHO) initiative to eradicate polio worldwide (*23*). First, the eradication of wild poliovirus, now at an advanced stage (*23*), must be completed as soon as possible. At the same time, it is imperative that immunity gaps in nonendemic countries are prevented, especially in tropical developing countries where the risk for poliovirus circulation is highest (*24*). After certification of wild poliovirus eradication, a carefully planned strategy for the orderly cessation of OPV use worldwide should be implemented. Finally, sensitive global poliovirus surveillance must be maintained for the foreseeable future, and emergency stockpiles of poliovirus vaccine established, for use in the event of any recurrent poliovirus transmission from chronic poliovirus excretors (*25*), a breach in poliovirus containment (*25*), or circulating VDPV.

References and Notes

1. F. C. Robbins, C. A. de Quadros, *J. Infect. Dis.* **175** (suppl. 1), S281 (1997).
2. C. A. de Quadros *et al.*, *J. Infect. Dis.* **175** (suppl. 1), S37 (1997).
3. J. K. Andrus, P. M. Strebel, C. A. de Quadros, J. M. Olive, *Bull. WHO* **73**, 33 (1995).
4. M. A. Drebot *et al.*, *Appl. Environ. Microbiol.* **63**, 519 (1997).
5. Pan American Health Organization, *EPI Newsl.* **18**, 4 (1996).
6. Supplementary figures and details of experimental procedures are available on *Science* Online at www.sciencemag.org/cgi/content/full/1068284/DC1.
7. L. De *et al.*, *J. Clin. Microbiol.* **33**, 562 (1995).
8. P. A. Patriarca, R. W. Sutter, P. M. Oostvogel, *J. Infect. Dis.* **175**, S165 (1997).
9. O. M. Kew, M. N. Mulders, G. Y. Lipskaya, E. E. da Silva, M. A. Pallansch, *Semin. Virol.* **6**, 401 (1995).
10. P. M. Strebel *et al.*, *Clin. Infect. Dis.* **14**, 568 (1992).
11. H.-M. Liu *et al.*, *J. Virol.* **74**, 11153 (2000).
12. L. M. Shulman *et al.*, *J. Clin. Microbiol.* **38**, 945 (2000).
13. M. S. Oberste, K. Maher, D. R. Kilpatrick, M. A. Pallansch, *J. Virol.* **73**, 1941 (1999).
14. A. R. Muzychenko *et al.*, *Virus Res.* **21**, 111 (1991).
15. N. Kawamura *et al.*, *J. Virol.* **63**, 1302 (1989).
16. M. J. Bouchard, D. H. Lam, V. R. Racaniello, *J. Virol.* **69**, 4972 (1995).
17. M. Gromeier, B. Bossert, M. Arita, A. Nomoto, E. Wimmer, *J. Virol.* **73**, 958 (1999).
18. P. D. Minor, *Curr. Top. Microbiol. Immunol.* **161**, 121 (1990).
19. H. G. A. M. van der Avoort *et al.*, *J. Clin. Microbiol.* **33**, 2562 (1995).
20. P. D. Minor, G. Dunn, *J. Gen. Virol.* **69**, 1091 (1988).
21. Centers for Disease Control and Prevention, *Morb. Mortal. Wkly. Rep.* **50**, 874 (2001).
22. ———, *Morb. Mortal. Wkly. Rep.* **50**, 41 (2001).
23. WHO, *Wkly. Epidemiol. Rec.* **76**, 126 (2001); for updates see www.who.int/vaccines-polio.
24. N. Nathanson, J. R. Martin, *Am. J. Epidemiol.* **110**, 672 (1979).
25. D. J. Wood, R. W. Sutter, W. R. Dowdle, *Bull. WHO* **78**, 347 (2000).

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Requirement for a Peptidoglycan Recognition Protein (PGRP) in Relish Activation and Antibacterial Immune Responses in *Drosophila*

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Components of microbial cell walls are potent activators of innate immune responses in animals. For example, the mammalian TLR4 signaling pathway is activated by bacterial lipopolysaccharide and is required for resistance to infection by Gram-negative bacteria. Other components of microbial surfaces, such as peptidoglycan, are also potent activators of innate immune responses, but less is known about how those components activate host defense. Here we show that a peptidoglycan recognition protein, PGRP-LC, is absolutely required for the induction of antibacterial peptide genes in response to infection in *Drosophila* and acts by controlling activation of the NF-κB family transcription factor Relish.

In response to infection, *Drosophila* activates the transcription of a battery of antimicrobial peptide genes in cells of the fat body (the insect analog of the liver). Two major branches of this humoral response have been identified; as in mammals, these responses require NF-κB transcription factors (*1*). One branch activates antifungal responses and requires the receptor Toll and the NF-κB family transcription factor Dif (*2–4*). The second branch, which is primarily antibacterial, requires the NF-κB protein Relish, an IκB kinase (IKK), a caspase, a mitogen-activated protein kinase kinase kinase, and the death-domain protein Imd (*5–11*).

We have taken a genetic approach to identifying genes required for the antibacterial response (*12, 13*). One gene that is absolutely required for the induction of the antibacterial response is *ird7* (*immune response deficient 7*). Two mutations in *ird7* identified in an ethylmethane sulfonate (EMS) mutagenesis screen (*12, 13*) prevented the induction of three antibacterial peptide genes, *Diptericin*, *Cecropin*, and *Defensin*, after infection by either Gram-negative or Gram-positive bacteria (Fig. 1, A and B). Three other antimicrobial peptide genes, *Attacin*, *Metchnikowin*, and *Drosomycin*, also failed to be induced to normal levels. The profile of antimicrobial gene expression observed in the *ird7* mutants was similar to that observed in *imd*, *DmIkkβ*/*ird5*, and *Relish* mutants after bacterial infection, but was distinct from that of *Toll* and *Dif* mutants (Fig. 1A). This pattern suggests that *ird7* is an essential component of the same signaling pathway that requires *imd* and *Relish*, but is not required for the Toll-Dif pathway. Both *ird7* mutants are homozygous viable and fertile, and blood cells from *ird7* mutants can phagocytose bacteria (*14*); these findings suggest that *ird7* is required specifically for the humoral immune response.

The transcription factor Relish directly activates antibacterial target genes in *Drosophila*. Relish is a compound protein similar to mammalian p100 and p105 (the precursors of the p52 and p50 subunits of NF-κB), with an NH_2-terminal Rel homology and a COOH-terminal ankyrin repeat domain similar to that of the NF-κB inhibitor IκB (*15*). In response to immune challenge, full-length Relish (REL-110) is endoproteolytically clipped to generate the NH_2-terminal REL-68 fragment, which translocates into the nucleus, and the COOH-terminal REL-49 ankyrin repeat fragment, which remains stable in the cytoplasm (*16*) (Fig. 1C). In contrast to wild-type animals, no processing of Relish was detected in *ird7* mutant larvae (Fig. 1C). The

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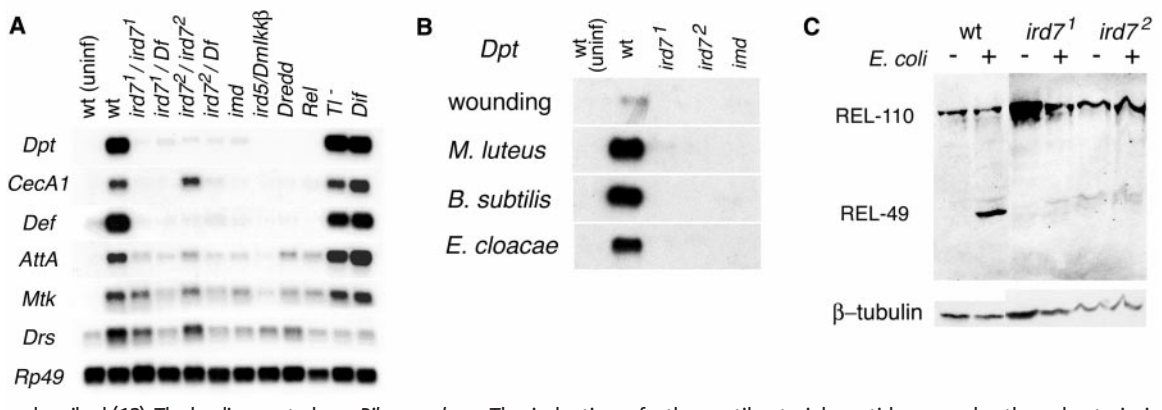

Fig. 1. Phenotypes of *ird7* mutants. (**A**) In *ird7* mutants, *Diptericin* (*Dpt*), *CecropinA1* (*CecA1*), *Defensin* (*Def*), *AttacinA* (*AttA*), *Metchnikowin* (*Mtk*), and *Drosomycin* (*Drs*) transcription is not induced normally after *E. coli* infection, as assayed by Northern hybridization. $ird7^1$ is a very strong or null allele, whereas $ird7^2$ behaves like a strong hypomorph. RNA was prepared from adult flies 6 hours after infection as described (*13*). The loading control was *Ribosomal protein49* (*Rp49*). Similar results were obtained in larvae (*25*). Genotypes: wt, wild type (the parental *P{w⁺ Dpt-lacZ} ca* stock); *Df*, *Df(3L)29A6*; *imd*, imd^1; *ird5/Dmlkk*β, $ird5^1$; *Dredd*, $Dredd^{D55}$; *Rel*, $Relish^{E20}$; *Tl*⁻, $Df(3R)Tl^{9QRX}/Df(3R)ro^{XB3}$; *Dif*, Dif^1. For quantitation, see (*17*). (**B**) *ird7* mutants fail to respond to both Gram-negative and Gram-positive bacteria. Adult flies were pricked with a sterile glass needle (wounding) or injected with *Micrococcus luteus*, *Bacillus subtilis* (Gram-positive), or *Enterobacter cloacae* (Gram-negative) and incu-bated for 6 hours, and total RNAs were prepared. *Rp49* was the loading control (*25*). The induction of other antibacterial peptide genes by these bacteria in *ird7* and *imd* mutants was also similar to that shown in (A) (*25*). (**C**) Relish is not endoproteolytically processed after infection in *ird7* mutants. Protein extracts from the wild-type parental stock ($P[w^+$ *Dpt-lacZ]ca*), $ird7^1$, and $ird7^2$ were prepared from uninfected (-) or infected (+) wandering third-instar larvae 30 min after *E. coli* injection (*16*). Protein from approximately 0.5 larva was loaded in each lane. After blotting, Relish processing was detected with a monoclonal antibody that recognizes the COOH-terminal ankyrin repeat domain of the protein. β-Tubulin was the loading control.

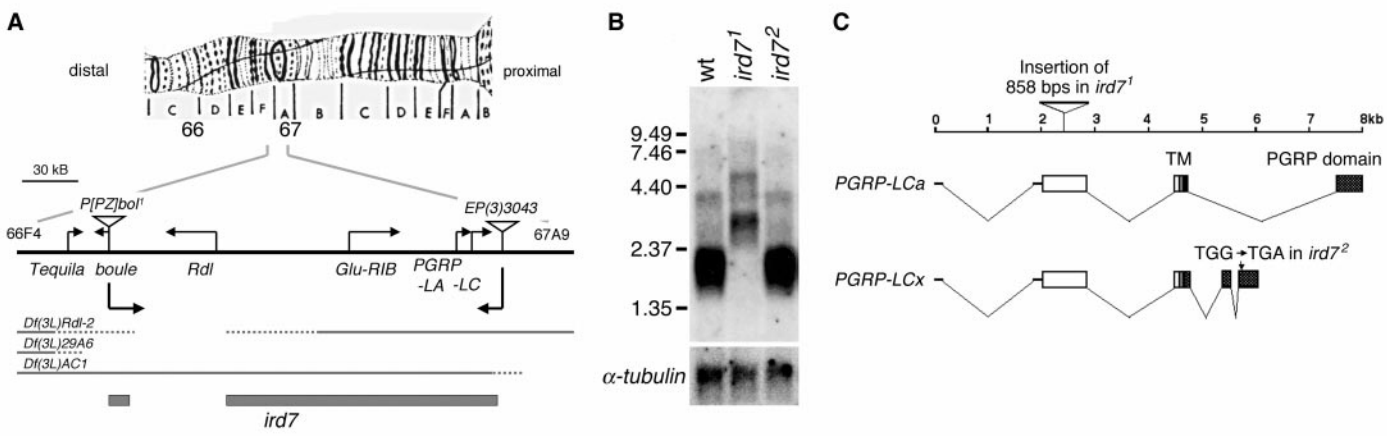

Fig. 2. Molecular identification of the *ird7* gene. (**A**) Genetic mapping of *ird7*. The *ird7* mutation failed to complement *Df(3L)29A6* but complemented *Df(3L)Rdl-2* and *Df(3L)AC1*. Deficiency breakpoints were defined by single-embryo polymerase chain reaction (PCR) (*26*). P element–induced male recombination mapping (*27*) placed the *ird7* locus between *boule* and *EP(3)3043*. Bars at bottom indicate the region that could include *ird7*. At all steps of mapping, X-Gal staining was used to monitor induction of *Dpt-lacZ* after *E. coli* infection. (**B**) Expression of *PGRP-LC* in wild-type and *ird7* mutants. Polyadenylated RNA (4 μg), prepared from wild-type ($P[w^+$ *Dpt-lacZ]ca*) and *ird7* adults, was loaded in each lane. Blots were hybridized with a radiolabeled probe from the second exon of *PGRP-LC*, which is common to both splice variants. α-Tubulin was the loading control. (**C**) Molecular lesions in *PGRP-LC* in *ird7* mutants. The $ird7^1$ allele is associated with an insertion of 858 bp in a common 5′ exon of *PGRP-LC* that introduces a stop codon and would generate a truncated cytoplasmic protein of 105 amino acids. The $ird7^2$ is associated with a nonsense mutation in the *x* PGRP domain of the *PGRP-LCx* isoform, which would truncate this isoform. Light gray bars represent the transmembrane domain. Dark gray bars represent peptidoglycan recognition domains. For cloning of *PGRP-LCx*, a larval-pupal cDNA library (LP library from Berkeley *Drosophila* Genome Project) was screened using a random-primed probe for putative exon *x* (*18*).

Rel domain of Relish failed to translocate to fat body nuclei in *ird7* mutants (*17*). These results indicate that *ird7* is required for Relish processing and nuclear translocation.

Recombination and deficiency mapping localized *ird7* to a small interval on the third chromosome, 66F5-67A9 (Fig. 2A). The *Drosophila* genome sequence annotation indicates the presence of 12 genes in this region, including two genes encoding peptidoglycan recognition protein (PGRP) domains, *PGRP-LA* and *PGRP-LC* (*18*). Peptidoglycan is a strong activator of innate immune responses in insects and mammals, and a PGRP was first identified in a silk moth (*Bombyx*) on the basis of its ability to bind peptidoglycan and activate one aspect of the immune response, the prophenoloxidase cascade (*19*). Later studies have implicated PGRPs in innate immune responses from arthropods to mammals (*20, 21*).

We identified sequence changes that would disrupt the function of *PGRP-LC* in both *ird7* alleles. The gene was represented by several expressed sequence tag clones that encode a single splice form, designated *PGRP-LCa*. In addition, sequences encoding two additional exons encoding PGRP domains ("*x*" and "*y*") were identified in an intron of *PGRP-LC* (*18*). We screened a larval-pupal cDNA library with the *x* and *y* exons and identified an alternatively spliced form of *PGRP-LC* that included the *x* exon; we call this isoform *PGRP-LCx*. Both *PGRP-LC* isoforms encoded type II transmembrane proteins with common NH_2-terminal cyto-

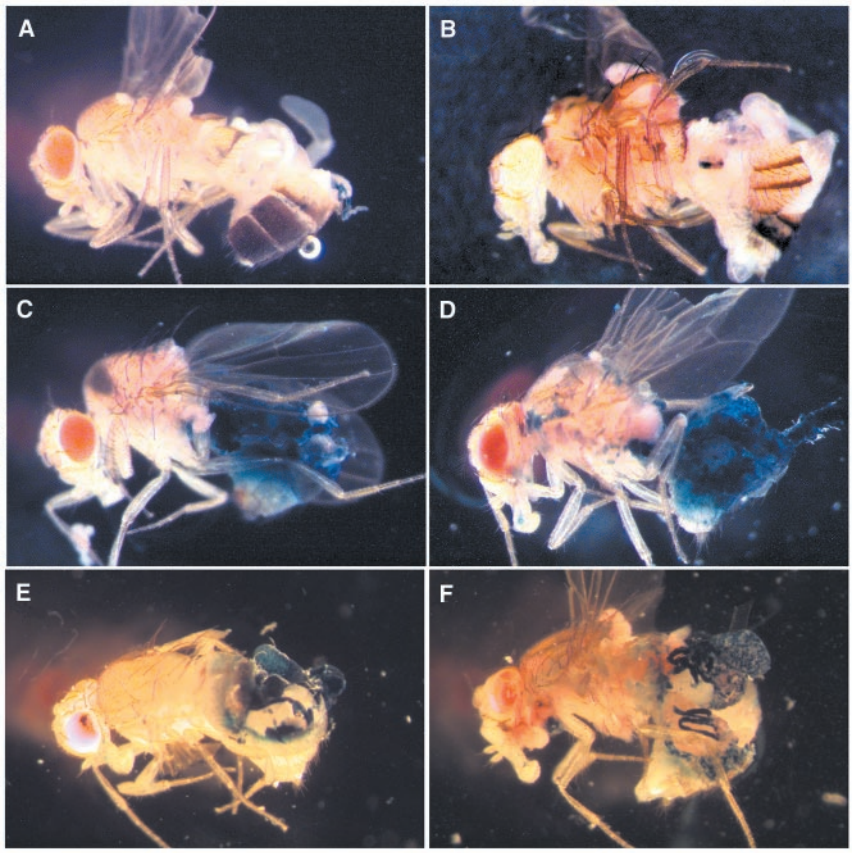

Fig. 3. Both *PGRP-LCa* and *PGRP-LCx* isoforms rescue induction of the *Dpt-lacZ* reporter gene in *ird7* mutants. Full-length *PGRP-LCa* and *PGRP-LCx* cDNAs were cloned into the pUAST (w^+) transformation vector (*28*) and introduced into *y w* flies by P element–mediated transformation (*29*). The second chromosome *c564-GAL4* line, which is expressed in the fat body and other tissues (*30*), was used to drive expression of the UAS construct. Flies of indicated genotypes were injected with *E. coli*, incubated for 6 hours, and assayed for β-galactosidase activity using X-Gal. (**A**) *c564-GAL4/CyO; ird7^1 Dpt-lacZ/ird7^1 Dpt-lacZ* (no UAS-cDNA) animals did not express the reporter gene. (**B**) *UAS-PGRP-LCx/CyO; ird7^1 Dpt-lacZ/ird7^1 Dpt-lacZ* (no GAL4 driver) did not express the reporter gene. The same result was obtained for *UAS-PGRP-LCa/CyO; ird7^1 Dpt-lacZ/ird7^1 Dpt-lacZ* animals. (**C**) *c564-GAL4/UAS-PGRP-LCa; ird7^1 Dpt-lacZ/ird7^1 Dpt-lacZ* expressed the reporter gene at high levels after infection, as did *c564-GAL4/UAS-PGRP-LCx; ird7^1 Dpt-lacZ/ird7^1 Dpt-lacZ* animals (**D**). The *GAL4*-driven transgenes also showed a low level of constitutive expression of *Dpt-lacZ* without *E. coli* injection: (**E**) *c564-GAL4/UAS-PGRP-LCa; ird7^1 Dpt-lacZ/ird7^1 Dpt-lacZ*. (**F**) *c564-GAL4/UAS-PGRP-LCx; ird7^1 Dpt-lacZ/ird7^1 Dpt-lacZ*. In four repetitions of this experiment, the level of X-Gal staining in animals carrying both *c564-GAL4* and the *UAS-PGRP-LC* transgene was greater in infected than in uninfected animals.

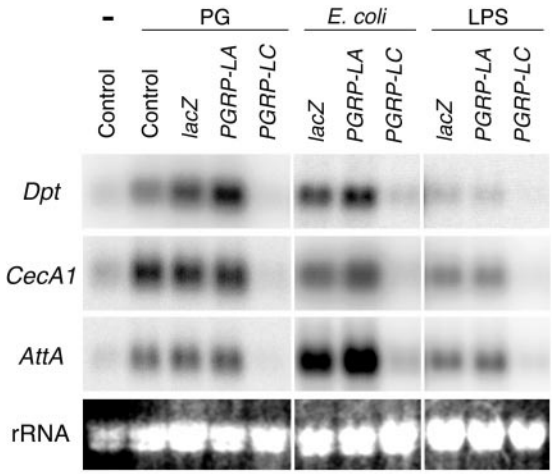

Fig. 4. Inactivation of *PGRP-LC* by transfection of dsRNA blocks induction of antibacterial gene expression in *mbn-2* cells. Northern blot detection of *Diptericin*, *Cecropin A1*, and *Attacin A* in *mbn-2* cells is shown after treatment with dsRNA from *PGRP-LC*, *PGRP-LA*, or *lacZ* and induction with the indicated elicitors. Ethidium bromide staining of ribosomal RNA was used as a loading control. *mbn-2* cells were plated at a density of 1 million cells/ml and transfected 1 day later with 10 μg of dsRNA (*31*). For *PGRP-LA* the dsRNA corresponded to 935 bp from exons 2 to 5; for *PGRP-LC* the dsRNA corresponded to 861 bp from the common exons 2 and 3. Three days after transfection, the cells were induced with insoluble peptidoglycan from *Micrococcus luteus* for 6 hours, live *E. coli* (*O55:B5*) for 6 hours, LPS from *E. coli* (*O55:B5*) for 2 hours, or sterile Ringer (-) as control. The pellet of an *E. coli* overnight culture was resuspended 1:100 in sterile Ringer, and 15 μl were used per induction. Peptidoglycan and LPS had a final concentration of 1 μg/ml. The cells were harvested after 2 or 6 hours, and total RNA was extracted. The loss of *PGRP-LA* and *PGRP-LC* mRNA due to RNAi was confirmed by reverse transcription PCR in a separate experiment. *Drosomycin* expression is not inducible in this *mbn-2* cell line, so the effect of *PGRP-LC* RNAi on its expression could not be assessed in this experiment.

plasmic and transmembrane domains but different extracellular domains. The extracellular PGRP domains of the two isoforms were only 38% identical (55 of 145 residues). Northern hybridization with a common *PGRP-LC* exon probe revealed transcripts about 2.0 kb in size in wild-type larvae, but no transcript of that size in *ird7^1* mutant animals; instead, a larger transcript of lower abundance was detected (Fig. 2B). Sequence analysis revealed an insertion of 858 base pairs (bp) of single-copy sequence into exon 2, which is the first coding exon in both isoforms, in the *ird7^1* allele (Fig. 2C). This insertion introduced a stop codon and would generate a truncated cytoplasmic protein. No sequence change in the *PGRP-LCa* isoform was identified in the *ird7^2* allele. However, there was a G to A substitution in the *x* PGRP domain in the *PGRP-LCx* isoform of *ird7^2*, which introduced a stop codon that makes a truncated protein lacking the last 107 amino acids of this isoform (Fig. 2C). Because the *ird7^2* allele alters only *PGRP-LCx* and has a profound effect on antimicrobial gene expression, this isoform must play a crucial role in vivo. The specific requirement for the *PGRP-LCx* isoform could be due to its ability to bind specific ligands or because its expression is limited to specific cell types by regulated RNA splicing. Overexpression of either of the *PGRP-LC* cDNAs rescued inducible expression of the *Diptericin-lacZ* reporter gene in homozygous *ird7^1* mutant animals (Fig. 3), confirming that the phenotype of *ird7* mutants was the result of the lack of *PGRP-LC* activity.

We used RNA interference (RNAi) to test the role of *PGRP-LC* in the response to bacterial components. Treatment of blood cells from the *mbn-2* line with peptidoglycan, *Escherichia coli*, or lipopolysaccharide (LPS) led to a robust induction of the antibacterial peptide genes. Introduction of double-stranded RNA (dsRNA) of *PGRP-LC*, but not *PGRP-LA*, effectively blocked induction of

Diptericin, *CecropinA1*, and *AttacinA* in response to all three stimuli (Fig. 4). Thus, PGRP-LC is required for the response to both peptidoglycan and LPS in these cells.

Because *PGRP-LC* is predicted to encode a transmembrane protein with an extracellular PGRP domain, PGRP-LC may act as a pattern recognition receptor that links recognition of microbial components with host immune responses (*22*). Because PGRP-LC is required for responses to both peptidoglycan and LPS, the extracellular domain of PGRP-LC may bind both peptidoglycan and LPS, and binding of either ligand may activate downstream signaling events. Alternatively, PGRP-LC may bind peptidoglycan (but not LPS) and may act as an essential subunit of a larger complex that includes other pattern recognition receptors that bind LPS. In mammals, signaling by Toll-like receptor 2 (TLR2) is activated by peptidoglycan (*23*). PGRP-LC might act in a complex with another transmembrane protein similar to TLR2.

Twelve PGRP genes have been identified in the *Drosophila* genome (*18*). Another *Drosophila* gene, *PGRP-SA*, encodes a soluble peptidoglycan recognition protein that is essential for activation of the Toll signaling pathway in response to infection by Gram-positive bacteria (*21*). Four PGRP genes have already been identified in the human genome (*24*). Given the evolutionary conservation of many proteins required for innate immune responses, it will be important to evaluate whether PGRPs function as a family of pattern recognition receptors in human innate immune responses.

References and Notes

1. R. S. Khush, F. Leulier, B. Lemaitre, *Trends Immunol.* **22**, 260 (2001).
2. B. Lemaitre, E. Nicolas, L. Michaut, J.-M. Reichhart, J. A. Hoffmann, *Cell* **86**, 973 (1996).
3. X. Meng, B. S. Khanuja, Y. T. Ip, *Genes Dev.* **13**, 792 (1999).
4. S. Rutschmann *et al.*, *Immunity* **12**, 569 (2000).
5. M. Hedengren *et al.*, *Mol. Cell* **4**, 827 (1999).
6. Y. Lu, L. P. Wu, K. V. Anderson, *Genes Dev.* **15**, 104 (2001).
7. M. Elrod-Erickson, S. Mishra, D. Schneider, *Curr. Biol.* **10**, 781 (2000).
8. S. Rutschmann *et al.*, *Nature Immunol.* **1**, 342 (2000).
9. F. Leulier, A. Rodriguez, R. S. Khush, J. M. Abrams, B. Lemaitre, *EMBO Rep.* **1**, 353 (2000).
10. S. Vidal *et al.*, *Genes Dev.* **15**, 1900 (2001).
11. P. Georgel *et al.*, *Dev. Cell* **1**, 503 (2001).
12. L. P. Wu, K. V. Anderson, *Nature* **392**, 93 (1998).
13. L. P. Wu, K.-M. Choe, Y. Lu, K. V. Anderson, *Genetics* **159**, 189 (2001).
14. K.-M. Choe, N. Matova, K. V. Anderson, unpublished data.
15. M. S. Dushay, B. Åsling, D. Hultmark, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 10343 (1996).
16. S. Stöven, I. Ando, L. Kadalayil, Y. Engström, D. Hultmark, *EMBO Rep.* **1**, 347 (2000).
17. See supplemental material on *Science* Online at www.sciencemag.org/cgi/content/full/1070216/DC1.
18. T. Werner *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 13772 (2000).
19. H. Yoshida, K. Kinoshita, M. Ashida, *J. Biol. Chem.* **271**, 13854 (1996).
20. D. Kang, G. Liu, A. Lundström, E. Gelius, H. Steiner, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 10078 (1998).
21. T. Michel, J. M. Reichhart, J. A. Hoffmann, J. Royet, *Nature* **414**, 756 (2001).
22. C. A. Janeway, *Cold Spring Harbor Symp. Quant. Biol.* **54**, 1 (1989).
23. O. Takeuchi *et al.*, *Immunity* **11**, 443 (1999).
24. C. Liu, Z. Xu, D. Gupta, R. Dziarski, *J. Biol. Chem.* **276**, 34686 (2001).
25. K.-M. Choe, K. V. Anderson, data not shown.
26. L. S. Hatton, K. O'Hare, *Elsevier Trends Journals Technical Tips Online* T01816, http://tto.trends.com (1999).
27. B. Chen, T. Chu, E. Harms, J. P. Gergen, S. Strickland, *Genetics* **149**, 157 (1998).
28. A. H. Brand, N. Perrimon, *Development* **118**, 401 (1993).
29. A. C. Spradling, in Drosophila*: A Practical Approach*, D. M. Roberts, Ed. (IRL, Oxford, 1986).
30. D. A. Harrison, R. Binari, T. S. Nahreini, M. Gilman, N. Perrimon, *EMBO J.* **14**, 2857 (1995).
31. S. M. Hammond, E. Bernstein, D. Beach, G. J. Hannon, *Nature* **404**, 293 (2000).

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