more than 5 × 10^8 cpm/μg by random priming [A. D. Feinberg and B. Vogelstein, *Anal. Biochem.* **137**, 266 (1984)], and unincorporated nucleotides were removed by centrifugation through a Sephadex G-50 column (Boehringer Mannheim). Probes were used at 1 × 10^6 cpm per milliliter of hybridization solution. Comparisons of the autoradiographs were facilitated by color coding [D. D. Dunigan, T. E. Smart, M. Zaitlin, *BioTechniques* **5**, 32 (1987)].

33. For Northern blots [P. S. Thomas, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5201 (1980)], RNA was isolated as described [P. Chomczynski and N. Sacchi, *Anal. Biochem.* **162**, 156 (1987)], and poly(A)$^+$ RNA was prepared (*32*). RNA samples were reacted with glyoxal, separated by electrophoresis, transferred to Nytran membrane (Schleicher & Schuell), and hybridized according to the manufacturer's instructions. The RNA was immobilized on the membrane by covalent cross-linking [G. M. Church and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1991 (1984)]. Each clone was analyzed at least two times.
34. Nuclear run-on assays were performed as described [C. L. Phillips *et al.*, *Mol. Endocrinol.* **2**, 641 (1988)] with the following modifications: The transcription reaction mixture contained 20 mM Hepes (pH 7.5), 100 mM potassium glutamate, 5 mM sodium fluoride, 10 mM magnesium chloride, 1 mM EGTA, 1 mM adenosine triphosphate, 1 mM cytidine triphosphate, 1 mM guanosine triphosphate, 10 mM creatine phosphate, creatine kinase (100 μg/ml), glycerol (20%), and 1 mM dithiothreitol. ^{32}P-labeled uridine triphosphate (3000 Ci/mmol) was added to give a final volume of 200 μl. Endonuclease-digested DNA samples (2 μg) were immobilized onto nitrocellulose by baking. The membrane was washed twice in 2× SSC and 0.1% SDS for 15 min at 65°C, twice in 2× SSC for 15 min at 65°C, once in 2× SSC and ribonuclease A (10 μg/ml) for 30 to 60 min at 37°C, and four times in 2× SSC and 0.1% SDS for 15 min at 65°C.
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Dual Role of the *Drosophila* Pattern Gene *tailless* in Embryonic Termini

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One of the first zygotically active genes required for formation of the terminal domains of the *Drosophila* embryo is *tailless* (*tll*). Expression of the *tll* gene is activated ectopically in gain-of-function mutants of the maternal terminal gene *torso* (*tor*); this suggests that *tor* normally activates the *tll* gene in the termini. Ectopic expression of *tll* under the control of an inducible promoter results in differentiation of ectopic terminal-specific structures, the Filzkörper, and leads to the activation of at least one gene, *hunchback*, that is required to form these structures. Ectopic expression of the *tll* gene also represses segmentation by repressing the gap genes *Krüppel* and *knirps* and probably also pair rule genes.

ESTABLISHMENT OF THE TERMINAL domains of the *Drosophila* embryo requires activity of the maternal terminal genes, including *tor, trunk* (*trk*), *torso-like* (*tsl*), and *l(1)polehole* [*l(1)ph*] (*1*). The key role of the *tll* gene in the terminal hierarchy is demonstrated by the almost complete overlap between the phenotype of loss-of-function mutants in maternal terminal genes and *tll*; both produce embryos that lack terminal structures, including parts of the brain and cephalopharyngeal skeleton in the anterior and the eighth abdominal segment (A8), posterior spiracles (including Filzkörper), anal pads, and hindgut in the posterior (*2–6*) (Fig. 1). Genetic and molecular data suggest that the termini of the *Drosophila* embryo are established by a pathway in which a uniformly distributed tyrosine kinase receptor (the product of the *tor* gene) is activated at the poles of the embryo by a ligand controlled by the *tsl* gene; the activated *tor* receptor in turn activates the D-raf protein (the *Drosophila* Raf-1 homolog, encoded by the *l(1)ph* gene), and this leads to localized activation of *tll* transcription at the termini (*4, 7, 8*). The *tll* gene product, a steroid receptor–like protein (*4*), likely regulates gene activity required for formation of the structures missing from the termini of both maternal-terminal and *tll* mutants.

To test whether the *tll* gene product is sufficient to activate programs leading to differentiation of terminal-specific structures, we prepared a construct in which *tll* is under the control of the heat-inducible *hsp70* promoter; lines carrying this construct are referred to as HS-*tll* lines. We examined the effect on cuticular differentiation of ectopic expression of *tll* in embryos from *tor trk* double mutant mothers; these embryos express little or no *tll* RNA in the posterior and do not make Filzkörper (*5, 9*) (example in Fig. 1C). Heat shock of HS-*tll* embryos from *tor trk* mothers leads to the formation of Filzkörper (example in Fig. 1D), indicating

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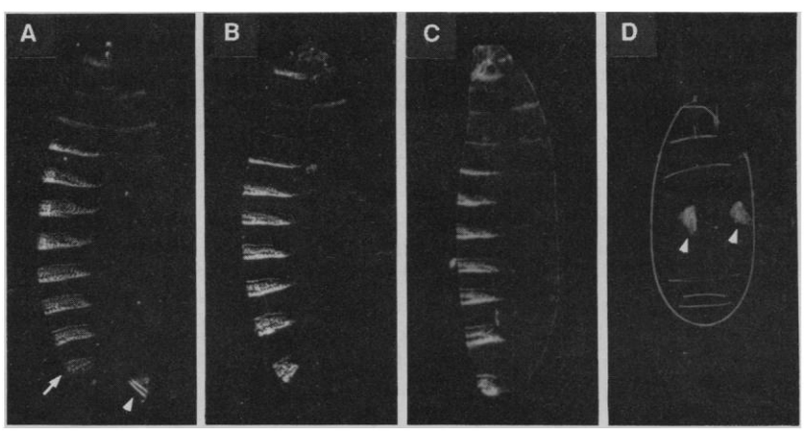

Fig. 1. Ectopic expression of the *tll* gene induces terminal structures. Dark-field photographs of cuticle preparations. (**A**) Wild-type (OreR), (**B**) tll^{149}/tll^{Pgx}, (**C**) embryo from a tor^{WK} trk^{RA} mother, and (**D**) embryo from a cross of tor^{WK} trk^{RA} females to HST3.1 males, heat-shocked for 20 min (*29*). Segment A8 and Filzkörper [indicated by arrow and arrowhead in the wild-type embryo in (A), respectively] are missing from the *tll* in (B) and tor^{WK} trk^{RA} in (C) embryos. Among 70 heat-shocked (10 to 20 min) HST3.1 embryos from tor^{WK} trk^{RA} mothers, 27 had Filzkörper, which ranged in appearance from the diffuse Filzkörper (arrowheads) shown in (D) to the well-organized Filzkörper seen in (A). In this experiment, more than two Filzkörper per embryo were occasionally seen, and in heat-shocked HST3.1 embryos up to eight Filzkörper per embryo were seen (Fig. 3B shows four Flizkörper). Among 184 heat-shocked (10 to 20 min) embryos from tor^{WK} trk^{RA} mothers, only four had a partial Filzkörper. The formation of partial Filzkörper in this situation is perhaps due to the fact that both *tor* and *trk* mutants display weaker phenotypes at higher temperatures (*5*).

that in the absence of the maternal terminal system and of the endogenous *tll* activity in the posterior, the ectopically expressed *tll* gene is sufficient to activate a pathway leading to differentiation of a terminal-specific structure. The appearance of ectopic Filzkörper in heat-shocked HS-*tll* embryos could be explained if *tll* activates the expression of specific genes required for Filzkörper formation. Altered expression of at least one such gene, *hunchback (hb)*, can be observed immediately following heat shock. The posterior stripe of *hb* expression depends on *tll* activity (*10*) and is required for the formation of posterior structures, including part of the Filzkörper (*11*). After 30 min of ectopic *tll* expression in a wild-type background, the posterior stripe of expression of the 3.2-kb *hb* transcript expands anteriorly but not posteriorly (Fig. 2); this supports the proposition that in the posterior *tll* activates *hb* expression, whereas *huckebein* represses it (*10*).

Another likely function for the *tll* gene product is the repression of segmentation genes. In *tll* mutants, the central segmented domain expands toward the termini (*2–4, 12–14*), and in *tor* gain-of-function mutants, the deletion of contiguous groups of segments from the central segmented domain depends on the presence of a wild-type copy of the *tll* gene (*15*). To test whether the *tll* gene product is sufficient to suppress segmentation, we expressed *tll* ectopically by heat shocking 1.5- to 2.5-hour-old HS-*tll* embryos. After 20 min of heat shock, virtually all embryos carrying the HS-*tll* construct showed a *tor* gain-of-function phenotype (Fig. 3G). With increasing duration of heat shock, the embryos showed an increasing repression of segmentation (Fig. 3, A through C) similar to that seen in embryos from mothers carrying the temperature-sensitive tor^{RL3} gain-of-function mutation (*15*). The observed repression of segmentation spreads from a central position at approximately 40% egg length in both HS-*tll* and tor^{RL3} (*15*) embryos, suggesting that segmented domains in the center of the embryo are more sensitive to suppression by *tll* than those closer to the termini. Because the maternal terminal system is not active in the central segmented domains of the embryo

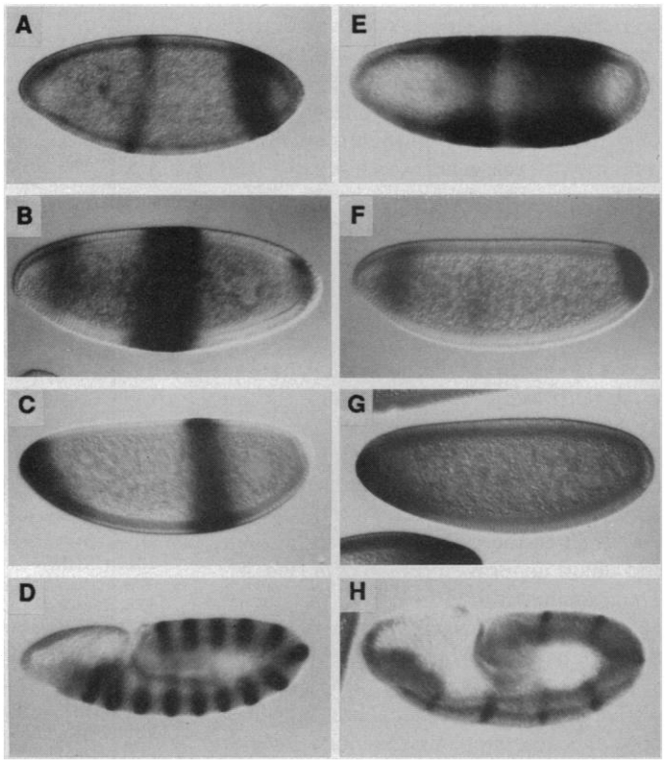

Fig. 2. Expression of segmentation genes in heat-shocked HS-*tll* embryos. The RNAs for *hb*, *Kr*, *kni*, and *en* were detected by in situ hybridization to whole embryos (*30, 31*). (**A** through **D**) Wild-type embryos (OreR). (**E** through **H**) Embryos from a cross of HST3.1 males to OreR females were heat shocked for 30 min. Embryos hybridized with a probe specific for the 3.2-kb *hb* transcript (A and E), a *Kr* probe (B and F), and a *kni* probe (C and G) were 1.5 to 2.5 hours old at time of initiation of heat shock and were fixed for hybridization 30 min after the heat shock. The embryos shown are at the blastoderm stage. Only the central expression domains of *Kr* and *kni* are repressed by ectopic *tll* expression. The repression of *Kr* by ectopic *tll* explains the posteriorward expansion of the anterior *hb* domain seen in (E) because this domain expands in *Kr* mutants (*32*). Embryos hybridized with the *en* probe (D and H) were 3.0 to 3.5 hours old at the time of initiation of heat shock and were fixed 30 min after the heat shock. Ectopic expression of *tll* in *tor* gain-of-function mutants or in HS-*tll* embryos heat shocked starting at 1.5 to 3.0 hours results in elimination of all *en* stripes.

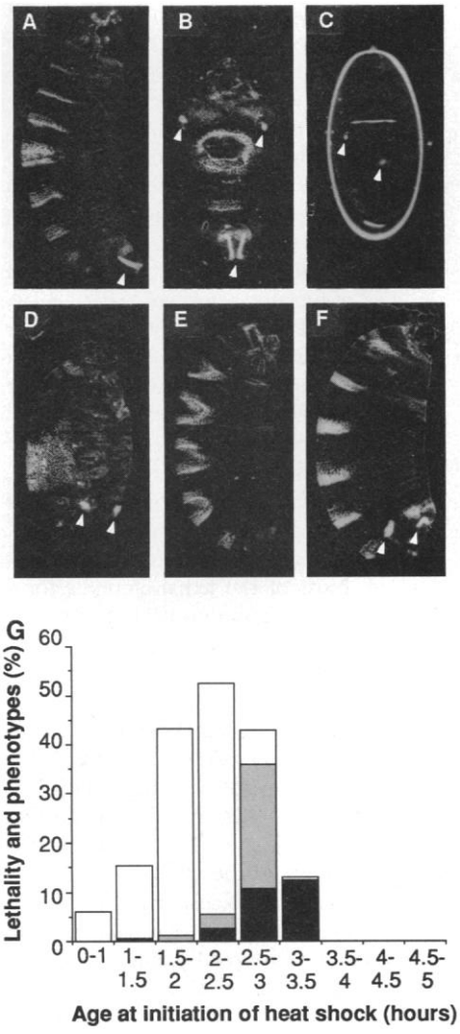

Fig. 3. Ectopic expression of the *tll* gene represses segmentation. The *tll* gene was expressed ectopically in embryos at different stages and for varying lengths of time during development as described in Fig. 1. (**A** through **C**) Cuticles of HS-*tll* embryos (from a cross of HST3.1 males to OreR females) aged 1.5 to 2.5 hours [nuclear cycles 9 through 13 (*33*) or embryonic stages 3 through 5 (*34*)] were heat-shocked for 15 min (A), 20 min (B), and 60 min (C). (**D** through **F**) Cuticular phenotypes of HS-*tll* embryos aged 2.5 to 3.0 hours [stages 5 to 6 (*34*)] (D) and 3.0 to 3.5 hours stages 7 to 8 (*34*)] (E and F) at time of initiation of 20-min heat shock. Filzkörper, both ectopic and those occurring in a normal position, are indicated by arrowheads. (**G**) We determined the time of maximal sensitivity to ectopic expression of *tll* by crossing HST3.1 males to w^{1118} females lacking the HS-*tll* construct, collecting embryos for 30-min intervals, aging the embryos to the indicated stages, and then heat shocking for 20 min. The fraction of unhatched embryos after this treatment was determined, and cuticles were prepared and analyzed from all unhatched embryos. The *tor* gain-of-function phenotype (white boxes) is any of the phenotypes falling in the range of those shown in (A) to (C), the lawn phenotype (shaded boxes) is that shown in (D), and the pair rule phenotype (black boxes) is that shown in (E) and (F). For each time point, an additional 3 to 8% of the total embryos failed to hatch and had either no apparent defects or a variety of unrelated defects, such as failures in head involution or cuticular differentiation. A similar proportion (3 to 5%) of the embryos from the control cross of ry^{506} males to w^{1118} females failed to hatch and showed similar wild-type or defective cuticles upon heat shock; none showed the segmentation defects seen in the HS-*tll* cross. For each time point, at least 100 embryos were heat shocked. No lethality (failure to hatch) above background was observed when the HS-*tll* embryos were heat shocked after 3.5 hours.

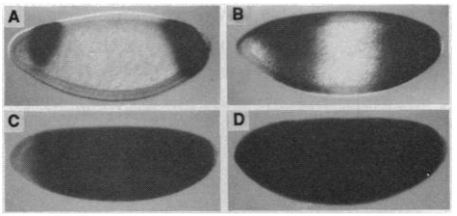

Fig. 4. Ectopic expression of *tll* in *tor* gain-of-function and HS-*tll* embryos. The *tll* RNA was detected by in situ hybridization to whole embryos (*30*). All embryos shown are at the blastoderm stage [nuclear cycle 14 (*33*) or embryonic stage 5 (*34*)]. (**A**) Wild-type embryo (OreR). (**B**) Embryo from a tor^{RL3} homozygous mother at 25°C. (**C**) Embryo from a tor^{D4021} heterozygous mother at 25°C. The strong staining throughout the embryo [except for the anterior tip because of a repressive effect of the *bicoid* gene (*9*)] suggests that the *tll* RNA is expressed in the ectopic location at an amount similar to that normally found in the termini. (**D**) Embryo from a cross of OreR females with HS-*tll* males, heat-shocked for 30 min as described in (*29*); after the heat shock, we allowed the embryo to continue development for 30 min before fixation.

(*16*) and a similar repression of segmentation is induced by the ectopic expression of *tll* in embryos from *tor trk* mothers (Fig. 1D), we conclude that *tll* is capable of repressing segmentation independently of other genes activated by the maternal terminal pathway.

The repression of segmentation that results from ectopic expression of *tll* (Fig. 3A through C) could occur if *tll* represses gap genes, which function at the top of the segmentation hierarchy. When 1.5- to 2.5-hour-old HS-*tll* embryos are heat-shocked for 30 min, expression of *Krüppel (Kr)* and *knirps (kni)* in the central segmented domain is repressed (Fig. 2, F and G). The terminal domains of *Kr* and the anterior domain of *kni*, which appear later than their respective central domains, are not repressed by ectopic *tll*, presumably because different constellations of transcription factors are present at these times and positions. Repression of *Kr* and *kni*, which together are required to establish segments T1 through A7 (*17*), is thus sufficient to explain the loss of contiguous groups of segments seen in HS-*tll* and *tor* gain-of-function embryos (Fig. 3, A through C and G). From these data, we infer that an important function of *tll* in the termini is to repress gap genes and thus to define the limits of the central segmented domain.

The phenotypes of HS-*tll* embryos heat shocked at slightly later times, however, suggest that ectopically expressed *tll* may also suppress other segmentation genes. When 2.5- to 3.0-hour-old embryos are heat shocked, most of the lethal embryos form cuticles with a continuous field of nonpolar denticles (the lawn phenotype) (Fig. 3, D and G). This could be explained by an effect of ectopic *tll* on either gap or pair rule gene expression (*18, 19*). Heat shock at 3.0 to 3.5 hours results in embryos in which T3 is fused to A1, A2 to A3, and so on (Fig. 3, E through G). This phenotype is by definition a pair rule phenotype (*17, 20*) and is not easily explained by the effect of *tll* on gap gene expression. Because the alternating odd and even stripes of *engrailed (en)* gene expression are activated by different combinations of pair rule genes (*21*), we used the *en* transcription pattern as an indicator of pair rule gene activity. When *tll* is expressed ectopically starting at 2.5 to 3.5 hours, the even-numbered *en* stripes, corresponding to the domains missing from the cuticles (Fig. 3, E and F), are missing (Fig. 2H). These results suggest that, when expressed ectopically in the central segmental domain, *tll* represses pair rule genes as well as gap genes.

The repression of the gap genes *Kr* and *kni* and the expansion of the late blastoderm stage *hb* domains seen after ectopic expression of *tll* closely mimic the effects of the *tor* gain-of-function alleles (*10, 13, 15*). The basis for the *tor* gain-of-function phenotypic series is revealed by the altered *tll* expression pattern in these mutants; in embryos from tor^{RL3} (a weak allele) mothers, the terminal domains of *tll* RNA expression are expanded, whereas in embryos from tor^{D4021} (a strong allele) mothers, the expanded domains frequently meet in the center of the embryo, resulting in high and almost uniform expression of *tll* (Fig. 4). These results support the notion that the *tll* gene is the crucial zygotic gene activated by the maternal terminal hierarchy.

The effects observed after ectopic expression of the *tll* gene indicate that it functions both as a repressor of segmentation genes and as an activator of genes required to establish the unique characteristics of the termini. This dual role could be explained if the product of the *tll* gene were a transcription factor capable of functioning as both an activator and a repressor. The coexistence of both functions in one protein is consistent with the sequence similarity between the *tll* protein and steroid receptors (*4*), some of which have been shown to act as both repressors and activators of transcription (*22*).

REFERENCES AND NOTES

1. K. V. Anderson, in *Genes and Embryos*, D. M. Glover and B. D. Hames, Eds. (IRL Press, Oxford, 1989), pp. 1–37.
2. T. R. Strecker, K. Kongsuwan, J. A. Lengyel, J. R. Merriam, *Dev. Biol.* **113**, 64 (1986).
3. T. R. Strecker, J. R. Merriam, J. A. Lengyel, *Development (Cambridge)* **102**, 721 (1988).
4. F. Pignoni *et al.*, *Cell* **62**, 151 (1990).
5. T. Schüpbach and E. Wieschaus, *Roux's Arch. Dev. Biol.* **195**, 302 (1986).
6. C. Nüsslein-Volhard, H. G. Frohnhöfer, R. Lehmann, *Science* **238**, 1675 (1987).
7. F. Sprenger, L. M. Stevens, C. Nüsslein-Volhard, *Nature* **338**, 478 (1989); L. M. Stevens, H. G. Frohnhöfer, M. Klingler, C. Nüsslein-Volhard, *ibid.* **346**, 660 (1990); L. Ambrosio, A. P. Mahowald, N. Perrimon, *ibid.* **342**, 288 (1989).
8. J. Casanova and G. Struhl, *Genes Dev.* **3**, 2025 (1989).
9. F. Pignoni and J. A. Lengyel, unpublished data.
10. J. Casanova, *Development (Cambridge)* **110**, 621 (1990).
11. R. Lehmann and C. Nüsslein-Volhard, *Dev. Biol.* **119**, 402 (1987).
12. P. A. Mahoney and J. A. Lengyel, *ibid.* **122**, 464 (1987).
13. M. J. Pankratz, M. Hoch, E. Seifert, H. Jäckle, *Nature* **341**, 337 (1989).
14. D. Weigel, G. Jürgens, M. Klingler, H. Jäckle, *Science* **248**, 495 (1990).
15. M. Klingler, M. Erdélyi, J. Szabad, C. Nüsslein-Volhard, *Nature* **335**, 275 (1988); M. Klingler, thesis, University of Tübingen, Tübingen, Germany (1989); T. R. Strecker, S. R. Halsell, W. W. Fisher, H. D. Lipshitz, *Science* **243**, 1062 (1989).
16. The restriction of the activity of the maternal terminal system to the termini is inferred from the exclusion of *Kr* expression from the termini in *bicoid oskar* (*bcd osk*) embryos but not in *bcd osk tor* embryos (*8*), from the loss of activation of *tll* at the termini in *tor* embryos (*9*), and from the expansion of *tll* expression into the central domain in *tor* gain-of-function mutants (Fig. 4).
17. C. Nüsslein-Volhard and E. Wieschaus, *Nature* **287**, 795 (1980).
18. C. Nüsslein-Volhard, J. Kluding, G. Jürgens, *Cold Spring Harbor Symp. Quant. Biol.* **50**, 145 (1985).
19. K. Harding, C. Rushlow, H. J. Doyle, T. Hoey, M. Levine, *Science* **233**, 953 (1986).
20. The phenotype of these embryos is similar to the phenotype seen in *odd-paired* mutant embryos (*23*) and to the pair-rule phenotype described for mutations in *en* (*17*) because the naked cuticle of segments T3, A2, A4, and A6 is missing.
21. P. W. Ingham, *Nature* **335**, 25 (1988).
22. K. Damm, C. C. Thompson, R. M. Evans, *ibid.* **339**, 593 (1989).
23. D. E. Coulter and E. Wieschaus, *Genes Dev.* **2**, 1812 (1988).
24. S. Schneuwly, R. Klemenz, W. J. Gehring, *Nature* **325**, 816 (1987).
25. A. C. Spradling and G. M. Rubin, *Science* **218**, 341 (1982); G. M. Rubin and A. C. Spradling, *ibid.*, p. 348.
26. E. Wieschaus and C. Nüsslein-Volhard, in Drosophila: *A Practical Approach*, D. B. Roberts, Ed. (IRL Press, Washington, DC, 1986), pp. 199–227.
27. D. Tautz *et al.*, *Nature* **327**, 383 (1987).
28. U. B. Rosenberg *et al.*, *ibid.* **319**, 336 (1986); U. Nauber *et al.*, *ibid.* **336**, 489 (1988); S. J. Poole, L. M. Kauvar, B. Drees, T. Kornberg, *Cell* **40**, 37 (1985).
29. We generated a construct carrying the *hsp70* promoter linked to the *tll* coding region by adding Kpn I linkers to the N4 cDNA (*4*) and cloning this into pHT4 (*24*). The resulting construct, pHST1, was introduced into ry^{506} flies by P element–mediated transformation (*25*). Four different transformant HS-*tll* (heat-shock *tll*) lines were obtained, three on the third (HST3.1, HST3.2, and HST3.3) and one on the second chromosome (HST2.1); all of these lines are homozygous lethal and are carried as balanced lines with only one copy of pHST1. For the experiment in Fig. 1D, HST3.1 males were crossed to $tor^{WK}trk^{RA}$ females, embryos were collected, transferred to a Nitex filter (TETKO, New York, NY), aged to 1.5 to 2.5 hours at 25°C, and then heat-shocked at 37°C by transfer of the filter to a warm plate. Cuticles were prepared as described (*26*). Except where otherwise stated, all flies and embryos were kept at 25°C, and all heat shocks were at 37°C.
30. D. Tautz and C. Pfeifle, *Chromosoma* **98**, 81 (1989).
31. We prepared a 465-nucleotide single-stranded probe that was specific for the 3.2-kb *hb* transcript (*27*) by the polymerase chain reaction using the cDNA clone J3 (J. Margolis and J. Posakony, unpublished data) as a template. The cDNAs used as probes for *Kr, kni*, and *en* were pcK2b, pcJ15, and c-2.4 (*28*), respectively.

32. H. Jäckle, D. Tautz, R. Schuh, E. Seifert, R. Lehmann, *Nature* **324**, 668 (1986).
33. V. E. Foe and B. M. Alberts, *J. Cell Sci.* **61**, 31 (1983).
34. J. A. Campos-Ortega and V. Hartenstein, *The Embryonic Development of* Drosophila melanogaster (Springer-Verlag, Berlin, 1985).
35. We thank U. Banerjee and K. V. Anderson for comments on the manuscript, J. Margolis and J. Posakony for the *hb* cDNA, H. Jäckle for the *Kr* and *kni* cDNAs, E. Gustavson and T. Kornberg for the *en* cDNA, and D. Krantz for pHT4 DNA. Supported by NIH grant 09948 and NSF grant 10451 to J.A.L. and by Ursula Mandel Fellowships to E.S. and F.P.

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Autocrine or Paracrine Inflammatory Actions of Corticotropin-Releasing Hormone in Vivo

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Corticotropin-releasing hormone (CRH) functions as a regulator of the hypothalamic-pituitary-adrenal axis and coordinator of the stress response. CRH receptors exist in peripheral sites of the immune system, and CRH promotes several immune functions in vitro. The effect of systemic immunoneutralization of CRH was tested in an experimental model of chemically induced aseptic inflammation in rats. Intraperitoneal administration of rabbit antiserum to CRH caused suppression of both inflammatory exudate volume and cell concentration by approximately 50 to 60 percent. CRH was detected in the inflamed area but not in the systemic circulation. Immunoreactive CRH is therefore produced in peripheral inflammatory sites where, in contrast to its systemic indirect immunosuppressive effects, it acts as an autocrine or paracrine inflammatory cytokine.

CRH WAS ORIGINALLY ISOLATED from the hypothalamus and identified by and named for its property to stimulate anterior pituitary secretion of adrenocorticotropic hormone (ACTH), the systemic hormone that regulates production of glucocorticoids by the adrenal cortex (*1*). CRH and its receptors are widely distributed in many extrahypothalamic sites of the central nervous system (*2*), and this peptide functions as a coordinator of the stress response (*3*). By activating glucocorticoid and catecholamine secretion, central nervous system CRH participates in the suppressive effects of stress on the immune or inflammatory system (*4*). CRH directly stimulates leukocytes to produce immunoregulatory pro-opiomelanocortin (POMC)–related peptides (β-endorphin, ACTH, and α-melanocyte-stimulating hormone) (*5, 6*) and to secrete interleukin 1 (IL-1) and IL-2 (*6, 7*). CRH also stimulates lymphocyte proliferation, enhances the proliferative response of leukocytes to lectins, and increases the expression of the IL-2 receptor on T lymphocytes (*8*). In addition, CRH-binding sites exist in various subpopulations of leukocytes (*9*), and CRH immunoreactivity and mRNA have been detected in resting subpopulations of human white blood cells (*10*). These data suggest that CRH might have local direct effects on immune or inflammatory processes. We have tested the effect of systemic immunoneutralization of CRH on the size of a quantifiable inflammatory response. Carrageenin, a seaweed polysaccharide, is known to elicit a chemical inflammatory response in rats (*11, 12*), which can be quantified in terms of exudate volume and cell concentration in the inflammatory exudate and is sensitive to dose-dependent suppression by glucocorticoids (*12*).

Male Sprague-Dawley rats were injected intraperitoneally with neutralizing rabbit antiserum to CRH (anti-CRH) 1 hour before they were subcutaneously injected with carrageenin (*13*). Both exudate volume and cell concentrations in the treated area were suppressed by approximately 50 to 60%, when compared with the responses in control rats treated first with normal saline, normal rabbit serum, or rabbit antiserum (anti-TSH) to thyroid-stimulating hormone (TSH) (Fig. 1). The specific suppression of the inflammatory response observed after immunoneutralization of CRH had an opposite effect to that expected from neutralization of CRH in the hypothalamic-hypophyseal portal system. The latter should have resulted in hypoglucocorticoidism, which enhances the inflammatory response (*12, 14*). Instead, the results are compatible with local inflammatory effects of CRH. Direct local administration of anti-CRH into the air pouch simultaneously with carrageenin suppressed the inflammatory response to carrageenin to the same extent as systemic administration of this antiserum (*15*). Neutralizing antiserum (anti-TNFα) to TNFα (tumor necrosis factor–α) also suppressed inflammation (Fig. 1). TNFα is a major autocrine or paracrine inflammatory cytokine, which stimulates inflammation directly and by inducing the secretion of IL-1 and IL-6 (*16*). The effects of anti-CRH and anti-TNFα were not additive, indicating that the two antisera might interfere with a

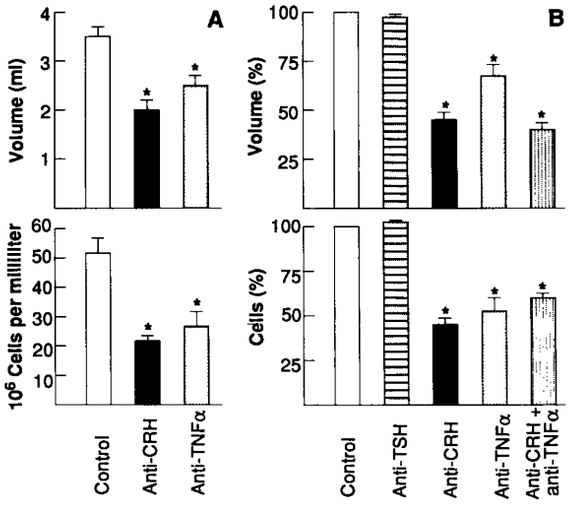

Fig. 1. (**A**) Effect of rabbit anti-CRH or anti-TNFα on the inflammatory response of Sprague-Dawley rats to subcutaneous administration of carrageenin (*11, 12*). The mean (± SEM) volume of the inflammatory exudate and concentration of extravasated leukocytes are shown for each treatment group. Rats were injected intraperitoneally with 1 ml of anti-CRH, anti-TNFα, or nonimmune rabbit serum (control) 1 hour before injection of carrageenin. (**B**) Effects of immunoneutralization of CRH and TNFα alone or in combination or treatment with anti-TSH, expressed as a percentage of control values. Data shown are one representative of three separate experiments (n = 9 to 10 animals per group per experiment) in (A) or combined results of three experiments in (B). Asterisks indicate significant differences from control (nonimmune rabbit serum) ($P < 0.05$), determined by analysis of variance.

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