15. M. R. Palmert *et al.*, *Biochem. Biophys. Res. Commun.* **165**, 182 (1989).
16. We constructed p770 by the insertion of the 2700-bp APP-770 cDNA insert into a puc 19–based vector that contained the SV40 promoter/enhancer upstream and SV40 small t splice and polyadenylation signals downstream of the cloning site. This insert included 150 bp of the 5′ untranslated region, the entire APP-770 coding region, and 280 bp of the 3′ untranslated sequence. p770Δ was constructed by insertion of a 65-bp fragment that encoded the six COOH-terminal amino acids of substance P with additional spacer sequences into the Bgl II site upstream of sequences that encode the APP transmembrane domain. p770SP, a recombinant molecule that replaced the COOH-terminal 17 amino acids of APP with 16 amino acids of substance P sequence, was prepared as follows. A full-length cDNA encoding APP-770 cloned in the Okayama-Berg vector, pCDv-1 [H. Okayama and P. Berg, *Mol. Cell. Biol.* **3**, 280 (1982)], was digested at the Spe I site in the 3′ untranslated region and then digested for varying periods of time with Exonuclease III. After blunt ending with S1 nuclease and secondary digestion with Hind III, molecules with lengths commensurate with deletions extending into the APP COOH-terminus were selected. The Hind III site delimits the 5′ end of the SV40 promoter/enhancer region of pCDv-1. These molecules were ligated with a vector molecule containing a Hind III site 5′ and a 3′ blunt-ended site directly upstream of substance P and SV40 small t splice and polyadenylation sequences. Recombinants were verified to be in-frame after double-stranded sequencing of plasmid templates (*17*) with a primer complementary to the substance P sequences.
17. E. Chen and P. H. Seeberg, *DNA* **4**, 165 (1985).
18. Subcloning of various APP regions was facilitated by polymerase chain reaction (PCR) techniques. Twenty-eight base "sense" primers, which encode regions NH_2-terminal to the APP transmembrane region or the transmembrane domain alone (in p770ΔM) and which contain a sequence CCGCTCGAG at the 5′ end, were synthesized and purified by gel electrophoresis. A 28-base "antisense" primer complementary to the sequence, which encodes five residues of the APP cytoplasmic domain and proximal transmembrane region and which contains the sequence CCGCTCGAG at its 5′ end, was also synthesized. The insert in plasmid p770Δcr2 was generated with a 28-base sense primer, which encodes a region upstream of the cr2 transmembrane domain, and a 28-base antisense primer, complementary to six amino acids of the cr2 cytoplasmic domain and the transmembrane region. These primers were used in a PCR in the presence of plasmid pBSCR2.1 [C. A. Lowell *et al.*, *J. Exp. Med.* **170**, 1931 (1989)], which encodes the full-length cr2 molecule. These primers also contained the sequence CCGCTCGAG at the 5′ end. To generate the insert in plasmid p770Δ11cr2M, we used a 60-base sense primer, which encoded 11 amino acids NH_2-terminal to the APP transmembrane domain and 6 amino acids of the cr2 transmembrane domain, in combination with the cr2 antisense primer in a PCR in the presence of plasmid pBSCR2.1. PCRs were carried out using a Thermal Cycler (Perkin-Elmer Cetus) with each of the sense oligonucleotides in conjunction with the antisense oligonucleotide in the presence of plasmid p770. PCR products were digested with Xho I and fractionated by polyacrylamide gel electrophoresis (PAGE). Fragments were electroeluted and ligated with plasmid p770Δ previously digested with Xho I and dephosphorylated with alkaline phosphatase (*19*). Test plasmids were verified by double-stranded sequencing of plasmid templates (*17*) with primers used in the PCR.
19. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
20. COS-1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). Plasmids were purified by double banding in CsCl-EtBr gradients (*19*). Cells were transfected by the $CaPO_4$ coprecipitation technique with minor modifications [F. L. Graham and A. J. Van der Eb, *Virology* **52**, 456 (1973); S. S. Sisodia, B. Sollner-Webb, D. W. Cleveland, *Mol. Cell. Biol.* **7**, 3602 (1987)] and were metabolically labeled with [^{35}S]methionine 36 hours after transfection. For short-term labeling, cells were washed and then incubated for 15 min with DMEM lacking methionine. Cells were labeled with 150 μCi [^{35}S]methionine (>800 Ci/mmol, Du Pont Biotechnology Systems) for 10 min in 1.6 ml of DMEM lacking methionine and containing 1% dialyzed FBS (DFBS). For long-term labeling studies, cells were washed with DMEM lacking methionine and then incubated for 4 hours with 150 μCi [^{35}S]methionine in DMEM lacking methionine and containing 1% DFBS.
21. For immunoprecipitation from cell pellets, cells were lysed in immunoprecipitation buffer (IPB) [1× IPB: 150 m*M* NaCl, 50 m*M* tris-HCl, *p*H 6.8, 0.5% NP-40, 0.5% sodium deoxycholate, 5 m*M* EDTA, pepstatin (50 μg/ml), leupeptin (50 μg/ml), aprotinin (10 μg/ml), and phenylmethylsulfonyl fluoride (PMSF) (0.25 m*M*)]. Nuclei and residual cytoskeleton were removed by centrifugation, and the soluble extract was made 0.35% SDS and then boiled for 3 min. For analysis of secreted products, 400 μl of conditioned media from cells labeled for 4 hours were made 1× in IPB after the addition of one-fifth volume of 5× IPB and then boiled in the presence of 0.35% SDS. Cell extract equivalent to ~10^5 cells or conditioned media was cleared for 15 min with 50 μl of a 10% suspension of Pansorbin (Calbiochem) and then incubated at 4°C for 3 to 4 hours with 1 to 2 μl of APP-specific polyclonal sera elicited against bacterially synthesized APP (*9*). After a short incubation with 40 μl of Pansorbin, immunocomplexes were layered onto a sucrose cushion (1*M*) prepared in IPB and then centrifuged for 2 to 3 min at 10,000*g*. The cushion was aspirated and the pellet resuspended in 40 μl of Laemmli sample buffer and then boiled for 5 min. After centrifugation, soluble materials were subject to SDS-PAGE. Gels were fixed, impregnated with 2,5-diphenyloxazole–dimethyl sulfoxide [W. Bonner and R. Laskey, *Eur. J. Biochem.* **46**, 83 (1974)], and exposed to Kodak X-Omat film at −70°C.
22. A. S. B. Edge *et al.*, *Anal. Biochem.* **118**, 131 (1981).
23. At 24 hours after transfection, media was replaced with a serum-free defined medium (MCDB 302, Sigma). Conditioned serum-free media was removed 72 hours later, briefly centrifuged to remove particulate material, and concentrated by ultrafiltration in Centricon 10 chambers (Millipore). The resulting material was fractionated by SDS-PAGE and electrophoretically transferred to nitrocellulose filter membranes. To detect substance P–containing polypeptides, we first modified blots by incubation in a solution (1*M*) of 1-ethyl-3-(3-aminopropyl) carbodiimide (EDC) (Sigma) in 0.5*M* ammonium chloride (*12*) to amidate the substance P COOH-terminal methionine residue. This modification is absolutely required for recognition by the monoclonal antibody to substance P, NCI/34. Substance P immunoreactivity on the blot was visualized after incubation for 16 hours with NCI/34 (Accurate Chemical & Scientific Corp), followed by incubation with rabbit antibody to rat H and L chain–specific immunoglobulin G (IgG) (Cappell Labs) and finally with ^{125}I-labeled protein A. To detect NH_2-terminal APP immunoreactivity, we incubated blots with an APP-specific monoclonal antibody, 22C11 (*9*), that recognizes an APP epitope residing between amino acids 60 and 100.
24. S. S. Sisodia, data not shown.
25. We thank D. Cleveland and G. Hart for helpful discussion and F. Davenport for excellent technical assistance. We are grateful to R. Ramabhadran and R. Bayney for providing the full-length APP cDNAs, A. Weidemann for providing APP-specific monoclonal and polyclonal antibodies, and J. Ahearn for providing a full-length type-2 complement receptor cDNA. Supported by grants from the USPHS (NIH AG 03359 and AG 05146), the American Health Assistance Foundation, and The Robert L. & Clara G. Patterson Trust. D.L.P. and E.H.K. are recipients of a Leadership and Excellence in Alzheimer's Disease (LEAD) award (NIA AG 07914). D.L.P. is the recipient of a Javits Neuroscience Investigator Award (NIH NS 10580).

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Two Gap Genes Mediate Maternal Terminal Pattern Information in *Drosophila*

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In *Drosophila* three maternal pattern organizing activities, the anterior, the posterior, and the terminal, establish the anterior-posterior body pattern of the embryo by initiating the spatially restricted activities of the gap class of zygotic segmentation genes. The activities of *tailless* (*tll*) and the newly identified gap gene *huckebein* (*hkb*) are specifically involved in mediating the maternal terminal information at the posterior end of the blastoderm embryo.

THE *Drosophila* GENE *torso* (*tor*) (*1–3*) is a key component of the terminal organizer system. It encodes a putative tyrosine kinase receptor (*4*). In embryos without maternal *tor* activity, all structures posterior to the seventh abdominal segment including the structures derived from the most posterior part of the blastoderm anlagenplan, hindgut and posterior midgut, fail to develop (*5, 6*). These mutant embryos deviate from wild-type development as early as gastrulation (*5*). Because the anlagen of hindgut and posterior midgut are absent, no corresponding invagination is formed at the posterior end of the embryo, and the germ band does not extend along the dorsal side of the embryo (Fig. 1). Genetic and phenotypic evidence indicates that one target of *tor*

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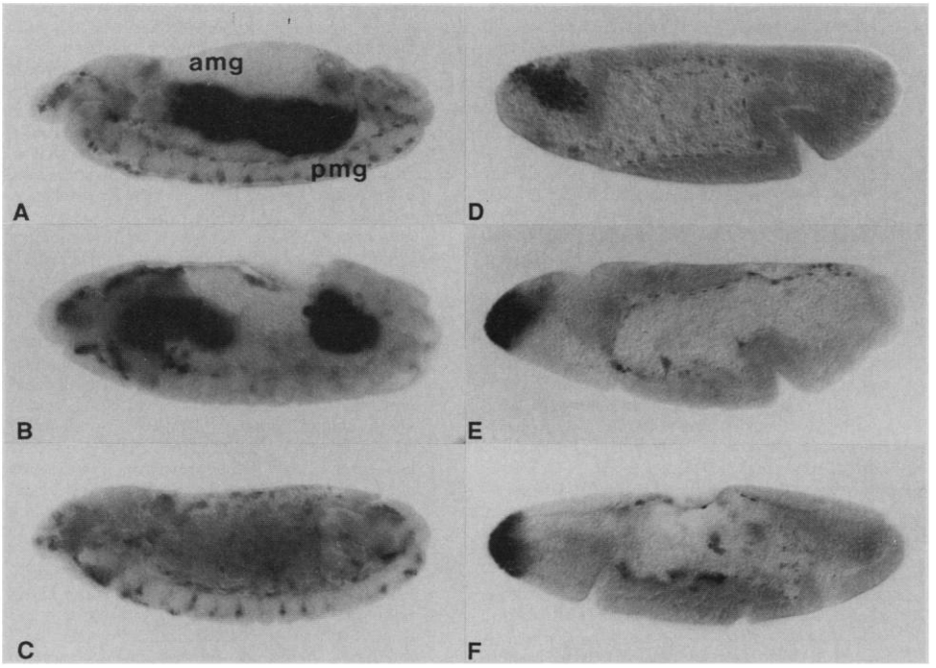

Fig. 1. Post-blastoderm phenotypes of different genetic combinations. (**A** to **C**) Development of the midgut in embryos after germ band retraction, as visualized with a tissue-specific marker. In the wild-type (A), anterior midgut (amg) and posterior midgut (pmg) are well developed. In the *tll*^{g} mutant embryo (B), the posterior midgut is reduced in size. Neither anterior nor posterior midgut are present in the *hkb*^{2} mutant embryo (C). (**D** to **F**) Embryos at germ band elongation stained with anti-fkh antibodies. (D) Embryo derived from a *tor*^{PM} loss-of-function mother (*15*). Note that the anterior *fkh* domain is present at this stage (compare to Fig. 2E). (E) *hkb*^{2} *tll*^{g} embryo derived from a wild-type mother. (F) *hkb*^{2} *tll*^{g} embryo derived from a *tor*^{4021} gain-of-function mother (*15*). In all three cases (D to F), the germ band does not elongate on the dorsal surface as in the wild type, but instead forms irregular folds due to the absence of the amnioproctodaeal invagination at the posterior pole.

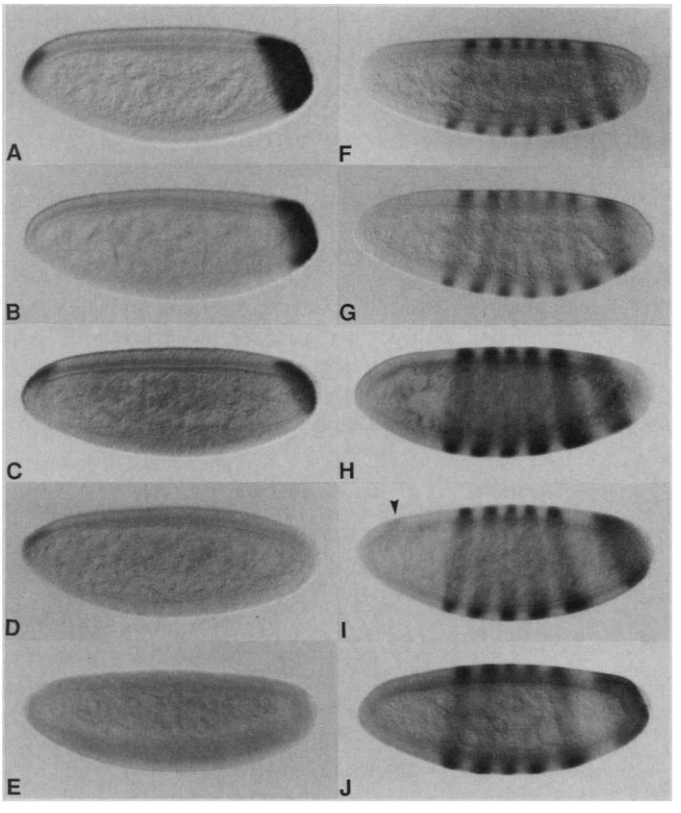

Fig. 2. The effect of *tor*, *hkb*, and *tll* on the central and the terminal regions of the anlagenplan as visualized by antibody to fkh (left panels) and antibody to ftz (right panels) in blastoderm or early gastrulation embryos. In the wild type, *ftz* and *fkh* are expressed at the posterior pole in a complementary fashion (*10*). (**A** and **F**) Wild type. (**B** and **G**) The *hkb*^{2} mutant. The posterior *fkh* domain is smaller than in wild type, the last *ftz* stripe is closer to the posterior pole. The anterior *fkh* domain is absent at this stage. (**C** and **H**) *tll*^{g}. The posterior *fkh* domain is much reduced in size. The number of *ftz* stripes is reduced to six. (**D** and **I**) *hkb*^{2} *tll*^{g}. The posterior *fkh* domain is completely missing. The seventh *ftz* stripe is missing as in the *tll*^{g} single mutant embryo, but the sixth *ftz* stripe is shifted from a subterminal position to the posterior pole. Ectopic *ftz* expression is indicated at the anterior end (arrowhead). (**E** and **J**) Embryos derived from *tor*^{PM} mothers. At the posterior end, these embryos look very similar to *hkb*^{2} *tll*^{g} embryos.

is the zygotic gap gene *tailless* (*tll*), as both *tor* and *tll* act posterior to the seventh abdominal segment (*5, 7*), and the phenotypic effects of *tor* gain-of-function alleles largely depend on the presence of the *tll* gene product (*2, 3*). In amorphic embryos that lack *tll*, the posterior midgut still develops, although it is reduced in size (*8*) (Fig. 1B). Consequently, a rudimentary invagination of future posterior midgut is formed at the posterior end, and germ band extension advances almost normally (*7*). Thus, in contrast to *tor*, *tll* mutations do not affect the most posterior part of the embryo, an indication that at least one other zygotic gap gene may be required to mediate maternal *tor* function. Examination of zygotic mutations affecting the development of the posterior midgut revealed that embryos mutant for the gene *huckebein* (*hkb*) lack the midgut completely (Fig. 1C), whereas all other exterior and interior pattern elements are present. The mutant phenotypes of *hkb* and *tll* suggest that the two genes act in adjacent, largely nonoverlapping domains that together comprise the posterior *tor* domain. This interpretation is supported by the finding that *hkb tll* double-mutant embryos lack an invagination of future hindgut and posterior midgut at the posterior end, and germ band extension is highly perturbed as in embryos lacking *tor* activity (Fig. 1, D and E).

The similarity between *tor* and *hkb tll* mutant phenotypes in older embryos led us to compare their effects on the blastoderm fate map. As a probe for the central segmented region of the embryo, we used antibodies to the protein of *ftz*, the *fushi tarazu* pair-rule gene (*9*). As a probe for the terminal unsegmented region of the embryo, we used antibodies to the protein of the homeotic gene *fork head* (*fkh*) (*10*). In embryos without *tor* activity, the posterior *fkh* domain was completely abolished (Fig. 2). In *tll* embryos, the posterior *fkh* domain is reduced, but it is not completely eliminated (Fig. 2C). The remaining *fkh*-positive cells constitute the anlage of the rudimentary posterior midgut which develops in *tll* embryos. In *hkb* embryos, the posterior *fkh* domain is also reduced (Fig. 2B), apparently because of the absence of the posterior midgut anlage. Correspondingly, the anlage of the segmented body region is expanded toward the pole as indicated by the altered expression of *ftz* (Fig. 2G). The *hkb tll* embryos behave almost like embryos without maternal *tor* activity in that the posterior *fkh* domain is completely eliminated (Fig. 2, D and E) and the posterior-most *ftz* stripe covers the posterior pole (Fig. 2, I and K). The *ftz* expression at the very posterior tip of *hkb tll* embryos, however, is weaker than in embryos without *tor* activity. The co-exten-

sive posterior realms of action of the maternal gene *tor* and the zygotic genes *hkb* and *tll* suggest that *hkb* and *tll* are required to mediate the maternal activity of *tor*. In addition, the almost identical posterior phenotypes of embryos without either *tor* or *hkb tll* activity suggest that, at some point within the terminal pathway, *hkb* and *tll* are specific factors required for executing *tor* function at the posterior end. This does not imply that the *tor* gene product acts directly on *hkb* and *tll* since genetic tests cannot show such a direct interaction. In fact, the finding that *tor* encodes a membrane-bound protein (*4*) suggests that other factors are involved in transmitting the localized signal transduced by the *tor* protein.

In order to test the hypothesis that *hkb* and *tll* are specifically involved in executing *tor* function at the posterior end, we introduced the *hkb tll* double mutant chromosome into the background of a dominant *tor* gain-of-function allele, tor^{4021}. Embryos derived from tor^{4021} mothers exhibit a phenotype opposite to that of embryos without *tor* activity. The terminal parts of the anlagenplan are expanded at the expense of the central segmented region of the anlagenplan (*2*). The tor^{4021} phenotype is likely to be caused by ectopic activity of the *tor* gene product which is normally restricted to the terminal regions (*2, 4*). This is reflected by the expression patterns of both *ftz* and *fkh* in embryos derived from tor^{4021} mothers. In particular, the posterior *fkh* domain is enlarged toward the anterior of the embryo and partly duplicated (Fig. 3A), whereas *ftz* expression is restricted to the center of the embryo (Fig. 3D). The *tor* gain-of-function phenotype is partially suppressed in embryos lacking *tll* wild-type activity (*2, 3*). Up to six *ftz* stripes reappear, which are, however, often incomplete. Particularly, the sixth *ftz* stripe is weak (Fig. 3E). The posterior *fkh* domain is greatly reduced, but is still clearly larger than in *tll* embryos derived from wild-type mothers (Fig. 3B). An almost complete suppression of the tor^{4021} phenotype is observed in *hkb tll* embryos. Six complete *ftz* stripes appear with the sixth *ftz* stripe covering the posterior pole (Fig. 3F). The posterior *fkh* domain is completely missing (Fig. 3C). This phenotype is almost identical to the one of *hkb tll* embryos derived from wild-type mothers (Fig. 2, D and I), and of embryos without *tor* activity (Fig. 2, E and K). Furthermore, in any of these three genotypes, that is, in embryos without *tor* activity, and in *hkb tll* embryos derived from either wild-type mothers or from tor^{4021} mothers, no invagination of future hindgut and posterior midgut is found (Fig. 1, D to F). Thus, a similar phenotype results from either lack of maternal *tor* activity, or from lack of zygotic *hkb tll* activity regardless of the functional state of the *tor* gene. The slight difference in phenotype between *hkb tll* embryos derived from wild-type mothers and from tor^{4021} mothers as indicated by the expression of *ftz* might be due to residual *hkb* wild-type activity in the strong hypomorph hkb^2 (see legend to Fig. 1). Since aberrant *tor* activity in tor^{4021} embryos is almost completely neutralized by the introduction of *hkb* and *tll* mutations, we propose that maternal *tor* activity in its posterior domain is mediated solely by the two zygotic gap genes, *hkb* and *tll*.

The gene *tor* also influences the expression of other central gap genes (*2, 11, 12*). If there is no *hkb tll*–independent terminal pathway, this aspect of *tor* function should also be mediated by *hkb* and *tll* at the posterior end. In the absence of a maternal gene of the posterior system such as *vasa* (*vas*), *tor* is responsible for repressing the gap gene *Krüppel* (*Kr*) in the posterior third of the embryo (*11, 13*) (Fig. 4A), since *Kr* expression expands to the posterior tip in embryos without *tor* activity (Fig. 4D). In *tll* embryos derived from *vas* mothers, *Kr* expression expands but does not cover the posterior pole (Fig. 4B). In contrast, in *hkb tll* embryos derived from *vas* mothers, *Kr* expression expands to the posterior tip as in embryos derived from *vas tor* mothers (Fig. 4C). This corroborates again our proposal that *hkb* and *tll* are sufficient to mediate all known aspects of posterior *tor* function.

All three genes, *tor, hkb*, and *tll*, act also at the anterior end. However, we have restricted our analysis to their posterior domains, because the situation at the anterior pole is more complex. The anterior *hkb* domain lies outside the anterior *tor* domain which does not include the anterior midgut (*5*) whereas

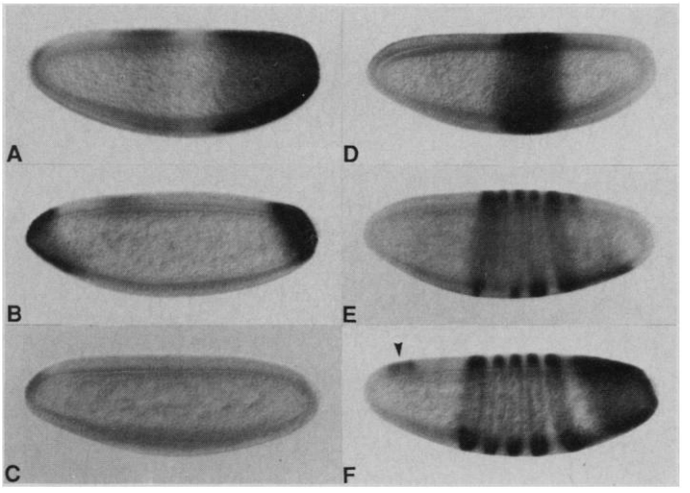

Fig. 3. Suppression of the *tor* dominant gain-of-function phenotype as visualized by anti-fkh (left panels) and anti-ftz (right panels) antibody staining. (**A** and **D**) hkb^+ tll^+ embryos derived from tor^{4021}/+ mothers. The posterior *fkh* domain is expanded and partly duplicated. The *ftz* gene is expressed only in a single, broad band in the middle of the embryo. (**B** and **E**) tll^8 embryos derived from tor^{4021}/+ mothers. The posterior *fkh* domain is reduced, but still larger than in tll^8 embryos derived from wild-type mothers (Fig. 2C). The anterior *fkh* domain is large; *ftz* expression is partially normalized. In most embryos, five somewhat well-developed stripes reappear. In the embryo shown, the second stripe is incomplete at the ventral side. The sixth stripe is always very weak and abnormal in shape (compare to Fig. 2H). (**C** and **F**) hkb^2 tll^8 embryos derived from tor^{4021}/+ mothers. The posterior *fkh* domain is completely absent. The first five *ftz* stripes are complete. The sixth *ftz* stripe covers the posterior pole, but is abnormally broad. Often the fifth and the sixth *ftz* stripe are fused ventrally. Ectopic *ftz* expression appears at the anterior pole (arrowhead, compare to Fig. 2I).

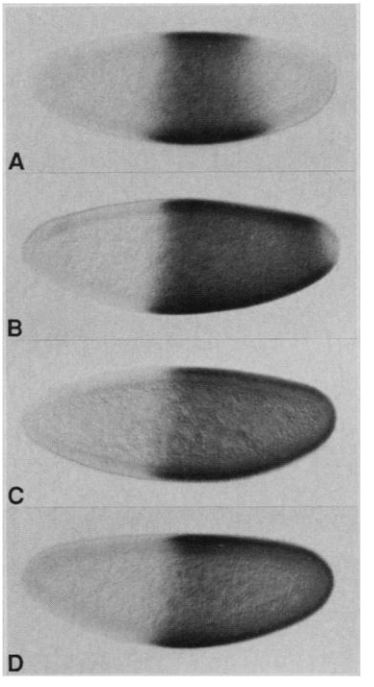

Fig. 4. Repression of *Kr* expression in the absence of posterior organizer activity by *tor, hkb*, and *tll* as visualized by antibody staining. (**A**) hkb^+ tll^+ embryo derived from a vas^{PD} mother. (**B**) tll^8 embryo derived from a *vas* mother. *Kr* expression expands to the posterior, but does not cover the posterior tip. (**C**) hkb^2 tll^8 embryo derived from a vas^{PD} mother. (**D**) Embryo derived from a vas^{PD} tor^{PM} mother. *Kr* expression in both embryos (C and D) expands completely to the posterior pole.

the anterior *tll* domain is contained within the anterior *tor* domain as deduced from both the cuticle phenotype and the expression pattern of the pair rule gene *hairy* (*8*, *14*).

REFERENCES AND NOTES

1. C. Nüsslein-Volhard, H. G. Frohnhöfer, R. Lehmann, *Science* **238**, 1675 (1987).
2. M. Klingler, M. Erdély, J. Szabad, C. Nüsslein-Volhard, *Nature* **335**, 275 (1988).
3. T. R. Strecker, S. R. Halsell, W. W. Fisher, H. D. Lipshitz, *Science* **243**, 1062 (1989).
4. F. Sprenger, L. M. Stevens, C. Nüsslein-Volhard, *Nature* **338**, 478 (1989).
5. T. Schüpbach and E. Wieschaus, *Wilhelm Roux's Arch. Dev. Biol.* **195**, 302 (1986).
6. M. Klingler, thesis, University of Tübingen (1989).
7. T. R. Strecker, K. Kongsuwan, J. A. Lengyel, J. R. Merriam, *Dev. Biol.* **113**, 64 (1986); U. Gaul and D. Weigel, unpublished data.
8. T. R. Strecker, J. R. Merriam, J. A. Lengyel, *Development* **102**, 721 (1988).
9. S. B. Carroll and M. P. Scott, *Cell* **47**, 113 (1986).
10. D. Weigel, G. Jürgens, F. Küttner, E. Seifert, H. Jäckle, *ibid.* **57**, 645 (1989).
11. U. Gaul and H. Jäckle, *Adv. Genet.*, in press.
12. M. Pankratz, H. Hoch, E. Seifert, H. Jäckle, *Nature* **341**, 337 (1989).
13. U. Gaul and H. Jäckle, *Cell* **51**, 549 (1987).
14. P. A. Mahoney and J. Lengyel, *Dev. Biol.* **122**, 464 (1987).

15. tor^{PM} is a loss-of-function mutation, presumably amorphic. tor^{4021} is the strongest dominant gain-of-function allele of the *tor* locus (*2*). The tll^{8} allele is associated with a small deletion and represents the amorphic condition (*8*). Two *hkb* alleles, hkb^{1} and hkb^{2}, have been isolated in a screen for mutations affecting head development using EMS as a mutagen (G. J. Anderson and K. V. Anderson, unpublished observations). The gene hkb^{2} exhibits the stronger phenotype of the two. In about 50% of hkb^{2} embryos, a small remnant of posterior midgut is present (not shown). In the other embryos, no posterior midgut develops, as shown in Fig. 1C. We have recovered two small deletions uncovering *hkb*. All embryos homozygous for one of these deletions have the phenotype shown in Fig. 1C (D. Weigel and B. Wild, unpublished data). We therefore conclude that hkb^{2} is a strong hypomorphic allele. The only defect observed in cuticle preparations of *hkb* mutant embryos is a failure in head involution. Since all pattern elements of the head skeleton and the esophagus are present, we interpret this effect as a secondary consequence of the absence of the anterior midgut. Staining of *hkb* embryos at later stages with anti-fkh antibodies reveals that, in contrast to the endodermally derived foregut and hindgut are almost normal (U. Gaul and D. Weigel, unpublished data). *hkb* maps to the third chromosome between the markers *ri* and *p*. Multiply mutant flies were obtained by recombination and standard crosses. As a marker for the anterior and posterior midgut, a P element transformant line containing a genomic DNA fragment from the *fkh* region in the enhancer detection vector pHZ50PL was used (D. Weigel, E. Seifert, H. Jäckle, *EMBO J.*, in press). Midgut-specific β-galactosidase expression in transformed embryos was visualized by antibody staining as described (*10*). Embryos were collected at 25°C.

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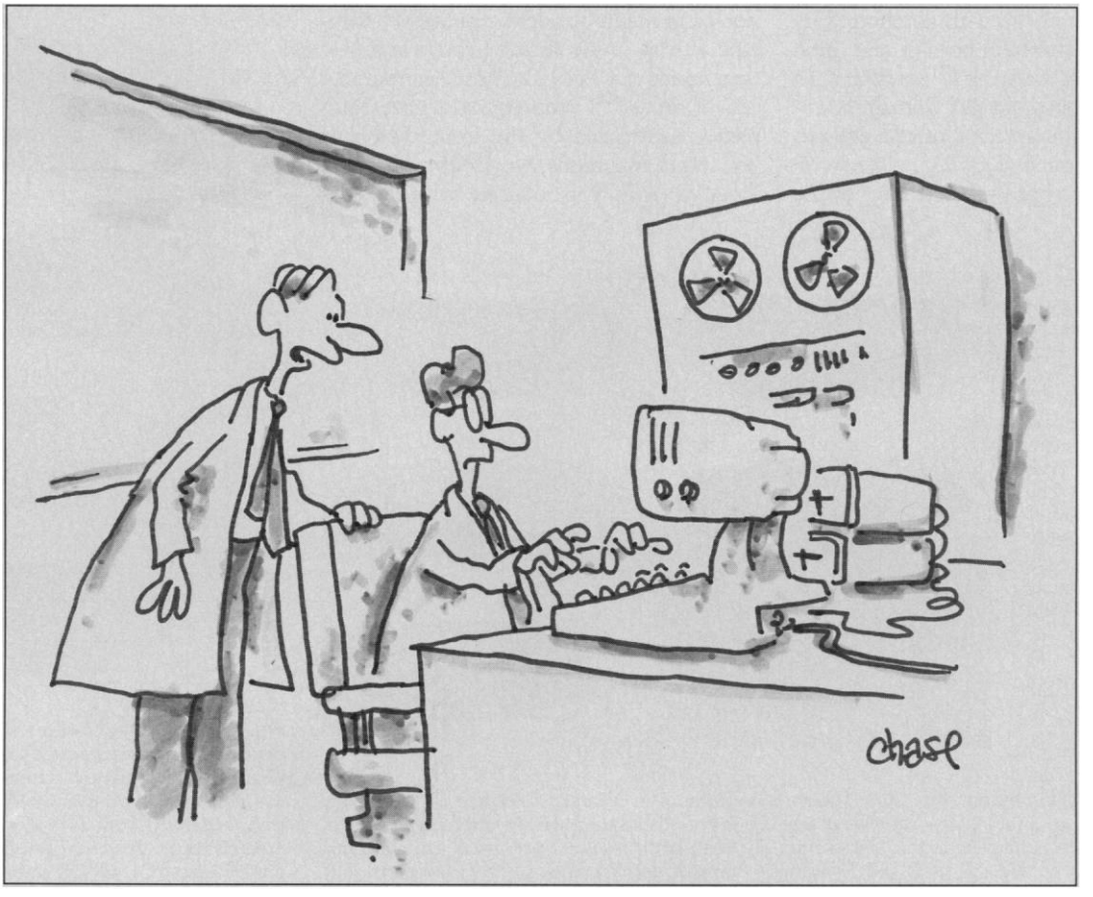

"Remember the days when analyzing software meant dissecting a frog?"