

We constructed a simple mathematical model to examine the relative effectiveness of SIT and several variants of RIDL (Fig. 1). Release of insects homozygous for one dominant female-specific lethal gene (DFL) was similarly effective to SIT, whereas enhanced versions of RIDL were more effective. Our model underestimates the relative effectiveness of RIDL compared to SIT because it does not account for advantages of RIDL, such as reduced production costs and the fitness advantage that transgenic males are likely to have over irradiated males. RIDL has additional advantages over SIT. Efficient removal of females from the released population, combined with marking the transgenic construct with an easily scored dominant marker, such as green fluorescent protein, would improve field trap data. The RIDL stock would produce no viable female progeny under normal environmental conditions, therefore the hazard posed by accidental release from a factory would be minimized. RIDL would also allow the factory strain to be released at any life-cycle stage, rather than requiring that the strain be grown to a late stage to allow sex separation and sterilization. We have demonstrated the system in *Drosophila*; the challenge now is to translate this to a pest of economic importance.

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9. Plasmids were constructed as follows. Yp3-tTa was constructed by cloning an Eco RI/Pvu II tTa fragment from pUHD 15-1neo (8) into Eco RI/Pvu II-digested Yp3 expression construct pFBE (10). tRe-Ras64B<sup>V12</sup> was constructed by cloning the Ras64B<sup>V12</sup> cDNA as an Eco RI/Not I fragment from the p(sevRas64B<sup>V12</sup>) (19) into WTP-2 (12). tRe-msl-2<sup>NOPU</sup> was constructed by cloning the msl-2 cDNA as a Not I/Xba I fragment from pM2 NOPU (19) into WTP-2.
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17. msl-2<sup>NOPU</sup> is a msl-2 transcript with ablated Sxl binding sites in the 5' and 3' untranslated regions (UTRs) (20). These sites in the UTRs normally restrict the Msl-2 protein to males, where it forms a complex

that hyperactivates the X chromosome as part of the dosage compensation mechanism (21).

18. For these experiments, recombinant second chromosomes were used, which carried the tTa and the tRe constructs, that is, Hsp26-tTa with tRe-msl-2<sup>NOPU</sup> or Yp3-tTa with tRe-msl-2<sup>NOPU</sup>.
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23. This model facilitates the comparison of SIT and various RIDL regimes under a set of simple assumptions, based on the original models of Knipling (7). We assume that the pest population is expanding as a new outbreak or recovering from a pesticide program. We have not included stochastic variation,

density-dependent factors, life-cycle mortality, or other factors that will vary from one insect species to another. These additional considerations have been discussed elsewhere, for example, (22). With these assumptions, our model provides the basis of a fair comparison of SIT and RIDL, but it cannot be used to predict population reduction for SIT or RIDL applied to a particular insect population.

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## *bicoid*-Independent Formation of Thoracic Segments in *Drosophila*

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The maternal determinant Bicoid (Bcd) represents the paradigm of a morphogen that provides positional information for pattern formation. However, as *bicoid* seems to be a recently acquired gene in flies, the question was raised as to how embryonic patterning is achieved in organisms with more ancestral modes of development. Because the phylogenetically conserved Hunchback (Hb) protein had previously been shown to act as a morphogen in abdominal patterning, we asked which functions of Bcd could be performed by Hb. By reestablishing a proposed ancient regulatory circuitry in which maternal Hb controls zygotic *hunchback* expression, we show that Hb is able to form thoracic segments in the absence of Bcd.

In *Drosophila*, a key component of the anterior maternal system is Bcd (1). Embryos from mothers mutant for *bicoid* (*bcd*) lack head, thorax, and some abdominal segments. The maternal *bcd* mRNA is localized to the anterior pole of the egg and early embryo, and its translation generates an anteroposterior gradient of the Bcd homeoprotein (2). In the syncytial environment of the early fly embryo, the Bcd protein appears to act as a morphogen in dictating distinct developmental fates by providing a series of concentration thresholds: At low levels, Bcd acts through high-affinity binding sites to activate target genes like *hunchback* (*hb*) for the formation of the thorax, whereas high levels of Bcd activate head gap genes, such as *orthodenticle* (*otd*), which contain low-affinity Bcd binding sites (3). However, *bcd* seems to be the result of a recent gene duplication event in the Hox cluster of flies, which would explain why no

*bcd* homologs have been identified outside higher Dipterans (4). Thus, different insect species may use other morphogens to pattern their embryos. One candidate morphogen that has been functionally conserved during evolution is the zinc-finger protein Hb (5–7).

In *Drosophila*, a maternal Hb gradient is established by the Nanos protein that blocks translation of the ubiquitously distributed *hb* mRNA in the posterior region of the embryo (8). This Hb gradient can by itself provide long-range polarity to the embryo and compensates for the absence of Bcd in the formation of abdominal segments (6). In addition, Hb synergizes with Bcd to pattern the anterior region of the embryo (9). However, the zygotic, *bcd*-dependent expression of *hb* (10) causes an intrinsic problem for the analysis of the specific roles of the two morphogens: Whenever Bcd activity is altered, Hb activity is changed (3). Thus, many of the effects attributed to Bcd might indirectly be caused by Hb. The *hb* gene is expressed from two independent promoters, P1 and P2 (Fig. 1A) (11): Maternal and late blastoderm expression in the central (parasegment 4, PS4) and posterior stripes are initiated at P1, whereas P2 mediates the zygotic, *bcd*-

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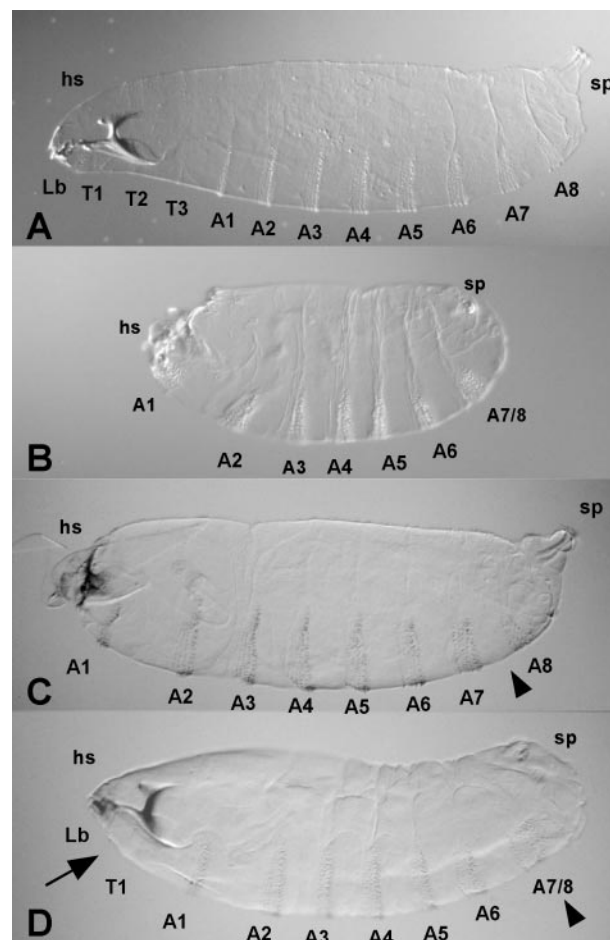
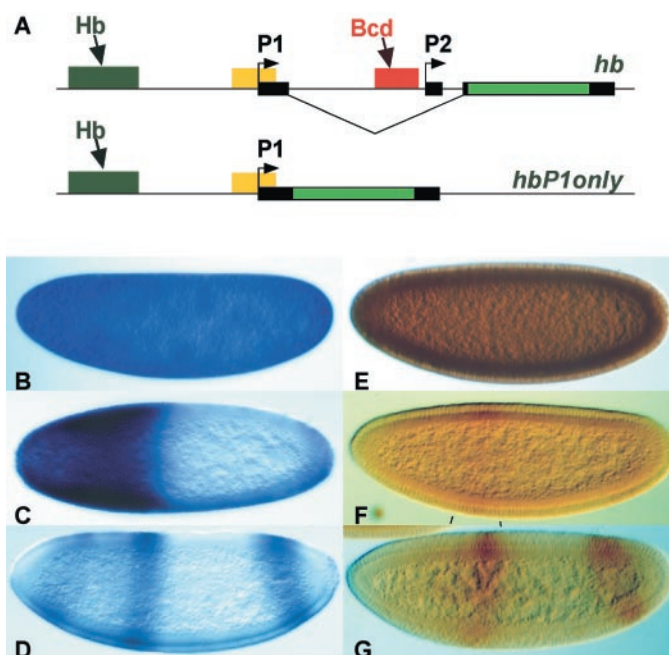
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dependent expression at the early blastoderm stage (Fig. 1, B to D) (12).

We have generated a functional *hb* transgene that is missing all P2 promoter sequences and relies solely on the P1 promoter (13) (*hbP1only*, Fig. 1A). *hbP1only* constructs (14) do not respond to *bcd* and do not mediate gene expression in the anterior cap domain (Fig. 1, E to G). Therefore, *hbP1only* uncouples the direct link between the Bcd and Hb morphogen systems. Zygotic *hb* mutants derived from heterozygous parents (14) do not develop labial or thoracic structures, and they also show a fusion of abdominal segments A7 and A8 (5) (Fig. 2, A and B). When one copy of the *hbP1only* transgene was provided zygotically to a *hb* mutant embryo (by the father), it rescued the posterior phenotype, and A7 and A8 developed normally (Fig. 2C). The labial/thoracic phenotype was not rescued. However, when *hbP1only* was provided as one copy by the mother to a *hb* mutant embryo, the posterior and part of the anterior phenotype were rescued. These embryos exhibited normal labial and prothoracic (T1) segments, and only lacked meso- and metathoracic segments (T2 and T3) (Fig. 3A). The anterior rescue is due to the maternal contribution of *hbP1only* because sibling embryos that did not inherit the *hbP1only* construct zygotically also exhibited the partial anterior (but not the posterior) rescue (Fig. 2D). This indicates that restoring high levels of maternal *hb* expression (i.e., two copies: one wild type plus one copy of *hbP1only*) is sufficient to rescue the labial and prothoracic segments in the zygotic *hb* mutant progeny (Fig. 2D) (6). Therefore, the lack of zygotic *hb* (Fig. 2D) leads only to the loss of T2 and T3 and to the fusion of A7 and A8, whereas the previously reported zygotic *hb* phenotype (Fig. 2B) (5) represents a combination of a haploinsufficient maternal plus a zygotic phenotype.

The loss of zygotic *hb* activity affects regions of the embryo that correspond to the two late stripes of zygotic *hb* expression: The A7-A8 fusion corresponds to the posterior stripe, whereas the loss of T2 and T3 corresponds to the PS4 stripe (Fig. 1D), which starts as a fairly wide domain covering the anlagen of T2 and T3 (11) (Fig. 1F). This correlation between the zygotic *hb* phenotype and the late stripe expression pattern led us to reconsider the importance of the early *bcd*-dependent anterior cap domain. Under some conditions, *hbP1only* (maternal *hb* contribution plus stripe expression) might suffice for normal segmentation of head and thorax, making superfluous the *bcd*-dependent anterior cap domain. Hence, we activated the *hb* PS4 stripe without *bcd*-dependent *hb* expression. This stripe is repressed by the *knirps* abdominal gap-gene product (15) and is activated by high levels of Hb itself, either directly or indirectly (through repression of *kni*) (16). We generated embryos that lacked the *bcd*-dependent *hb* cap domain but contained an

**Fig. 1.** *hb* genomic organization and expression pattern. (A) *hb* transcription is initiated at two different promoters, P1 and P2. Early embryonic expression is controlled by three separable enhancer elements: An oogenesis element (yellow box) (11) leads to the maternal contribution (B), the *bcd*-dependent element (red) (3) directs anterior cap expression (C), and a stripe element (green) (11) controls the central *hb*-dependent PS4 and the posterior stripes (D). In (B) to (D), wild-type *hb* expression was detected by in situ hybridization (12). (E to G) *hbP1AB* was constructed by replacing the *hb* coding region of *hbP1only* with a *lacZ* gene (13); embryos were immunostained using antibody to  $\beta$ -galactosidase (12). When provided maternally, this construct leads to strong expression throughout the embryo (E). When provided only zygotically, no anterior cap domain is observed, but a wide stripe forms (F, borders indicated by two lines) that later refines to the PS4 stripe. In the late blastoderm, both the PS4 and the posterior stripes are formed (G).



**Fig. 2.** Rescue of zygotic *hb* mutants by *hbP1only* (14). (A) Wild-type cuticle (hs, head skeleton; Lb, labial; T1, prothoracic; T2, mesothoracic; T3, metathoracic; A1 to A8, abdominal segments; sp, spiracles). (B) *hb* zygotic mutant embryo derived from heterozygous *hb* mother. (C) *hb* zygotic mutant with A7 and A8 rescued (arrowhead) by the *hbP1only* transgene provided paternally. (D) *hb* zygotic mutant with labial and T1 segments (arrow) rescued by maternal expression of *hbP1only* in addition to one wild-type maternal copy of *hb*.



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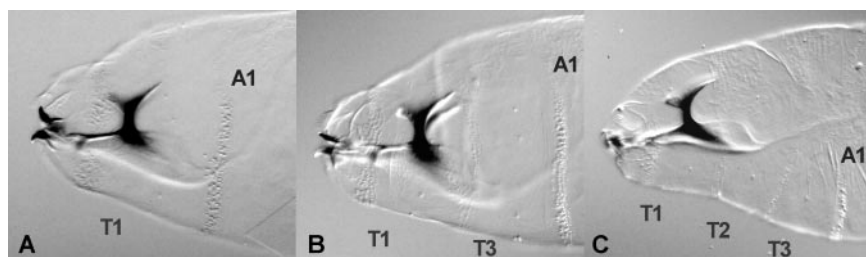
increased maternal *hb* contribution (to four copies) and *kni* reduced to one copy (14). These embryos displayed a range of partially rescued *hb* phenotypes, including embryos (5 to 10%) with a full set of head and thoracic segments (Fig. 3). Thus, *bcd*-dependent *hb* expression can in principle be dispensable for embryonic segmentation, and the only critical anterior domain of zygotic *hb* expression appears to be the PS4 stripe, with the *bcd*-dependent cap domain serving to activate this stripe. This role is likely

achieved by the maternal *hb* contribution in species where zygotic *hb* is not under the control of *bcd* or where a *bcd* homolog might not exist (17, 18).

The rescue of T2 and T3 structures by *bcd*-independent *hb* expression raised the question of whether these structures could develop in a completely *bcd*-independent manner. Embryos derived from *bcd* mutant mothers develop ectopic tail structures that replace head and thorax and exhibit a disruption of some abdominal

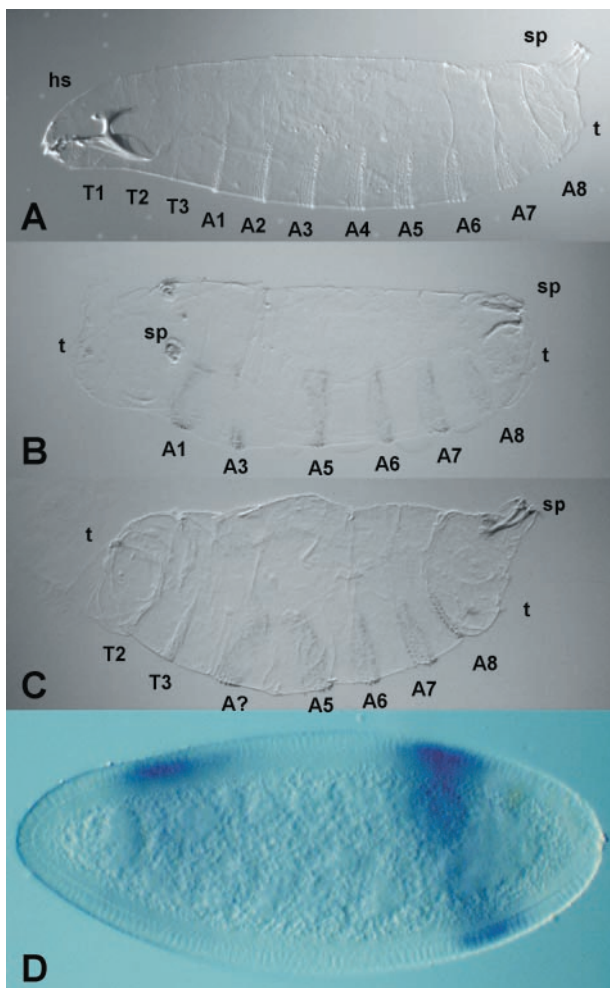
segments (Fig. 4, A and B). Although previous work (6) has shown that, in the absence of *bcd*, high levels of maternal *hb* can rescue a normal abdomen and some thoracic structures, no complete thoracic segments could be induced. We therefore introduced into a *bcd* mutant background a *bcd*-independent source for high levels of zygotic *hb* expression (Fig. 4) (14). By establishing this artificial zygotic Hb gradient, we obtained, with variable expressivity, two notable results: First, about 20% of the embryos exhibited rescued T2 and T3 segments (Fig. 4C). The maintenance of high Hb levels that lead to the rescue of thoracic segments is likely due to the activation of the *hb* stripe element (Fig. 1A) because the *hbP1AB* reporter (13) is activated as a stripe where T2 and T3 form (Fig. 4D). Second, most of the ectopic tail structures that are anteriorly duplicated in *bcd* mutants were suppressed, suggesting further redundancy between Hb and Bcd. However, Hb and Bcd must act at different levels in suppressing these tail structures, which depend on the activity of the *caudal* (*cad*) gene (19): Bcd acts by repressing *cad* mRNA translation (20), whereas Hb does not (19) but might instead interfere with Cad protein function. This *bcd*-independent suppression of *cad* function might be important in organisms where the Cad gradient only forms late (21) and represents another variation as to how *cad* activity is suppressed at the anterior of the embryo.

Different species use various strategies for repression of Cad function: In *Drosophila*, translational repression of *cad* mRNA involves the Bcd homeoprotein (19), whereas in *Caenorhabditis elegans* repression involves the KH-domain protein MEX-3 (22). In vertebrates, a mutually antagonistic relation between *otd*-like and *cad*-like genes has been proposed to reflect an ancestral system to pattern the anteroposterior axis of the embryo (23). In arthropods, ancestral head determinants are probably encoded by *otd*-like genes as well. Thus, in the beetle *Tribolium*, where no *bcd* homologs but Bcd-like activities have been found (18), these activities are probably also covered by Otd or KH-domain proteins. This is consistent with the Otd-like DNA binding specificity of Bcd, which is atypical for a factor encoded by a gene duplication in the Hox cluster. This change in specificity was probably crucial for Bcd to acquire its key role in anterior patterning, as it allowed Bcd to function both as an RNA binding protein (20) and as an Otd-like transcription factor. In this respect, it is not surprising that the zinc-finger protein Hb cannot completely replace Bcd in the head region. Even the highest levels of Hb obtained in our experiments were not able to induce head formation in the absence of Bcd. However, Hb is required for the posterior head region (maxillary and labial segment) (5) and supports anterior head development by synergizing with Bcd (9). It will be interesting to see whether such a synergism can



**Fig. 3.** Rescue of thorax without *bcd*-dependent *hb* expression (12, 14). (A) In 30% of embryos, only the maternal rescue of the labial and T1 segments is seen (compare with Fig. 2D). Most embryos, however, show additional rescue of thoracic segments, varying from an additional set of thoracic sensory organs to an additional thoracic denticle belt, or both (B). These structures are most likely derived from the T3 segment, which is less sensitive than the T2 segment to lack of *hb* function (5). (C) In 5 to 10% of the embryos, a complete thorax (consisting of T1, T2, and T3 segments) is seen.

**Fig. 4.** Rescue of T2 and T3 segments in the absence of *bcd* activity (13, 14). (A) Wild-type cuticle preparation (t, tuft; other abbreviations as in Fig. 2). (B) Embryo derived from a *bcd* mutant mother. At the anterior end of the embryo, the head and thorax are replaced by tail structures (sp and t). The embryo also lacks abdominal segments A2 and A4. (C and D) Embryos derived from a cross between *bcd* mutant mothers carrying four copies of the *nos-GAL4GCN4-3'**bcd* driver and fathers carrying *UAS-HB* transgenes homozygous on both the second and third chromosomes. In (C), most of the anterior duplicated tail structures, except for the most terminal structure (t), are suppressed by the induced zygotic *hb* expression. Moreover, thoracic structures are rescued to a variable extent, up to a complete rescue of the T2 and T3 segments. In (D), anterior expression of zygotically provided *hbP1AB*, detected by *lacZ* in situ hybridization (11) in a blastoderm-stage embryo, indicates that zygotic *hb* expression is mediated in this thoracic rescue situation by regulatory elements upstream of the P1 promoter.



also take place between Hb and other more ancestral head determinants.

Our results indicate that the two morphogenetic systems Bcd and Hb do not need to be directly linked. Hence, the direct regulation of *hb* by Bcd might represent a recent evolutionary addition to the insect body plan (17, 18). In *Drosophila*, the abundance of *bcd*-dependent *hb* expression eventually rendered superfluous the maternal *hb* contribution (8), which is widespread within arthropods (24). Consistent with the idea that the *bcd*-dependent *hb* expression represents a recent evolutionary acquisition, the P2 promoter contains only activator sites that allow the direct response to a specific threshold level of a morphogen (3, 16). This might be a unique situation, given that most other developmentally regulated promoters contain, in addition to activator sites, repressor elements for setting the exact borders of gene expression (25). By tinkering with the rather plastic mechanisms of early development, we could change the ontogeny of *Drosophila* toward an inferred ancestral state where maternal Hb controls zygotic *hb*. This change could be brought about by altering patterns and levels of gene expression, which presents the most likely variation on which evolutionary processes are based (26).

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12. Procedures for whole-mount in situ hybridization, immunohistochemistry, and cuticle preparations were as described (27). DNA probes for *hb* and *lacZ* were generated using the 2.4-kb insert from *phbXba2.4* (17) and a 3.6-kb fragment from *pCHABΔSal* (27). Rabbit antibody to  $\beta$ -galactosidase (Cappel) was used at 1:5000 dilution. For Fig. 3, fully developed *hb* mutant embryos were selected under a Leica MZ12 stereomicroscope for green fluorescent protein (GFP) expression before cuticle preparations.
13. The *hb* transgenes were constructed as follows: A 4-kb genomic Eco RI fragment containing the *hb* upstream sequences including the P1 promoter was isolated from *phbXho10.5* (17) and cloned into the Eco RI site of *pCHABΔXba* (27), thereby creating *pChbP1*. The complete open reading frame for *hb* was excised by Not I and partial Eco RI digests from the cDNA clone J3, which was derived from a transcript originating at the P1 promoter (17). The cDNA fragment was inserted into *pChbP1* that was opened by a complete Not I and partial Eco RI digest to fuse the genomic and cDNA at the Eco RI site within the 5' untranslated leader, resulting in *pChbP1only*. To assess the expression pattern of the transgene, we replaced the *hb* coding region (including its 3' untranslated region) by the *lacZ* gene. *pChbP1only* was opened by an Xba I digest and religated to generate *pChbP1ΔXba*, into which a 3.6-kb Xba I fragment containing the AUG- $\beta$ -gal (AB) gene derived from *pCHABΔSal* (27) was cloned to create *pChbP1AB*. A *pUAST-HB* construct was generated by cloning the 2.4-kb Xba I fragment containing the *hb* coding region from *phbXba2.4* (17) into *pUAST* (28). These constructs were introduced into the fly genome as described (27) to generate several fly strains carrying the transgenes *hbP1only*, *hbP1AB*, and *UAS-HB*.
14. Fly strains were generated as follows: The *hbP1only* transgenes were recombined or crossed into the amorphic *hb<sup>FB</sup>* mutant background. For the experiments described in Fig. 2, crosses were carried out to generate amorphic *hb<sup>FB</sup>/hb<sup>14F</sup>* trans-heterozygotes, which were identified on the basis of anterior or posterior *hb* phenotype. For Fig. 3, the rescued *hb* mutant embryos derived from *hb* heterozygous mothers that carry three copies of the *hbP1only* transgene to increase the maternal Hb contribution. Assuming that each dose of the *hbP1only* transgenes is approximately equivalent to a normal dose of *hb*, these mothers provide twice as much maternal Hb as the wild type. The maternally inherited *hb* mutant chromosome was marked with an *UAS-GFP* transgene, whereas the paternally inherited double mutant chromosome carrying the amorphic alleles *hb<sup>7M</sup>* and *kni<sup>1D</sup>* was marked with a *wg-GAL4* transgene. Homozygous *hb* mutant embryos were identified by their expression of GFP in the *wg* pattern (12). All embryos with a fully rescued thorax also show disturbance of abdominal segments A3 and A4, which are most sensitive to partial loss of *kni* (15). For Fig. 4, the amorphic *bcd<sup>E1</sup>* allele was used and third chromosomal *UAS-HB* and *hbP1AB* transgenes were recombined. The *GAL4* driver lines were *nos-GAL4GCN4-3' bcd* (29) and *wg-GAL4*.
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30. We thank P. Beaufils for *UAS-GFP* strains; M. Boyle, N. Dostatni, U. Gaul, F. Janody, and J. Posakony for materials; and V. Schaeffer, I. Brun, J. Burr, and the members of the Desplan, DiNardo, and Gaul labs for helpful discussions. E.A.W. was the recipient of a Human Frontier Science Program long-term fellowship. Supported by the Howard Hughes Medical Institute at Rockefeller University and by NSF grant IBN-9817981.

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## Necessity for Afferent Activity to Maintain Eye-Specific Segregation in Ferret Lateral Geniculate Nucleus

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In the adult mammal, retinal ganglion cell axon arbors are restricted to eye-specific layers in the lateral geniculate nucleus. Blocking neuronal activity early in development prevents this segregation from occurring. To test whether activity is also required to maintain eye-specific segregation, ganglion cell activity was blocked after segregation was established. This caused desegregation, so that both eyes' axons became concentrated in lamina A, normally occupied only by contralateral afferents. These results show that an activity-dependent process is necessary for maintaining eye-specific segregation and suggest that activity-independent cues may favor lamina A as the target for arborization of afferents from both eyes.

Development of mammalian visual pathways is characterized by activity-dependent sculpting of precise neuronal connections from ini-

tially diffuse projections. In the developing lateral geniculate nucleus (LGN), retinal ganglion cell inputs from the two eyes initially