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 (*11*) and a 3.6-kb fragment from pCHABΔSal (*27*). Rabbit antibody to β-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 (*11*) 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 (*11*). 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-β-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 (*11*) 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^{FB} mutant background. For the experiments described in Fig. 2, crosses were carried out to generate amorphic hb^{FB}/hb^{14F} 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^{7M} and kni^{IID} 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^{E1} 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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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 initially diffuse projections. In the developing lateral geniculate nucleus (LGN), retinal ganglion cell inputs from the two eyes initially

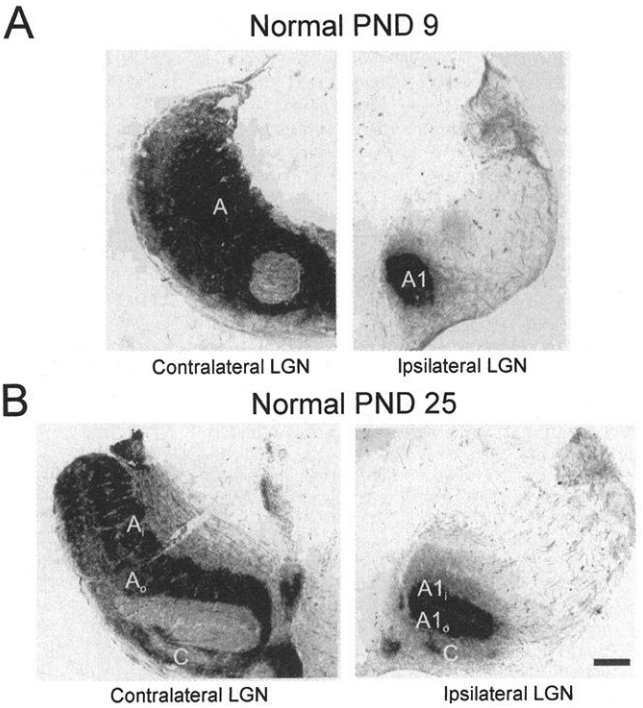

Fig. 1. Normal development of the LGN. Retinogeniculate afferents from the right eye were labeled by anterograde transport of WGA-HRP. (**A**) Horizontal sections from PND 9 ferret LGN show good segregation of afferents from the two eyes into lamina A (contralateral) and lamina A1 (ipsilateral). (**B**) PND 25 ferret afferents show both eye-specific segregation into lamina A and lamina A1 and ON/OFF segregation into inner (A_i and $A1_i$) and outer (A_o and $A1_o$) leaflets. C laminae are also seen. Bar = 100 μm.

overlap and only later segregate into eye-specific layers (*1, 2*). This eye-specific segregation in the LGN is activity dependent; it is prevented by blocking action potentials in the LGN (*3*) or by binocular blockade of spontaneous retinal activity (*4, 5*).

The failure to develop eye-specific segregation in the absence of activity appears to be due to a specific role for neuronal activity in a competitive process. Activity is not necessary for the general growth and development of axons. Activity blockade does not freeze individual axons in an immature, sparsely branched state. Instead, in the absence of activity, retinogeniculate axons continue to grow and elaborate their axonal arbors, albeit without spatial specificity (*3*). Monocular deprivation paradigms have suggested that the activity-dependent processes that cause segregation of eye-specific layers in the LGN involve competition between afferents serving the two eyes. If the normal balance of

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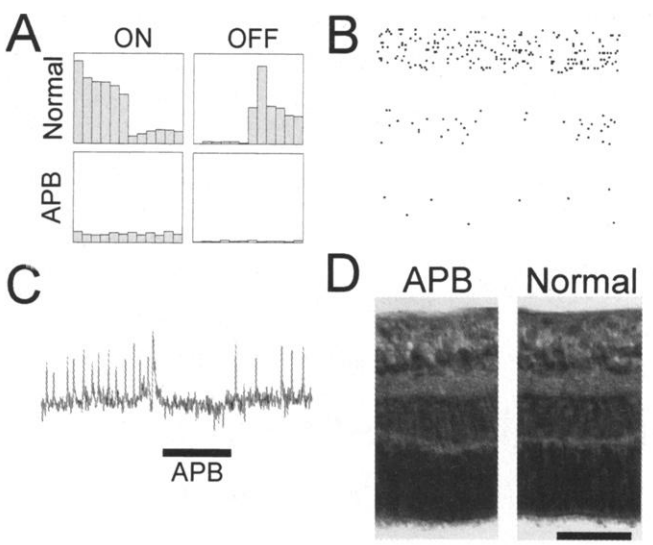

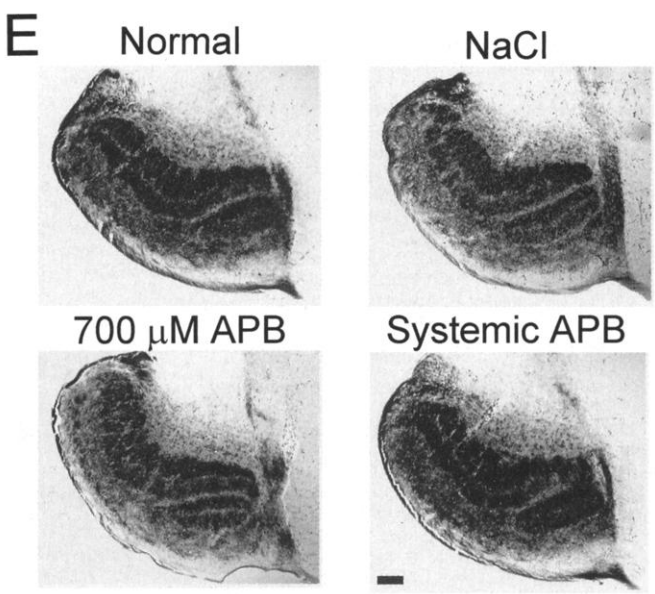

Fig. 2. Physiological effects of APB treatment and controls for nonspecific effects of eye injections and possible systemic APB effects. (**A**) APB (3.5 mM) abolishes both ON and OFF LGN responses. Poststimulus time histograms of multiunit LGN activity in response to flashing light and dark circles (white, 1.5 s; black, 1.5 s). (Top left) Normal PND 30 multiunit ON response from lamina A. (Bottom left) Recording from the same location 20 min after contralateral eye APB injection. (Top right) Normal PND 30 multiunit OFF response from lamina A1. (Bottom right) Recording from the same location 20 min after ipsilateral eye APB injection. *y* axis, 700 spikes per second; *x* axis, 3 s. APB was found to block all ON and OFF activity in the PND 30 LGN for 24 to 28 hours in three ferrets. (**B**) APB (3.5 mM) blocks spontaneous LGN activity in PND 30 ferrets. Ten-second raster plots of spontaneous LGN activity. (Top) Normal spontaneous activity in layer A outer. (Middle) Activity at same location 10 min after contralateral eye APB injection. (Bottom) Thirty min after injection. (**C**) Effects of 1 μM APB on spontaneous retinal activity in PND 7 ferret. In vitro calcium imaging shows complete blockade of retinal ganglion cell activity. *y* axis, nM Ca^{2+}; *x* axis, 40 min. APB wasapplied at 19 min and washed out at 29 min. Data graciously provided by Wong and Wong (*16*). (**D**) APB does not disrupt normal development of gross morphology in the retina. Thionin-stained sections from ferret retina treated with 3.5 mM APB from PND 9 to 25 (right) and from a normal PND 25 animal (left). Bar = 50 μm. (**E**) Eye injections of saline or low concentration APB or systemic injections of high-concentration APB do not affect retinogeniculate afferent segregation. Binocular WGA-HRP injections reveal the normal PND 25 pattern of eye-specific layers and ON/OFF sublaminae in ferret LGN. Daily eye injections of 0.9% NaCl or 700 μM APB PND 9 to 25 do not alter the projection pattern. Note that 700 μM APB is sufficient to block ON-center cells in PND 30 ferrets for approximately 24 hours, suggesting that OFF-center activity alone may be sufficient to drive axonal segregation in the LGN. Systemic daily (PND 9 to 25) injections of APB directly into the cisterna magna in three ferrets had no effect. The concentration of APB used was the same as that used for eye injections, but the volume was doubled so the total amount of APB administered in cisternal injections was equal to that administered in binocular eye injections. Bar = 100 μm.

activity between the two eyes is disrupted pharmacologically by monocularly silencing either retinal waves (*4*) or retinal ganglion cell action potentials (*5*), the silenced eye loses territory in the LGN, while the normal eye's projection expands.

Previous studies have concentrated on the initial establishment of segregation. If binocular activity blockade is begun early in development when the afferents serving the two eyes still have overlapping projection patterns, eye-specific axonal segregation is prevented or retarded (*3–5*). To investigate whether activity-dependent competition is necessary not only for establishment of eye-specific segregation but also for its maintenance, we blocked binocular retinal activity beginning after the initial formation of eye-specific layers in the LGN.

In the ferret LGN, eye-specific segregation of retinal axons is complete by postnatal day (PND) 9 (*2*) (Fig. 1A). Further segregation of afferents into ON- and OFF-center sublaminae is complete by PND 25 (*2*) (Fig. 1B). Binocular activity from PND 9 to PND 25 was blocked in six ferrets by daily binocular intravitreal injections of a high concentration of the glutamate analog 2-amino-4-phosphonobutyric acid (APB) (*6*). To ensure full blockade of both ON- and OFF-center retinal ganglion cell activity, a retinal concentration of 3.5 mM APB was used. This concentration was found to block all activity in the LGN of even relatively mature (PND 30) ferrets for 24 hours or more (Fig. 2, A and B) and much lower doses of APB block spontaneous activity in the retina of young ferrets (*7*) (Fig. 2C). The chronic APB treatment did not appear to produce any morphological damage to the developing retina (*8*) (Fig. 2D). Daily eye injections of saline or of relatively low dosages of APB (700 μM) (*9*) or systemic injections of APB did not affect development of the retinogeniculate afferent projection (Fig. 2E).

After the APB-induced binocular activity blockade from PND 9 to 25, the pattern of retinal ganglion cell arborization in the LGN (*10*) was very different from that seen in normal PND 25 animals (compare Fig. 3A and Fig. 1B). Instead of the normal pattern of eye-specific segregation into lamina A (contralateral) and lamina A1 (ipsilateral), the LGNs of APB-treated ferrets show spatially identical contralateral and ipsilateral projection patterns, indicating complete overlap of the axons from the two eyes (Fig. 3). This intermingling of inputs from the two eyes cannot represent the preservation of an immature, unsegregated projection pattern, because the afferents were already segregated into eye-specific layers at PND 9 (Fig. 1A). Instead, activity blockade caused a desegregation of afferents that were previously segregated.

APB-treated LGNs showed a lack of the interlaminar space (*11*) (Fig. 4) similar to that seen when animals were binocularly (*12*) or monocularly (*13*) enucleated before development of LGN lamination. In the present experiments, however, a faint interlaminar space is already present at PND 9 (*2*) (Fig. 4A) when the APB treatment began, indicating that maintenance of the interlaminar space, like that of axon segregation, is activity dependent.

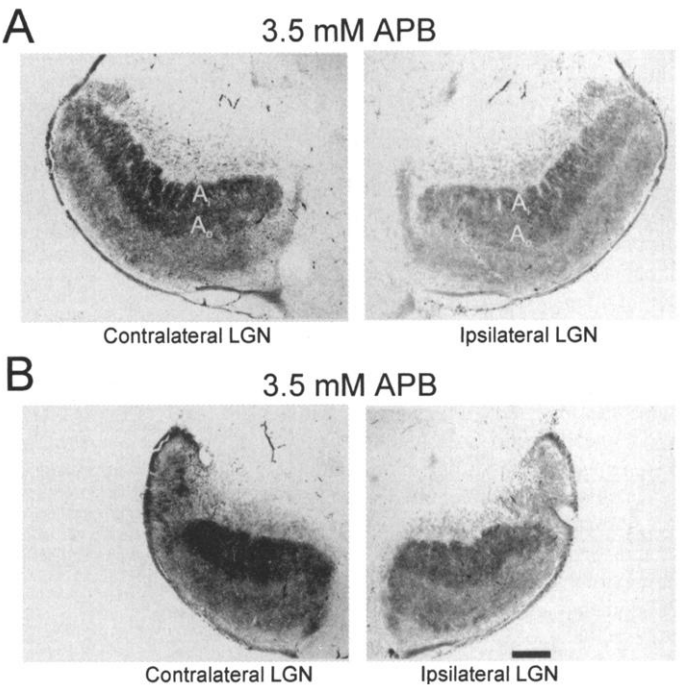

Fig. 3. Effects of APB-induced retinal activity blockade on eye-specific segregation of afferents in the LGN. Retinogeniculate afferents were labeled by anterograde transport of WGA-HRP. (**A**) Horizontal sections from the LGN contralateral (left) and ipsilateral (right) to the injected eye show the same pattern of afferent projection. Cross-sectional areas of the ipsilateral and contralateral projections to lamina A measured in 10 horizontal sections through the middle of LGNs from four APB-treated animals were statistically indistinguishable (two-tailed t test; $P < 0.001$) from each other and from the area occupied by the contralateral projection to lamina A in similar sections from four normal ferrets [APB contralateral, 0.56 ± 0.06 mm^2; APB ipsilateral, 0.49 ± 0.08 mm^2; normal contralateral, 0.52 ± 0.07; normal ipsilateral (lamina A1), 0.18 ± 0.04]. Faint segregation of afferents into inner (A_i) and outer (A_o) leaflets can be seen in both eyes' projections. Staining was always darker contralateral to the injected eye, suggesting that the contralateral projection remains stronger than the ipsilateral projection. (**B**) Sections from the ventral third of the LGN of another ferret again show identical projection patterns contralateral (left) and ipsilateral (right) to the injected eye. This abnormal lack of segregation was found in all six animals studied throughout the depth of the LGN. Bar = 100 μm.

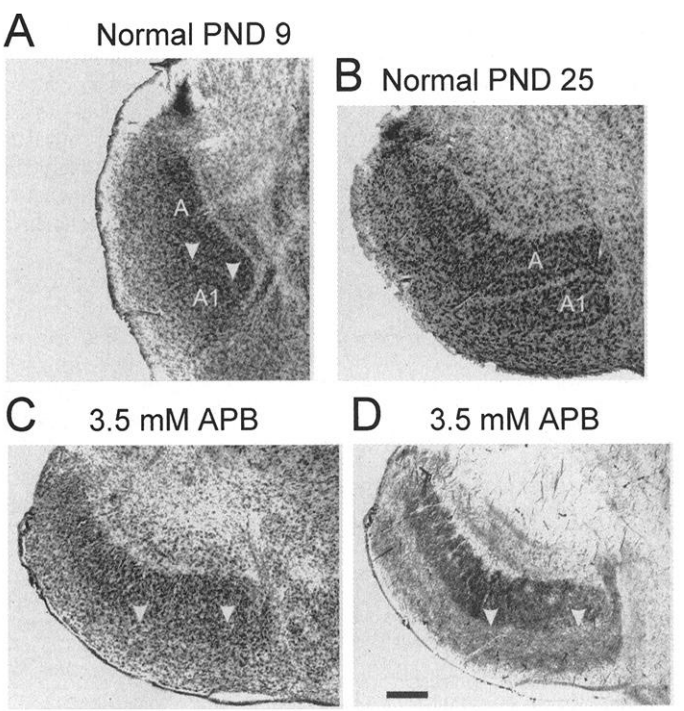

Fig. 4. APB-induced retinal activity blockade disrupts development of the interlaminar space in ferret LGN. (**A**) Faint cell-sparse space between lamina A and lamina A1 can be seen in normal PND 9 ferret LGN (arrows). (**B**) By PND 25, the interlaminar space is clear. (**C**) APB-treated ferrets show no interlaminar space on PND25. Nissl-stained section with arrows marking the apparent A/A1 border, as shown by the pattern of the afferent projection seen in an adjacent WGA-HRP section from the same LGN (**D**). Bar = 100 μm.

Despite the lack of eye-specific segregation of afferents after PND 9 to 25 activity blockade, the afferent projections were stereotyped in their patterning and did not represent a random diffuse projection to the entire LGN as when activity is blocked before development of segregation. Instead, the projections from both eyes were concentrated in a region similar to the normal location of lamina A (Fig. 4, C and D) and appeared to avoid lamina A1, indicating a substantial expansion and relocation of the afferent projection from the ipsilateral eye. This spatially restricted but unsegregated pattern suggests that there might be activity-independent cues in lamina A that are relatively attractive to axons from both eyes. In normal animals, activity-dependent competition occurs, and contralateral axons appear to have a competitive advantage, allowing them to take over the attractive real estate of lamina A and force the ipsilateral axons into lamina A1. Without competition, both eyes' axons would have equal ability to arborize in lamina A and select this region preferentially over lamina A1. The preferential arborization of axons in lamina A seen in this study was not observed in previous studies in which the development of segregation was prevented or retarded by activity blockade or enucleation before establishment of eye-specific layers (*3–5, 12, 13*). This difference could be explained if a preference for lamina A is established during the initial axonal segregation (PND 0–9) and requires a period of normal neuronal activity.

This study indicates that activity-dependent competition is vital not only for initial establishment of specific connections in the mammalian visual system but also for maintenance of these connections at least for some time during development. The possibility of attractive molecular cues or gradients in lamina A and the interactions between such cues and activity-dependent competition in normal development remain important open questions.

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6. Concerns about toxicity in the very young ferrets made the use of the sodium channel blocker tetrodotoxin impractical as a method for blocking retinal ganglion cell activity. All surgeries were performed according to protocols approved by the University of California at Davis Animal Care and Use Committee. Daily (24 ± 2 hours) eye injections of APB were done. Isofluorane anesthesia was used. Injections were made just posterior to the scleral margin with a 33-gauge needle on a Hamilton syringe. All subsequent injections were made into the same hole. APB dosages were calculated as described elsewhere (*9*). Injection volumes ranged from 1 to 2.8 μl. After the injections, animals received antibiotic ophthalmic ointment (Chloroptic) and prophylactic broad-spectrum antibiotics intramuscularly (Flocillin). The animals were returned to their mothers and littermates as soon as they awoke from the anesthesia (3 to 5 min).
7. In the adult retina, APB is a quite specific blocker of the ON-center pathway (*14*). However, in very young ferrets (PND 5 to 7) calcium imaging in vitro demonstrates that even very small concentrations of APB (1 μM) block all retinal ganglion cell spontaneous activity (Fig. 2). By PND 21, low concentrations of APB begin to have a more selective effect, but concentrations that fully block ON-center ganglion cells still decrease activity in OFF-center cells (*15*). The mechanisms by which APB blocks OFF-center activity in the young ferret retina are unknown.
8. APB-injected and normal age-matched control animals were euthnanized and perfused with 4% paraformaldehyde. Retinae were dissected out of the eyes, 1-mm "punches" were embedded in 5% agar, and 30-μm cross sections were cut on a vibratome. Sections were mounted on slides, stained for Nissl substance with thionin, cover-slipped, and photographed.
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10. Five microliters of 5% wheat germ agglutinin–horseradish peroxidase (WGA-HRP) (Sigma) was injected on PND 23 over a period of 5 min, following the same injection protocol used for the APB injections. Animals were euthanized 48 hours after the WGA-HRP injections and perfused with 4% paraformaldehyde. LGNs were embedded in gelatin/albumin and 50-μm vibratome sections were cut. Floating sections were reacted for HRP with a TMB protocol. Sections were mounted on slides, dried overnight, dehydrated, cover-slipped, and photographed. Ipsilateral and contralateral projections were measured with Scion Image software.
11. Animals were euthanized and perfused with 4% paraformaldehyde. LGNs were embedded in gelatin/albumin and 50-μm vibratome sections were cut. Sections were mounted on slides, dried overnight, dehydrated, Nissl-stained with cresyl violet, cover-slipped, and photographed.
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Structure of the RNA Polymerase Domain of *E. coli* Primase

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All cellular organisms use specialized RNA polymerases called "primases" to synthesize RNA primers for the initiation of DNA replication. The high-resolution crystal structure of a primase, comprising the catalytic core of the *Escherichia coli* DnaG protein, was determined. The core structure contains an active-site architecture that is unrelated to other DNA or RNA polymerase palm folds, but is instead related to the "toprim" fold. On the basis of the structure, it is likely that DnaG binds nucleic acid in a groove clustered with invariant residues and that DnaG is positioned within the replisome to accept single-stranded DNA directly from the replicative helicase.

All known polymerases synthesize nucleic acid in a 5′ to 3′ direction. This feature requires that the two antiparallel strands of DNA be replicated asymmetrically: The "leading" strand is made continuously at the replication fork, whereas the "lagging" strand is formed discontinuously. Because DNA polymerases are incapable of de novo initiation, cells use other mechanisms to prime DNA synthesis throughout replication. In 1971, Kornberg and co-workers proposed that DNA replication initiation required RNA transcription (*1*). Since then, replication-priming RNA polymerases (primases) have proven central to cellular and many viral replication mechanisms. Primases initiate leading-strand synthesis once and lagging-strand synthesis multiple times during the course of replication. Depending on the organism, primases exist either as individual proteins or as primase-helicase polyproteins; in almost all cases their activities are coupled to the replisome by protein-protein interactions with other replication factors (*2*).

Escherichia coli primase (DnaG) interacts with the replicative DnaB helicase, single-

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