Eds. (Springer-Verlag, New York, 1986), pp. 412–424.
15. V. Siegel and P. Walter, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1801 (1988); X. Liao, P. Brennwald, J. A. Wise, *ibid.* **86**, 4837 (1989).
16. Y. Endo, Y. L. Chan, A. Lin, K. Tsurugi, I. G. Wool, *J. Biol. Chem.* **263**, 7917 (1988).
17. The GCAA and GAAA hairpins were synthesized and purified as described [J. F. Milligan, D. R. Groebe, G. W. Witherell, O. C. Uhlenbeck, *Nucleic Acids Res.* **15**, 8783 (1987); H. A. Heus and A. Pardi, *J. Mol. Biol.* **217**, 113 (1991)] and dialyzed in 10 mM sodium phosphate, 100 mM sodium chloride, 0.2 mM EDTA, pH 7.0.
18. We thank O. C. Uhlenbeck for numerous discussions, F. Jucker for purification of the GCAA hairpin, M. Rance for collection of several of the NMR spectra, P. Yip for structure calculations, and L. Moon-McDermott for preparation of some of the 7-deazaadenine RNA oligomers. Supported by NIH grants AI 27026 and AI 30726. The NMR spectrometer was purchased with partial support from NIH grant RR03283. We also thank the W. M. Keck Foundation for support of RNA science at the University of Colorado at Boulder. This report is dedicated to the memory of Peter (Piet) H. Van Knippenberg, who devoted much of his career to the study of a member of the GNRA family, the GGAA hairpin loop.

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Organizer-Specific Homeobox Genes in *Xenopus laevis* Embryos

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The dorsal blastopore lip of the early *Xenopus laevis* gastrula can organize a complete secondary body axis when transplanted to another embryo. A search for potential gene regulatory components specifically expressed in the organizer was undertaken that resulted in the identification of four types of complementary DNAs from homeobox-containing genes that fulfill this criterion. The most abundant of these encodes a DNA-binding specificity similar to that of the *Drosophila melanogaster* anterior morphogen *bicoid*. The other three are also homologous to developmentally significant *Drosophila* genes. These four genes may participate in the regulation of the developmental potential of the organizer.

THE DORSAL BLASTOPORE LIP FROM an early salamander gastrula, when implanted into the ventral side of a recipient gastrula, can organize the formation of a secondary body axis consisting of both host and graft-derived tissue (*1*). The dorsal blastopore lip was called the "organizer" to reflect its ability to recruit or organize host cells to form a secondary axis with appropriate anterior-posterior and dorsal-ventral polarity. The anterior-posterior extent of the secondary axis induced by the transplanted dorsal lips differed as gastrulation proceeded; dorsal lips from early gastrulae could induce nearly complete axes including heads, whereas dorsal lips from late gastrulae induced axes consisting of trunk and tail (*2*). The biochemical basis of the organizer phenomenon has, however, remained elusive, despite intensive investigation (*3*).

Experiments have shown that peptide growth factors related to transforming growth factor–β (TGFβ, XTC-MIF, activin) and basic fibroblast growth factor can induce mesoderm formation in uncommitted ectoderm and even confer organizer activity on treated ectoderm in transplantation experiments (*4*). Activin protein can organize body axis in uncommitted ectoderm and activin mRNA, injected into a single vegetal blastomere, can organize a secondary body axis (*5*). Although it is not known whether any of these molecules is a natural inducer in vivo, they are believed to be, or be related to, the actual molecules involved in mesoderm induction. The exact relation between mesoderm induction and the organizer phenomenon is not clear at present. However, these growth factors have been shown to influence the expression of several position-specific homeobox genes (*6*). Therefore, it has been postulated that mesoderm-inducing growth factors may provide positional information along the anterior-posterior axis by the regulation of the expression of homeobox genes (*6*).

Although single growth factors may induce a variety of mesodermal structures and even confer organizer-like activity on uncommitted ectoderm or microinjected blastomeres, the organizer's actual mode of action is likely to involve a number of molecules that jointly provide the spectrum of activities necessary for the induction of a body axis. That localized positional information exists in the organizer is indicated by experiments in which the salamander dorsal lip was divided into a number of fragments and cultured separately. The types of tissues formed by each fragment depended on its original location along the longitudinal axis in the dorsal lip (*7*). The quantity of organizer tissue in a *Xenopus laevis* embryo is directly proportional to the extent of anterior development (*8*). Thus, it can be inferred that the inductive potential of the organizer is dependent on both regional specialization in, and the overall size of, the dorsal blastopore lip.

We investigated the molecular basis of the organizer by isolating mRNA from excised dorsal lips [at stage (st) 10.25 gastrula when the dorsal lips are capable of inducing a complete secondary axis] (Fig. 1A). We then constructed a cDNA library from this mRNA, which is presumably enriched in molecules encoding proteins that provide positional information specific for the organizer, and screened for molecules that are likely to be involved in conferring organizer activity. As a first step in understanding what underlies regional specialization in the organizer, we decided to study a well-characterized family of genes known to be developmentally significant—those containing a homeobox. The homeobox is a DNA se-

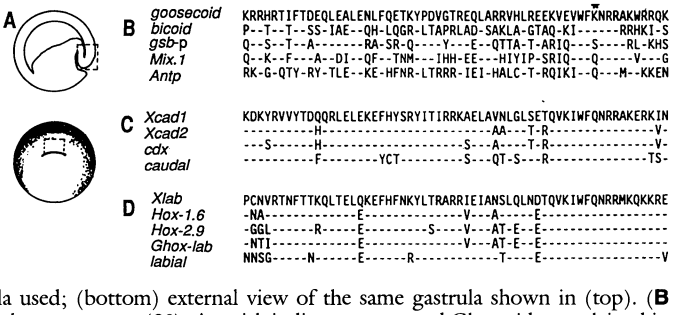

Fig. 1. Identification of the *Xenopus* organizer-specific homeobox. (**A**) Diagrammatic representation of the area used in the construction of the dorsal lip library. Dashed area indicates the extent of the dissected area used in the construction of the library. (Top) Cross-sectional view of the st 10.25 gastrula used; (bottom) external view of the same gastrula shown in (top). (**B** through **D**) Dorsal lip homeobox sequences (*29*). Asterisk indicates conserved Glu residue explained in text. Except where indicated, homeobox sequences used for comparison were derived from Scott and co-workers (*11*). (B) The relations among the products of *goosecoid* and related genes: *Mix.1* (*15*), *gooseberry*-proximal, *bicoid*, and *Antennapedia*. (C) The relations among the products of *Xcad1*, *Xcad2*, and the related genes *caudal* and *cdx* (*18*). (D) The products of *Xlab* and the related genes *Hox-1.6*, *Hox-2.9* (*37*), *labial*, and *GHox-lab* (*19*).

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quence, first found in homoeotic genes of *Drosophila melanogaster*, that encodes a conserved sequence of 60 amino acids called the homeodomain (*9*). Proteins containing a homeodomain are transcription factors that are expressed in temporally and spatially restricted patterns during development and have been implicated in the regulation of cell identity (*10, 11*). If they were found specifically in the early gastrula dorsal lip, they would represent good candidates for providing or interpreting positional information in the organizer.

We screened the dorsal lip cDNA library with a degenerate oligonucleotide that recognizes a variety of homeobox genes (*12*). We identified 30 cDNAs containing recognizable homeoboxes and classified them according to their amino acid sequence (*13*). The most abundant type (23 out of 30 clones) was named *goosecoid* to reflect its resemblance, in regions of the homeodomain, to two *Drosophila* genes, the segmentation gene *gooseberry* and the anterior morphogen *bicoid* (*14*) (Fig. 1B). The *goosecoid* homeobox is most similar to that of *gooseberry* (57% identical) but divergent from the *Antennapedia* homeobox (32% identical). The *goosecoid* homeobox is similar to the homeobox of *Mix.1* (53% identical), which is one of the first *Xenopus* genes activated after the onset of zygotic transcription and is expressed as a primary response (there is no need for ongoing protein synthesis) to growth factor treatment in isolated animal cap ectoderm (*15*). There is no obvious similarity between *goosecoid* and any other gene outside of the homeobox (*16*). An analysis of the 1.3-kb *goosecoid* mRNA expression shows that it is first detectable at st 8.5, peaks in quantity at st 10.5, and is much reduced by the beginning of neurulation (st 13) (Fig. 2A) (*16*). The *goosecoid* mRNA is enriched in manually dissected dorsal lips (Fig. 2A, right panel). The temporal pattern of *goosecoid* mRNA expression is similar to that of *Mix.1*, and, like *Mix.1*, *goosecoid* expression is a primary response to growth factor treatment (*17*); however, *Mix.1* is not localized to the dorsal lip but rather is expressed predominantly in the prospective endoderm at this stage (*15*).

Six cDNA clones were isolated that correspond to two different *Xenopus* genes similar to the *Drosophila* gene *caudal*. Four compose one class that is most similar to the mouse *cdx* gene (*18*); this class is designated *Xcad1* (Fig. 1C). The other two cDNAs define a different class of *caudal*-related genes and are designated *Xcad2*. The 2.3-kb *Xcad1* and the 4.5-kb *Xcad2* mRNAs are first detectable by Northern analysis in the early gastrula (st 10.5), reach maximal quantities at about st 13, and decrease to low amounts after st 15 (Fig. 2C). Both *Xcad1* and *Xcad2* mRNAs are enriched in manually dissected dorsal lips when compared with the rest of the gastrula (Fig. 2C, right panel).

One cDNA was isolated that is related to the *Drosophila* gene *labial*, the mouse *Hox-2.9* and *Hox-1.6* genes, and the chicken gene *GHox-lab* (Fig. 1D). We have designated this gene *Xlab*. *Xlab* mRNA is first detectable by Northern analysis at st 10.5, peaks at midgastrula (st 11.5), and is also enriched in the dorsal lip (Fig. 2B). The temporal and spatial distribution of *Xlab* is similar to that of *GHox-lab*, which is expressed in high amounts by the midgastrula stage in Hensen's node (the organizer equivalent of the chicken embryo) and in the primitive streak (*19*). *Xlab* is not similar enough to any single vertebrate *labial*-related gene to allow its assignment to a particular *Hox* cluster.

The homeobox-containing cDNAs that we isolated from the dorsal lip library all appear to be enriched in the dorsal blastopore lip (Fig. 2). This is not an artifact of the methods used because we have isolated other, nonhomeobox cDNAs from this library whose mRNAs are expressed during gastrulation but are not limited to the dorsal lip (*16*). We did not isolate cDNAs encoding other *Xenopus* homeobox genes reported to be expressed at this stage of gastrulation [*Mix.1* (*15*), *Xhox1A*, and *Xhox3* (*20*)]. It is not known where *Xhox1A* and *Xhox3* mRNAs are localized in the early gastrula, whereas *Mix.1* does not appear to be localized to the dorsal lip (*15*). Although our screen could have missed rare cDNAs, this result provides further molecular evidence that molecules encoding potential positional information are spatially restricted in the early *Xenopus* gastrula and probably reflects the special nature of the organizer, as compared with the rest of the embryo.

The majority of homeodomain proteins isolated to date contain a Gln residue at position 50 of the homeodomain (indicated by an asterisk in Fig. 1B). At this position, the *bicoid* protein has a Lys residue that causes its DNA-binding specificity to differ from that of other homeodomains (*21*). The substitution of Lys by Gln at this position in the *bicoid* protein homeodomain is sufficient to replace the *bicoid* protein DNA-binding specificity with that of the *Antennapedia* protein (*21*). Because the *goosecoid* protein homeodomain contains a Lys residue at position 50, we investigated whether the *goosecoid* protein could bind to a *bicoid* protein–binding site (*22*). A fusion protein containing the *goosecoid* protein homeodomain specifically bound to an oligonucleotide containing the *bicoid* protein–binding site (Fig. 3), which suggests that the

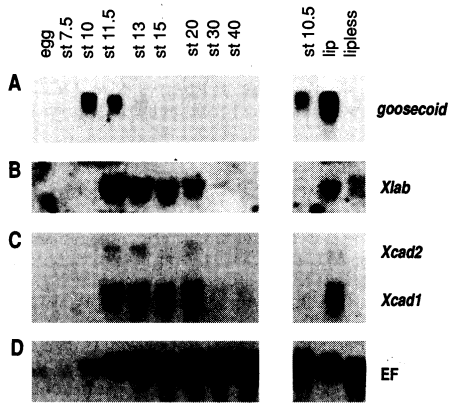

Fig. 2. Developmental expression of *Xenopus* organizer-specific homeobox genes. Northern analysis of *Xenopus* RNA from specific developmental stages probed with homeobox gene probes. Equal amounts of total RNA were loaded on each lane. The probes used were the following: (**A**) *goosecoid*, (**B**) *Xlab*, (**C**) *Xcad1* and *Xcad2*, and (**D**) the gene for *Xenopus* EF-1α. All of the homeobox transcripts are localized in a the dorsal lip at st 10.5; however, *Xlab* and *Xcad* mRNAs are just beginning to accumulate at this stage, whereas the quantity of *goosecoid* mRNA peaks at this stage. In each panel one blot was sequentially hybridized with the indicated probes. Egg, unfertilized egg; st 7.5, early blastula; st 10, early gastrula; st 11.5, midgastrula; st 13, early neurula; st 15, midneurula; st 20, late neurula; st 30, tailbud; st 40, swimming tadpole; st 10.5, gastrula; lip, from the dorsal lip; and lipless, from all areas of the gastrula except the dorsal lip.

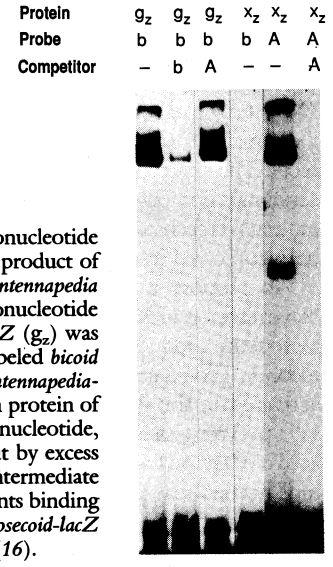

Fig. 3. The *goosecoid* protein binds preferentially to an oligonucleotide containing a *bicoid* protein DNA-binding site. The fusion protein product of *goosecoid*, but not of the *XlHbox1* protein (which contains an *Antennapedia* type homeodomain), retards the *bicoid* product–binding site oligonucleotide (b). The retardation by the fusion protein product of *goosecoid-lacZ* (g_z) was competed out by the addition of a 500-fold molar excess of unlabeled *bicoid* competitor oligonucleotide but not by an equal amount of *Antennapedia*-binding site oligonucleotide (A). Equivalent amounts of the fusion protein of *XlHbox1-lacZ* (x_z) were capable of binding the *Antennapedia* oligonucleotide, but not the *bicoid* oligonucleotide. This binding was competed out by excess unlabeled *Antennapedia* oligonucleotide. The retarded band of intermediate mobility present in the *XlHbox1-lacZ* preparation probably represents binding by a partially degraded protein. The fusion protein product of *goosecoid-lacZ* binds the *Antennapedia* oligonucleotide weakly, but reproducibly (*16*).

goosecoid and *bicoid* proteins can bind to similar DNA target sequences.

The similarity between *goosecoid* and *bicoid* may be significant, considering the role of *bicoid* in *Drosophila* development. The *bicoid* mRNA is localized to the anterior pole of the *Drosophila* egg, and its translation after fertilization results in a gradient of *bicoid* protein (*23*). The different threshold responses of subordinate genes to the concentration of *bicoid* protein are responsible for the generation of pattern in the anterior of the embryo (*24*). The injection of synthetic *bicoid* mRNA into *Drosophila* eggs can lead to the formation of an ectopic anterior signal, resulting in the formation of secondary anterior structures, including heads (*24*). These properties underscore the significance of the *bicoid* protein in development. Despite investigation at a number of laboratories, no vertebrate *bicoid* homolog has yet been reported. To our knowledge, *goosecoid* is the first example of a vertebrate gene that shows any functional similarity to *bicoid*. The *bicoid* protein is a maternal gene product and exerts its concentration-dependent morphogenetic effects in the syncytial *Drosophila* embryo, before the formation of cells interferes with the diffusion of macromolecules. In contrast, *goosecoid* is presumably expressed in a small subset of cells beginning at the blastula stage. Thus, its concentration and any associated dependent morphogenetic effects cannot be explained by a diffusion model as for the *bicoid* protein. However, the early onset of *goosecoid* mRNA expression, its localization to the organizer region, and its functional similarity to *bicoid* lead us to believe that *goosecoid* plays a central role in the initial events of axis formation in vertebrates.

The homologies between homeobox genes expressed predominantly in the *Xenopus* dorsal lip and anteriorly expressed (*bicoid* and *labial*) and posteriorly expressed (*caudal*) *Drosophila* genes may reflect the different inductive capacities of certain regions of the invaginating dorsal lip. The *Xenopus* embryo may first specify the ends of the body axis in the dorsal lip and later interpolate between these ends to generate the remainder of the axis as gastrulation proceeds. The expression during axis formation of vertebrate homeobox genes suggests that the molecular mechanism to specify position has been conserved in evolution.

We propose a mechanism for body axis determination in *Xenopus*. As a result of dorsal mesoderm induction, early-response homeobox genes, such as *goosecoid*, would be activated in the future location of the dorsal lip. The *goosecoid* gene would, in turn, activate various subordinate genes, including homeobox genes such as *Xlab* and *Xcad*, that further refine the axis. Observations that overexpression of homeobox genes in uncommitted embryonic cells can give the cells axis-forming activity (*25*) are consistent with this hypothesis.

REFERENCES AND NOTES

1. H. Spemann and H. Mangold, *Wilhelm Roux Arch. Entwicklungsmech. Org.* **100**, 599 (1924).
2. H. Spemann, *ibid.* **123**, 389 (1931).
3. O. Nakamura and S. Toivonen, Eds., *Organizer: A Milestone of a Half Century From Spemann* (Elsevier North-Holland, New York, 1978); V. Hamburger, *The Heritage of Experimental Embryology* (Oxford Univ. Press, New York, 1988); J. B. Gurdon, *Development (Cambridge)* **99**, 285 (1987).
4. J. C. Smith, *Development (Cambridge)* **105**, 665 (1989); I. B. Dawid, T. D. Sargent, F. Rosa, *Curr. Top. Dev. Biol.* **24**, 261 (1989); A. Ruiz i Altaba and D. A. Melton, *Trends Genet.* **6**, 57 (1990).
5. S. Sokol, G. G. Wong, D. A. Melton, *Science* **249**, 561 (1990); G. Thomsen *et al.*, *Cell* **63**, 485 (1990).
6. A. Ruiz i Altaba and D. A. Melton, *Nature* **341**, 33 (1989); K. W. Y. Cho and E. M. De Robertis, *Genes Dev.* **4**, 1910 (1990).
7. H. B. Holtfreter, thesis, University of Rochester (1965).
8. R. M. Stewart and J. C. Gerhart, *Development (Cambridge)* **109**, 363 (1990).
9. W. McGinnis, M. S. Levine, E. Hafen, A. Kuriowa, W. J. Gehring, *Nature* **308**, 428 (1984); M. P. Scott and A. J. Weiner, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4115 (1984).
10. C. V. E. Wright, K. W. Y. Cho, G. Oliver, E. M. De Robertis, *Trends Biochem. Sci.* **14**, 52 (1989); J. B. Jaynes, J.-P. Vincent, P. H. O'Farrell, in *Genetics of Pattern Formation and Growth Control*, A. P. Mahowald, Ed. (Wiley-Liss, New York, 1990), pp. 47–64.
11. M. P. Scott, J. W. Tamkun, G. W. Hartzell III, *Biochim. Biophys. Acta* **989**, 25 (1989).
12. Dorsal lips, 30° of arc containing superficial and deep mesoderm, were isolated from st 10.25-10.5 gastrulae (*26*) (Fig. 1A). Three hundred dorsal lips yielded 80 μg of total RNA. Total RNA (50 μg) was annealed to an oligonucleotide [ACTAGTGCGGCCGCCTAGGCCTCGAG$(T)_{15}$], and we prepared first strand cDNA with reverse transcriptase from avian myeloblastosis virus (Seikagaku America) in the presence of 5-methyl–deoxycytidine triphosphate (to protect internal Xho I sites) (*27*) and converted the cDNA to double-stranded cDNA (*28*). The cDNA was made blunt-ended with T4 DNA polymerase (Pharmacia), internal Eco RI sites were protected with Eco RI methylase (Pharmacia), and Eco RI linkers were attached. After Eco RI and Xho I digestion and chromatography on Sepharose CL-4B (Pharmacia) to remove linkers and small molecules, we ligated the cDNA to λZAPII (Stratagene) digested with Eco RI and Xho I. We then packaged the phage in vitro with Gigapack II Gold Stratagene). The library consists of 6.5×10^6 independent clones, of which 3×10^6 were amplified. We screened 50,000 unamplified clones with a mixture of 1024 oligonucleotides [C(G,T)(A,C,G,T)C(G,T)(A,G)TT(C,T)T(G,T)(A,G)AACCA(A,G)AT(C,T)TT] that are complementary to all possible variations of the DNA sequence encoding the conserved amino acid sequence KIWF(Q/K)NRR (*29*); we used the tetramethylammonium chloride method (*30*), plaque-purified the clones, and converted them to plasmids by the automatic excision process (*31*). We sequenced plasmid DNA with the use of a shorter oligonucleotide [C(G,T)(A,C,G,T)C(G,T)(A,G)TT(C,T)T(G,T)(A,G)AACCA], corresponding to [WF(Q/K)NRR] (*29*) as a primer, and then further characterized the DNA by sequencing with an oligonucleotide located 5′ to the homeobox to read back across the homeobox. For RNA-blot analysis, 20 μg of total RNA from embryos of specific stages were denatured with 10 mM methyl mercuric hydroxide, separated by electrophoresis in 1% agarose–0.66 M formaldehyde gels, and transferred to nitrocellulose filters. Filters were sequentially hybridized with ^{32}P-labeled probes corresponding to individual cloned dorsal lip cDNA sequences and *Xenopus* elongation factor (EF) 1α (*32*) in 0.5 M sodium phosphate (pH 7.2), 7% SDS, and 5% w/v dextran sulfate at 65°C; washed at 65°C in 0.015 M sodium chloride, 0.0015 M sodium citrate, and 0.2% SDS; and autoradiographed at −70°C with two intensifying screens.
13. The sequences reported here have been submitted to the GenBank/EMBL data bank and are available under the following accession numbers: *goosecoid* (complete), M63782; *Xlab* (complete), M63873; *Xcad1* (partial), M63874; and *Xcad2* (partial), M63875.
14. D. Bopp, M. Burri, S. Baumgartner, G. Frigerio, M. Noll, *Cell* **47**, 1033 (1986); G. Frigerio, M. Burri, D. Bopp, S. Baumgartner, M. Noll, *ibid.*, p. 735.
15. F. M. Rosa, *ibid.* **57**, 965 (1989).
16. K. W. Y. Cho, B. Blumberg, E. M. De Robertis, unpublished data.
17. ———, in preparation.
18. P. Duprey *et al.*, *Genes Dev.* **2**, 1647 (1988).
19. O. H. Sundin, H. G. Busse, M. B. Rogers, L. J. Gudas, G. Eichele, *Development (Cambridge)* **108**, 47 (1990); O. H. Sundin and G. Eichele, *Genes Dev.* **4**, 1267 (1990).
20. R. P. Harvey, C. J. Tabin, D. A. Melton, *EMBO J.* **5**, 1237 (1986); A. Ruiz i Altaba and D. A. Melton, *Development (Cambridge)* **106**, 173 (1989).
21. S. D. Hanes and R. Brent, *Cell* **57**, 1275 (1989); J. Treisman, P. Gonczy, M. Vashishtha, E. Harris, C. Desplan, *ibid.* **59**, 553 (1989).
22. The construction and isolation of the fusion protein from the *XlHbox1* were previously described (*33*). We constructed a fusion protein from the *goosecoid* gene in a similar manner by fusing the *goosecoid* homeobox to the vector pTRBO (*34*). Gel retardation assays were performed exactly as described (*35*). The *Antennapedia*-type oligonucleotide recognized by the fusion protein of the *XlHbox1* gene is GATCGCAATTAAACTATAAAGCAATT and was derived from a binding site for the *XlHbox1* protein in *Xenopus Hox*-complex DNA (*33*). The double-stranded *bicoid* oligonucleotide was GATCCCATCTAATCCCTTGACG, which contains the highest affinity DNA-binding site for the *Drosophila bicoid* protein in the promoter of the *Drosophila* gap gene *hunchback* (*36*).
23. W. Driever and C. Nüsslein-Volhard, *Cell* **54**, 83 (1988).
24. ———, *ibid.*, p. 95; W. Driever, V. Siegel, C. Nüsslein-Volhard, *Development (Cambridge)* **109**, 811 (1990).
25. K. W. Y. Cho, E. A. Morita, C. V. E. Wright, E. M. De Robertis, *Cell* **65**, 1 (1991).
26. P. O. Nieuwkoop and J. Faber, *A Normal Table of* Xenopus laevis *(Daudin)* (North-Holland, Amsterdam, 1967).
27. J. H. Han and W. J. Rutter, *Nucleic Acids Res.* **16**, 11837 (1988).
28. U. Gubler and B. J. Hoffman, *Gene (Amsterdam)* **25**, 263 (1983).
29. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
30. T. R. Bürglin, M. Finney, A. Coulson, G. Ruvkun, *Nature* **341**, 239 (1989).
31. J. Short, J. Fernandez, J. Sorge, W. Atuse, *Nucleic Acids Res.* **16**, 7583 (1988).
32. P. A. Krieg, S. M. Varnum, M. Wormington, D. A. Melton, *Dev. Biol.* **133**, 93 (1989).
33. K. W. Y. Cho *et al.*, *EMBO J.* **7**, 2139 (1988).
34. T. R. Bürglin and E. M. De Robertis *ibid.* **6**, 2617 (1987).
35. C. V. E. Wright, K. W. Y. Cho, J. Hardwicke, R. H. Collins, E. M. De Robertis, *Cell* **59**, 81 (1989).
36. W. Driever and C. Nüsslein-Volhard, *Nature* **337**, 138 (1989).
37. M. A. Frohman, M. Boyle, G. Martin, *Development (Cambridge)* **110**, 589 (1990).

38. We thank J. Lengyel, M. Carey, J. Fessler, H. Dewes, D. Blumberg, and D. Bittner for critically reading the manuscript and T. R. Bürglin for the oligonucleotide-screening protocol. Supported by postdoctoral fellowships from the NIH (HD-07273) and the Lucille Markey Charitable Trust (to B.B.); C.V.E.W. was an American Cancer Society Senior Fellow; K.W.Y.C. was a fellow of the UCLA Clinical Genetics Program; and supported by a grant (HD-21502) from the NIH (to E.M.D.R.).

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