J. Physiol. 385, 243 (1987); D. Langosch, L. Thomas, H. Betz, Proc. Natl. Acad. Sci. U.S.A., in press.
28. R. Hedrich and E. Neher, Nature 329, 833 (1987).
29. B. Katz, The Release of Neural Transmitter Substances (Thomas, Springfield, IL, 1969), The Sherrington Lectures, vol. 10, pp. 1–60.
30. As the cytoplasmic region of synaptophysin binds Ca^{2+} (9), coupling could be triggered by a depolarization-induced Ca^{2+} influx [B. Katz and R. Miledi, J. Physiol. (London) 203, 689 (1969); T. Pozzan, G. Gatti, N. Dozio, L. M. Vicentini, J. Meldolesi, J. Cell Biol. 99, 628 (1984)]. Alternatively, Ca^{2+} may affect the channel activity of synaptophysin. However, we have not been able to demonstrate a direct effect of Ca^{2+} on reconstituted synaptophysin preparations.
31. We thank B. Wiedenmann for collaboration and the SY38 antibody, E. Harbich and C. Grund for technical assistance, C.-M. Becker and E. Gundelfinger for reading of the manuscript, and J. Rami and I. Baro for help during its preparation. Supported by the Bundesministerium für Forschung und Technologie (BCT 381-5) and, in part, by the Deutsche Forschungsgemeinschaft (SFB 317 and 169). D.L. is the recipient of a predoctoral fellowship of the Fonds der Chemischen Industrie.

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The Presence of Fibroblast Growth Factor in the Frog Egg: Its Role as a Natural Mesoderm Inducer

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A complementary DNA clone corresponding to a 4.2-kilobase transcript that is present in the Xenopus oocyte and newly transcribed in the neurula stages of development has been isolated. This messenger RNA encodes a 155–amino acid protein that is 84% identical to the human basic fibroblast growth factor (bFGF). When expressed in Escherichia coli and purified, the Xenopus FGF induced mesoderm in animal cell blastomeres as measured by muscle actin expression. Immunoblots with an antibody to a Xenopus FGF peptide show that the oocyte and early embryo contain a store of the FGF polypeptide at high enough concentrations to induce mesoderm. The presence of FGF in the oocyte, together with the apparent lack of a secretory signal sequence in the protein, suggest that the regulation of mesoderm induction may involve novel mechanisms that occur after the translation of FGF.

MESODERM IN VERTEBRATE EMbryos is induced in the ectoderm by signals emanating from the underlying endoderm (1). Such inductive signals are probably responsible for much of the tissue patterning in vertebrate organisms. Although these inductive interactions are well documented by transplantation and ablation experiments, the specific signals have been very difficult to characterize. Homologs of several growth factors may be the signaling molecules; basic and acidic fibroblast growth factors (bFGF and aFGF, respectively), transforming growth factor–β2 (TGF-β2), and a partially purified low molecular weight growth factor are potent inducers of mesoderm in Xenopus (2–4). At physiological concentrations these growth factors induce the synthesis of muscle gene products and the formation of morphologically identifiable mesodermal tissues in ectodermal cells of the animal cap. Transcripts encoding molecules related to FGF and TGF-β could be found in the early embryo

(3, 5). One of these transcripts encodes a protein (Vg-1) that is a member of the TGF-β superfamily but is distinct from either TGF-β1, which can enhance the FGF-mediated induction of mesoderm (3), or TGF-β2. Early embryonic transcripts also encode a peptide that is very similar to a COOH-terminal portion of the mammalian bFGFs used in the mesoderm induction
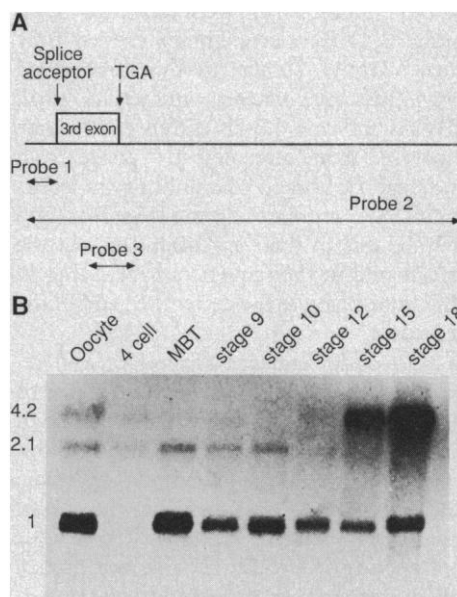
assays. Complementary DNA (cDNA) clones containing FGF sequences, however, all appeared to be copies of unspliced messages, so it was unclear if the encoded protein sequence was indeed translated as part of a complete FGF-like molecule (3).

Below we describe a cDNA clone, corresponding to a 4.2-kb transcript present in the Xenopus oocyte and at later stages, encoding a 155-residue protein that is closely related to human bFGF and that can induce the synthesis of muscle actin mRNA in animal cap explants. In addition, we show that the unfertilized egg contains an FGF polypeptide, suggesting that the regulation of induction may involve post-translational events.

We previously isolated a Xenopus oocyte cDNA that contained sequences similar to the human and bovine bFGF genes (3). This clone hybridizes to a 1-kb RNA that is present in the oocyte and in the midblastula to neurula stages. Although this RNA is large enough to encode a protein the size of bFGF, it lacks sequences similar to the first and second exons of human bFGF (6) and contains only a short open reading frame encoding a polypeptide domain homologous to the third exon of mammalian bFGFs [Fig. 1A, (3)]. It is unlikely that this RNA could encode a truncated form of FGF, since there are no in-frame potential translation initiation (ATG) codons upstream of the small coding region. In RNA blot analysis, a probe derived from the presumed noncoding sequences 5' of the FGF third exon (Fig. 1A, probe 1) hybridized to the 1-kb mRNA, as expected if the cDNAs represent copies of this mRNA. The existence of this major noncoding RNA is intriguing and is currently the subject of further study.

With a probe corresponding to the entire

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Fig. 1. (A) DNA probes used for RNA blots and screening of the cDNA libraries. The 1.3-kb cDNA isolated previously (3) is shown, with the potential splice acceptor site and the open reading frame region homologous to the third exon of the mammalian bFGFs indicated. The TGA signifies the stop codon, coincident with the site of translation termination in human and bovine bFGF (6, 9). Probe 1 is a 105-bp Mnl I fragment. Probe 2, which consists of the entire cDNA clone, is a 1.3-kb Eco R1 fragment. Probe 3 is a 175-bp Pvu II–Ssp I fragment. (B) Analysis of RNA from various stages of Xenopus development. Polyadenylated RNA was isolated from 50 oocytes or embryos at different stages (22). The RNA was separated on a 1% formaldehyde-agarose gel and blotted onto Hybond-N (Amersham). The membrane was hybridized as previously described (3) with probe 3 labeled with ^{32}P (3000 Ci/mmol), and the filter was washed and exposed for 2 weeks. The sizes of the three transcripts (4.2, 2.1 and 1 kb) were determined relative to λ Hind III markers loaded in a parallel lane on the gel and separately hybridized to a λ DNA probe.

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- - - - - - - - - ⌐A L P┐ - - - ⌐S G A┐ - ⌐P┐ - - ⌐H┐ - - - - - - - - - -
M A A G S I T T L P│T E S│E D G G│N T P│F│S│P G│S│F K D P K R L Y C K
                 10      20        30


- - - - - - - ⌐H P┐ - - - - - - ⌐V┐ - ⌐E┐ - - - ⌐P┐ - ▼ - - - - - ⌐E┐ - - -
N G G F F L R I│N S│D G R V D G│S│R│D│K S D│S│H I K L Q L Q A│V│E R G
        40                50              ▲                         70


- - - - - - ⌐V C┐ - - - - - - - - - - - - ⌐L A S K┐ - ⌐V┐ - - - - - - -
                                                   ▼
V V S I K G│I T│A N R Y L A M K E D G R L│T S L R│C│I│T D E C F F F E
          80                  90                ▲        100


- - - ⌐S┐ - - - - - - - - - - - ⌐T┐ - - - - - - - - - - - - ⌐L┐ - - ⌐K┐ - -
R L E│A│N N Y N T Y R S R K Y│S│S W Y V A L K R T G Q Y K│N│G S│S│T G
    110                  120                  130                      140


- - - - - - - - - - - - - - - -
P G Q K A I L F L P M S A K S
            150
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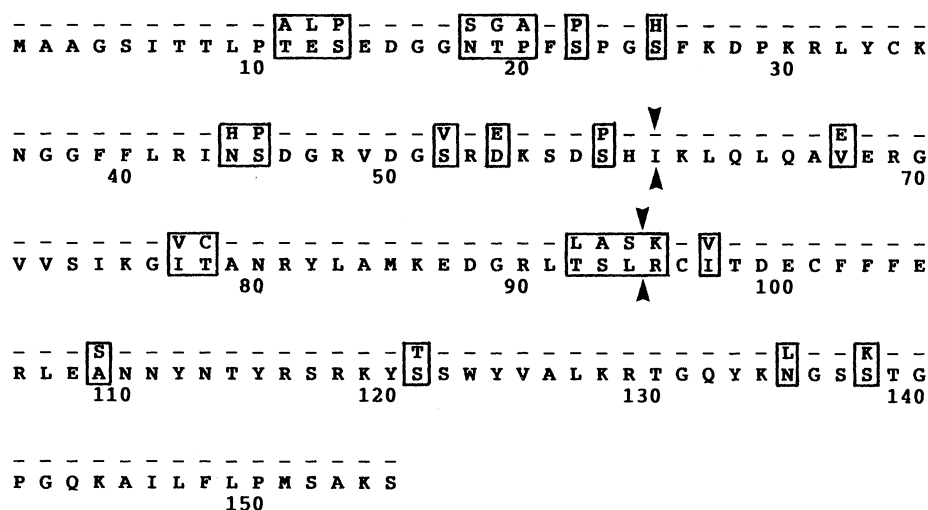
Fig. 2. Comparison of *Xenopus* bFGF with human bFGF. The amino acid sequence of the 155-residue form of human bFGF (*6*) is aligned above the sequence encoded by the *Xenopus* cDNA clone; a dash in the human sequence indicates that the amino acid at that position is identical to the residue in the *Xenopus* sequence. Differences in the two sequences are boxed. The arrowheads indicate the locations of the introns so far identified in the genomic sequences of the two species. The sequence of the 1115-bp Eco R1–Hind III fragment containing the FGF coding region from phage λ40 has been deposited at GenBank and given the accession number M21092.

cDNA (Fig. 1A, probe 2) we only detected the 1-kb message on Northern blots, but with a probe that contained only the COOH-terminal half of the "third exon" coding region (Fig. 1A, probe 3) we detected two new mRNA species (Fig. 1B): a 2.1-kb transcript present in the oocyte and continuously thereafter until stage 12; and a 4.2-kb transcript that was present in the oocyte and then reappeared during the neurula stages. Probe 2 may have hybridized predominantly to the small mRNA (*3*) because the 3' region of the small transcript is different than that of the larger mRNAs (*7*). This difference could arise by differential splicing in the 3' noncoding region.

To obtain cDNA copies of the larger mRNAs, *Xenopus* oocyte and stage 17 cDNA libraries (*8*) were screened with probe 3. The oocyte library yielded only small cDNAs, similar to those recovered previously (*3*), whereas numerous small cDNAs and one 4.3-kb cDNA (λ40) were recovered from screening 10⁶ phage from the stage 17 library. The difficulty in isolating cDNAs representing the large messages may be due to their low abundance in the oocyte and to the sequences hybridizing to the probe being at the extreme 5' end of the transcript.

Nucleotide sequence analysis of λ40 revealed an ATG codon at nucleotides 335 to 337 that precedes an open reading frame of 154 codons. An in-frame translation termination codon is located six codons upstream and thus this ATG is probably the site of translation initiation, yielding a 155-residue polypeptide (~17 kD). Bovine and human bFGF were initially proposed to be translat-

ed as 155–amino acid polypeptides (*6*, *9*). Forms of bFGF longer than 155 residues, however, have been described (*10*), indicating that either (i) the true translation initiation site for bFGF in mammals lies upstream from the site initially proposed or (ii) multiple sites of translation initiation exist. In this regard we note that the NH₂-terminus of the putative *Xenopus* 155-residue bFGF aligns exactly with that of the 155-residue product initially proposed for bovine and human bFGF (Fig. 2).

The *Xenopus* protein is remarkably homologous (84% conserved residues) to human bFGF (Fig. 2). Except for a substitution of Ser¹²¹ for Thr¹²¹, the *Xenopus* bFGF conserves a region (residues 115 to 129) proposed to be involved in receptor and heparin binding of bFGF (*11*). Cys⁷⁸ in the human protein is not essential for biological activity (*12*) and is not conserved in *Xenopus*. Comparison of the cDNA sequence with *Xenopus* bFGF genomic clones (*13*) showed two introns in the gene sequence that align exactly with the two introns in the human bFGF gene (*6*). When DNA blots of genomic *Xenopus* DNA were probed with fragments of the *Xenopus* bFGF clones, two hybridizing sequences for both the first and third exons were detected, suggesting that the *Xenopus* genome may contain two bFGF-like genes (*13*).

Like bovine and human bFGF, there is no strong indication of a classical secretory signal sequence in *Xenopus* bFGF. The in vitro translation product of the *Xenopus* cDNA clone could not be secreted from a dog pancreas–stripped microsome system under conditions in which preprolactin was

efficiently translocated (*14*). Furthermore, in mouse NIH 3T3 and chinese hamster ovary (CHO) cells, the mammalian FGF protein is not secreted when produced from a transfected gene (*15*, *16*) but could be secreted when a signal sequence from human growth hormone was spliced to the FGF coding region (*16*). Therefore both *Xenopus* bFGF and the mammalian homologs may be released from cells by a route different from that classically described for secretory proteins.

The functional evidence that bFGF is the natural mesoderm inducer relies on the activity of purified heterologous factors. We have tested the activity of *Xenopus* bFGF by expressing it in *Escherichia coli* using a T7 expression system (*17*). The induced protein was highly purified by heparin-Sepharose chromatography (*18*); the majority of the bacterial proteins that bound to heparin in the 0.5*M* NaCl loading buffer were eluted at a lower concentration of NaCl than was the bFGF polypeptide (Fig. 3A). We tested the mesoderm inducing activity of the purified *Xenopus* bFGF by measuring induction of cardiac actin mRNA with mid-blastula stage animal cap explants. This assay measures the conversion of cells from their normal ectodermal fate to a mesodermal pathway by the use of a muscle specific gene (*3*, *19*). With no bFGF, or with a fraction from the heparin-Sepharose column enriched in bacterial proteins (Fig. 3A, lane 5), no cardiac actin mRNA was detected (Fig. 3B, lanes 1 and 2). In contrast, increased amounts of bFGF (from the fraction in Fig. 3A, lane 8) enhanced the expression of the cardiac actin gene (Fig. 3B, lanes 3 to 7); the amount of expression was comparable to that induced by bovine bFGF (Fig. 3B, compare lanes 4 and 8). Thus *Xenopus* and bovine bFGFs are similarly effective mesodermal inducers for animal cap cells. We have also found that *Xenopus* bFGF, like human bFGF, is a potent mitogen for cultured bovine adrenal cortex capillary endothelial cells (*20*).

The presence in the oocyte of a basic FGF mRNA suggested that the protein product would also be present and that it could have therefore been synthesized long before mesoderm induction occurs. We determined when the FGF polypeptide appeared during early embryogenesis by immunoblotting with a rabbit polyclonal antibody to residues 141 to 155 (a peptide that is completely conserved between *Xenopus* and human bFGF). To enrich for FGF-like peptides, an extract of eggs or embryos was bound to heparin-Sepharose, eluted with high salt, and electrophoretically fractionated on a polyacrylamide gel; the protein was then visualized by immunoblotting (Fig. 4). A single protein of approximately 15 kD was detected in both unfertilized eggs and in

mid-blastula stage (MBT) embryos; in fact there is a relatively constant amount of FGF present from the oocyte to stage 17 embryos (7). Binding of the antibody to this protein was blocked when the antibody was first preincubated with the 141 to 155 peptide (7). One caveat is that the antibody used in these experiments weakly binds human acidic FGF. Since both basic and acidic FGF induce mesoderm when added to animal hemisphere cells (2), and both bind to heparin-Sepharose, the possibility remains that either is the natural mesodermal inducer. However, because we find a transcript in the oocyte that can encode bFGF, we argue that this is most likely the polypeptide detected in our samples.

We can estimate from Western blots that there is approximately 100 pg of FGF per egg, or a total concentration of approximately 200 ng/ml. Since *Xenopus* bFGF can effectively induce mesoderm at concentrations of 20 to 50 ng/ml (Fig. 3B), the egg appears to contain four to ten times more protein than is necessary to cause induction even if FGF were uniformly distributed throughout the embryo. Although this falls short of proof that FGF is the natural mesoderm inducer, since proof would require experiments eliminating FGF from the oocyte, the presence of FGF at the correct time and in sufficient amounts to induce mesoderm strongly suggests that FGF is a natural agent for mesoderm induction. Further support for the natural role of FGF comes from an experiment in which the animal and vegetal hemispheres were physically separated in a way that still permitted inductive signals to pass between them (2). In this experiment, addition of heparin to the region between the two hemispheres blocked mesoderm induction, suggesting that a heparin binding protein must pass between the two hemispheres for induction to occur.

From what is known about the timing of mesoderm induction, if the FGF in the oocyte is to function in this process it must be stored and released at a later time. Although the exact times at which induction occurs are not yet known, the vegetal hemisphere can induce mesoderm formation in the animal hemisphere at least as early as stage 6.5 (64-cell) and as late as stage 10.5 (early gastrula) (21). Induction cannot be possible before the eight-cell stage, at which time the animal and vegetal blastomeres first separate. Therefore if the FGF stored in the egg is to participate in mesoderm formation it must be sequestered until sometime after the eight-cell stage and most likely until much later stages, although the means by which FGF is sequestered are unknown.

If the FGF polypeptides are stored globally in the early embryo, then a specific inductive agent from the vegetal hemisphere might signal the release of FGF from the animal blastomeres. Alternatively FGF may be released directly by the vegetal blastomeres. The ability of FGF to induce a mesodermal response may also be controlled by other factors such as regulation of the FGF receptor or second messenger system, inhibitors of the FGF response, or other molecules that are required to elicit mesodermal induction. For example, TGF-β1– and TGF-β2–like molecules may modulate
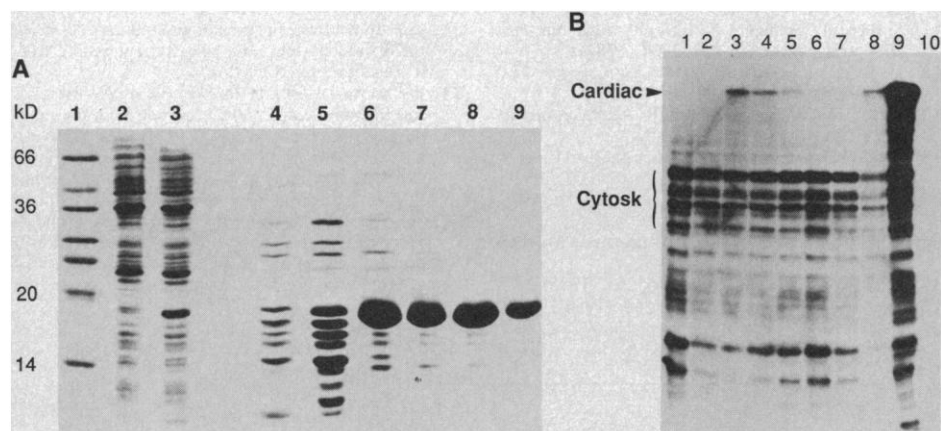

Fig. 3. Induction of cardiac actin mRNA by a recombinant *Xenopus* bFGF. (**A**) The *Xenopus* bFGF cDNA was engineered to remove sequences 5′ of the initiating ATG and then cloned into a T7 expression plasmid (17). Bacterial cells (LYS S strain) containing this plasmid were grown to $OD_{600} = 0.6$, induced with isopropyl-β-D-thiogalactopyranoside (IPTG), incubated for 2 hours, then lysed by sonication in a lysis buffer containing 20 mM tris-HCl, pH 7.5, 1 mM EDTA, and 1 μM phenylmethylsulfonylfluoride. The lysate was centrifuged and the supernatant collected. NaCl was added to 0.5M and the sample was loaded onto a 4-ml heparin-Sepharose column (Pharmacia). The column was washed with lysis buffer containing 0.5M NaCl, and the bound material was eluted with the lysis buffer containing a gradient of NaCl from 0.5M to 2.5M. Fifty microliters of each fraction were precipitated with methanol, separated on a 17.5% polyacrylamide gel, and stained with Coomassie Brilliant blue. (Lane 1) Markers, (lane 2) total cell lysate from uninduced bacteria, (lane 3) total cell lysate from IPTG-induced cells, (lanes 4 to 9) the first 6 of 15 fractions eluted from the column. The molecular weights of four of the marker proteins are indicated (in kilodaltons). (**B**) Induction of cardiac actin from mid-blastula stage explants. *Xenopus* animal hemispheres were prepared (3) at 6 hours after fertilization. The following samples were added to the explants in 1-ml cultures: (lane 1) no addition; (lane 2) a sample of the material loaded in (A), lane 5 (23); (lanes 3 to 7) 80, 40, 20, 8, and 1.6 ng, respectively, of the material loaded in (A), lane 8; (lane 8) 40 ng of bovine pituitary bFGF. RNA was prepared at the equivalent of stage 17, hybridized to a cardiac actin RNA probe, and digested with ribonuclease (RNase) A and T1 (3). (Lane 9) A sample to which stage 17 whole embryo RNA was added; (lane 10) a control in which only transfer RNA was added to the RNase protection. The protected cardiac actin and cytoskeletal actin bands are indicated.

Fig. 4. FGF is present in the *Xenopus* egg. Three thousand unfertilized eggs or mid-blastula transition stage (MBT) embryos were placed in 5 ml of a lysis buffer (10 mM tris-HCl, pH 7.5, 1 mM EDTA, 25 μg/ml aprotinin, 25 μg/ml leupeptin, 1 mM benzamidine, 0.5 mM phenylmethylsulfonylfluoride, 10 μg/ml pepstatin) containing 0.5M NaCl, sonicated, then centrifuged. The supernatant was collected and bound to 0.4 ml of heparin-Sepharose for 30 min at 4°C. The resin was loaded into a column, then washed with the lysis buffer containing 0.5M NaCl. The bound material was eluted with the lysis buffer containing 1.0M NaCl, then with the lysis buffer containing 3.0M NaCl. The eluates were precipitated with 10% trichloroacetic acid, redissolved in SDS-sample buffer, and then half of this material was separated on a 17.5% polyacrylamide gel. After electrophoresis the proteins were transferred to nitrocellulose, which was then blocked with 5% nonfat dry milk (NFDM) in tris-buffered saline (TBS) and hybridized with antibody to the 141 to 155 bFGF peptide in TBS containing 2% NFDM, and 2% ovalbumin. The filter was washed, hybridized with ^{125}I–protein A in TBS containing 2% NFDM and 2% ovalbumin, washed again, and exposed to film. No hybridizing protein was detected in the 1M NaCl eluate; therefore only the 3M NaCl eluate is shown. The human FGF lane was loaded with 1 μg of bacterially produced, 154-residue human bFGF. The total protein loaded in the MBT lane was 60% that loaded in the unfertilized lane.
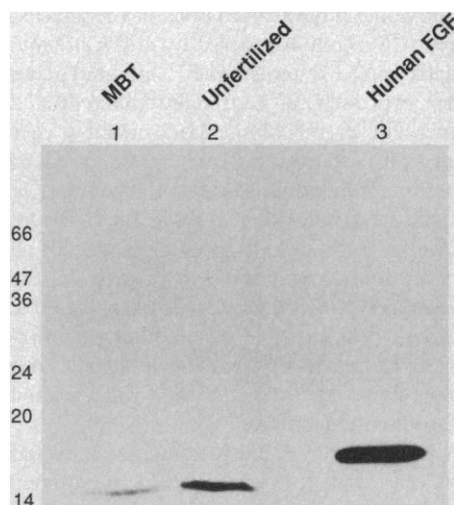
the effects of FGF in the embryo (3, 4). It is increasingly important to know what parts of the embryo contain FGF, what parts release it, and what parts are capable of responding to FGF. The pattern of release and response should explain many of the features of signaling during gastrulation and neurulation.

REFERENCES AND NOTES

1. P. D. Nieuwkoop, A. G. Johnen, B. Albers, *The Epigenetic Nature of Early Chordate Development. Inductive Interaction and Competence* (Cambridge Univ. Press, Cambridge, 1985).
2. J. M. W. Slack, B. G. Darlington, J. K. Heath, S. F. Godsave, *Nature* **326**, 197 (1987).
3. D. Kimelman and M. Kirschner, *Cell* **51**, 869 (1987).
4. F. Rosa *et al.*, *Science* **239**, 783 (1988); J. C. Smith, *Development* **99**, 3 (1987); J. Cooke, J. C. Smith, E. J. Smith, M. Yaqoob, *ibid.* **101**, 893 (1987).
5. D. L. Weeks and D. A. Melton, *Cell* **51**, 861 (1987).
6. J. A. Abraham *et al.*, *EMBO J.* **5**, 2523 (1986).
7. D. Kimelman, unpublished observations.
8. M. R. Rebagliati, D. L. Weeks, R. P. Harvey, D. A. Melton, *Cell* **42**, 769 (1985); C. R. Kintner and D. A. Melton, *Development* **99**, 311 (1987).
9. J. A. Abraham *et al.*, *Science* **233**, 545 (1986).
10. A. Sommer *et al.*, *Biochem. Biophys. Res. Commun.* **144**, 543 (1987); D. Moscatelli, J. Joseph-Silverstein, R. Manejias, D. B. Rifkin, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5778 (1987); M. Klagsbrun, J. Sasse, R. Sullivan, J. A. Smith *ibid.* **83**, 2448 (1986).
11. A. Baird, D. Schubert, N. Ling, R. Guillemin, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2324 (1988).
12. M. Seno *et al.*, *Biochem. Biophys. Res. Commun.* **151**, 701 (1988).
13. D. Kimelman, T. Haaparanta, R. Feldman, unpublished observations.
14. D. Kimelman and K. Matlack, unpublished observations.
15. S. Rogelj, R. A. Weinberg, P. Fanning, M. Klagsbrun, *Nature* **331**, 173 (1988).
16. S. B. Blam *et al.*, *Oncogene*, in press; R. Mitchell, personal communication.
17. A. H. Rosenberg *et al.*, *Gene* **56**, 125 (1987).
18. Y. Shing *et al.*, *Science* **223**, 1296 (1984); R. R. Lobb and J. W. Fett, *Biochemistry* **23**, 6295 (1984); D. Gospodarowicz, J. Cheng, G-M. Lui, A. Baird, P. Bohlen, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6963 (1984); T. Maciag, T. Mehlman, R. Friesel, A. B. Schreiber, *Science* **225**, 932 (1984).
19. J. B. Gurdon, S. Fairman, T. J. Mohun, S. Brennan, *Cell* **41**, 913 (1985).
20. S. Silver and J. Abraham, unpublished results.
21. E. A. Jones and H. R. Woodland, *Development* **101**, 557 (1987).
22. P. A. Krieg and D. A. Melton, *Nucleic Acids Res.* **12**, 7057 (1984).
23. One percent of the eluted fraction was added in this case. In contrast, the maximum amount of *Xenopus* bFGF added (Fig. 3B, lane 3) represented only 0.1% of the eluted fraction.
24. We thank D. Melton for *Xenopus* cDNA libraries and helpful ideas, L. Vitto for help with immunoblotting, R. Feldman and T. Crisp for DNA sequencing work, A. Protter, J. Whang, and J. Hatch for characterization of the anti-peptide antiserum, D. Gospodarowicz for providing bovine pituitary bFGF and G. Martin, D. Gospodarowicz and J. Fiddes for critical review of this work. D.K. is a postdoctoral fellow of the National Institutes of Health. Supported by grants to M.K. from the NIH.

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Activation of Developmentally Mutated Human Globin Genes by Cell Fusion

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Human fetal globin genes are not expressed in hybrid cells produced by the fusion of normal human lymphocytes with mouse erythroleukemia cells. In contrast, when lymphocytes from persons with globin gene developmental mutations (hereditary persistence of fetal hemoglobin) are used for these fusions, fetal globin is expressed in the hybrid cells. Thus, mutations of developmental origin can be reconstituted in vitro by fusing mutant lymphoid cells with differentiated cell lines of the proper lineage. This system can readily be used for analyses, such as globin gene methylation, that normally require large numbers of pure nucleated erythroid cells, which are difficult to obtain.

D URING HUMAN ONTOGENY THERE is a switch from embryonic to fetal globin production early in development and a switch from fetal to adult globin production around birth (1). In a group of genetic mutations known as hereditary persistence of fetal hemoglobin (HPFH), the latter switch is never completed, thus resulting in fetal globin production in adult life. HPFH mutations are of two general types: those characterized by deletions in the globin cluster (deletion HPFH) and those that consist of point mutations in the promoters of the fetal globin genes (promoter mutation HPFH) (1).

The mechanism whereby HPFH mutations activate fetal hemoglobin production in adult red cells is not yet known, and the

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functional molecular analysis of the affected cells is severely hampered by a lack of amenable model systems. HPFH-mutated genes are expressed in terminally differentiated cells, the erythroblasts; bone marrow cells and purified erythroblasts are required to perform functional studies of chromatin or structural studies such as DNA methylation. The same limitations apply to studies of all other human mutations expressed in the terminally differentiated cells of inaccessible tissues. For example, for studies of mutations expressed in the liver or the nervous system, hepatic or neural cells are required for functional studies.

Certain quiescent genes can be activated by cell fusion (2). With HPFH as a model, we used this approach to activate human developmental mutations and thereby facilitate their analysis. After transfer into mouse erythroleukemia (MEL) cells, normal lymphoid chromosomes express only adult and not fetal globin (3). We therefore examined whether lymphoid chromosomes containing HPFH mutations would express the fetal globin genes after chromosomal transfer into MEL cells.

We used two deletion HPFH mutations, HPFH-1 and HPFH-2 (1). In both cases there are large deletions in the β globin gene cluster (>150 kb), which remove the δ and
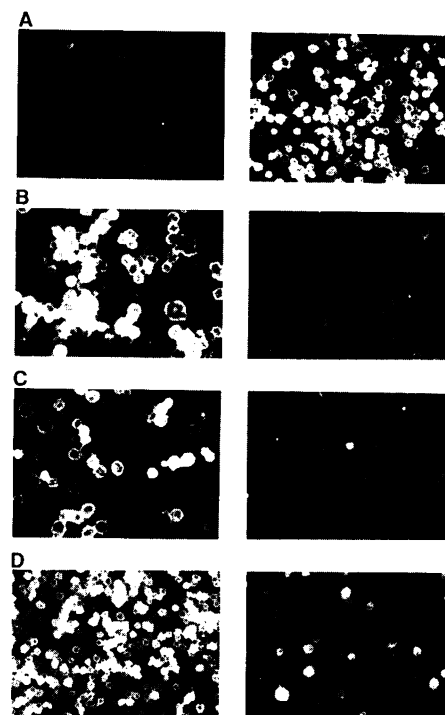
Fig. 1. Immunofluorescent labeling of lymphoid × MEL hybrid cells with monoclonal antibodies to the γ and β chains. Hybrids contain (A) the normal human β globin locus, (B) the HPFH-2 mutation locus, (C) the HPFH-1 locus from a HPFH-1/HPFH-2 compound heterozygote, and (D) the −117 ^Aγ mutation locus.