MOLECULAR AND CELLULAR BIOLOGY, May 1991, p. 2481–2488
0270-7306/91/052481-08$02.00/0

Vol. 11, No. 5

cDNA Cloning and Developmental Expression of Fibroblast Growth Factor Receptors from *Xenopus laevis*

ROBERT FRIESEL†* AND IGOR B. DAWID

Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, Bethesda, Maryland 20892

Received 25 May 1990/Accepted 13 February 1991

The heparin-binding growth factors constitute a family of homologous polypeptides including basic and acidic fibroblast growth factors (FGFs). These factors participate in a variety of processes, including wound healing, angiogenesis, neuronal survival, and inductive events in the early amphibian embryo. We have isolated three closely related species of cDNA clones for *Xenopus* FGF receptors. One of these, designated XFGFR-A1, encodes an open reading frame of 814 amino acids. A second class encodes an identical amino acid sequence with the exception of an 88-amino-acid deletion near the 5′ end. This species probably arises through alternative splicing. A third class of cDNA corresponding to the shorter form of XFGFR-A1 was isolated and shown to be 95% homologous and is designated XFGFR-A2. *Xenopus* FGF receptors are similar to FGF receptors from other species in that they contain a transmembrane domain, a tyrosine kinase domain split by a 14-amino-acid insertion, and a unique conserved stretch of eight acidic residues in the extracellular domain. Overexpression of *Xenopus* FGF receptor protein by transfection of COS1 cells with the corresponding cDNA in a transient expression vector leads to the appearance of new FGF binding sites on transfected cells, consistent with these cDNAs encoding for FGF receptors. RNA gel blot analysis demonstrates that *Xenopus* FGF receptor mRNA is a maternal message and is expressed throughout early development. When blastula-stage ectoderm is cultured in control amphibian salt solutions, *Xenopus* FGF receptor mRNA declines to undetectable levels by late neurula stages. However, when cultured in the presence of FGF of XTC mesoderm-inducing factor, *Xenopus* FGF receptor RNA expression is maintained.

The heparin-binding growth factors (HBFGs) constitute a family of homologous genes that includes the genes for acidic (aFGF) and basic (bFGF) fibroblast growth factors (1, 22), *hst*/KS (kFGF) (3, 60, 64), *int*-2 (8), FGF-5 (65), KGF (12), and FGF-6 (33). Although these genes are related structurally to one another, the highest degree of similarity is between aFGF and bFGF. aFGF and bFGF were originally purified from a variety of tissues on the basis of their high affinity for heparin (31, 55). Both polypeptides have been shown to be potent mediators of mitogenesis, angiogenesis, and differentiation (reviewed in references 4 and 13). The biological activities of aFGF and bFGF are mediated through high-affinity cell surface receptors of 150 and 130 kDa (6, 15, 21, 24, 30, 34, 36, 40) that have an associated tyrosine kinase activity (6, 14, 20). Experiments using radiolabeled aFGF and bFGF suggest that these ligands interact with the same or very similar receptors (9, 23, 36, 40). Recently, a bFGF receptor (FGFR) was purified and cloned from chicken embryonic tissue (29) and shown to be identical to the developmentally regulated tyrosine kinase gene *cek*-1 (42). Both the chicken FGFR (CFGFR) and *cek*-1 are homologous to the human tyrosine kinase gene *flg* (50). *flg* has subsequently been shown to encode the human FGFR (9, 49). In addition, two FGFR-like genes have been cloned from the mouse. One is homologous to the human FGFR (44, 51); the other one (called *bek* [27]), although distinct, has extensive homology to the other FGFRs and may be a member of an FGFR family of genes.

During normal amphibian embryonic development, signals originating from the vegetal hemisphere of the embryo specify cells of the equatorial marginal zone to become mesoderm (38; reviewed in references 7 and 58). Explanted animal pole ectoderm, which in control culture forms only epidermis, can be induced to form mesoderm when placed in close proximity to vegetal pole endoderm (18, 38). Mesoderm induction may be scored by morphological criteria (38, 56) and by molecular markers such as activation of α-actin mRNA expression (18, 26, 53). Several growth factors from homologous or heterologous sources can mimic the mesoderm-inducing effect of the vegetal pole cells in this assay system (reviewed in references 7 and 58). These factors include bovine aFGF and bFGF (26, 56) and bFGF from *Xenopus laevis* (25, 57). In addition, two FGF-like proto-oncogenes, kFGF and *int*-2, also possess mesoderm-inducing activity (43). Transforming growth factor β1 (TGF-β1) has been reported to potentiate the mesoderm-inducing activity of bFGF while having no mesoderm inducing activity alone (26). Porcine and *Xenopus* TGF-β2 and recombinant TGF-β3 are able to induce mesoderm in animal explants without the presence of FGF, although a modest synergism has been observed (45, 47). The most potent mesoderm inducer known is the factor (MIF) secreted by XTC cells which has been shown recently to be a frog homolog of mammalian activin A (59). Activin A is a member of the TGF-β superfamily of factors (59). Thus, mesoderm inducers can be divided into two classes, FGF-like and TGF-β-like. There are not only differences in effectiveness in certain induction assays between these inducers but also qualitative differences: FGF class inducers generate ventral mesoderm like mesenchyme and some muscle (17, 56), while TGF-β-like inducers yield more dorsoanterior derivatives, including large amounts of muscle and notochord (17, 45, 47). There are also differences in molecular responses to the different

* Corresponding author.
† Present address: Jerome H. Holland Laboratory for the Biomedical Sciences, American Red Cross, 15601 Crabbs Branch Way, Rockville, MD 20855.

inducing factors: the homeobox gene *Mix*.1 is activated by XTC MIF (i.e., activin A) but not by FGF (46), whereas the homeobox gene *Xhox*3 is strongly activated by FGF and only weakly by XTC MIF (activin A) (48). Thus, there is good reason to believe that both classes of inducing factors are involved in vivo in mesoderm induction and establishment of polarity in the embryo.

Recently, FGFRs have been identified in the developing *Xenopus* embryo by ligand binding assays and affinity cross-linking experiments (16). Here we report cDNA cloning and expression during embryogenesis of FGFR RNAs in *X. laevis*. We find that the *Xenopus* FGFR (XFGFR) mRNA is expressed throughout early development and that expression can be regulated by growth factors in animal cap explants.

MATERIALS AND METHODS

Library screening. A cDNA library containing approximately 10^6 recombinants was constructed by using poly(A)$^+$ RNA isolated from the *Xenopus* XTC cell line and inserted into the λ Zap vector (Stratagene). A synthetic degenerate oligonucleotide corresponding to a conserved region of the intracellular domain of the CFGFR (29, 42) and *flg* (50) sequences was used as a probe. The nucleotide sequence is 5′-TA(C/T) ATG ATG ATG (C/A)GG GA(C/T) TGG CAT GC(A/T) GTC CC-3′. A second oligonucleotide probe, upstream from the first probe, was used to confirm clones as being members of the tyrosine kinase family: 5′-GTG ACA GAG (G/A)AC AA(T/C) GTG ATG AA(G/A) AT(A/G/T) GCA GA(C/T) TT(T/C) GG-3′. The oligonucleotide probes were labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP (>5,000 Ci/mmol; Amersham) and used to screen the library as described by Maniatis et al. (32) at 42°C for 16 h. Filters were washed sequentially in 6× SSC–0.1% SDS at room temperature for 30 min, 42°C for 30 min, and 50°C for 30 min and received a final wash at 65°C for 30 min. Filters were dried and exposed to Kodak XAR-5 film at −70°C with a Dupont Cronex intensifying screen.

Analysis of clones. Positive clones were purified according to published procedures (32). Phagemid clones were rescued into plasmid form in XL-1 Blue cells as instructed by the manufacturer (Stratagene). Clones were mapped with several restriction enzymes, and several overlapping clones were identified. Nucleotide sequencing was done by the dideoxy-chain termination method (52), using a Sequenase kit (United States Biochemicals) and [α-^{35}S]dATP (>1,000 Ci/mmol; Amersham) on double-stranded templates (28). For sequencing clones >2 kb, sets of nested deletions were prepared with exonuclease III and S1 nuclease (Erase-a-base; Promega). Second-strand sequencing was performed by using synthetic oligonucleotide primers and Sequenase. Sequences were compiled and analyzed with the University of Wisconsin Genetics Computer Group sequence analysis package and PC Gene (Intelligenetics).

RNA gel blot analysis. RNAs were prepared from *Xenopus* embryos at different stages of development, from XTC cells, and from ectodermal explants as described previously (53). RNA was denatured with glyoxal, separated by electrophoresis through 1.0% agarose gels, and blotted as described previously (61) except that Nytran (Schleicher & Schuell) was substituted for nitrocellulose. Hybridization of filter-bound RNA to DNA probes prepared by random primer labeling of full-length XFGFR cDNAs (11) was carried out by the method of Church and Gilbert (5) in buffer containing 0.5 M sodium phosphate (pH 7.0), 7% sodium dodecyl sulfate (SDS), 1% bovine serum albumin, and 10 mM EDTA at 65°C for 16 h. Filters were washed in 0.2× SSPE at 65°C for 30 min, dried, and exposed to X-ray film as described above. In some instances, blots were rehybridized with a probe corresponding to *X. laevis* cardiac α-actin (pSPAC-9) cDNA. Labeling, hybridization, and washing were performed as described above. To confirm that equivalent amounts of RNA were loaded, blots were hybridized to an oligonucleotide probe corresponding to a sequence of *X. laevis* 18S rRNA. The sequence of the 18S oligonucleotide probe is 5′-GGT CAG CGC TCG TCG GCA TGT AAT AG-3′. Blots were hybridized as described above and washed in 2× SSPE for 30 min at 22°C.

***Xenopus* eggs and embryos.** Embryos were staged according to Nieuwkoop and Faber (39). Culture of embryos, preparation of ectodermal explants, and induction with conditioned medium from XTC cells (XTC-CM) were carried out essentially as described previously (46, 47). Growth factors and other compounds were obtained from the following sources: recombinant human bFGF, Amgen; retinoic acid and phorbol 12-myristate 13-acetate (PMA), Sigma. Retinoic acid was prepared as a 1 mM stock in ethanol and stored at −70°C protected from light. PMA was prepared as a 1-mg/ml stock in dimethyl sulfoxide and stored at −20°C.

In vitro transcription and translation. A full-length cDNA clone encoding the two-immunoglobulinlike (Ig-like)-domain form of XFGFR was linearized by digestion with *Bam*HI, which cuts within the multiple cloning site of Bluescript at the 3′ end of the cDNA insert. In vitro transcripts were obtained by using the Riboprobe system (Promega) and T7 RNA polymerase. In vitro-synthesized RNA transcripts (~1 μg) of the XFGFR construct were translated in a rabbit reticulocyte lysate system (Promega) in the presence of [^{35}S]methionine (Amersham). Samples were dissolved in SDS sample buffer and run on 10% SDS-polyacrylamide gels. The gels were stained, destained, treated with Enhance (New England Nuclear), dried, and subjected to autoradiography.

Cell culture and DNA transfection. COS1 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Irvine Scientific) supplemented with 10% fetal bovine serum (FBS) (Irvine Scientific). A full-length cDNA clone corresponding to the two-Ig-like-domain form of the receptor was cloned into a *Xho*I-*Not*I site of the mammalian expression vector CDM8 (54). The clone was confirmed to be in the correct orientation relative to the cytomegalovirus promoter of the vector by restriction enzyme mapping. The construct was designated CDM8-28.1. For transient expression, COS1 cells were plated at 5×10^5 cells per dish into 10-cm culture dishes. The next day, plasmid DNAs were transfected into cells by the DEAE-dextran method (32). After transfection, the cells were incubated in DMEM–10% fetal bovine serum for 2 days at 37°C.

Covalent ligand affinity cross-linking. Recombinant human bFGF was iodinated by published procedures (34). For affinity cross-linking experiments, monolayer of COS1 cells transfected with either the control plasmid (CDM8) or XFGFR construct (CDM8-28.1) were washed with cold DMEM containing 25 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES; pH 7.4), heparin (10 μg/ml), and 0.1% bovine serum albumin (binding buffer). Cells were incubated at 4°C for 1 h in the presence of binding buffer containing 600 pM ^{125}I-bFGF in the presence or absence of a 200-fold excess of unlabeled ligand. Cells were washed three times with ice-cold binding buffer and exposed to disuccinimidyl suberate at a final concentration of 0.3 mM in phosphate-

```
                                                    ▼
XFGFR-A1    MFSGRSLLLWGVLLGAALSVARPPSTLPDEVAPKTKTEVEPYSARPGDTVTLQCRLREDV    60
XFGFR-A2        M                                  ------------------------------

                                                                        ▼
XFGFR-A1    QSISWVKNGVQLLETNRTRITGEEIQISNAGPEDNGLYACVTIGPSGTYTVLFSINVSDA   120
XFGFR-A2    ------------------------------------------------------------

XFGFR-A1    QPSAEDDDDDDDNSSSEEKASENSKPNRPFWSHPEKMEKKLHAVPAAKTVKFRCPANGTP   180
XFGFR-A2    L       E           A        L

XFGFR-A1    SPALRWLKNGKEFRPDQRIGGYKVRSQTWSLIMDSVVPSDKGNYTCIVENKYGTLNHTYQ   240
XFGFR-A2    T T       RA QQ                                          AI

XFGFR-A1    LDVVERSPHRPILQAGLPANTSVTVGSTAEFSCKVYSDPQPHIQWLRHIEINGSRVASDG   300
XFGFR-A2                            T

XFGFR-A1    FPYVEILKTAGVNTSDKDMEVLHLRNVTFEDAGQYTCLAANSIGISHHSAWLTVLEVEDD   360
XFGFR-A2                                                            K   N

XFGFR-A1    KPALLASPLQLEIIIYCTGAAFVSAMVVTIIIFKMKHPSKKSDFNSQLAVHKLAKSIPLR   420
XFGFR-A2

XFGFR-A1    RQVTVSGDSNSSMHSGVILVRPSRLSSSGTPMLSGVSEYELPEDPRWEVARDRLILGKPL   480
XFGFR-A2              S   N       --            L

XFGFR-A1    GEGCFGQVVMAEAIGLDKEKPNRVTKVALKMLKSDANEKDLSDLISEMEMMKMIGKHKNI   540
XFGFR-A2                          K     V       S

XFGFR-A1    INLLGACTQDGPLYVIVEYASKGNLREYLRARRPPGMEYCYNPMCAPDQLLSFKDLVSCA   600
XFGFR-A2                        T               A       T V

XFGFR-A1    YQVARGMEYLASKKCIHRDLAARNVLVTEDNVMKIADFGLARDIHHIDYYKKTTNGRLPV   660
XFGFR-A2          D                           I       R

XFGFR-A1    KWMAPEALFDRIYTHQSDVWSFGVLLWEIFTLGGSPYPGVPMEELFKLLKEGHRMDKPTN   720
XFGFR-A2

XFGFR-A1    CTNELYMMMKDCWHAMPSQRPTFNQLVEDLDRILALSSNQEYLDLSMPVNQYSPCFPDTR   780
XFGFR-A2

XFGFR-A1    SSTCSSGEDSVFSHDPLPDEPCLPKYSNGGLKKR     814
XFGFR-A2
```

FIG. 1. Predicted amino acid sequences of XFGFR-A1 and XFGFR-A2. The sequences were aligned by using PC Gene (Intelligenetics). Gaps in the sequence are positioned with dashes. The arrowheads indicate deleted regions found in some XFGFR-A1 clones and correspond to the first Ig-like domain. Amino acids are numbered at the right.

buffered saline. After 20 min at 4°C, the cross-linking reagent was removed and cells were solubilized in SDS sample buffer and heated to 95°C for 5 min. Samples were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) and autoradiography.

Nucleotide sequence accession number. The DNA sequences for XFGFR-A1 and -A2 are in the GenBank and EMBL data bases under accession numbers M55163 and M62322.

RESULTS

Isolation and sequences of XFGFR cDNAs. To isolate *Xenopus* homologs of the CFGFR and the human tyrosine kinase gene *flg*, 10^6 clones from an XTC cDNA library were screened with degenerate oligonucleotide probes corresponding to two regions of the cytoplasmic domain of the CFGFR (29, 42) and human *flg* (50). Fourteen clones were isolated that hybridized to both probes, and restriction enzyme analysis showed that several of these were overlapping. Sequence analysis of several clones revealed three closely related species of cDNAs. One of these, designated XFGFR-A1 (Fig. 1), encodes an open reading frame of 814 amino acids. A second class has an identical amino acid sequence with the exception of an 88-amino-acid (264-bp) deletion (indicated by arrowheads in Fig. 1). The shorter cDNA species probably correspond to an alternative RNA splicing product, as discussed below. A third class of cDNA corresponding to the shorter form of XFGFR-A1 was sequenced and shown to be 95% homologous; it is designated XFGFR-A2. The presence of two highly homologous cDNAs such as XFGFR-A1 and XFGFR-A2 most likely is the result of a complete genome duplication during the evolution of *X. laevis*, as previously suggested (2).

The deduced amino acid sequence of XFGFR shows overall identities of 82, 80, and 79% to the chicken, mouse, and human FGFRs, respectively (Fig. 2). The highest degree of sequence similarity between species lies in the tyrosine kinase domain, with lower levels of homology in the Ig-like domains.

The XFGFR has several features that are characteristic of tyrosine kinase receptors in general and FGFRs in particular. These include a stretch of hydrophobic amino acids corresponding to a putative transmembrane domain. Like the CFGFR, the extracellular domain is arranged into Ig-like domains with two variant forms, one consisting of three Ig-like domains and a second containing two Ig-like domains (29, 42). The XFGFR has 10 consensus sequences for asparagine-linked glycosylation in the extracellular domain. Nine of these sites are conserved between *Xenopus*, chicken, mouse, and human FGFRs. The 10th site arises as a result of an asparagine insertion immediately following a stretch of eight acidic residues in the extracellular domain; this asparagine is not found in the chicken, mouse, or human FGFR (Fig. 2). The XFGFR has a unique stretch of eight acidic residues in the extracellular domain, a feature that is conserved among all FGFR sequences reported so far (9, 23, 29, 42, 44, 51). The XFGFR contains the sequence GXGXXG, a known consensus sequence for tyrosine kinases (9, 23, 29, 42, 44, 51), and contains a 14-amino-acid insertion within the tyrosine kinase catalytic domain, a characteristic of many receptor tyrosine kinases (9, 23, 29, 42, 44, 51).

```
X FGF-R MFSGRSLLLWGVLLGAALSVARPPSTLPDEVAPKTKT.EVEPYSARPGDTVTLQCRLRED   60
C FGF-R   TW C I  A  VT T  A   AP    QAL  ANI.   SH  H   LLQ R    D
M FGF-R  WGWKC  F A  VT T CT   AP    EQAQ WGVPV   SLLVH   LLQ R    D
H FGF-R  W WKC  F A  VT T CT   SP    EQAQ WGAPV   SFLVH   LLQ R    D

X FGF-R VQSISWVKNGVQLLETNRTRITGEEIQISNAGPEDNGLYACVTIGPSGTYTVLFSINVSD  120
C FGF-R     N  RD    P N         VEVRD V   S     M NS   SE TY  V
M FGF-R     N LRD    V S         VEVRDSI A S       SS   SD TY  V
H FGF-R     N LRD    A S         VEVQDSV A S       SS   SD TY  V

X FGF-R AQPSAEDDDDDDDNSSSEEKASENSKPNR....PFWSHPEKMEKKLHAVPAAKTVKFRCP  180
C FGF-R  L        E  .      EAD T    QAVA Y TY                   K
M FGF-R  L  S     .        ETD T    RPVA Y TS                   K
H FGF-R  L  S     .        ETD T    MPVA Y TS                   K

X FGF-R ANGTPSPALRWLKNGKEFRPDQRIGGYKVRSQTWSLIMDSVVPSDKGNYTCIVENKYGTL  240
C FGF-R SG   N T          K  H        YA   I                     SI
M FGF-R SS   N T          K  H        YA   I                  E  SI
H FGF-R SS   N T          K  H        YA   I                  E  SI

X FGF-R NHTYQLDVVERSPHRPILQAGLPANTSVTVGSTAEFSCKVYSDPQPHIQWLRHIEINGSR  300
C FGF-R                          KT AL  NV  V                K  V  K
M FGF-R                 HPS      KT AL  NV  M                K  V  K
H FGF-R                          KT AL  NV  M                K  V  K

X FGF-R VASDGFPYVEILKTAGVNTSDKDMEVLHLRNVTFEDAGQYTCLAANSIGISHHSAWLTVL  360
C FGF-R IGP NL   Q         T  E        S    E    G
M FGF-R IGP NL   Q         T  E        S    E    G    L
H FGF-R IGP NL   Q         T  E        S    E    G    L

X FGF-R EVEDDKPALLASPLQLEIIIYCTGAAFVSAMVVTIIIFKMKHPSKKSDFNSQLAVHKLAK  420
C FGF-R  ATEQS  MMT   Y          FLI C    V  Y   STT  T
M FGF-R  ALEER  VMT   Y          FLI C LGSV  Y   SGT    H  M
H FGF-R  ALEER  VMT   Y          FLI C  GSV VY   SGT    H  M

X FGF-R SIPLRRQVTVSGDSNSSMHSGVILVRPSRLSSSGTPMLSGVSEYELPEDPRWEVARDRLI  480
C FGF-R           A  S   N   M                A             LP
M FGF-R           A  SA  N   L                A             LP    V
H FGF-R           A  SA  N   L                A             LP    V

X FGF-R LGKPLGEGCFGQVVMAEAIGLDKEKPNRVTKVALKMLKSDANEKDLSDLISEMEMMKMIG  540
C FGF-R              L       D        V       T
M FGF-R              L       D        V       T
H FGF-R              L       D        V       T

X FGF-R KHKNIINLLGACTQDGPLYVIVEYASKGNLREYLRARRPPGMEYCYNPMHCAPDQLLSFKD  600
C FGF-R                                  Q             TRI EEQ
M FGF-R                                  Q       L     SHN EEQ  S
H FGF-R                                  Q       L     SHN EEQ  S

X FGF-R LVSCAYQVARGMEYLASKKCIHRDLAARNVLVTEDNVMKIADFGLARDIHHIDYYKKTTN  660
C FGF-R
M FGF-R
H FGF-R

X FGF-R GRLPVKWMAPEALFDRIYTHQSDVWSFGVLLWEIFTLGGSPYPGVPMEELFKLLKEGHRM  720
C FGF-R                                M             V
M FGF-R                                              V
H FGF-R                                              V

X FGF-R DKPTNCTNELYMMMKDCWHAMPSQRPTFKQLVEDLDRILALSSNQEYLDLSMPVDQYSPC  780
C FGF-R    S         R    V       K        V MT        V LD    G
M FGF-R    S         R    V       K       H V  T       I LD    S
H FGF-R    S         R    V       K         V  T         LD    S

X FGF-R FPDTRSSTCSSGEDSVFSMDPLPDEPCLPK....YSNGGLKKR    822
C FGF-R   A                          RCPPH. H A  RH
M FGF-R                     E   E    RNPTQLA S   R
H FGF-R                     E   E    RNPAQLA     RR
```

FIG. 2. Amino acid sequence comparisons of *Xenopus*, chicken, mouse, and human FGFRs. Sequences were compared and aligned with the GAP program of the University of Wisconsin Genetics Computer Group sequence analysis package. The XFGFR-A1 sequence is shown on the top line. For the chicken, mouse, and human receptors, only the differences are shown. Gaps have been introduced to maximize alignment and are indicated by dots. Amino acids are numbered at the right.

Functional expression of XFGFR. To confirm the coding potential of XFGFR cDNAs, we transcribed full-length sense transcripts from one of them (XFGFR-A2) with T7 RNA polymerase and translated them in vitro with a rabbit reticulocyte lysate system. Transcripts prepared from this cDNA direct the synthesis of a major translation product of ~85 kDa (Fig. 3). This value is consistent with the molecular mass predicted from the open reading frame of this clone.

The XFGFR-A2 cDNA was cloned into the CDM8 vector (54) and transiently expressed by transfection into COS1 cells by the DEAE-dextran method (32). Expression of the XFGFR was tested by the ability of transfected cells to bind ^{125}I-bFGF in ligand affinity cross-linking assay. COS1 cells transfected with the CDM8 vector alone served as controls. ^{125}I-bFGF can be cross-linked to a protein of ~120 kDa that is present in COS1 cells transfected with CDM8-28.1 (Fig. 4, lane 3) but not in cells transfected with control CDM8 (lane 1). Upon longer autoradiographic exposure of these gels (>5 days), a cross-linked band of ~145 kDa can be detected in control CDM8-transfected cells and probably represents low levels of endogenous FGFR. However, these cells do not express the 120-kDa band seen in COS1 cells transfected with the XFGFR cDNA. Cross-linking of ^{125}I-bFGF to XFGFR-transfected COS1 cells is specific, since cross-linking to this band is absent in the presence of a 200-fold excess of unlabeled bFGF (lane 4) and unlabeled aFGF (not shown). The size of the protein expressed in transfected COS1 cells is larger than that shown by the in vitro translation product but can be accounted for by the presence of multiple glycosylation sites predicted from the cDNA se-

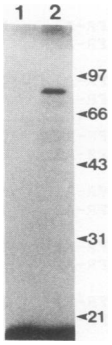

FIG. 3. In vitro translation of XFGFR cDNA. The XFGFR-A2 cDNA was linearized and transcribed with T7 polymerase and translated in a reticulocyte lysate system. Ten-microliter samples were run on 10% SDS-polyacrylamide gels. Lanes: 1, no RNA; 2, XFGFR-A2 RNA. Migration of the molecular weight standards is indicated on the right in kilodaltons.

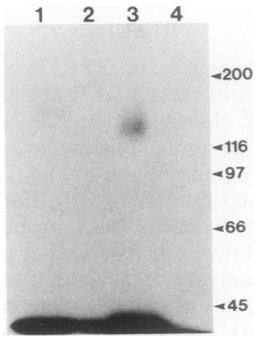

FIG. 4. Cross-linking of ^{125}I-bFGF to COS1 cells expressing XFGFR. COS1 cells transfected with control plasmid CDM8 (lanes 1 and 2) or with XFGFR construct CDM8-28.1 (lanes 3 and 4) were exposed to ^{125}I-bFGF in the presence (lanes 2 and 4) or absence (lanes 1 and 3) of excess unlabeled bFGF at 4°C for 1 h. Cells were washed and exposed to the cross-linking reagent disuccinimidyl suberate at a final concentration of 0.3 mM for 20 min at 4°C. Cells were solubilized in SDS sample buffer and subjected to 7.5% SDS-PAGE and autoradiography. Migration of the molecular weight standards is shown at the right in kilodaltons.

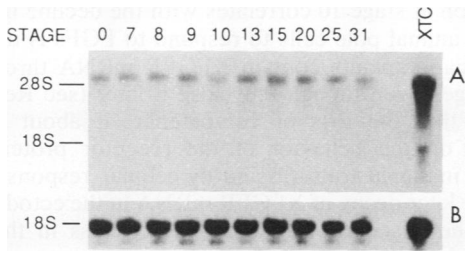

FIG. 5. RNA gel blot analysis of XFGFR mRNA in frog embryos and XTC cells. (A) Total RNAs of fertilized eggs, embryos at the stages indicated, and XTC cells were subjected to gel blot analysis and hybridized with a full-length XFGFR probe. One embryo equivalent, or 10 μg of XTC cell RNA, was loaded per lane. Positions of 28S and 18S rRNAs are indicated at the left. (B) Blots were rehybridized to an 18S rRNA oligonucleotide probe to show equivalence of RNAs loaded as described in Materials and Methods.

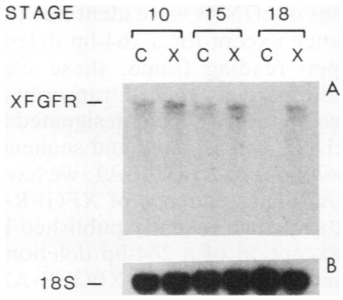

FIG. 6. Regulation of expression of XFGFR mRNA by XTC-CM in explanted animal caps. (A) Animal pole ectoderm was dissected from stage 8 embryos and incubated in 67% L-15 medium containing 0.1% bovine serum albumin in the presence (X) or absence (C) of a 1:10 dilution of XTC-CM. Animal caps (four per lane) were collected at control stage stages 10, 15, and 18, and total RNA was isolated. RNAs were subjected to RNA gel blot analysis and hybridized with a labeled XFGFR probe. (B) Blots were rehybridized to an 18S rRNA oligonucleotide probe to show equivalence of RNA loads.

quence. These results are consistent with the isolated XFGFR cDNAs encoding functional FGF receptors.

Developmental expression of XFGFR RNA. Expression of XFGFR was assessed by RNA gel blot analysis using total RNA from *Xenopus* embryos at different stages of development (Fig. 5). A major transcript of about 4.3 kb was detected in the unfertilized egg and at all embryonic stages up to the swimming tadpole stage. There is a reproducible decline ranging from 30 to 50% in the expression of this mRNA at about stage 10 (beginning of gastrulation), as determined by densitometric analysis of the autoradiograms (data not shown). High levels of expression were also detected in the *Xenopus* XTC cell line from which the RNA was obtained to derive the cDNAs. Rehybridization of these blots to a synthetic oligonucleotide probe corresponding to a sequence of *Xenopus* 18S rRNA demonstrates that approximately equal amounts of RNA were loaded in each lane. Although not well resolved, a doublet band was often detected, consistent with the use of alternative splicing or alternative polyadenylation signals; evidence from cDNA sequencing supports the former hypothesis.

Regulation of XFGFR expression by mesoderm inducers. To determine whether XFGFR mRNA expression is regulated by polypeptide growth factors that are also mesoderm inducers, we incubated stage 8 ectodermal explants in control medium or in diluted XTC-CM. At the indicated control embryonic stages, animal caps were harvested and RNA was isolated and subjected to RNA gel blot analysis. Animal caps were shown to express XFGFR mRNA at the time of explantation (data not shown). XFGFR mRNA expression was maintained in control explants through early neurula stages but declined to undetectable levels by late neurula stages (Fig. 6). However, animal caps that were cultured in the presence of XTC-CM maintained expression of XFGFR mRNA through late neurula stages. To determine what other inducers or morphogenic substances would also maintain XFGFR expression, stage 8 ectodermal explants were cultured in the presence of the indicated concentrations of bFGF, XTC-CM, retinoic acid, or PMA to control stage 20, and RNA was isolated and analyzed (Fig. 7A). Both bFGF and XTC-CM maintained approximately constant levels of XFGFR mRNA in ectodermal explants up to control stage 20, a stage at which control explants no longer express detectable levels of this mRNA. Retinoic acid and PMA were also tested since they have been shown to have morphogenic effects on early *Xenopus* embryos although not directly related to mesoderm induction (10, 41); neither retinoic acid nor PMA showed any effect on the maintenance of XFGFR expression. Rehybridization with an α-actin probe showed that XTC-CM induced a high level, FGF induced a low level, and retinoic acid and PMA had no detectable α-actin mRNA (Fig. 7B). Interestingly, at the concentrations tested, bFGF and XTC-CM maintained similar levels of XFGFR expression despite the superior ability of XTC-CM to induce α-actin mRNA (17).

DISCUSSION

We have isolated several cDNAs from the *Xenopus* XTC cell line that exhibit structural similarities to the receptors for the FGF family of growth factors. These features include a signal peptide, a conserved stretch of acidic residues in the extracellular domain, a transmembrane domain, and a tyrosine kinase domain that is split by a 14-amino-acid inser-

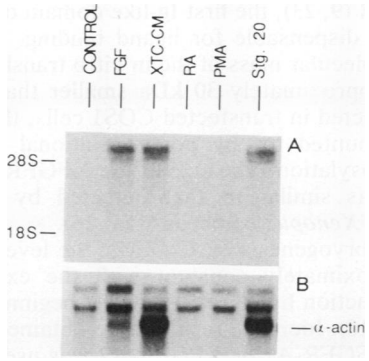

FIG. 7. Effects of mesoderm inducers and other morphogens on XFGFR mRNA expression in explanted animal caps. (A) Animal pole ectoderm was dissected and incubated essentially as described for Fig. 6. Animal caps were incubated either in XTC-CM or in the presence of bFGF (50 ng/ml), a 1:10 dilution of XTC-CM, 10^{-6} M retinoic acid (RA), or PMA (100 ng/ml). Animal caps (eight per lane) were collected at control stage 20 (Stg. 20), and RNA was isolated and subjected to RNA gel blot analysis with a labeled XFGFR probe. (B) Blots were reprobed with a labeled α-actin probe as described in Materials and Methods. The cardiac α-actin band is indicated.

tion. Two classes of cDNAs were identified that are identical by DNA sequence except for a 264-bp deletion near the 5′ end of the open reading frame; these classes probably correspond to alternatively spliced transcripts from the same gene. These two classes we have designated XFGFR-A1. A third class of cDNA was isolated and sequenced and shown to be 95% homologous to XFGFR-A1; we have designed this class XFGFR-A2. The sequence of XFGFR-A2 is identical to the XFGFR sequence recently published by Musci et al. (35) with the exception of a 264-bp deletion. Therefore, it seems likely that XFGFR-A1 and XFGFR-A2 both give rise to alternatively spliced variants. The isolation and sequencing of two XFGFR genes is not surprising, since in all cases thus far examined two or more copies of a given gene are found in the *X. laevis* haploid genome, the result of a genomic duplication event about 30 million years ago (2).

The deleted region of 88 amino acids (31 to 118) near the amino terminus of the XFGFR corresponds to a deletion at the same position in the mouse and human FGFRs, both of which are thought to arise through alternative splicing (23, 44). Since it has been postulated that the FGFRs belong to the immunoglobulin supergene family (29), the deleted region found in *Xenopus*, mouse, and human FGFRs would correspond to the Ig-like domain nearest the amino terminus of the receptor protein. The proposed splicing mechanism would give rise to FGFRs that would be identical in their intracellular domains but differ in consisting of either three or two Ig-like domains in their extracellular domains. It is tempting to speculate that the deletion of the amino-terminal Ig-like domain may alter the affinity of the FGFR for its ligand(s). In this regard, it has recently been shown that human FGFRs expressed as either the three- or two-Ig-like-domain form bind both aFGF and bFGF (23). Whether these binding specificities extend to other FGFs remains to be determined.

The expression of new binding sites for FGF on the surface of COS1 cells transiently transfected with XFGFR cDNAs indicates that these cDNAs encode functional FGFRs. In addition, our data demonstrate that the two-Ig-like-domain form of the XFGFR is able to bind FGF. Recently, it was demonstrated that the three-Ig-like-domain form of the XFGFR is able to bind FGF (35). Thus, as in the human FGFR (9, 23), the first Ig-like domain of the XFGFR seems to be dispensable for ligand binding. Although the estimated molecular mass of the in vitro translation product (Fig. 3) is approximately 30 kDa smaller than that of the receptor detected in transfected COS1 cells, the differences may be accounted for by posttranslational modifications such as glycosylation. The size of the XFGFR expressed in COS1 cells is similar to that detected by cross-linking ^{125}I-bFGF to *Xenopus* embryos (15a, 16).

During embryogenesis in *X. laevis*, the levels of XFGFR remain approximately constant with the exception of a transient reduction by 30 to 50% at the beginning of gastrulation (stage 10). Identical results were obtained when either full-length XFGFR-A1 or XFGFR-A2 was used as a probe. It is possible that the decline in XFGFR mRNA around stage 10 reflects the decay of maternally derived RNAs, while accumulation of newly transcribed XFGFR mRNAs has not yet become significant. These data are generally consistent with those of Gillespie et al. (16), who demonstrated that ^{125}I-aFGF binds to high-affinity receptors in the early *Xenopus* embryo. FGF binding to early embryos was reported to follow a temporal pattern consistent with the period during which animal pole cells are competent to be induced to mesoderm by FGF. Whether the decline in XFGFR mRNA expression at stage 10 correlates with the decline in competence of animal pole cells to respond to FGFs is uncertain. Animal cap explants contain XFGFR mRNA through gastrula stages even though they lose it later (see Results). It appears that the loss of competence at about stage 10 depends on the behavior of the receptor protein or on changes in signal transmission or cellular responses; however, the later decay of XFGFR mRNA in the ectoderm may ensure the absence of FGF responsiveness in this tissue during subsequent stages of development.

The ability of growth factors with mesoderm-inducing activity (XTC MIF and FGF) to maintain XFGFR mRNA expression in ectodermal explants (Fig. 7) suggests that continued expression of XFGFR may be important for the progressive development of mesodermal derivatives. Both XTC MIF and FGF were able to maintain XFGFR mRNA expression at approximately the same level, whereas XTC MIF was more potent an inducer of α-actin expression, as had been previously reported (17, 57). This finding suggests that there are both common and different elements in the signal transduction and cellular response mechanisms of XTC MIF and FGF in embryonic ectoderm. These data are largely consistent with those recently published by Musci et al. (35) demonstrating regulation of expression of XFGFR mRNA by mesoderm-inducing factors.

The fact that the XFGFR is a tyrosine kinase would indicate that activation of tyrosine kinase activity by ligands such as FGF plays a role in mesoderm induction in early *Xenopus* development. Support for this notion was obtained recently by the induction of mesoderm in explanted animal caps derived from embryos that had been injected with polyomavirus middle-T antigen mRNA and incubated in the absence of exogenously added inducer (62). Middle-T antigen presumably interacts with c-*src* and c-*yes*, two cytoplasmic tyrosine kinases, causing an increase in their tyrosine kinase activities. Thus, available evidence suggests that tyrosine phosphorylation is an important component of the signalling mechanism of mesoderm induction.

The identification of several new members of the HBGF family of growth factors and the identification of two (9, 27, 29, 51) or more (42, 44) different genes encoding receptors for these growth factors suggest that differential regulation and expression of these genes may be important to growth and development. In this regard, Hebert et al. (19) recently reported that several members of the HBGF family of growth factors are differentially expressed in the developing mouse embryo. The cloning and analysis of other HBGFs and their receptors from *X. laevis* should facilitate an understanding of their role in embryonic inductive interactions. We have recently cloned the *Xenopus* homolog of *bek* (unpublished data), another FGFR-like gene (9, 27); this suggests that HBGFs other than bFGF may play a role in embryonic development in *X. laevis*.

ACKNOWLEDGMENTS

We thank Patricia Bray, Peter Good, Frederic Rosa, and Tom Sargent for the gift of various materials and helpful discussions throughout the course of this work. R. F. thanks Sharron Brown for excellent technical assistance. We thank Rudy Pozzati and Mora Mattingly for the gift of COS1 cells and Tim Hla for the CDM8 vector. We thank Sally Young for assistance in preparation of the manuscript.

R.F. was supported by a National Research Council research associateship.

REFERENCES

1. **Abraham, J. A., A. Mergia, J. L. Whang, A. Tumolo, J. Friedman, K. A. Hjerrild, D. Gospodarowicz, and J. C. Fiddes.** 1986. Nucleotide sequence of a bovine clone encoding the angiogenic protein, basic fibroblast growth factor. Science **233:** 545–548.
2. **Bisbee, C. A., M. A. Baker, A. C. Wilson, I. Hadji-Adzimi, and M. Fischberg.** 1977. Albumin phylogeny for clawed frogs (*Xenopus*). Science **195:**785–787.
3. **Bovi, P. D., A. M. Curatola, F. G. Kern, A. Greco, M. Ittmaun, and C. Basilico.** 1987. An oncogene isolated by transfection of Kaposi's sarcoma DNA encodes a growth factor that is a member of the FGF family. Cell **50:**729–737.
4. **Burgess, W. H., and T. Maciag.** 1989. The heparin-binding (fibroblast) growth factor family of proteins. Annu. Rev. Biochem. **58:**575–606.
5. **Church, G. M., and W. Gilbert.** 1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA **81:**1991–1995.
6. **Coughlin, S. R., P. J. Barr, L. S. Cousens, L. J. Fretto, and L. T. Williams.** 1988. Acidic and basic fibroblast growth factors stimulated tyrosine kinase activity *in vivo*. J. Biol. Chem. **263:**988–993.
7. **Dawid, I. B., T. D. Sargent, and F. Rosa.** 1990. The role of growth factors in embryonic induction in amphibians, p. 261–288. *In* M. Nilsen-Hamilton (ed.), Role of growth factors in development. Academic Press, Orlando, Fla.
8. **Dickson, C., and G. Peters.** 1987. Potential oncogene product related to growth factors. Nature (London) **326:**833.
9. **Dionne, C. A., G. Crumley, F. Bellot, J. M. Kaplow, G. Searfoss, M. Ruta, W. H. Burgess, M. Jaye, and J. Schlessinger.** 1990. Cloning and expression of two distinct high-affinity receptors cross-reacting with acidic and basic fibroblast growth factors. EMBO J. **9:**2685–2692.
10. **Durston, A. J., J. P. M. Timmermans, W. J. Hage, H. F. J. Hendriks, N. J. DeVries, M. Heideveld, and P. D. Nieuwkoop.** 1989. Retinoic acid causes an anteroposterior transformation in the developing central nervous system. Nature (London) **340:** 140–144.
11. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction fragments to high specific activity. Anal. Biochem. **132:**6–13.
12. **Finch, P. W., J. S. Rubin, T. Miki, D. Ron, and S. A. Aaronson.** 1989. Human KGF is FGF-related with properties of a paracrine effector of epithelial cell growth. Science **245:**752–755.
13. **Folkman, J., and M. Klagsbrun.** 1987. Angiogenic factors. Science **235:**442–447.
14. **Friesel, R., W. H. Burgess, and T. Maciag.** 1989. Heparin-binding growth factor 1 stimulates tyrosine phosphorylation in NIH 3T3 cells. Mol. Cell. Biol. **9:**1857–1865.
15. **Friesel, R., W. H. Burgess, T. Mehlman, and T. Maciag.** 1986. The characterization of the receptor for endothelial cell growth factor by covalent ligand attachment. J. Biol. Chem. **261:**7581–7584.

15a. **Friesel, R., and I. B. Dawid.** Unpublished data.

16. **Gillespie, L. L., G. D. Paterno, and J. M. W. Slack.** 1989. Analysis of competence: receptors for fibroblast growth factor in early *Xenopus* embryos. Development **106:**203–208.
17. **Green, J. B. A., G. Howes, K. Symes, J. Cooke, and J. C. Smith.** 1990. The biological effects of XTC-MIF: quantitative comparison with *Xenopus* bFGF. Development **108:**173–183.
18. **Gurdon, J. B., S. Fairman, J. J. Mohun, and S. Brennan.** 1985. Activation of muscle specific actin genes in *Xenopus* development by an induction between animal and vegetal cells of a blastula. Cell **41:**317–323.
19. **Hebert, J. M., C. Basilico, M. Goldfarb, O. Haub, and G. R. Martin.** 1990. Isolation of cDNAs encoding four mouse FGF family members and characterization of their expression patterns during embryogenesis. Dev. Biol. **138:**454–463.
20. **Huang, S. S., and J. S. Huang.** 1986. Association of bovine brain-derived growth factor receptor with protein tyrosine kinase activity. J. Biol. Chem. **261:**9568–9571.
21. **Imamura, T., Y. Tokita, and Y. Mitsui.** 1988. Purification of basic FGF receptors from rat brain. Biochem. Biophys. Res. Commun. **155:**583–590.
22. **Jaye, M., R. Howk, W. H. Burgess, G. A. Ricca, I. M. Chiu, M. W. Ravera, S. J. O'Brien, W. S. Modi, T. Maciag, and W. Drohan.** 1986. Human endothelial cell growth factor: cloning, nucleotide sequence, and chromosomal localization. Science **233:**541–545.
23. **Johnson, D. E., P. L. Lee, J. Lu, and L. T. Williams.** 1990. Diverse forms of a receptor for acidic and basic fibroblast growth factors. Mol. Cell. Biol. **10:**4728–4736.
24. **Kan, M., D. DiSorbo, J. Hou, H. Hoshi, P. E. Mansson, and W. L. McKeehan.** 1988. High and low affinity binding of heparin-binding growth factor to a 130-kDa receptor correlates with stimulation and inhibition of growth of a differentiated human hepatoma cell. J. Biol. Chem. **263:**11306–11313.
25. **Kimelman, D., J. A. Abraham, T. Haaparanta, T. M. Palisi, and M. W. Kirschner.** 1988. The presence of fibroblast growth factor in the frog egg: its role as a natural mesoderm inducer. Science **242:**1053–1056.
26. **Kimelman, D., and M. W. Kirschner.** 1987. Synergistic induction of mesoderm by FGF and TGF-β and the identification of an mRNA coding for FGF in the early *Xenopus* embryo. Cell **51:**869–877.
27. **Kornbluth, S., K. E. Paulson, and H. Hanafusa.** 1988. Novel tyrosine kinase identified by phosphotyrosine antibody screening of cDNA libraries. Mol. Cell. Biol. **8:**5541–5544.
28. **Korneluk, R. G., F. Quan, and R. A. Gravel.** 1985. Rapid and reliable dideoxy sequencing of double-stranded DNA. Gene **40:**317–323.
29. **Lee, P. L., D. E. Johnson, L. S. Cousens, V. A. Fried, and L. T. Williams.** 1989. Purification and complementary DNA cloning of a receptor for basic fibroblast growth factor. Science **245:**57–60.
30. **Libermann, T. A., R. Friesel, M. Jaye, R. Lyall, B. Westermark, W. Drohan, A. Schmidt, T. Maciag, and J. Schlessinger.** 1987. An angiogenic growth factor is expressed in human glioma cells. EMBO J. **6:**1627–1632.
31. **Maciag, T., T. Mehlman, R. Friesel, and A. B. Schreiber.** 1984. Heparin binds endothelial cell growth factor: the principal endothelial cell mitogen in bove brain. Science **225:**932–935.
32. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Marics, I., J. Adelaide, F. Raybaud, M. G. Mattei, F. Coulier, J. Planche, O. de Lapeyriere, and D. Birnbaum.** 1989. Characterization of the HST-related FGF.6 gene, a new member of the fibroblast growth factor gene family. Oncogene **4:**335–340.
34. **Moenner, M., B. Chevallier, J. Badet, and D. Barritault.** 1986. Evidence and characterization of the receptor to eye-derived growth factor I, the retinal form of basic fibroblast growth factor, on bovine lens epithelial cells. Proc. Natl. Acad. Sci. USA **83:**5024–5028.
35. **Musci, J. J., E. Amaya, and M. Kirschner.** 1990. Regulation of the fibroblast growth factor receptor in early *Xenopus* embryos. Proc. Natl. Acad. Sci. USA **87:**8365–8369.
36. **Neufeld, G., and D. Gospodarowicz.** 1985. The identification and partial characterization of the fibroblast growth factor receptor of baby hamster kidney fibroblasts. J. Biol. Chem. **260:**13860–13866.
37. **Neufeld, G., and D. Gospodarowicz.** 1986. Basic and acidic fibroblast growth factors interact with the same cell surface receptors. J. Biol. Chem. **261:**5631–5637.
38. **Nieuwkoop, P. D.** 1969. The formation of mesoderm in urodelean amphibians. I. Induction by the endoderm. Wilhelm Roux's Dev. Biol. **162:**341–373.
39. **Nieuwkoop, P. D., and J. Faber. (ed.).** 1967. Normal table of *Xenopus laevis* (Daudin), 2nd ed. Elsevier/North-Holland Publishing Co., Amsterdam.
40. **Olwin, B. B., and S. D. Hauschka.** 1986. Identification of the fibroblast growth factor receptor on Swiss 3T3 cells and mouse skeletal muscle myoblasts. Biochemistry **25:**3487–3492.
41. **Otte, A. P., C. H. Koster, G. T. Snoek, and A. J. Durston.** 1988. Protein kinase C mediates neural induction in *Xenopus laevis*. Nature (London) **334:**618–620.
42. **Pasquale, E. B., and S. J. Singer.** 1989. Identification of a

developmentally regulated protein-tyrosine kinase by using anti-phosphotyrosine antibodies to screen a cDNA expression library. Proc. Natl. Acad. Sci. USA **86**:5449–5453.

43. **Paterno, G. D., L. Gillespie, M. Dixon, J. M. W. Slack, and J. K. Heath.** 1989. Mesoderm inducing properties of *int*-2 and kFGF: two oncogene encoded growth factors related to FGF. Development **106**:79–84.
44. **Reid, H. H., A. F. Wilks, and O. Bernard.** 1990. Two forms of the basic fibroblast growth factor receptor-like mRNA are expressed in the developing mouse brain. Proc. Natl. Acad. Sci. USA **87**:1596–1600.
45. **Roberts, A. B., P. Kondaiah, F. Rosa, S. Wantanabe, P. Good, D. Danielpour, N. Roche, M. Rebbert, I. B. Dawid, and M. B. Sporn.** 1990. Mesoderm induction in *Xenopus laevis* distinguishes between various TGF-β isoforms. Growth Factors **3**:277–286.
46. **Rosa, F. M.** 1989. Mix.1, a homeobox mRNA inducible by mesoderm inducers, is expressed mostly in the presumptive endodermal cells of *Xenopus* embryos. Cell **57**:965–974.
47. **Rosa, F., A. B. Roberts, D. Danielpour, L. L. Dart, M. B. Sporn, and I. B. Dawid.** 1988. Mesoderm induction in amphibians: the role of $TGF\beta_2$-like factors. Science **239**:783–785.
48. **Ruiz i Altaba, A., and D. A. Melton.** 1989. Interaction between peptide growth factors and homeobox genes in the establishment of antero-posterior polarity in frog embryos. Nature (London) **341**:33–38.
49. **Ruta, M., W. Burgess, D. Givol, J. Epstein, N. Neiger, J. Kaplow, G. Crumley, C. Dionne, M. Jaye, and J. Schlessinger.** 1989. Receptor for acidic fibroblast growth factor is related to the tyrosine kinase encoded by the *fms*-like gene (FLG). Proc. Natl. Acad. Sci. USA **86**:8722–8726.
50. **Ruta, M., R. Howk, G. Ricca, W. Drohan, M. Zabelshansky, G. Laureys, D. E. Barton, U. Francke, J. Schlessinger, and D. Givol.** 1988. A novel protein tyrosine kinase gene whose expression is modulated during endothelial cell differentiation. Oncogene **3**:9–15.
51. **Safran, A., A. Aviv, A. Orr-Urteneger, G. Neufeld, P. Lonai, D. Givol, and Y. Yarden.** 1990. The murine *flg* gene encodes a receptor for fibroblast growth factor. Oncogene **5**:635–643.
52. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
53. **Sargent, T. D., M. Jamrich, and I. B. Dawid.** 1986. Cell interactions and the control of gene activity during early development of *Xenopus laevis*. Dev. Biol. **114**:238–246.
54. **Seed, B.** 1987. An LFA-3 cDNA encodes a phospholipid-linked membrane protein homologous to its receptor CD2. Nature (London) **329**:840–842.
55. **Shing, Y., J. Folkman, R. Sullivan, C. Butterfield, J. Murray, and M. Klagsbrun.** 1984. Heparin affinity: purification of a tumor-derived capillary endothelial cell growth factor. Science **223**:1296–1299.
56. **Slack, J. M. W., B. G. Darlington, J. K. Heath, and S. F. Godsave.** 1987. Mesoderm induction in the early *Xenopus* embryo by heparin-binding growth factors. Nature (London) **326**: 197–200.
57. **Slack, J. M. W., and H. V. Isaacs.** 1989. Presence of fibroblast growth factor in the early *Xenopus* embryo. Development **105**:147–154.
58. **Smith, J. C.** 1989. Mesoderm induction and mesoderm-inducing factors in early amphibian development. Development **105**:665–677.
59. **Smith, J. C., B. M. J. Price, K. Van Nimmen, and D. Huylebroeck.** 1990. XTC-MIF, a potent mesoderm inducing factor, is activin A. Nature (London) **345**:729–731.
60. **Taira, M., T. Yoshida, K. Miyagawa, H. Sakamoto, M. Terada, and T. Sugimura.** 1987. cDNA sequence of human transforming gene *hst* and identification of the coding sequence required for transforming activity. Proc. Natl. Acad. Sci. USA **84**:2980–2984.
61. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
62. **Whitman, M., and D. A. Melton.** 1989. Induction of mesoderm by a viral oncogene in early *Xenopus* embryos. Science **244**: 803–806.
63. **Winkles, J. A., R. Friesel, W. H. Burgess, R. Howk, T. Mehlman, R. Weinstein, and T. Maciag.** 1987. Human vascular smooth muscle cells both express and respond to heparin binding growth factor-I (endothelial cell growth factor). Proc. Natl. Acad. Sci. USA **84**:7124–7128.
64. **Yoshida, T., K. Miyawawa, H. Odagiri, H. Sakamoto, P. F. R. Little, M. Terada, and T. Sugimura.** 1987. Genomic sequence of *hst*, a transforming gene encoding a protein homologous to fibroblast growth factors and the *int*-2-encoded protein. Proc. Natl. Acad. Sci. USA **84**:7305–7309.
65. **Zhan, X., B. Bates, X. Hu, and M. Goldfarb.** 1988. The human FGF-5 oncogene encodes a novel protein related to fibroblast growth factors. Mol. Cell. Biol. **8**:3487–3495.