Low-Molecular-Weight Protein Tyrosine Phosphatase Is a Positive Component of the Fibroblast Growth Factor Receptor Signaling Pathway

Eui Kyun Park,¹ Neil Warner,² Kathleen Mood,¹ Tony Pawson,² and Ira O. Daar^{1*}

Regulation of Cell Growth Laboratory, National Cancer Institute—Frederick, Frederick, Maryland 21702,¹ and Program in Molecular Biology and Cancer, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, M5G 1X5 Toronto, Ontario, Canada²

Received 28 November 2001/Returned for modification 7 January 2002/Accepted 12 February 2002

Low-molecular-weight protein tyrosine phosphatase (LMW-PTP) has been implicated in the regulation of cell growth and actin rearrangement mediated by several receptor tyrosine kinases, including platelet-derived growth factor and epidermal growth factor. Here we identify the *Xenopus laevis* homolog of LMW-PTP1 (XLPTP1) as an additional positive regulator in the fibroblast growth factor (FGF) signaling pathway during *Xenopus* development. XLPTP1 has an expression pattern that displays substantial overlap with FGF receptor 1 (FGFR1) during *Xenopus* development. Using morpholino antisense technology, we show that inhibition of endogenous XLPTP1 expression dramatically restricts anterior and posterior structure development and inhibits mesoderm formation. In ectodermal explants, loss of XLPTP1 expression dramatically blocks the induction of the early mesoderm gene, *Xbrachyury* (*Xbra*), by FGF and partially blocks *Xbra* induction by Activin. Moreover, FGF-induced activation of mitogen-activated protein (MAP) kinase is also inhibited by XLPTP1 morpholino antisense oligonucleotides; however, introduction of RNA encoding XLPTP1 is able to rescue morphological and biochemical effects of antisense inhibition. Inhibition of FGF-induced MAP kinase activity due to loss of XLPTP1 is also rescued by an active Ras, implying that XLPTP1 may act upstream of or parallel to Ras. Finally, XLPTP1 physically associates only with an activated FGFR1, and this interaction requires the presence of SNT1/FRS-2 (FGFR substrate 2). Although LMW-PTP1 has been shown to participate in other receptor systems, the data presented here also reveal XLPTP1 as a new and important component of the FGF signaling pathway.

A cascade of tyrosine phosphorylation induced by growth factors controls many aspects of developmental processes, including proliferation, differentiation, survival, and migration. Tyrosine phosphorylation of signaling molecules is tightly regulated by the coordinated actions of protein tyrosine kinases and protein tyrosine phosphatases (PTPs) (34). While much emphasis has been placed on the role of protein tyrosine kinases in biological processes, the targets and mechanisms of PTPs in receptor tyrosine kinase (RTK) signaling pathways are beginning to emerge.

PTPs can be classified into four subfamilies based on function, structure, and sequence: classic tyrosine-specific phosphatases, VH1-like dual specificity PTPs, the Cdc25 phosphatase, and the low-molecular-weight phosphatase (21). Low-molecular-weight protein tyrosine phosphatases (LMW-PTPs) are cytoplasmic enzymes found in most species from prokaryotes to mammals (65, 66). LMW-PTPs have a conserved signature motif, CXXXXXRS/T, in their active site (75) but lack most other sequences conserved among PTPs. Several isoforms of LMW-PTP have been identified in mammals and are believed to result from alternative splicing (18, 19, 46, 50, 53, 78). These isoforms may have different kinetic characteristics and substrate specificities (15, 71).

LMW-PTP has been shown to interact with several RTKs, including epidermal growth factor receptor (64), platelet-de-

rived growth factor receptor (PDGFR) (9, 10), and an Eph receptor (74). Upon PDGF stimulation of fibroblasts, a cytoplasmic pool of LMW-PTP interacts with and dephosphorylates the PDGFR, resulting in reduction of proliferation (9, 10). However, the insoluble LMW-PTP pool acts on cytoskeletal proteins such as Rho-GAP, suggesting that LMW-PTP plays multiple roles in PDGFR-mediated mitogenesis (14). While LMW-PTP is generally considered to inhibit growth factor-induced cell proliferation, in some instances LMW-PTP functions as a positive regulator. For example, in v-Ha-Ras-transformed cells, LMW-PTP increases the cell proliferation rate (65, 66). LMW-PTP has also been implicated as a positive regulator in the Eph receptor system, in which the recruitment of LMW-PTP to Eph receptor complexes was shown to be important for promotion of endothelial capillary-like assembly and cell adhesion. Mutation of the LMW-PTP binding site in Eph B1 causes failure of endothelial cells to adhere to fibronectin, suggesting that interaction of LMW-PTP with Eph B1 may be necessary for cell adhesion and plays a positive role in this event (74).

A number of growth factors have been implicated in establishing the primary germ layers during early *Xenopus laevis* development. Members of the fibroblast growth factor (FGF) family control mesoderm production and maintenance as well as morphogenetic movements during gastrulation in *Xenopus* embryos. Several lines of evidence support this concept. For example, in ectodermal explant tissue, overexpression of embryonic FGF (eFGF) or treatment with basic FGF (bFGF) induces mesoderm (38, 73). In embryos, a C-terminally trun-

* Corresponding author. Mailing address: Building 560, Room 22-3, National Cancer Institute—Frederick, Frederick, MD 21702. Phone: (301) 846-1667. Fax: (301) 846-1666. E-mail: daar@ncifcrf.gov.

cated FGF receptor 1 (FGFR1) (XFD) has been demonstrated to inhibit the formation of most mesodermal tissue and to cause gastrulation defects (2). In contrast, constitutively activated forms of FGFR1 induce mesoderm in ectodermal explants (animal caps) (57). Mesoderm is maintained via a positive feedback loop in which *Xbra*, a pan-mesodermal marker induced by FGF signaling, activates eFGF expression, which in turn aids in the maintenance of *Xbra* expression (35, 72). After mesoderm induction, FGFR is implicated in a series of coordinated cell movements involving the three established germ layers that leads to the extension of the anterior-posterior (A-P) axis (convergent extension) (2, 3, 37, 40).

FGF triggers dimerization and autophosphorylation of FGFR, and this leads to the recruitment of several SH2-containing molecules such as phospholipase C- γ (54), Crk (45), and possibly the Src kinase (44, 89). However, association of the FGFR with the docking protein SNT1/FRS-2 is not dependent upon receptor phosphorylation (39, 84). These associated signaling molecules play specific roles in FGFR activity. Activation of phospholipase C- γ (55) and Crk (27) does not appear to affect mesoderm induction in *Xenopus* ectodermal explants. Upon tyrosine phosphorylation, SNT1/FRS-2 promotes the activation of Ras via recruitment of SH2-containing signaling molecules such as Grb2, which associates with Sos, Gab1, and SHP-2 (60). Phosphatidylinositol 3 kinase enters the complex by association with Gab1 (61). Dominant-negative forms of the SH2/SH3 adapter Grb2 and Nck inhibit FGF-induced mesoderm in ectodermal explants (27). Moreover, expression of a dominant-negative variant of the PTP SHP-2 causes severe posterior truncations of *Xenopus* embryos, a similar phenotype to that induced by XFD (79). Once Ras is activated, it in turn activates the Raf-MEK-mitogen-activated protein (MAP) kinase pathway. Both the Raf-MEK-MAP kinase and phosphatidylinositol 3 kinase pathways are essential for proper mesoderm development (7, 24, 29, 41, 48, 82, 86).

While significant progress has been made in delineating the FGFR signaling pathway, the number of components in the pathway has not been completely elucidated, with additional positive and negative regulators acting between the receptor and Ras still being identified. For example, in *Drosophila melanogaster*, Dof (downstream of FGFR) is necessary for MAP kinase activation and acts upstream of Ras (83), while Sprouty potentially blocks activation of Ras by binding to Drk and Gap1 (8). In *Xenopus*, however, Sprouty2 is reported to inhibit FGF-induced calcium release and Activin-induced convergent extension while having no effect on MAP kinase activity or mesoderm induced by FGF (59). There may also be additional protein tyrosine kinases involved downstream of FGFR activation. For example, Laloo is a Src-like kinase that acts downstream of the FGFR and is an essential component of the FGF signaling pathway (85).

In this study, we have isolated two isoforms of XLPTP1, whose expression is remarkably similar to that of FGFR1. A loss-of-function strategy employing antisense morpholino oligonucleotides revealed that XLPTP1 is required for FGF-induced mesoderm formation in *Xenopus* ectodermal explants and during embryogenesis. Further, we present evidence that XLPTP1 is a functional component of the FGFR1 complex and acts as a positive regulator of the Ras-MAP kinase pathway.

MATERIALS AND METHODS

Library screening and plasmid construction. A stage 30 *Xenopus* embryo cDNA library (ZapII) (a generous gift from R. Harland) was screened by using degenerate PCR products. The degenerate PCR primers used were as follows: forward, 5'-CCA AGT C(G/A/C)G TGC TGT T(C/T)G TGT G-3'; and reverse, 5'-CTC AGA TTG CT(C/T) TCA TCC ATA C-3'. The PCR product was cloned into the pCR4-TOPO plasmid (Invitrogen) and sequenced. Using this PCR product as a probe, we isolated six strongly hybridizing phage clones and sequenced them. The cDNAs encoding XLPTP1a and -1b were subcloned into pCS2+ by PCR (*pfx* platinum polymerase; Gibco BRL). A hemagglutinin (HA) tag was introduced into the 3' end of both XLPTP1 isoforms by PCR. Following PCR, the products were inserted into *EcoRI* and *XhoI* restriction sites of pCS2+. To generate XLPTP1a Δ 5'UTR and XLPTP1b Δ 5'UTR, a Kozak consensus sequence was introduced by PCR at the start codon of both XLPTP1a and -1b using the primer 5'-CGA ATT CGC CAC CAT GGC GCA GCA GGG GAA CGG C-3'. All PCR products were verified by sequencing.

Preparation of *Xenopus* embryos, oocytes, synthetic RNA, and animal cap explants. For all embryo injections and manipulations, wild-type *X. laevis* embryos were obtained by artificial insemination after induction of females with 500 IU of human chorionic gonadotropin (49). Developmental stages were designated according to the method of Nieuwkoop and Faber (58). All capped mRNA was made by using the SP6 mMessage mMachine kit as specified by the manufacturer (Ambion). pCS2+ plasmids containing XLPTP1a and -1b, XLPTP1a Δ 5'UTR and XLPTP1b Δ 5'UTR, eFGF, FGFR1WT (wild type), and FGFR1K562E (an activated FGFR1) were linearized with *NorI*. pSP64T containing FGFR1C289R (an activated FGFR1) was linearized with *XbaI*. FGFR1KD in pSP64T3 was linearized with *BamHI*. Constitutively activated Ras and Raf in pSP64T were linearized with *BamHI* and *EcoRI*, respectively. Constitutively activated Mek in pSP64A was linearized with *EcoRI*. All FGF-related cDNAs were generous gifts from R. Friesel. Control morpholino antisense and XLPTP antisense morpholino oligonucleotides complementary to the 5' untranslated region (UTR) of XLPTP1 cDNAs were obtained from Gene Tools, LLC. Embryos were injected into both blastomeres at the two-cell stage with indicated amounts of RNAs or antisense morpholino oligonucleotides. Oocytes were prepared as described previously (20). For stimulation of FGFR1WT, human recombinant bFGF (Promega) was applied to oocyte medium (300 ng/ml). Animal cap explants were made using forceps or a Gastromaster (Xenotek Engineering) at stage 8. The explants were cultured as described previously (49). For Activin treatment, explants were incubated in 0.5 $\times$ modified Barth's solution with human recombinant Activin (50 ng/ml) until the indicated stages.

Whole-mount in situ hybridization. A pBluescript SK(+) plasmid containing XLPTP1b was linearized with *EcoRI*, and digoxigenin-labeled riboprobe was synthesized using T3 polymerase (MegaScript kit; Ambion). Plasmids containing *Xenopus Ap-2* (*Xap-2*) (87) and *Nrp-1* (67) were linearized (*Xap-2* with *HindIII* and *Nrp-1* with *BamHI*), and digoxigenin-labeled riboprobes were synthesized using T7 and T3 polymerase, respectively. Whole-mount in situ hybridization was essentially done as described by Harland (28), with a modification (49). For detection, BM Purple (Roche) was used as a chromogenic substrate. When staining became apparent, the reaction was stopped and the embryos were refixed in MEMFPA (0.1 M MOPS [pH 7.4], 2 mM EGTA, 1 mM MgSO₄, and 4% paraformaldehyde) for 1 h. When pigmented embryos were used, embryos were bleached with a solution containing 1% H₂O₂, 5% formamide, and 0.5 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (52) after staining. Photographs were taken under a dissecting microscope (Nikon SMZ1500) with a DKC-5000 digital camera system (Sony), and digital images were processed using Adobe Photoshop.

Whole-mount immunostaining. Whole-mount immunostaining was done essentially as described by Hemmati-Brivanlou and Harland (31). Embryos fixed with MEMFPA were blocked with 10% goat serum in phosphate-buffered saline containing 0.1% Tween 20 (PTw). The 12/101 antibody (developmental studies hybridoma bank) and Tor-70 antibody (a generous gift from R. Harland) were used at a 1:500 dilution. Incubation with the secondary antibody, a goat anti-mouse immunoglobulin G-horseradish peroxidase conjugate (UBI), was done at a 1:100 dilution at 4°C overnight. After extensive washing with PTw, embryos were placed in 0.5 mg of diaminobenzidine tetrahydrochloride solution per ml. The colorimetric reaction was stopped by replacing diaminobenzidine tetrahydrochloride solution with PTw. Embryos stained with Tor-70 were cleared with benzotriazole-benzyl alcohol (1:2 [vol/vol]).

RT-PCR assay. Total RNA from staged embryos was extracted using Trizol (Gibco BRL). cDNA was synthesized from 1 μ g of total RNA using SuperScriptII (Gibco BRL). PCR for XLPTP1a and XLPTP1b was performed in 50 μ l of solution containing 1 μ l of cDNA, 1 $\times$ Taq buffer, 1.5 mM MgCl₂, 0.2 mM (each)

FIG. 1. Comparison of deduced amino acid sequences of LMW-PTPs. (a) Sequence alignment of XLPTP1a with those of other species as follows: human (HLPTP1a) and *Drosophila* (DLPTP1). (b) Sequence comparison of XLPTP1a and -1b. Sequences were aligned using ClustalW multiple sequence alignment (<http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html>). Identical amino acid residues are highlighted in black and highly conserved residues are highlighted in gray. Gaps are shown by dashes.

dNTPs, 350 ng of each isoform-specific primer, and 1 U of AmpliTaq DNA polymerase (Perkin Elmer). Following PCR, 10 μ l of sample was loaded on a 1% agarose gel. Primers and cycle number used for XLPTP1a and XLPTP1b were as follows: XLPTP1a, 5'-AGC CTG CAT GAA GAA GCA TG-3' and 5'-GAG CAT CGG ATA CAT TGC TG-3' for 30 cycles at 58°C; and XLPTP1b, 5'-CAT AGA CAG CGC TGC AAC TTC-3' and 5'-CTG CAC CTG GCT ACC TCT TC-3' for 27 cycles at 62°C. Extraction of total RNA from animal cap explants and reverse transcription (RT)-PCR assays were performed as described previously (49). The primer sequences and conditions for *Xbra* and *EF-1 α* (32) have been previously published. Histone H4 primer sequences were posted at the Xenbase web page (<http://cbmed.ucalgary.ca/pvize/html/methods/RT-PCR.html>).

Immunoprecipitation and Western blot analysis. Embryo or oocyte lysates were prepared with ice-cold lysis buffer (20 mM Tris [pH 8.0], 150 mM NaCl, 1% Nonidet P-40 containing 1:50-diluted Calbiochem Protease Inhibitor Cocktail Set III) as previously described (12), followed by extraction with Freon (Sigma) at a 1:1 (vol/vol) ratio. Immunoprecipitation was conducted on 20 oocyte equivalents with the indicated antibodies for 4 h and protein-A/G agarose (Santa Cruz) for an additional 1 h. Following three washes with lysis buffer, immunoprecipitates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (8%). After the proteins were transferred to a membrane (Immobilon-P; Millipore), the membrane was blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween 20 and was incubated with primary antibodies anti-HA (Covance), anti-phosphoMAP kinase (Sigma), and anti-FGFR1 (a generous gift from R. Friesel). Proteins were visualized using an appropriate secondary antibody coupled to horseradish peroxidase (UBI), followed by application of enhanced chemiluminescence reagents as specified by the manufacturer (Amersham). For phosphorylated MAP kinase (phosphoMAP kinase) analysis, lysates were prepared with buffer A (42). One embryo equivalent was examined for direct lysate analysis.

Nucleotide sequence accession numbers. XLPTP1a (accession number AY039214) and XLPTP1b (accession number AY039215) cDNA sequences have been submitted to GenBank.

RESULTS

Isolation of XLPTP1a and -1b. LMW-PTP has been implicated in regulating RTK signaling pathways (65, 66, 74). In an effort to understand the roles of PTPs during *Xenopus* development, we have isolated two *Xenopus* isoforms of LMW-PTP from a *Xenopus* stage 30 ZapII cDNA library using a degenerate PCR product. The degenerate PCR primers were designed from conserved sequences among other species (18, 50, 51, 88). Using the PCR product as a probe, we isolated

two complete cDNAs that were designated XLPTP1a and XLPTP1b. These cDNAs show the highest degree of amino acid identity with human LMW-PTPs (Fig. 1a; 78 and 73% for XLPTP1a and -1b, respectively). These cDNAs display approximately 40% identity with the *Drosophila* proteins. XLPTP1a and -1b are 159-amino-acid proteins containing the conserved signature motif, CXXXXXRS/T, diagnostic of the PTP active site, spanning amino acids 14 to 22. XLPTP1a and XLPTP1b differ from each other only in an internal 36-amino-acid region (positions 42 to 77) (Fig. 1b), suggesting that the two isoforms originate from alternative splicing (46, 71).

Expression of XLPTP1 overlaps with FGFR1 during *Xenopus* development. The temporal regulation of XLPTP1a and -1b transcripts during *Xenopus* development was examined by RT-PCR analysis. The expression of XLPTP1a and -1b was observed to remain relatively constant throughout development, displaying only a slight transient decrease during gastrula and neurula stages (stage 10 to 13) (Fig. 2a). We next examined the spatial expression pattern of XLPTP1 during development by whole-mount in situ hybridization. At the blastula stage, XLPTP1 transcripts are predominantly localized in the animal pole of the embryos (Fig. 2b). During early neurulation, XLPTP1 is expressed broadly in the anterior neural plate and to a lesser degree in the lateral posterior region of the embryo (Fig. 2c). By stage 17, XLPTP1 staining expands toward the posterior region with prominent expression along the neural fold (Fig. 2d). As development proceeds (stage 22-23), the optic placode, branchial arches, and somites express XLPTP1 (Fig. 2e). At stage 28, faint staining is detected in the otic vesicle and pronephric anlage. Expression in the optic vesicle, branchial arches, and somites becomes prominent at this stage (Fig. 2f). At later developmental stages (stage 36-37), staining in the otic vesicle, pronephros, and pronephric duct is robust. XLPTP1 expression is also found in the forebrain, midbrain, hindbrain, midbrain-hindbrain junction, olfactory placode, branchial arches, proctodeum, and ventral tail bud (Fig. 2g to j).

FIG. 2. Spatial and temporal expression of XLPTP1 mRNAs during development. (a) RNA was extracted from the indicated stages as described in Materials and Methods. RT-PCR was performed using XLPTP1a- and -1b-specific primers. Histone H4 primers were used for the loading control. (b to j) Whole-mount in situ hybridization of XLPTP1 transcripts. (b) Stage 8, lateral view of embryo. Note the staining in the animal pole (top). (c) Stage 13, dorsal view of embryo. Note the broad staining in the anterior (left). (d) Stage 17, dorsal view, anterior is left. Note the staining in the neural fold. (e) Stage 21-22, lateral view, anterior is left. Arrow heads indicate branchial arches. (f) Stage 28, lateral view. Arrow heads indicate the branchial arches. (g) Stage 36, lateral view. (h) Dorsal view of the head region from panel g. (i and j) Pronephros and somites, respectively. (k and l) Whole-mount in situ hybridization of FGFR1 transcripts at stage 17 (k) and stage 28 (l). Nf, neural fold; mhj, midbrain-hindbrain junction; ov, otic vesicle; fb, forebrain; mb, midbrain; hb, hindbrain; pn, pronephros; pd, pronephric duct; sm, somites.

Studies from cell culture systems suggest that LMW-PTP may be involved in a few RTK signaling pathways, including the PDGFR (9), macrophage colony-stimulating factor receptor (70), and insulin receptor (77) pathways. Since the FGFR signaling pathway is a well-characterized RTK pathway during *Xenopus* development, we tried to determine whether a possible relationship between LMW-PTP and the FGF signaling pathway exists during *Xenopus* development. We found that the overall expression pattern of XLPTP1 had striking similarity to that of FGFR1 (Fig. 2k and l) (23) and provided the first indication of a possible link to the FGF signaling pathway.

Loss of XLPTP1 function causes posterior truncation and

shortening of the A-P axis. Before determining whether a relationship exists between XLPTP1 and FGFR1 signaling, it is important to understand the role of endogenous XLPTP1 during *Xenopus* development. A loss-of-function strategy was undertaken using XLPTP1 antisense morpholino oligonucleotides (XLPTP-AS) corresponding to the 5' UTR of XLPTP1 (Fig. 3a). Targeting this region should block the translation of all XLPTP1 isoforms. These oligonucleotides were injected into the animal pole region of both blastomeres of two-cell-stage embryos (70 ng/embryo) and control morpholino oligonucleotides were also injected (70 ng/embryo). Embryos injected with XLPTP-AS developed normally until gastrulation and later showed severe truncation of posterior structures and shortening of the A-P axis in a dose-dependent manner (Fig. 3e). The gross anterior structures, including the cement gland, appeared to develop normally (Fig. 3c and e). Embryos injected with control morpholino oligonucleotides developed normally (Fig. 3b and e). To confirm that the XLPTP-AS-induced phenotype is specifically caused by blocking the XLPTP1 signal, we performed a morphological and biochemical rescue by coinjecting XLPTP-AS with XLPTP1 RNAs lacking the 5' UTR sequence that is complementary to the antisense oligonucleotides (XLPTP1a Δ 5'UTR or XLPTP1b Δ 5'UTR). Either single expression (not shown) or coexpression of XLPTP1a Δ 5'UTR and XLPTP1b Δ 5'UTR (Fig. 3d) efficiently rescued elongation of the A-P axis and posterior structures that were blocked by XLPTP-AS (XLPTP1a rescue, 74% [$n = 50$]; XLPTP1b rescue, 78% [$n = 48$]; XLPTP1a and -1b rescue, 81% [$n = 47$]). Specificity of XLPTP-AS was also biochemically confirmed by injecting HA-tagged XLPTP1a or -1b RNAs alone or with XLPTP-AS. The embryos were harvested at stage 9 and the expression of XLPTP1 was analyzed by Western blot analysis with anti-HA antibody. XLPTP-AS efficiently blocked expression of both XLPTP1 isoforms (Fig. 3f, left panel), while expression of XLPTP1a Δ 5'UTR or XLPTP1b Δ 5'UTR remained unaffected (Fig. 3f, right panel). One additional specificity control was the use of one other antisense morpholino oligonucleotide complementary to the sequence 5' of XLPTP-AS which yielded the same phenotype (not shown). These results indicate that XLPTP-AS specifically and efficiently blocks XLPTP1 function.

Inhibition of XLPTP1 causes deficiencies in neural- and mesoderm-derived tissues. Morphological analysis of XLPTP-AS showed poor development in the trunk and posterior structure. To further analyze the defects caused by XLPTP-AS, whole-mount in situ hybridization and whole-mount immunostaining of neural and mesodermal gene markers were performed. Uninjected embryos or those injected with 70 ng of XLPTP-AS were stained for *Nrp-1* (a pan-neural marker), *Xap-2* (a cephalic neural crest marker), 12/101 (an antibody against muscle actin), and Tor-70 (an antibody against a notochord-specific antigen) as described in Materials and Methods. In XLPTP-AS-injected embryos, the trunk and caudal expression of *Nrp-1* was dramatically repressed. Expression in anterior neural tissue such as the brain and eye was detectable, but those tissues were poorly developed (Fig. 4a and b). The cephalic neural crest marker, *Xap-2*, was only partially suppressed by XLPTP-AS; however, the expression pattern, particularly in the branchial arches, was very disorganized (Fig. 4c

FIG. 3. Morphological defects caused by XLPTP-AS and rescue of defects by XLPTP1. (a) Corresponding nucleotide sequence for XLPTP-AS is designated by an arrow. (b to d) Embryos were injected into both blastomeres at the two-cell stage and cultured until stage 28-29. (b) Control morpholino oligos (70 ng/embryo); (c) XLPTP-AS (70 ng/embryo); (d) XLPTP-AS plus both XLPTP1aΔ5'UTR and XLPTP1bΔ5'UTR. (e) XLPTP-AS or control morpholino oligonucleotides (CTL) were injected at the two-cell stage in the indicated amount. Truncated embryos were scored by displaying 40% reduction of axis length at stage 28-29. (f) Western blot analysis showing specificity of XLPTP-AS. Embryos were injected with XLPTP-AS (70 ng/embryo) with or without HA-tagged full-length XLPTP1a or -1b (2 ng of each per embryo) (left panel), or embryos were injected with XLPTP-AS (70 ng/embryo) with or without HA-tagged XLPTP1aΔ5'UTR and XLPTP1bΔ5'UTR (2 ng of each per embryo) (right panel) and cultured until stage 8-9. Embryonic extracts were prepared and run on a gel (sodium dodecyl sulfate–16% polyacrylamide gel electrophoresis gel), and Western blot analysis was performed with an anti-HA antibody.

and d). We reasoned that the XLPTP-AS-induced reduction of neural tissue might be the result of poorly developed mesodermal tissues. To examine the mesoderm-derived tissue, we performed whole-mount immunostaining with 12/101 and Tor-70. In XLPTP-AS-injected embryos, muscle tissue structure was suppressed and disorganized, as evidenced by the weak antibody staining and disruption of the chevron pattern of the somites (Fig. 4e and f). Notochord formation was also dramatically reduced in XLPTP-AS-injected embryos, in which the tubular pattern of the notochord was greatly disrupted (Fig. 4g and h). We tested whether expression of XLPTP1 could rescue this structure. XLPTP-AS (70 ng/embryo) was injected into two-cell-stage embryos alone or with XLPTP1aΔ5'UTR and XLPTP1bΔ5'UTR RNAs. Whole-mount immunostaining for Tor-70 clearly indicates that exogenous XLPTP1 can restore notochord formation (Fig. 4i and j). These results suggest that XLPTP1 may be involved in the development of mesoderm-derived structures.

Loss of XLPTP1 represses FGF-mediated mesoderm induction and elongation of ectodermal explants. It has been well established that FGF and Activin, a member of the transforming growth factor beta (TGF-β) family, can induce mesoderm induction in ectodermal explants (26, 38, 73). Since the main-

tenance and proper formation of mesoderm-derived tissue was disrupted by the XLPTP-AS in whole embryos, we tested the requirement for XLPTP1 during mesoderm induction in ectodermal explants. XLPTP-AS (70 ng/embryo) was coinjected with either eFGF (5 pg/embryo) or an activated FGFR1 (FGFR1 act) (50 pg/embryo) into both blastomeres of two-cell-stage embryos. Animal cap explants were excised at stage 8 and analyzed at stage 11.5 for the expression of *Xbra*. Both eFGF and FGFR1 act prominently induced *Xbra* expression (Fig. 5a) (35, 57); however, this expression was dramatically reduced by XLPTP-AS (Fig. 5a). Animal caps injected with XLPTP-AS displayed a substantial, but not complete, reduction of *Xbra* expression in response to human Activin (50 ng/ml) (Fig. 5b). These results indicate that XLPTP1 function plays a role in FGF-induced mesoderm gene expression.

Once mesoderm is formed, extensive movements of the germ layers are required for proper elongation of the body axis. Since XLPTP-AS-injected embryos have a truncated axis, we investigated whether XLPTP-AS could also block the elongation movements induced by FGF in animal cap explants. Embryos were left uninjected or were injected with XLPTP-AS (70 ng/embryo) alone or with FGFR1 act (100 pg/embryo). Animal cap explants were excised at stage 8 and cultured until

FIG. 4. Effect of XLPTP-AS on neural, neural crest, and mesoderm markers. XLPTP-AS (70 ng/embryo) was injected into two-cell-stage embryos and cultured until the tail bud stage. In situ hybridization of *Nrp-1* (pan neural marker) (a and b) and *Xap-2* (neural crest marker) (c and d) and immunostaining of 12/101 (muscle tissue marker) (e and f) and Tor-70 (notochord marker) (g and h) are shown. (i and j) Embryos were injected with XLPTP-AS (70 ng/embryo) with or without XLPTP1 Δ 5'UTR and XLPTP1b Δ 5'UTR (XLPTP1 Δ 5'UTR [2 ng of each per embryo]), cultured until stage 26, and immunostained for Tor-70. Arrow indicates the restored notochord. a to d, i, and j, lateral view; e to h, dorsal view; anterior is left in all embryos.

stage 14. XLPTP-AS inhibited the elongation of animal caps expressing the activated form of FGFR1 (Fig. 5c). While the FGFR-induced elongation was not completely blocked, these movements were dramatically reduced in XLPTP-AS-injected animal caps. Collectively, these results indicate that loss of XLPTP1 function inhibits FGF-induced mesoderm induction.

XLPTP1 function is essential for FGF-induced MAP kinase activation. Having established that XLPTP1 makes a valuable contribution to the morphological structures and gene expression associated with FGFR activity, we tested the biochemical events linked to FGF signaling. Since the Ras-MAP kinase pathway plays a critical role in FGF-mediated mesoderm induction (24, 41, 48, 86), we tested whether XLPTP-AS could influence FGF-induced activation of MAP kinase. Embryos were injected with eFGF (5 pg/embryo) or FGFR1 act RNA

(50 pg/embryo) either alone or with XLPTP-AS (70 ng/embryo). Whole embryonic lysates were prepared at stage 7 because this represents a period when endogenous MAP kinases are not activated (13, 17, 25). Since, phosphorylation of MAP kinase correlates with its activity, the phosphorylation state of MAP kinase was assessed by Western blotting with anti-phosphoMAP kinase antibody. Consistent with *Xbra* expression, activation of MAP kinase in response to eFGF and FGFR1 act was almost completely blocked by XLPTP-AS (Fig. 6a). Moreover, this MAP kinase inhibition was rescued by the reintroduction of XLPTP1a Δ 5'UTR and XLPTP1b Δ 5'UTR RNAs (Fig. 6b), again suggesting that XLPTP-AS specifically blocks translation of the endogenous XLPTP1. To further define the position of XLPTP1 in the FGF signaling pathway, activated Ras was tested for the ability to rescue the XLPTP-AS block of eFGF-mediated MAP kinase activation. eFGF RNA was coinjected into embryos with activated Ras (Ras act) (50 pg/embryo) plus XLPTP-AS. Activated Ras (Fig. 6b) rescued the XLPTP-AS-mediated inhibition of MAP kinase. As expected, XLPTP-AS did not inhibit activation of MAP kinase induced by activated Raf (Raf act) (500 pg/embryo) or Mek (Mek act) (700 pg/embryo) (Fig. 6c). We also tested whether there was an obvious effect of overexpressing XLPTP1 or inhibiting

FIG. 5. Inhibition of FGF-mediated mesoderm induction and animal cap elongation by XLPTP-AS. (a) Two-cell-stage embryos were left uninjected or injected with eFGF (5 pg) or FGFR1 act (50 pg) RNAs with or without XLPTP-AS into two blastomeres. Animal caps were excised at stage 8 and cultured until stage 11.5. Expression of *Xbra* was analyzed by RT-PCR. (b) Animal caps from embryos left uninjected or injected with XLPTP-AS were treated with or without Activin (50 ng/ml) and incubated until stage 11.5. *Xbra* expression was analyzed by RT-PCR. For both panels a and b, EF-1 α was used as a loading control. (c) Embryos were left uninjected or injected with FGFR1 act (100 pg) RNA with or without XLPTP-AS into two blastomeres at the two-cell stage. Animal cap explants were excised at stage 8 and cultured until stage 14. WE, whole embryo; RT(-), reaction without reverse transcriptase.

FIG. 6. Effect of XLPTP-AS on FGF-induced MAP kinase activation. (a) Inhibition of FGF-mediated MAP kinase phosphorylation by XLPTP-AS. eFGF (5 pg) or FGFR1 act (50 pg) RNAs were injected with or without XLPTP-AS (70 ng) into two blastomeres at the two-cell stage. (b) Rescue of MAP kinase inhibition by XLPTP1 or activated Ras. Combination of eFGF (5 pg), XLPTP-AS (70 ng), and XLPTP1a Δ 5'UTR and XLPTP1b Δ 5'UTR (XLPTP1 Δ 5'UTR [2 ng of each per embryo each]) or activated Ras (Ras act [50 pg]) RNAs were injected into two blastomeres at the two-cell stage as indicated. Embryonic extracts were prepared from stage 7 embryos. Western blot was performed with anti-phosphoMAP kinase (1:1,000). Anti-Erk-2 (1:1,000) blotting was used as a loading control. (c) Effect of XLPTP-AS on MAP kinase activation induced by activated Raf and Mek. Activated Raf (Raf act [500 pg/embryo]) or Mek (Mek act [700 pg/embryo]) was injected with or without XLPTP-AS. Phosphorylated MAP kinase was analyzed as for panel b. (d) Effect of XLPTP-AS or XLPTP1 on tyrosine phosphorylation of FGFR1. Embryos were injected with FGFR1 act (2 ng/embryo) alone or FGFR1 act and either XLPTP-AS or XLPTP1a plus -1b (2 ng of each per embryo). FGFR1 was immunoprecipitated and tyrosine phosphorylation was analyzed by Western blot with antiphosphotyrosine antibody.

XLPTP1 expression on tyrosine phosphorylation of FGFR1. Embryos were injected with FGFR1 act (2 ng/embryo) alone or with XLPTP-AS (70 ng/embryo) or XLPTP1a plus -1b (2 ng of each per embryo). Whole lysates were immunoprecipitated with anti-FGFR1 antibody and tyrosine phosphorylation of FGFR1 was analyzed by Western blotting with antiphosphotyrosine antibody. Loss or overexpression of XLPTP1 did not change the observed amount of tyrosine phosphorylated FGFR1 (Fig. 6d), suggesting that FGFR1 may not be the substrate of XLPTP1. Collectively, the above results suggest that XLPTP1 may serve as a positive component of FGF signaling that acts upstream of or parallel to Ras and downstream of FGFR1.

XLPTP1 physically interacts with FGFR1. The data presented above indicated that XLPTP1 acts at a point between FGFR1 and Ras. Since SNT1/FRS-2 constitutively associates with FGFR and acts as a docking platform for essential components of the Ras-MAP kinase pathway, we thought it was necessary to isolate the *Xenopus* SNT1/FRS-2 cDNA in an effort to determine whether XLPTP1 can physically associate with the FGFR1 complex. A full-length *Xenopus* SNT1/FRS-2 (XSNT1) clone was isolated from a *Xenopus* cDNA library, and the amino acid sequence shows 81 and 39% identity to human SNT1/FRS-2 and SNT2/FRS-3, respectively. These data clearly indicate that the cDNA represents XSNT1. A more detailed characterization of XSNT1 is published elsewhere (1). To test for possible interactions between XLPTP1 and FGFR1, *Xenopus* oocytes were injected with RNA encoding an activated FGFR1 (FGFR1 act) (14 ng/oocyte), a kinase-dead FGFR1 (FGFR1KD) (14 ng/oocyte), XSNT1 (14 ng/oocyte), and HA-tagged XLPTP1a plus -1b (14 ng/oocyte) as indicated. Oocytes were lysed and subjected to immunoprecipitation with an anti-HA antibody, and XLPTP1 immune complexes were analyzed by Western blotting with anti-FGFR1. We found that XLPTP1 interacted with only an activated FGFR1 in an XSNT1-dependent manner (Fig. 7a). XLPTP1 did not form complexes with FGFR1 act unless XSNT1 was coexpressed, and no complexes were observed for FGFR1KD, regardless of the presence of XSNT1 (Fig. 7a). The association between XLPTP1 and FGFR1 was further confirmed using wild-type FGFR1 stimulated with bFGF. RNAs encoding FGFR1WT (14 ng/oocyte) and HA-tagged XLPTP1a plus -1b (14 ng/oocyte) were injected into oocytes alone or with XSNT1 (14 ng/oocyte) RNA and then cultured in the presence or absence of bFGF (300 ng/ml) overnight. Immunoprecipitation of HA-tagged XLPTP1 was followed by Western blotting with anti-FGFR1. Consistent with the results of the activated FGFR1 mutant (Fig. 7a), XLPTP1 interacted with wild-type FGFR1 when stimulated with bFGF, and this interaction required the presence of XSNT (Fig. 7b). These observations suggest that XLPTP1 can interact with the FGFR1 signaling complex but that receptor activation and the association with XSNT1 are required for this interaction.

DISCUSSION

There is a wealth of biochemical data characterizing the LMW-PTPs, but the *in vivo* function of LMW-PTPs is less well understood. Recent overexpression and dominant-negative studies using cell culture systems have implicated the LMW-

FIG. 7. Interaction of XLPTP1 with FGFR1. (a) RNAs encoding HA-tagged XLPTP1a and -1b (XLPTP1 [14 ng each]), FGFR1 act (14 ng), FGFR1KD (14 ng), or XSNT1 (14 ng) were injected into oocytes as indicated. (b) HA-tagged XLPTP1a and -1b (XLPTP1 [14 ng each]), FGFR1WT (14 ng), FGFR1KD (14 ng), or XSNT1 (14 ng) RNAs were injected into oocytes and treated with bFGF (300 ng/ml) as indicated. Oocyte extracts were immunoprecipitated with an anti-HA antibody and Western blot analysis was performed with an anti-FGFR1 antibody (top panels). The middle panels show the expression of FGFR1 and the lower panels show expression of XLPTP1 in each extract. Note that XLPTP1 can interact only with an activated FGFR1 through XSNT1.

PTPs as negative, and in some cases, positive regulators of RTK signaling (65, 66, 74). To investigate the *in vivo* role of LMW-PTP, we isolated two *Xenopus* homologs of LMW-PTP. The expression pattern of XLPTP1 displayed substantial overlap with that of FGFR1, suggesting a possible involvement of XLPTP1 in the FGF signaling pathway (Fig. 2d, f, k, and l). We employed a loss-of-function approach based upon the use of antisense oligonucleotides to block translation of endogenous XLPTP1 transcripts. Morpholino-modified antisense oligonucleotides have been shown to hinder translation of target messages with high specificity and stability (76). This strategy has been successfully applied to recapitulate phenotypes of established genetic mutations and to understand the novel function of genes in several systems, including *Xenopus* (30, 36, 56). Embryos receiving XLPTP-AS showed restriction of trunk and posterior structures in a dose-dependent manner (Fig. 3c and e), and the A-P axis was significantly shortened, implicating gastrulation defects (Fig. 3c). Reintroduction of XLPTP1 RNA lacking the 5' UTR sequences that are complementary to the oligonucleotide was able to rescue the XLPTP-AS phenotype (Fig. 3d). This provides an excellent indication of the specificity and nontoxicity of the antisense oligonucleotides because the rescue is solely dependent upon reexpression of the XLPTP1 protein. Embryos rescued by the XLPTP1a and -1b isoforms still showed some subtle defects in fin mesenchyme and pharyngeal pouch formation (Fig. 3d). Although we cannot preclude the possibility that these subtle defects are nonspecific, some remaining defects may be expected since

XLPTP-AS should inhibit all XLPTP1 isoforms and we have only reintroduced two isoforms.

Appropriate elongation of the A-P body axis is dependent upon coordinated cell movements, and a possible role for XLPTP1 in cell migration is supported by a previous study in NIH 3T3 fibroblasts. In that study, LMW-PTP has been proposed to dephosphorylate tyrosine phosphorylated p190Rho-GAP in response to PDGF stimulation, leading to activation of Rho (11). Rho has been suggested to control actin rearrangement (80) and thus cell movement and shape changes during gastrulation in *Xenopus* and *Drosophila* (5, 62). Therefore, increased tyrosine phosphorylation of p190Rho-GAP caused by the depletion of XLPTP1 may down-regulate Rho, resulting in disruption of the actin rearrangement and impairment of cell movement. Whether this proposed signaling cascade is playing a role during *Xenopus* development will require further studies.

Disruption of FGF or PDGF signaling causes posterior truncation of *Xenopus* embryos. For example, expression of a truncated FGFR1 suppresses mesoderm induction and inhibits convergent extension during gastrulation, resulting in posterior truncation and lack of blastopore closure (2, 3). Loss of XLPTP1 causes a trunk and posterior truncation phenotype, but blastopore closure was not completely prevented (Fig. 3c). One possible explanation is that loss of XLPTP1 may also modulate the other RTK signaling pathways such as PDGFR, allowing embryos to close the blastopore. Evidence in support of this idea comes from studies in fibroblasts showing that

LMW-PTP can down-regulate PDGFR activity. During *Xenopus* gastrulation, PDGF signaling has been shown to regulate adhesion of involuting mesoderm to the overlying ectoderm (4). Therefore, loss of XLPTP1 may inappropriately modulate the PDGFR during gastrulation, which may compensate for inhibition of the FGFR signaling. Another possibility may be that other XLPTP1-related PTPs are not inhibited by XLPTP-AS and these proteins may provide partial redundancy. Regardless of the molecular events that allow blastopore closure in the presence of XLPTP-AS oligonucleotides, the morphological and molecular data presented here strongly suggest that loss of XLPTP1 causes A-P shortening and posterior truncations.

Although the loss of XLPTP1 in embryos caused certain phenotypic defects that are consistent with reduced FGF signaling (reduction of posterior neural tissue and mesoderm-derived tissues), other defects are also observed, like poor development of anterior neural tissue and partial blastopore closure (Fig. 4). These additional phenotypic defects suggest that XLPTP1 may play a significant role in other signal transduction pathways and may not be restricted to a role only in the FGF signaling pathway. Regardless, the similarity in expression patterns between XLPTP1 (Fig. 2b to j), FGFR1 (Fig. 2k and l) (23), and XLPTP-AS-induced disruption of mesoderm-derived tissues in embryos (Fig. 4e to h) suggested the possibility that XLPTP1 might play a role in mesoderm induction in response to FGF. Using ectodermal explants, we have determined that loss of XLPTP1 expression inhibits eFGF or activated FGFR1-induced *Xbra* expression (Fig. 5a) and partially inhibits Activin induction of this mesodermal gene marker (Fig. 5b). These results support a role for XLPTP1 in FGF signaling. Most, but not all, mesoderm differentiation is lost in the absence of FGF signaling (2, 3), while blocking most TGF- β family signals prevents the formation of any mesoderm (33). Interestingly, inhibition of FGF signaling prevents continuous expression of most other mesodermal properties in response to Activin, and FGF signaling may provide a permissive environment in which Activin can fully elicit and maintain its signals (43). The partial reduction in Activin-induced *Xbra* expression caused by XLPTP-AS may be due to incomplete inhibition of all FGFR signaling, since only a basal level of activity is needed to allow Activin-induced mesoderm formation (43). Alternatively, *Xbra* may be transiently induced in the absence of XLPTP1 function, similar to other studies showing that mesoderm-specific marker genes are transiently activated by Activin in the presence of a truncated FGFR. These studies suggest that FGF has a role in mesoderm maintenance rather than induction in response to Activin (35, 72). Additional support for this concept comes from a recent study showing that the transmembrane protein, Sef, inhibits *Xbra* expression in response to FGF but not Activin (81).

In addition to mesoderm induction, FGF causes animal cap tissue to elongate. Loss of XLPTP1 function blocks mesoderm formation in caps and therefore may block the corresponding elongation movements. However, recent data indicate that the pathways controlling mesoderm induction and elongation in response to FGF may be at least partially separable. Activated forms of SHP-2 preferentially activate a pathway(s) downstream of the *Xenopus* FGFR that leads to animal cap elongation (62), and Xsprouty-2, an endogenous inhibitor of FGF

signaling, blocks elongation but not *Xbra* or MAP kinase activation (59). We provide evidence that loss of XLPTP1 function substantially reduces the elongation movements induced by the FGF signal transduction pathway (Fig. 5c).

Since loss of XLPTP1 inhibits FGF-induced mesoderm induction and accompanying elongation movements, it is important to consider the functional position of XLPTP1 in the FGF signal transduction pathway. The induction of mesoderm via FGF is dependent upon signals from the Ras-MAP kinase pathway (44). The introduction of XLPTP-AS efficiently blocked FGF-induced MAP kinase activation (Fig. 6a), suggesting that XLPTP1 may function at a point between the FGFR1 and MAP kinase. Introduction of activated Ras completely rescued the XLPTP-AS-induced block to FGF-mediated MAP kinase activation (Fig. 6b and c). Tyrosine phosphorylation of FGFR1 was not obviously affected by XLPTP-AS or XLPTP1 (Fig. 6d). These data provide evidence that XLPTP1 may act as a positive component, upstream or parallel to Ras and downstream of FGFR1, in the FGF signaling pathway.

Evidence placing XLPTP1 in a complex with FGFR1 came from experiments in the *Xenopus* oocyte expression system showing that XLPTP1 physically interacts with FGFR1, but this interaction requires an activated receptor and the presence of XSNT1 (Fig. 7a and b). *Xenopus* oocytes were used in this experiment because the full mediation of FGF signaling by endogenous SNT1 is somewhat limited in the oocyte when compared to the embryo (K. Mood and I. O. Daar, unpublished results). We have taken advantage of this system because it allows us to test whether the association between XLPTP1 and FGFR1 is dependent upon SNT1. Interestingly, SNT1/FRS-2 has been shown to constitutively associate with FGFR1 independent of receptor activation (60). However, recruitment of other signaling molecules requires phosphorylation induced by FGFR. Growing evidence suggests that SNT1/FRS-2 has a docking function much like that of the insulin receptor substrate (63), and the above data suggest that XLPTP1 may complex with XSNT1 or one of the recruited proteins. While it is formally possible that XSNT1 activates a downstream signal that allows XLPTP1 to directly bind the FGFR1, evidence suggests that this is unlikely. In embryos coexpressing an activated FGFR1 and XLPTP1, no detectable decrease in tyrosine phosphorylation of FGFR1 was observed (Fig. 6d). It is also possible that XLPTP1 may modulate the activity and/or recruitment of a protein within the FGFR1 complex. SNT1 is unlikely to be such a protein because we observe no obvious change in the tyrosine phosphorylation state of SNT1 in the presence of an activated FGFR1, regardless of whether XLPTP1 is present (data not shown). These data do not support a model in which FGFR1 is the target of XLPTP1 but do support the concept that XLPTP1 acts as a positive component of the FGF signaling pathway. However, we cannot exclude the possibility that XLPTP1 may act at other points in the signaling pathway and play a subsequent role in termination of FGF signaling.

In the case of another PTP, SHP-2, there is evidence supporting both a positive and negative role in PDGF signaling (69). For example, the SHP-2 binding site (Tyr1009) of PDGFR- β is important for down-regulation of the PDGFR (16, 47), suggesting a negative role. However, SHP-2 has also

been shown to act as a positive component of PDGF signaling, where mutations in the SHP-2 binding sites, Tyr763 and Tyr1009, inhibited chemotaxis and reduced PDGF-induced Ras and MAP kinase activation (68). Interestingly, an in vitro study has shown that LMW-PTP binds to and efficiently dephosphorylates a phosphopeptide containing Tyr1009 derived from PDGFR- β (6), suggesting that SHP-2 and LMW-PTP may share a common target(s). SHP-2 also has been shown to play a positive role in FGF-mediated mesoderm induction during *Xenopus* development (62, 79).

Our finding that XLPTP1 plays a positive role in the FGF signaling pathway identifies another component of this signaling pathway. However, the precise mechanism by which XLPTP1 and SHP-2 act as positive regulators in the FGF signal transduction pathway remains to be determined. A small but growing number of positive and negative regulators that act between FGFR and Ras are being identified, for instance, Sprouty, Dof, Laloo, and Sef (22, 81). Defining the protein target(s) of XLPTP1 and understanding the relationship of XLPTP to the other identified regulators will be a necessary step toward understanding the FGF signaling pathway.

ACKNOWLEDGMENTS

We thank Richard Harland, Igor Dawid, Tom Sargent, and Robert Friesel for generously providing reagents. We also thank Mark Lewandoski, Alan Perantoni, and Jaebong Kim for critical reading of the manuscript and for discussion. Activin A was obtained through NHPP, NIDDK, and Parlow. We apologize to many of our colleagues whose work we were unable to cite due to space considerations.

REFERENCES

1. Akagi, K., E. K. Park, K. Mood, and I. O. Daar. 2002. The docking protein SNT1 is a critical mediator of fibroblast growth factor signaling during *Xenopus* embryonic development. *Dev. Dyn.* **223**:216–228.
2. Amaya, E., T. J. Musci, and M. W. Kirschner. 1991. Expression of a dominant negative mutant of the FGF receptor disrupts mesoderm formation in *Xenopus* embryos. *Cell* **66**:257–270.
3. Amaya, E., P. A. Stein, T. J. Musci, and M. W. Kirschner. 1993. FGF signalling in the early specification of mesoderm in *Xenopus*. *Development* **118**:477–487.
4. Ataliotis, P., K. Symes, M. M. Chou, L. Ho, and M. Mercola. 1995. PDGF signalling is required for gastrulation of *Xenopus laevis*. *Development* **121**:3099–3110.
5. Barrett, K., M. Leptin, and J. Settlement. 1997. The Rho GTPase and a putative RhoGEF mediate a signaling pathway for the cell shape changes in *Drosophila* gastrulation. *Cell* **91**:905–915.
6. Bucciantini, M., M. Stefani, N. Taddei, F. Chiti, S. Rigacci, and G. Ramponi. 1998. Sequence-specific recognition of peptide substrates by the low Mr phosphotyrosine protein phosphatase isoforms. *FEBS Lett.* **422**:213–217.
7. Carballada, R., H. Yasuo, and P. Lemaire. 2001. Phosphatidylinositol-3 kinase acts in parallel to the ERK MAP kinase in the FGF pathway during *Xenopus* mesoderm induction. *Development* **128**:35–44.
8. Casci, T., J. Vinos, and M. Freeman. 1999. Sprouty, an intracellular inhibitor of Ras signaling. *Cell* **96**:655–665.
9. Chiarugi, P., P. Cirri, G. Raugi, G. Camici, F. Dolfi, A. Berti, and G. Ramponi. 1995. PDGF receptor as a specific in vivo target for low M(r) phosphotyrosine protein phosphatase. *FEBS Lett.* **372**:49–53.
10. Chiarugi, P., P. Cirri, G. Raugi, G. Manao, L. Taddei, and G. Ramponi. 1996. Low M(r) phosphotyrosine protein phosphatase interacts with the PDGF receptor directly via its catalytic site. *Biochem. Biophys. Res. Commun.* **219**:21–25.
11. Chiarugi, P., P. Cirri, L. Taddei, E. Giannoni, G. Camici, G. Manao, G. Raugi, and G. Ramponi. 2000. The low M(r) protein-tyrosine phosphatase is involved in Rho-mediated cytoskeleton rearrangement after integrin and platelet-derived growth factor stimulation. *J. Biol. Chem.* **275**:4640–4646.
12. Chong, L. D., E. K. Park, E. Latimer, R. Friesel, and I. O. Daar. 2000. Fibroblast growth factor receptor-mediated rescue of x-ephrin B1-induced cell dissociation in *Xenopus* embryos. *Mol. Cell. Biol.* **20**:724–734.
13. Christen, B., and J. M. Slack. 1999. Spatial response to fibroblast growth factor signalling in *Xenopus* embryos. *Development* **126**:119–125.
14. Cirri, P., P. Chiarugi, L. Taddei, G. Raugi, G. Camici, G. Manao, and G. Ramponi. 1998. Low molecular weight protein-tyrosine phosphatase tyrosine phosphorylation by c-Src during platelet-derived growth factor-induced mitogenesis correlates with its subcellular targeting. *J. Biol. Chem.* **273**:32522–32527.
15. Cirri, P., T. Fiaschi, P. Chiarugi, G. Camici, G. Manao, G. Raugi, and G. Ramponi. 1996. The molecular basis of the differing kinetic behavior of the two low molecular mass phosphotyrosine protein phosphatase isoforms. *J. Biol. Chem.* **271**:2604–2607.
16. Cossette, L. J., O. Hoglinger, L. Mou, and S. H. Shen. 1996. Localization and down-regulating role of the protein tyrosine phosphatase PTP2C in membrane ruffles of PDGF-stimulated cells. *Exp. Cell Res.* **223**:459–466.
17. Curran, K. L., and R. M. Grainger. 2000. Expression of activated MAP kinase in *Xenopus laevis* embryos: evaluating the roles of FGF and other signaling pathways in early induction and patterning. *Dev. Biol.* **228**:41–56.
18. Dissing, J., and A. H. Johnsen. 1992. Human red cell acid phosphatase (ACP1): the primary structure of the two pairs of isozymes encoded by the ACP1*A and ACP1*C alleles. *Biochim. Biophys. Acta* **1121**:261–268.
19. Dissing, J., A. H. Johnsen, and G. F. Sensabaugh. 1991. Human red cell acid phosphatase (ACP1). The amino acid sequence of the two isozymes Bf and Bs encoded by the ACP1*B allele. *J. Biol. Chem.* **266**:20619–20625.
20. Fabian, J. R., D. K. Morrison, and I. O. Daar. 1993. Requirement for Raf and MAP kinase function during the meiotic maturation of *Xenopus* oocytes. *J. Cell Biol.* **122**:645–652.
21. Fauman, E. B., and M. A. Saper. 1996. Structure and function of the protein tyrosine phosphatases. *Trends Biochem. Sci.* **21**:413–417.
22. Furthauer, M., W. Lin, S. L. Ang, B. Thisse, and C. Thisse. 2002. Sef is a feedback-induced antagonist of Ras/MAPK-mediated FGF signalling. *Nat. Cell Biol.* **4**:170–174.
23. Golub, R., Z. Adelman, J. Clementi, R. Weiss, J. Bonasera, and M. Servetnick. 2000. Evolutionarily conserved and divergent expression of members of the FGF receptor family among vertebrate embryos, as revealed by FGFR expression patterns in *Xenopus*. *Dev. Genes Evol.* **210**:345–357.
24. Gotoh, Y., N. Masuyama, A. Suzuki, N. Ueno, and E. Nishida. 1995. Involvement of the MAP kinase cascade in *Xenopus* mesoderm induction. *EMBO J.* **14**:2491–2498.
25. Graves, L. M., J. L. Northrop, B. C. Potts, E. G. Krebs, and D. Kimelman. 1994. Fibroblast growth factor, but not activin, is a potent activator of mitogen-activated protein kinase in *Xenopus* explants. *Proc. Natl. Acad. Sci. USA* **91**:1662–1666.
26. Green, J. B., and J. C. Smith. 1990. Graded changes in dose of a *Xenopus* activin A homologue elicit stepwise transitions in embryonic cell fate. *Nature* **347**:391–394.
27. Gupta, R. W., and B. J. Mayer. 1998. Dominant-negative mutants of the SH2/SH3 adapters Nck and Grb2 inhibit MAP kinase activation and mesoderm-specific gene induction by eFGF in *Xenopus*. *Oncogene* **17**:2155–2165.
28. Harland, R. M. 1991. In situ hybridization: an improved whole-mount method for *Xenopus* embryos. *Methods Cell Biol.* **36**:685–695.
29. Hartley, R. S., A. L. Lewellyn, and J. L. Maller. 1994. MAP kinase is activated during mesoderm induction in *Xenopus laevis*. *Dev. Biol.* **163**:521–524.
30. Heasman, J., M. Kofron, and C. Wylie. 2000. Beta-catenin signaling activity dissected in the early *Xenopus* embryo: a novel antisense approach. *Dev. Biol.* **222**:124–134.
31. Hemmati-Brivanlou, A., and R. M. Harland. 1989. Expression of an engrailed-related protein is induced in the anterior neural ectoderm of early *Xenopus* embryos. *Development* **106**:611–617.
32. Hemmati-Brivanlou, A., and D. A. Melton. 1994. Inhibition of activin receptor signaling promotes neuralization in *Xenopus*. *Cell* **77**:273–281.
33. Hemmati-Brivanlou, A., and D. A. Melton. 1992. A truncated activin receptor inhibits mesoderm induction and formation of axial structures in *Xenopus* embryos. *Nature* **359**:609–614.
34. Hunter, T. 1995. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell* **80**:225–236.
35. Isaacs, H. V., M. E. Pownall, and J. M. Slack. 1994. eFGF regulates Xbra expression during *Xenopus* gastrulation. *EMBO J.* **13**:4469–4481.
36. Joukov, V., J. Chen, E. A. Fox, J. B. Green, and D. M. Livingston. 2001. Functional communication between endogenous BRCA1 and its partner, BARD1, during *Xenopus laevis* development. *Proc. Natl. Acad. Sci. USA* **98**:12078–12083.
37. Keller, R. 1991. Early embryonic development of *Xenopus laevis*. *Methods Cell Biol.* **36**:61–113.
38. Kimelman, D., and M. Kirschner. 1987. Synergistic induction of mesoderm by FGF and TGF-beta and the identification of an mRNA coding for FGF in the early *Xenopus* embryo. *Cell* **51**:869–877.
39. Kouhara, H., Y. R. Hadari, T. Spivak-Kroizman, J. Schilling, D. Bar-Sagi, I. Lax, and J. Schlessinger. 1997. A lipid-anchored Grb2-binding protein that links FGF-receptor activation to the Ras/MAPK signaling pathway. *Cell* **89**:693–702.
40. Kroll, K. L., and E. Amaya. 1996. Transgenic *Xenopus* embryos from sperm nuclear transplantations reveal FGF signaling requirements during gastrulation. *Development* **122**:3173–3183.
41. LaBonne, C., B. Burke, and M. Whitman. 1995. Role of MAP kinase in

- mesoderm induction and axial patterning during *Xenopus* development. *Development* **121**:1475–1486.
42. LaBonne, C., and M. Whitman. 1997. Localization of MAP kinase activity in early *Xenopus* embryos: implications for endogenous FGF signaling. *Dev. Biol.* **183**:9–20.
 43. LaBonne, C., and M. Whitman. 1994. Mesoderm induction by activin requires FGF-mediated intracellular signals. *Development* **120**:463–472.
 44. Landgren, E., P. Blume-Jensen, S. A. Courtneidge, and L. Claesson-Welsh. 1995. Fibroblast growth factor receptor-1 regulation of Src family kinases. *Oncogene* **10**:2027–2035.
 45. Larsson, H., P. Klint, E. Landgren, and L. Claesson-Welsh. 1999. Fibroblast growth factor receptor-1-mediated endothelial cell proliferation is dependent on the Src homology (SH) 2/SH3 domain-containing adaptor protein Crk. *J. Biol. Chem.* **274**:25726–25734.
 46. Lazaruk, K. D., J. Dissing, and G. F. Sensabaugh. 1993. Exon structure at the human ACP1 locus supports alternative splicing model for f and s isozyme generation. *Biochem. Biophys. Res. Commun.* **196**:440–446.
 47. Lechleider, R. J., S. Sugimoto, A. M. Bennett, A. S. Kashishian, J. A. Cooper, S. E. Shoelson, C. T. Walsh, and B. G. Neel. 1993. Activation of the SH2-containing phosphotyrosine phosphatase SH-PTP2 by its binding site, phosphotyrosine 1009, on the human platelet-derived growth factor receptor. *J. Biol. Chem.* **268**:21478–21481.
 48. MacNicol, A. M., A. J. Muslin, and L. T. Williams. 1993. Raf-1 kinase is essential for early *Xenopus* development and mediates the induction of mesoderm by FGF. *Cell* **73**:571–583.
 49. Maeda, R., K. Mood, T. L. Jones, J. Aruga, A. M. Buchberg, and I. O. Daar. 2001. Xmeis1, a protooncogene involved in specifying neural crest cell fate in *Xenopus* embryos. *Oncogene* **20**:1329–1342.
 50. Magherini, F., E. Giannoni, G. Raugé, P. Cirri, P. Paoli, A. Modesti, G. Camici, and G. Ramponi. 1998. Cloning of murine low molecular weight phosphotyrosine protein phosphatase cDNA: identification of a new isoform. *FEBS Lett.* **437**:263–266.
 51. Manao, G., L. Pazzagli, P. Cirri, A. Caselli, G. Camici, G. Cappugi, A. Saeed, and G. Ramponi. 1992. Rat liver low M(r) phosphotyrosine protein phosphatase isoenzymes: purification and amino acid sequences. *J. Protein Chem.* **11**:333–345.
 52. Mayor, R., R. Morgan, and M. G. Sargent. 1995. Induction of the prospective neural crest of *Xenopus*. *Development* **121**:767–777.
 53. Modesti, A., R. Marzocchini, G. Raugé, F. Chiti, A. Sereni, F. Magherini, and G. Ramponi. 1998. Cloning, expression and characterisation of a new human low Mr phosphotyrosine protein phosphatase originating by alternative splicing. *FEBS Lett.* **431**:111–115.
 54. Mohammadi, M., A. M. Honegger, D. Rotin, R. Fischer, F. Bellot, W. Li, C. A. Dionne, M. Jaye, M. Rubinstein, and J. Schlessinger. 1991. A tyrosine-phosphorylated carboxy-terminal peptide of the fibroblast growth factor receptor (Flg) is a binding site for the SH2 domain of phospholipase C-gamma 1. *Mol. Cell. Biol.* **11**:5068–5078.
 55. Muslin, A. J., K. G. Peters, and L. T. Williams. 1994. Direct activation of phospholipase C-gamma by fibroblast growth factor receptor is not required for mesoderm induction in *Xenopus* animal caps. *Mol. Cell. Biol.* **14**:3006–3012.
 56. Nasevicius, A., and S. C. Ekker. 2000. Effective targeted gene “knockdown” in zebrafish. *Nat. Genet.* **26**:216–220.
 57. Neilson, K. M., and R. Friesel. 1996. Ligand-independent activation of fibroblast growth factor receptors by point mutations in the extracellular, transmembrane, and kinase domains. *J. Biol. Chem.* **271**:25049–25057.
 58. Nieuwkoop, P. D., and J. Faber. 1967. Normal table of *Xenopus laevis*. Daudine: North-Holland, Amsterdam, The Netherlands.
 59. Nutt, S. L., K. S. Dingwell, C. E. Holt, and E. Amaya. 2001. *Xenopus* Sprouty2 inhibits FGF-mediated gastrulation movements but does not affect mesoderm induction and patterning. *Genes Dev.* **15**:1152–1166.
 60. Ong, S. H., G. R. Guy, Y. R. Hadari, S. Laks, N. Gotoh, J. Schlessinger, and I. Lax. 2000. FRS2 proteins recruit intracellular signaling pathways by binding to diverse targets on fibroblast growth factor and nerve growth factor receptors. *Mol. Cell. Biol.* **20**:979–989.
 61. Ong, S. H., Y. R. Hadari, N. Gotoh, G. R. Guy, J. Schlessinger, and I. Lax. 2001. Stimulation of phosphatidylinositol 3-kinase by fibroblast growth factor receptors is mediated by coordinated recruitment of multiple docking proteins. *Proc. Natl. Acad. Sci. USA* **98**:6074–6079.
 62. O'Reilly, A. M., S. Pluskey, S. E. Shoelson, and B. G. Neel. 2000. Activated mutants of SHP-2 preferentially induce elongation of *Xenopus* animal caps. *Mol. Cell. Biol.* **20**:299–311.
 63. Powers, C. J., S. W. McLeskey, and A. Wellstein. 2000. Fibroblast growth factors, their receptors and signaling. *Endocr. Relat. Cancer* **7**:165–197.
 64. Ramponi, G., G. Manao, G. Camici, G. Cappugi, M. Ruggiero, and D. P. Bottaro. 1989. The 18 kDa cytosolic acid phosphatase from bovine liver has phosphotyrosine phosphatase activity on the autophosphorylated epidermal growth factor receptor. *FEBS Lett.* **250**:469–473.
 65. Ramponi, G., and M. Stefani. 1997. Structural, catalytic, and functional properties of low M(r) phosphotyrosine protein phosphatases. Evidence of a long evolutionary history. *Int. J. Biochem. Cell Biol.* **29**:279–292.
 66. Ramponi, G., and M. Stefani. 1997. Structure and function of the low Mr phosphotyrosine protein phosphatases. *Biochim. Biophys. Acta* **1341**:137–156.
 67. Richter, K., P. J. Good, and I. B. Dawid. 1990. A developmentally regulated, nervous system-specific gene in *Xenopus* encodes a putative RNA-binding protein. *New Biol.* **2**:556–565.
 68. Ronnstrand, L., A. K. Arvidsson, A. Kallin, C. Rorsman, U. Hellman, U. Engstrom, C. Wernstedt, and C. H. Heldin. 1999. SHP-2 binds to Tyr763 and Tyr1009 in the PDGF beta-receptor and mediates PDGF-induced activation of the Ras/MAP kinase pathway and chemotaxis. *Oncogene* **18**:3696–3702.
 69. Ronnstrand, L., and C. H. Heldin. 2001. Mechanisms of platelet-derived growth factor-induced chemotaxis. *Int. J. Cancer* **91**:757–762.
 70. Rovida, E., S. Rigacci, A. Paccagnini, P. D. Sbarba, and A. Berti. 1998. The low-molecular-weight phosphotyrosine protein phosphatase, when overexpressed, reduces the mitogenic response to macrophage colony-stimulating factor and tyrosine phosphorylation of its receptor. *Biochem. Biophys. Res. Commun.* **253**:300–304.
 71. Rudbeck, L., J. Dissing, K. D. Lazaruk, and G. Sensabaugh. 2000. Human 18 kDa phosphotyrosine protein phosphatase (ACP1) polymorphism: studies of rare variants provide evidence that substitutions within or near alternatively spliced exons affect splicing result. *Ann. Hum. Genet.* **64**:107–116.
 72. Schulte-Merker, S., and J. C. Smith. 1995. Mesoderm formation in response to brachyury requires FGF signalling. *Curr. Biol.* **5**:62–67.
 73. Slack, J. M., B. G. Darlington, J. K. Heath, and S. F. Godsave. 1987. Mesoderm induction in early *Xenopus* embryos by heparin-binding growth factors. *Nature* **326**:197–200.
 74. Stein, E., A. A. Lane, D. P. Cerretti, H. O. Schoeckmann, A. D. Schroff, R. L. Van Etten, and T. O. Daniel. 1998. Eph receptors discriminate specific ligand oligomers to determine alternative signaling complexes, attachment, and assembly responses. *Genes Dev.* **12**:667–678.
 75. Stone, R. L., and J. E. Dixon. 1994. Protein-tyrosine phosphatases. *J. Biol. Chem.* **269**:31323–31326.
 76. Summerton, J. 1999. Morpholino antisense oligomers: the case for an RNase H-independent structural type. *Biochim. Biophys. Acta* **1489**:141–158.
 77. Taddei, M. L., P. Chiarugi, P. Cirri, D. Talini, G. Camici, G. Manao, G. Raugé, and G. Ramponi. 2000. LMW-PTP exerts a differential regulation on PDGF- and insulin-mediated signaling. *Biochem. Biophys. Res. Commun.* **270**:564–569.
 78. Tailor, P., J. Gilman, S. Williams, and T. Mustelin. 1999. A novel isoform of the low molecular weight phosphotyrosine phosphatase, LMPTP-C, arising from alternative mRNA splicing. *Eur. J. Biochem.* **262**:277–282.
 79. Tang, T. L., R. M. Freeman, Jr., A. M. O'Reilly, B. G. Neel, and S. Y. Sokol. 1995. The SH2-containing protein-tyrosine phosphatase SH-PTP2 is required upstream of MAP kinase for early *Xenopus* development. *Cell* **80**:473–483.
 80. Tapon, N., and A. Hall. 1997. Rho, Rac and Cdc42 GTPases regulate the organization of the actin cytoskeleton. *Curr. Opin. Cell Biol.* **9**:86–92.
 81. Tsang, M., R. Friesel, T. Kudoh, and I. B. Dawid. 2002. Identification of Sef, a novel modulator of FGF signalling. *Nat. Cell Biol.* **4**:165–169.
 82. Umbhauer, M., C. J. Marshall, C. S. Mason, R. W. Old, and J. C. Smith. 1995. Mesoderm induction in *Xenopus* caused by activation of MAP kinase. *Nature* **376**:58–62.
 83. Vincent, S., R. Wilson, C. Coelho, M. Affolter, and M. Leptin. 1998. The *Drosophila* protein Dof is specifically required for FGF signaling. *Mol. Cell* **2**:515–525.
 84. Wang, S., L. Tabernero, M. Zhang, E. Harms, R. L. Van Etten, and C. V. Stauffacher. 2000. Crystal structures of a low-molecular weight protein tyrosine phosphatase from *Saccharomyces cerevisiae* and its complex with the substrate *p*-nitrophenyl phosphate. *Biochemistry* **39**:1903–1914.
 85. Weinstein, D. C., J. Marden, F. Carnevali, and A. Hemmati-Brivanlou. 1998. FGF-mediated mesoderm induction involves the Src-family kinase Laloo. *Nature* **394**:904–908.
 86. Whitman, M., and D. A. Melton. 1992. Involvement of p21ras in *Xenopus* mesoderm induction. *Nature* **357**:252–254.
 87. Winning, R. S., L. J. Shea, S. J. Marcus, and T. D. Sargent. 1991. Developmental regulation of transcription factor AP-2 during *Xenopus laevis* embryogenesis. *Nucleic Acids Res.* **19**:3709–3714.
 88. Wo, Y. Y., M. M. Zhou, P. Stevis, J. P. Davis, Z. Y. Zhang, and R. L. Van Etten. 1992. Cloning, expression, and catalytic mechanism of the low molecular weight phosphotyrosyl protein phosphatase from bovine heart. *Biochemistry* **31**:1712–1721.
 89. Zhan, X., C. Plourde, X. Hu, R. Friesel, and T. Maciag. 1994. Association of fibroblast growth factor receptor-1 with c-Src correlates with association between c-Src and cortactin. *J. Biol. Chem.* **269**:20221–20224.