Direct Activation of Phospholipase C- γ by Fibroblast Growth Factor Receptor Is Not Required for Mesoderm Induction in *Xenopus* Animal Caps

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Members of the fibroblast growth factor (FGF) family induce mesoderm formation in explants of *Xenopus* embryonic ectoderm (animal caps). Recent studies have been directed at determining signaling pathways downstream of the FGF receptor that are important in mesoderm induction. We have recently shown that a point mutation in the FGF receptor changing tyrosine 766 to phenylalanine (Y/F mutation) abolishes phospholipase C- γ (PLC γ) activation in mammalian cells. To explore the role of PLC γ activation in FGF-stimulated mesoderm induction, we constructed two chimeric receptors, each consisting of the extracellular portion of the platelet-derived growth factor β receptor, with one having the transmembrane and intracellular portions of the wild-type FGF receptor 1 (PR-FR wt) and the other having the corresponding region of the Y/F766 mutant FGF receptor 1 (PR-FR Y/F766). When expressed in *Xenopus* oocytes, only PR-FR wt was able to mediate PLC γ phosphorylation, inositol-1,4,5-trisphosphate accumulation, and calcium efflux in response to platelet-derived growth factor stimulation. However, both receptors mediated mesoderm induction in *Xenopus* animal caps as measured by cap elongation, muscle-specific actin mRNA induction, and skeletal muscle formation. These results demonstrate that PLC γ activation by the FGF receptor is not required for FGF-stimulated mesoderm induction.

Mesoderm induction can be mediated in explants of *Xenopus* embryonic ectoderm (animal caps) by members of the fibroblast growth factor (FGF) and transforming growth factor β families (14, 32, 35). Studies employing dominant-negative forms of the FGF and activin receptors have provided compelling evidence that these growth factors induce mesoderm in vivo (1, 13). The signal transduction pathways that mediate growth factor-stimulated mesoderm induction are now beginning to be examined. Recently, the use of dominant-negative forms of the signaling molecules Ras and Raf-1 has provided data indicating that both of these molecules are required for FGF-stimulated mesoderm induction in frogs (22, 39).

Another intracellular signaling molecule, phospholipase C- γ (PLC γ), associates with and is activated by several growth factor receptors (5, 10, 24, 28, 38), including the FGF receptor (27). PLC γ mediates the hydrolysis of phosphatidylinositol biphosphate to inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (2), which, in turn, lead to the mobilization of Ca²⁺ and to the activation of protein kinase C (2, 4).

Phosphoinositide (PI) hydrolysis has been postulated to play a critical role in vertebrate embryonic axis formation and in mesoderm induction (3, 4, 16, 17). In *Xenopus* embryos, IP3 accumulation increases at the time of mesoderm induction (23), and when early *Xenopus* embryos are treated with lithium, an inhibitor of PI metabolism, presumptive ventral mesodermal tissues are respecified to dorsal cell fates (8, 17). This "dorsalization" of lithium-treated embryos could be rescued by cotreatment with myoinositol or a diacylglycerol

analog (6). Taken together, these results strongly suggest that PI turnover is important in ventral mesoderm induction.

Previous studies have provided substantial evidence that a member of the FGF family is a natural inducer of mesoderm and that FGF induces mesoderm that has posterior and ventral features, such as mesenchyme and blood (12, 32). This is in contrast to activin, which induces mesoderm having more anterior and dorsal features, such as facial mesoderm and notochord (12, 28, 34, 36). Given the dramatic effects of lithium on embryonic pattern formation, we predicted that PLC γ activation by the FGF receptor would be important for mesoderm induction by FGF.

Recently we have shown that an FGF receptor with a single point mutation changing tyrosine 766 to phenylalanine (Y/F766) failed to associate with or phosphorylate PLC γ . As a result the mutant FGF receptor was unable to stimulate PI turnover or Ca²⁺ mobilization, yet it retained the ability to stimulate mitogenesis and chemotaxis in cultured mammalian cells (26, 31). To determine whether PLC γ activation by the FGF receptor is required for mesoderm induction in *Xenopus* animal caps, we made chimeric growth factor receptors, consisting of the extracellular portion of the platelet-derived growth factor β (PDGF- β) receptor and the intracellular portion of either the wild-type FGF receptor or the Y/F766 mutant FGF receptor. Since PDGF does not induce mesoderm in animal caps (32), the chimeric receptors allowed us to study mesoderm induction by the FGF receptor without stimulating endogenous FGF receptors.

MATERIALS AND METHODS

Antibodies. A rabbit polyclonal antibody was raised against the murine FGF receptor type 1 (Ab 50) (31). The murine monoclonal anti-PLC antibody was obtained from Upstate Biotechnology Inc. (UBI). A murine monoclonal antiphospho-

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FIG. 1. Chimeric receptor constructs used in this study. (A) PR-FR wt construct consisting of the extracellular portion of the murine PDGF- β receptor and the transmembrane and intracellular portions of the human FGF receptor 1. (B) PR-FR Y/F766 construct, which is identical to PR-FR wt except for a point mutation at codon 766 resulting in a phenylalanine-for-tyrosine substitution.

tyrosine antibody was raised by using phosphotyramine as an immunogen. The murine monoclonal anti-*Xenopus* muscle-specific antibody 12/101 was obtained from the Developmental Studies Hybridoma Bank (Johns Hopkins University).

Plasmid constructs and RNA transcription. Y/F766 was constructed as described previously (31). The chimeric PDGF/FGF receptor constructs were generated by subcloning cDNA fragments encoding the extracellular domain of the murine PDGF- β receptor and the transmembrane and cytoplasmic domains of either the human wild-type FGF receptor 1 (PR-FR wt) or the Y/F766 mutant FGF receptor 1 (PR-FR Y/F766) (15) into a modified pSP64T vector containing the 5' and 3' untranslated sequences of the *Xenopus* β -globin gene (21). For in vitro transcription, all plasmids were linearized with *Eco*RI and transcribed with SP6 RNA polymerase (Boehringer Mannheim) as previously described (25).

Oocyte injections. Large oocytes were obtained from mature female frogs by established techniques. Oocytes were treated with collagenase and maintained in $1 \times$ MBSH (37) with 1 mg of Ficoll per ml, 1 mg of bovine serum albumin (BSA) per ml, and antibiotics at 19°C . Oocytes were injected with 5 to 10 nl of RNA solution with glass needles as described previously (37).

IP3 assay. Oocytes were injected with wild-type or Y/F766 RNA and incubated for 24 h at 18°C . Oocytes were incubated in 20 mM LiCl in $1 \times$ MBSH for 15 min, and then some oocytes were exposed to 2 nM PDGF BB for 30 min at room temperature in the continued presence of LiCl. Acid extracts were made with 1 M trichloro-1,2,2-trifluoroethane and triethylamine (Aldrich). The DuPont NEN [^3H]IP3 radioreceptor assay kit was utilized to measure IP3 levels in acid extracts.

Calcium efflux assays. Oocytes were injected with 2.5 ng of PR-FR wt or PR-FR Y/F766 RNA and were incubated for 24 h at 19°C . Oocytes were loaded with $^{45}\text{Ca}^{2+}$ and stimulated with 2 nM PDGF (Chiron). Calcium efflux was measured as previously described (37).

Isolation and analysis of protein. Oocytes, whole embryos, and animal caps were lysed in cold Nonidet P-40 buffer (22). Each oocyte, whole embryo, or animal cap was lysed in 10 μl of buffer. Insoluble material and lipid were separated by centrifugation at $13,000 \times g$ for 10 min at 4°C . Lysates were run on sodium dodecyl sulfate (SDS)-polyacrylamide gels and transferred to 0.2- μm -pore-size nitrocellulose filters (Schleicher and Schuell). The filters were blocked with 2% BSA. Following incubation with primary antibodies, bound antibodies were visualized with alkaline phosphatase-conjugated secondary antibody and color developing reagents (Promega).

Embryo injections. Mature females were injected with 700 U of human chorionic gonadotropin (Sigma). The next morning, eggs were manually expelled and fertilized with minced testes.

Embryos were dejellied with 2% L-cysteine and washed in $0.2 \times$ MBSH. At the two-cell stage the embryos were transferred to $1 \times$ MBSH with 5% Ficoll and antibiotics. Each blastomere was injected with 5 to 10 nl of RNA solution. After 1 h, embryos were transferred to $0.2 \times$ MBSH with 5% Ficoll and antibiotics at 19°C .

Animal cap assays. Animal caps were dissected from stage 8 embryos (29) and incubated in agarose-lined culture dishes at room temperature in $1 \times$ MBSH with 1 mg of BSA per ml, antibiotics, and added growth factors. Recombinant basic FGF (bFGF) (UBI) was used at a concentration of 50 ng/ml, recombinant activin A (Ajinomoto Co.) was used at a concentration of 30 ng/ml, and recombinant PDGF BB (Chiron Corp.) was added at a concentration of 2 nM. The animal caps were allowed to develop until control embryos were at late-neurula stages, at which time they were scored for changes in morphology. The animal caps were fixed in 4% paraformaldehyde for either histology or immunohistochemistry.

RNAse protection assays. Two-cell embryos were injected with wild-type or Y/F766 RNA as described above and animal caps were obtained from stage 8 embryos. Some caps were exposed to 2 nM PDGF BB and incubated at room temperature for 3 h (*goosecoid*) or 18 h (muscle actin). RNA was extracted from caps as described previously (22). RNAse protection assays were performed with either a muscle actin probe (22) or a *goosecoid* probe (7) as described previously.

Immunohistochemistry. Animal caps were fixed at 4°C with 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.5. Specimens were dehydrated and embedded in paraffin and then sectioned on a rotary microtome. Sections were deparaffinized, rehydrated, and incubated with the muscle-specific antibody 12/101. Bound antibody was visualized with alkaline phosphatase-conjugated second antibody and Vector Red color substrate (Vector Laboratories). Sections were counterstained with hematoxylin.

RESULTS

Chimeric PDGF/FGF receptors are expressed in *Xenopus* oocytes and mediate ligand-induced signaling. Two chimeric growth factor receptors were constructed and inserted into the frog expression vector pSP64T (21). PR-FR wt was constructed with the extracellular portion of the murine PDGF- β receptor and the transmembrane and intracellular portions of the wild-type human FGF receptor 1 (15) (Fig. 1). PR-FR Y/F766 was constructed with the extracellular portion of the wild-type murine PDGF- β receptor and the transmembrane and intracellular portions of the Y/F766 mutant human FGF receptor 1. Given that both FGF receptors and PDGF receptors are

FIG. 2. Biochemical properties of chimeric receptors in *Xenopus* oocytes. (A) Both chimeric receptors are expressed in *Xenopus* oocytes. Oocytes were injected with 2.5 ng of RNA encoding either chimeric receptor. One day later, lysates were obtained. Proteins were separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and blotted with anti-FGF receptor antibody (Ab 50). Two bands were visualized for each construct, at 155 and 175 kDa, presumably representing the precursor and cell surface glycosylated forms of the receptor, respectively (18). (B) PLC γ is phosphorylated by ligand stimulation of PR-FR wt but not PR-FR Y/F766. Oocytes were injected with 2.5 ng of RNA encoding either chimeric receptor. One day later, oocytes were stimulated for 10 min with 2 nM PDGF BB. Protein lysates were made, and PLC immunoprecipitates were blotted with antiphosphotyrosine antibody as shown in the upper panel. The blot was reblotted with anti-PLC antibody as shown in the lower panel. (C) IP $_3$ concentration increases in response to ligand stimulation of PR-FR wt but not PR-FR Y/F766. Oocytes were injected with RNA as described above. Oocytes were preincubated in 10 mM LiCl and then stimulated with 2 nM PDGF BB. Acid extracts of oocytes were assayed for IP $_3$ concentrations. Each column represents the mean $\pm$ the standard error of four such determinations. (D) Calcium efflux occurs in response to ligand stimulation of PR-FR wt but not PR-FR Y/F766. Oocytes were injected with RNA as described above. Oocytes were loaded with $^{45}\text{Ca}^{2+}$ and stimulated with 2 nM PDGF BB. Calcium efflux was measured as described above.

initially activated by ligand-induced dimerization, these chimeras allowed us to study FGF receptor signaling utilizing PDGF as a ligand.

Immature *Xenopus* oocytes were injected with 2.5 ng of RNA encoding PR-FR wt or PR-FR Y/F766. After 24 h, lysates from the injected oocytes were probed with a rabbit polyclonal antibody directed against the intracellular portion of the FGF receptor (Ab 50), demonstrating significant expression of both receptor proteins (Fig. 2A). When PLC γ immunoprecipitates from injected oocytes stimulated with PDGF were immunoblotted with an antiphosphotyrosine antibody, PLC γ was phosphorylated in oocytes expressing PR-FR wt but not in oocytes expressing PR-FR Y/F766 (Fig. 2B). PDGF stimulation also induced IP $_3$ accumulation and calcium efflux in oocytes expressing PR-FR wt but not in oocytes expressing PR-FR Y/F766 when they were stimulated with PDGF (Fig. 2C and D). These findings demonstrate that the PR-FR wt

chimera could phosphorylate and activate *Xenopus* PLC γ and that the PR-FR Y/F766 chimera was deficient in these abilities.

PDGF-stimulated mesoderm induction occurs in animal caps expressing both chimeric receptors. Two-cell embryos were injected with 2.5 ng of PR-FR wt or PR-FR Y/F766 RNA, and protein lysates were obtained at stage 8 (29). When such lysates were examined by immunoblotting with Ab 50, receptor expression was detected for each construct (Fig. 3A). Two-cell embryos were injected as described above, and animal caps were isolated at stage 8. Caps were stimulated with 2 nM PDGF BB for 10 min, and PLC γ immunoprecipitates were made. Phosphotyrosine blots of PLC γ immunoprecipitates from PDGF-stimulated caps showed that PLC γ was phosphorylated in the animal caps expressing the PR-FR wt chimera but not in the animal caps expressing the PR-FR Y/F766 chimera (Fig. 3B). Since tyrosine phosphorylation is required for PLC γ activation (11, 19, 30), these data indicate that, in *Xenopus*

FIG. 3. Biochemical properties of chimeric receptors in *Xenopus* embryos. (A) Expression of chimeric receptors in blastula-stage *Xenopus* embryos. Two-cell embryos were injected with RNA encoding chimeric receptor PR-FR wt or PR-FR Y/F766. When embryos reached stage 8, protein lysates were made. Proteins were separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and blotted with antireceptor antibody (Ab 50). (B) PLC γ is phosphorylated by ligand stimulation of PR-FR wt but not PR-FR Y/F766. Two-cell embryos were injected with 2.5 ng of RNA encoding either chimeric receptor. When embryos reached stage 8, animal caps were dissected and stimulated for 10 min with 2 nM PDGF BB. Protein lysates were made, and PLC immunoprecipitates were blotted with antiphosphotyrosine antibody as shown in the upper panel. The blot was reblotted with anti-PLC antibody as shown in the lower panel.

animal caps as in *Xenopus* oocytes, PLC γ is activated by the PR-FR wt chimera but not by the PR-FR Y/F766 chimera. In contrast to the situation with oocytes, basal PLC γ phosphorylation was noted in animal caps expressing the PR-FR wt chimera; however, this phosphorylation increased in response to ligand stimulation (Fig. 3B).

When control embryos reached late-neurula stages, animal caps were scored for elongation, a marker of mesoderm induction (35). Caps from uninjected embryos did not exhibit elongation when stimulated with PDGF but did exhibit elongation when stimulated with bFGF. Caps derived from embryos injected with either PR-FR wt or PR-FR Y/F766 exhib-

FIG. 4. Animal cap elongation in response to growth factor treatment. Two-cell embryos were injected with RNA encoding chimeric receptor PR-FR wt or PR-FR Y/F766. Animal caps were isolated from preinjected stage 8 embryos, and some were exposed to 2 nM PDGF BB. Animal caps from uninjected control embryos were exposed to 2 nM PDGF BB or 50 ng of bFGF per ml. Caps were incubated overnight at room temperature and scored for elongation as previously described (31).

ited marked elongation in response to PDGF (Fig. 4). These elongation responses of animal caps from embryos injected with PR-FR wt and PR-FR Y/F766 were similar.

To further assess the ability of the chimeric receptors to mediate mesoderm induction, we isolated RNA from neurula-stage animal caps and measured the induction of muscle-specific actin by RNase protection. Protected bands representing muscle-specific actin were quantitated with a PhosphorImager (Mo-

FIG. 5. Muscle actin expression in animal caps as determined by RNase protection assay. Two-cell embryos were injected with RNA encoding receptor PR-FR wt or PR-FR Y/F766. Animal caps were isolated from preinjected stage 8 embryos and were exposed to 2 nM PDGF BB. Animal caps from uninjected control embryos were exposed to 50 ng of bFGF per ml. Caps were incubated for 18 h at room temperature, RNA was extracted, and RNase protection assays were performed with a muscle actin probe. Protected bands were quantified with a PhosphorImager with ImageQuant software. Each muscle actin band volume was divided by the corresponding uppermost cytoskeletal actin band volume to normalize for RNA loading. Each column represents the mean $\pm$ the standard error of two such determinations.

FIG. 6. Muscle expression in animal caps as determined by immunohistochemical staining. Two-cell embryos were injected with 2.5 ng of RNA encoding chimeric receptor PR-FR wt or PR-FR Y/F766. Animal caps were isolated from preinjected stage 8 embryos, and some were exposed to 2 nM PDGF BB. Animal caps from uninjected control embryos were exposed to 50 ng of bFGF per ml or 30 ng of activin A per ml. Caps were incubated for 3 days at room temperature, fixed in paraformaldehyde, and processed for immunocytochemistry using muscle-specific monoclonal antibody 12/101. Arrowheads indicate skeletal muscle and *n* indicates notochord. (A) Cap from an uninjected embryo not exposed to growth factor. (B) Cap from an uninjected embryo exposed to bFGF. (C) Cap from an uninjected embryo exposed to activin A. (D) Cap from a PR-FR wt-injected embryo not exposed to growth factor. (E) Cap from a PR-FR wt-injected embryo exposed to PDGF. (F) Cap from a PR-FR Y/F766-injected embryo exposed to PDGF.

lecular Dynamics Inc.) and standardized against a cytoskeletal actin band that appears in the same assay (22). The chimeric receptors induced approximately equal amounts of muscle-specific actin (Fig. 5). Neither chimera induced expression of the *goosecoid* gene, a gene induced by activin but not FGF (7). The results of this analysis confirmed that both the PR-FR wt

chimera and the PR-FR Y/F766 chimera could mediate mesoderm induction and suggested that the mesoderms induced by both chimeras were of similar natures.

To further examine the nature of the mesoderm induced by the chimeric receptors, animal caps from injected and control embryos were analyzed histologically and by immunocyto-

TABLE 1. Growth factor-induced mesoderm induction in animal caps^a

Injected RNA	Growth factor	No. of caps with induced tissue/total no. of caps		
		Notochord	Neural	Muscle
None (control)	None	0/9	0/9	0/9
None (control)	bFGF	0/9	0/9	8/9
PR-FR wt	PDGF	0/12	0/12	9/12
PR-FR Y/F766	PDGF	0/11	0/11	8/11

^a Two-cell embryos were injected with RNA encoding chimeric receptor PR-FR wt or PR-FR Y/F766. Animal caps were isolated from preinjected stage 8 embryos, and some were exposed to 2 nM PDGF BB. Animal caps from uninjected control embryos were exposed to 50 ng of bFGF per ml. Caps were incubated for 3 days at room temperature and fixed, stained with hematoxylin, and sectioned. Sections were scored for the presence of skeletal muscle, notochord, and neural tissue.

chemistry with a skeletal muscle-specific antibody (20). These experiments revealed that both chimeric receptors induced skeletal muscle development in response to PDGF (Fig. 6). Large blocks of skeletal muscle and occasional mesenchymal cells were detected in caps expressing PR-FR wt or PR-FR Y/F766, as is characteristic of FGF-induced mesoderm induction. In no instance was notochord, neural tissue, eye, or cement gland noted in animal caps expressing either chimera (Table 1). Therefore, there was no evidence that the PR-FR Y/F766 chimera specified induction of mesoderm of a composition different from the composition of that induced by the PR-FR wt chimera.

DISCUSSION

Given the dramatic effects of lithium on embryonic pattern formation, we hypothesized that PLC γ activation by the FGF receptor would be important for FGF-stimulated mesoderm induction. In order to test the role of PLC γ in mesoderm induction by the FGF receptor, we developed a novel approach using chimeric receptors consisting of the extracellular portion of the PDGF- β receptor and the transmembrane and intracellular portion of the FGF receptor 1 expressed in *Xenopus* animal caps. Two chimeras were used, PR-FR wt and PR-FR Y/F766. Since PDGF does not induce mesoderm in uninjected *Xenopus* animal caps, these chimeric receptors allowed us to specifically examine the importance of PLC γ activation by the FGF receptor in mesoderm induction without stimulating endogenous receptors.

The chimeric constructs were first tested in *Xenopus* oocytes. These studies revealed that although both chimeric receptors were expressed, only the PR-FR wt chimera mediated PLC γ phosphorylation, IP₃ accumulation, and calcium efflux. However, when animal caps were isolated from injected stage 8 embryos and treated with PDGF, mesoderm induction as measured by cap elongation and skeletal muscle actin induction was mediated by both the PR-FR wt and PR-FR Y/F766 chimeras. When such caps were examined histologically, skeletal muscle and mesenchyme were noted with both constructs, while no evidence of notochord, brain, or cement gland was seen in any cap. These results suggest that PLC γ activation by the FGF receptor is not critical for FGF receptor-stimulated mesoderm induction. Furthermore, FGF receptor signaling in the absence of PLC γ activation does not result in the specification of different types of mesoderm.

The present experiments, however, do not prove that PI turnover is not important for early embryonic pattern formation. The dorsalized lithium phenotype can be rescued by

cotreatment with myoinositol or a diacylglycerol analog (6). Moreover, *Xenopus* animal caps made from lithium-treated embryos are hyperresponsive to mesoderm induction by both FGF and activin and produce larger amounts of dorsal-type mesoderm (9, 17, 33). On the basis of these data, Maslanski and coworkers have suggested that PI turnover acts as a negative feedback signal during early embryonic patterning, perhaps by attenuating the formation of dorsal mesoderm and allowing the formation of ventral and posterior mesoderm (23). Thus, one might have expected to see a higher proportion of dorsal mesoderm induced by the PR-FR Y/F766 chimera than by the wild-type chimera. However, since PLC γ can be activated by a variety of growth factors and is only one of a number of isozymes that catalyze PI hydrolysis, it is conceivable that PI turnover mediated by an FGF-independent or PLC γ -independent pathway is sufficient to permit ventral mesoderm induction by the FGF receptor.

We have previously shown that a dominant-negative Raf-1 kinase blocks mesoderm induction in response to FGF but has little effect on mesoderm induction by activin (22). This result suggests that Raf-1 kinase activation is required for mesoderm induction in response to FGF and that other pathways are involved in mesoderm induction in response to activin. In mammalian cells the Y/F766 FGF receptor mutant retains the ability to activate the Raf-1 and MAP kinases (30a), showing that PLC γ activation and PI turnover are not required for the activation of these kinases. Thus, to fully understand mesoderm induction by the FGF receptor, it will be important to elucidate the mechanisms by which the FGF receptor activates Raf-1 kinase. Studying mesoderm induction by using other mutant chimeric receptors in *Xenopus* animal caps should provide insight into the nature of the signals between the FGF receptor and Raf-1 kinase activation.

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REFERENCES

- 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.
- Berridge, M. J. 1987. Inositol trisphosphate and diacylglycerol: two interacting second messengers. *Annu. Rev. Biochem.* 56:159-193.
- Berridge, M. J. 1993. Inositol trisphosphate and calcium signalling. *Nature (London)* 361:315-325.
- Berridge, M. J., C. P. Downes, and M. R. Hanley. 1989. Neural and developmental actions of lithium: a unifying hypothesis. *Cell* 59:411-419.
- Burgess, W. H., C. A. Dionne, J. Kaplow, R. Mudd, R. Freisel, A. Zilberstein, J. Schlessinger, and M. Jaye. 1990. Characterization and cDNA cloning of phospholipase C- γ , a major substrate for heparin-binding growth factor 1 (acidic fibroblast growth factor)-activated tyrosine kinase. *Mol. Cell. Biol.* 10:4770-4777.
- Busa, W. B., and R. L. Gimlich. 1989. Lithium-induced teratogenesis in frog embryos prevented by a polyphosphoinositide cycle intermediate or a diacylglycerol analog. *Dev. Biol.* 132:315-324.
- Cho, K. W. Y., B. Blumberg, H. Steinbeisser, and E. M. DeRobertis. 1991. Molecular nature of Spemann's organizer: the role of the *Xenopus* homeobox gene *gooseoid*. *Cell* 67:1111-1120.
- Cooke, J., and E. J. Smith. 1988. The restrictive effect of early exposure to lithium upon body pattern in *Xenopus* development,

- studied by quantitative anatomy and immunofluorescence. *Development* **102**:85–99.
9. Cooke, J., K. Symes, and E. J. Smith. 1989. Potentiation by the lithium ion of morphogenetic responses to a *Xenopus* inducing factor. *Development* **105**:549–558.
 10. Downing, J. R., B. L. Margolis, A. Zilberstein, R. A. Ashman, A. Ullrich, C. J. Scherr, and J. Schlessinger. 1990. Phospholipase C- γ , a substrate for PDGF receptor kinase, is not phosphorylated on tyrosine during the mitogenic response to CSF-1. *EMBO J.* **8**:3345–3350.
 11. Goldschmidt-Clermont, P., J. W. Kim, L. M. Machesky, S. G. Rhee, and T. D. Pollard. 1991. Regulation of phospholipase C- γ 1 by profilin and tyrosine phosphorylation. *Science* **251**:1231–1233.
 12. Green, J. B. A., H. V. New, and J. C. Smith. 1992. Responses of embryonic *Xenopus* cells to activin and FGF are separated by multiple dose thresholds and correspond to distinct axes of the mesoderm. *Cell* **71**:731–739.
 13. Hemmati-Brivanlou, A., and D. A. Melton. 1992. A truncated activin receptor inhibits mesoderm induction and formation of axial structures in *Xenopus* embryos. *Nature (London)* **359**:609–614.
 14. Jessel, T. M., and D. A. Melton. 1992. Diffusible factors in vertebrate embryonic induction. *Cell* **68**:257–270.
 15. 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.
 16. Kao, K. R., and R. P. Elinson. 1988. The entire mesodermal mantle behaves as Spemann's organizer in dorsoanterior enhanced *Xenopus laevis* embryos. *Dev. Biol.* **127**:64–77.
 17. Kao, K. R., and R. P. Elinson. 1989. Dorsalization of mesoderm induction by lithium. *Dev. Biol.* **132**:81–90.
 18. Keating, M. T., C. C. Harryman, and L. T. Williams. 1989. Platelet-derived growth factor receptor inducibility is acquired immediately after translation and does not require glycosylation. *J. Biol. Chem.* **264**:9129–9132.
 19. Kim, H. K., J. W. Kim, A. Zilberstein, B. Margolis, J. G. Kim, J. Schlessinger, and S. G. Rhee. 1991. PDGF stimulation of inositol phospholipid hydrolysis requires PLC γ 1 phosphorylation on tyrosine residues 783 and 1254. *Cell* **65**:435–441.
 20. Kintner, C. R., and J. P. Brockes. 1984. Monoclonal antibodies identify blastemal cells derived from dedifferentiating muscle in newt limb regeneration. *Nature (London)* **308**:67–69.
 21. Krieg, P. A., and D. A. Melton. 1984. Functional messenger RNAs are produced by SP6 in vitro transcription of cloned cDNAs. *Nucleic Acids Res.* **12**:7057–7070.
 22. 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.
 23. Maslanski, J. A., L. Leshko, and W. B. Busa. 1992. Lithium-sensitive production of inositol phosphates during amphibian mesoderm induction. *Science* **256**:243–245.
 24. Meisenhelder, J., P.-G. Suh, S. G. Rhee, and T. Hunter. 1989. Phospholipase C- γ is a substrate for the PDGF and EGF receptor protein-tyrosine kinases in vivo and in vitro. *Cell* **57**:1108–1122.
 25. Melton, D. A., P. A. Krieg, M. R. Rebagliatti, T. Maniatis, K. Zinn, and M. R. Green. 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res.* **12**:7035–7056.
 26. Mohammadi, M., C. A. Dionne, W. Li, T. Spivak, A. M. Honegger, M. Jaye, and J. Schlessinger. 1992. Point mutation in FGF receptor eliminates phosphatidylinositol hydrolysis without affecting mitogenesis. *Nature (London)* **358**:681–684.
 27. 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- γ 1. *Mol. Cell. Biol.* **11**:5068–5078.
 28. Morrison, D. K., D. R. Kaplan, S. G. Rhee, and L. T. Williams. 1990. Platelet-derived growth factor (PDGF)-dependent association of phospholipase C- γ with the PDGF receptor signaling complex. *Mol. Cell. Biol.* **10**:2359–2366.
 29. Nieuwkoop, P. D., and J. Faber. 1967. Normal table of *Xenopus laevis* (Daudin). North-Holland, Amsterdam.
 30. Nishibe, S., M. I. Wahl, S. M. T. Hernandez-Sotomayor, N. K. Tonks, S. G. Rhee, and G. Carpenter. 1990. Increase of the catalytic activity of phospholipase C- γ 1 by tyrosine phosphorylation. *Science* **250**:1253–1256.
 - 30a. Peters, K. G., and D.-S. Duan. Unpublished data.
 31. Peters, K. G., J. Marie, E. Wilson, H. E. Ives, J. Escobedo, M. Del Rosario, D. Mirda, and L. T. Williams. 1992. Point mutation of an FGF receptor abolishes phosphatidylinositol turnover and Ca²⁺ flux but not mitogenesis. *Nature (London)* **358**:678–681.
 32. Slack, J. M. W., B. G. Darlington, J. K. Heath, and S. F. Godsave. 1987. Mesoderm induction in early *Xenopus* embryos by heparin-binding growth factors. *Nature (London)* **326**:197–200.
 33. Slack, J. M. W., H. V. Issacs, and B. G. Darlington. 1988. Inductive effects of fibroblast growth factor and lithium ion on *Xenopus* blastula ectoderm. *Development* **103**:581–590.
 34. Smith, J. C., B. M. Price, K. Van Nimmen, and D. Huylebroeck. 1990. Identification of a potent *Xenopus* mesoderm-inducing factor as a homologue of activin A. *Nature (London)* **345**:729–731.
 35. Symes, K., and J. C. Smith. 1987. Gastrulation movements provide an early marker of mesoderm induction in *Xenopus laevis*. *Development* **101**:339–349.
 36. Thomsen, G., T. Woolf, M. Whitman, S. Sokol, J. Vaughn, W. W. Vale, and D. A. Melton. 1990. Activins are expressed early in *Xenopus* embryogenesis and can induce axial mesoderm and anterior structures. *Cell* **63**:485–493.
 37. Ueno, H., M. Gunn, K. Dell, A. Tseng, and L. T. Williams. 1992. A truncated form of fibroblast growth factor receptor 1 inhibits signal transduction by multiple types of fibroblast growth factor receptor. *J. Biol. Chem.* **267**:1470–1476.
 38. Wahl, M. I., N. E. Olashaw, S. Nishibe, S. G. Rhee, W. J. Pledger, and G. Carpenter. 1989. Platelet-derived growth factor induces rapid and sustained tyrosine phosphorylation of phospholipase C- γ in quiescent BALB/c 3T3 cells. *Mol. Cell. Biol.* **9**:2934–2943.
 39. Whitman, M., and D. A. Melton. 1992. Involvement of p21^{ras} in *Xenopus* mesoderm induction. *Nature (London)* **357**:252–254.