The docking protein SNT1/FRS2 (fibroblast growth factor receptor substrate 2) is implicated in the transmission of extracellular signals from the fibroblast growth factor receptor (FGFR), which plays vital roles during embryogenesis. Activating FGFR mutations cause several craniosynostoses and dwarfism syndromes in humans. Here we show that the Xenopus homolog of mammalian FRS-2 (XFRS2) is essential for the induction of oocyte maturation by an XFGFR1 harboring an activating mutation (XFGFR1act). Using a dominant-negative form of kinase suppressor of Ras, we show the Mek activity is required for germinal vesicle breakdown (GVBD) induced by co-expression of XFGFR1act and XFRS2, but this activity is not required for progesterone-induced GVBD. Furthermore, Mek/MAPK activity is critical for the induction and/or maintenance of H1 kinase activity at metaphase of meiosis II in progesterone-treated oocytes. An activated XFGFR1 containing a mutation in the phospho-binding site (XFGFR1act Y672F) displayed a reduced ability to induce cell-cycle progression in oocytes, suggesting phospholipase Cγ may not be necessary but that it augments XFGFR signaling in this system. Oocytes co-expressing XFGFR1act and XFRS2 showed substantial H1 kinase activity, but this activity was blocked when the oocytes were treated with the phosphatidylinositol 3-kinase inhibitor LY294002. Although phosphatidylinositol 3-kinase activity is essential for XFGFR1act/XFRS2-induced oocyte maturation, this activity is not required for maturation induced by progesterone. Finally, ectopic expression of Xspry2, a negative regulator of XFGFR signaling, greatly reduced MAPK activation and GVBD induced by the expression of either XFGFR1act plus XFRS2 or activated Ras (H-RasV12). In contrast, Xspry2 did not prevent GVBD induced by an activated form of Raf1, suggesting that Xspry2 exerts its inhibitory function upstream or parallel to Raf and downstream of Ras.

Fibroblast growth factor (FGFs) represent a large family of at least 22 different growth factors. FGFs play a critical role in the control of multiple biological processes, including mitogenesis, angiogenesis migration, differentiation, and mesoderm induction (1, 2). Upon activation, the FGFR receptor dimersize and undergoes rapid autophosphorylation on numerous tyrosine residues. Autophosphorylation of the cytoplasmic domain recruits SH2-containing molecules, such as phospholipase Cγ (PLCγ), Crk, and possibly Sre, whereas a docking protein, SNT1/FRS2 (hereafter referred to as FRS2), associates with the FGFR receptor (FGFR) in a tyrosine phosphorylation-independent manner (2, 3).

Upon tyrosine phosphorylation, FRS2 (3, 4) engages the SH2 domains of Grb2 and Shp2 (5). In response to FGF stimulation in cultured cells, SNT1/FRS-2 and Gab1 associate indirectly via Grb2, resulting in tyrosine phosphorylation of Gab1 and activation of the PI 3-kinase/Akt pathway (6, 7). FRS-2 has been shown to bind and link FGFR, TrkA, and the Ret receptors to MAP kinase (8–14). The FGFR receptor signaling pathway has been extensively studied in cultured cells, whereas the role of associated signaling components during development are beginning to emerge.

Targeted disruption of FGFs and FGFR receptors indicates a crucial role for these polypeptides during mouse development. Mice lacking the FGFR1 or FGF-8 have severe gastrulation defects (15–17), and mice lacking FGF4 or the docking protein FRS2 exhibit early embryonic lethality (18, 19). Mice null for FGF-9 display mesenchymal defects resulting in lung hypoplasia and neonatal death (20). Loss of the FGF3 gene causes defects in skeletal morphogenesis (21), and FGF2 null mice demonstrate that FGF2 is required for trophectoderm development, lung-branching morphogenesis, and limb outgrowth (22, 23).

Mutations in the human FGFRs have also helped elucidate the development role of FGFRs. For example, mutations in FGFR3 are associated with various forms of dwarfism (for review, see Ref. 24), and those in the FGFR1 and FGFR2 have been associated with craniofacial and limb deformities (24). In the mouse system, creation of gain-of-function mutations in FGFR2 and -3 cause similar phenotypes to the human syndromes (24, 25). These genetic mutations provide evidence that FGFRs are involved in bone and limb growth as well as morphogenesis (24).

The Xenopus embryo system has also provided valuable information regarding the role of FGFs and their receptors during development. Members of the FGF family control mesoderm production and maintenance as well as morphogenetic movements during gastrulation in Xenopus embryos. In ectodermal explant tissue, overexpression of FGF or treatment
with FGF induces mesoderm (26, 27). In embryos, a carboxyl-terminally truncated FGF (XFD) has been demonstrated to inhibit the formation of most mesoderm and cause gastrulation defects (28, 29). In contrast, constitutively activated forms of FGFR1 induce mesoderm in ectodermal explants (animal caps) (30).

Studies examining the role of FGFR-associated signaling molecules reveal that Grb-2 and Shp2 play critical roles in FGF-induced mesoderm tissue in explants as well as formation of posterior structures in embryos (31, 32). In contrast, the ability to bind PLCγ or mediate a signal through Crk is not required for mesoderm induction in explants (31, 32). A Shp2 mutant blocks FGF-induced mesoderm as well as MAP kinase required for mesoderm induction in explants (31, 32). A Shp2 phosphodiesterase, maturation induced by progesterone does not (42, 53, 55). Phospho-MAP kinase (p42, 44)–mediated signal transduction by progesterone and RTKs results in the synthesis of Mos and the induction of MPF, the two pathways differ in several aspects. Although maturation in- 

The Xenopus oocyte system has also proved useful for examining the events of signal transduction. Fully grown Xenopus oocytes are arrested in prophase of meiosis I and are induced to mature upon exposure to progesterone. Progesterone stimulates the synthesis of the Mos protooncogene product, pp39mos, and leads to the activation of maturation-promoting factor (MPF), an activity responsible for coordinating the biochemical events of meiosis I and II (40–46). During meiosis I, the rise in MPF activity is coincident with phosphorylation and activation of MAPK (47–49).

Several RTKs have been shown to induce Xenopus oocyte maturation when activated constitutively or in response to growth factors. For example, germinal vesicle breakdown (GVBD) is induced by receptors for insulin-like growth factor (42, 50), epidermal growth factor (51), nerve growth factor (52), and activated forms of hepatocyte growth factor (Tpr-Met) (53) and the glial cell-derived neurotrophic factor (Ret) (54). Although signal transduction by progesterone and RTKs results in the synthesis of Mos and the induction of MPF, the two pathways differ in several aspects. Although maturation induced by Tpr-Met and IGF-1 requires the stimulation of a phosphodiesterase, maturation induced by progesterone does not (42, 53, 55–59). Furthermore, maturation-signaling cascades induced by IGF-1, but not those induced by progesterone, require the specific involvement of p21ras, GAP, and protein kinase Cζ (60–62).

To further investigate the signaling events elicited by an activated Xenopus FGFR1 harboring mutations identified in human dysmorphic syndromes, we expressed these activated mutant receptors in Xenopus oocytes. Our results indicate that the active receptor can induce cell-cycle progression only when co-expressed with FRS2. Furthermore, Mek activity and PI 3-kinase activity are essential for GVBD mediated by an activated XFGFR1 and XFRS2, whereas PLCγ assists but is not required for this event. Moreover, we show that the natural inducer of oocyte maturation, progesterone, does not require Mek activity or PI 3-kinase for GVBD. Finally, overexpression of Xsprouty2, a negative regulator of RTK signaling, can block GVBD and MAPK activation by the activated FGFR1 but not progesterone. This block appears to influence a point upstream or parallel to Raf and downstream of Ras.
tiated in 76% of oocytes (Table I, Fig. 1A). Oocytes displayed the hallmark “white spot” on the animal pole, and H1 kinase assays performed on extracts from these oocytes confirmed the morphological data. H1 kinase activity is only present in extracts from oocytes co-injected with XFRS2 and XFGFR1act RNA (Fig. 1B). Western analysis demonstrates that approximately equivalent levels of the normal and mutant forms of XFGFR1 are expressed in the oocytes (Fig. 1C). These results suggest that the expression of XFRS2 is sufficient to allow only activated forms of XFGFR1 to induce oocyte maturation.

These data are in contrast to a previous study (65) showing that expression of the Pleurodeles FGFR1 in Xenopus oocytes induces maturation upon exposure to FGF-1 and when a platelet-derived growth factor receptor (PDGF) extracellular domain/Pleurodeles FGFR1 fusion protein is expressed and activated by PDGF. It may be possible that the differences in results may be attributed to species (Pleurodeles or Xenopus) differences, receptor expression levels, or possibly due to an intrinsic activity difference in XFGFR1 receptors that are activated as a result of mutations found in human disorders. To explore whether an intrinsic difference between the wild-type and activated XFGFR1 exists in the oocyte system, we examined whether the wild-type or mutant Xenopus FGFR1 can induce oocyte maturation upon ligand stimulation in the absence or presence of Xenopus FRS2. Oocytes were injected with RNA encoding XFGFR1 or XFGFR1act alone (14 ng) or co-injected with XFRS2 (7 ng) and either left untreated or treated with basic FGF (200 ng/ml). Oocytes co-expressing XFGFR1 and XFRS2 underwent GVBD (80%; Table II) after stimulation with bFGF, whereas the unstimulated oocytes or bFGF-stimulated oocytes that were only injected with XFGFR1 RNA or XFGFR1kd plus XFRS2 RNA did not mature (Table II). As expected, oocytes injected with XFGFR1act and XFRS2 displayed GVBD regardless of whether the oocytes were treated with bFGF (93% ± bFGF; Table II), suggesting that there is no obvious intrinsic difference between the constitutively active or ligand-activated XFGFR1 regarding induction of oocyte maturation.

FGFR-induced tyrosine phosphorylation of FRS-2 causes activation of the Ras/MAP kinase pathway via recruitment of Grb-2, SOS, and Shp-2. It has also been recently shown that in response to FGF stimulation, FRS2 and Gab1 associate indirectly via Grb2, resulting in tyrosine phosphorylation of Gab1 and activation of the PI 3-kinase/Akt pathway (7, 19). We tested whether expression of XFRS2 allowed the XFGFR1act or ligand-stimulated XFGFR1 to activate MAPK in the oocyte. Western analysis demonstrates that roughly equivalent levels of XFGFR1act or XFGFR1 are present in the extracts (Fig. 2). Phosphorylated MAPK, which correlates with MAPK activity, was also examined. A basal level of MAPK phosphorylation is detected upon bFGF stimulation of oocytes expressing XFGFR1 or XFGFR1act in the absence of XFRS2 and when XFGFR1 and XFRS2 are co-expressed in oocytes in the absence of bFGF (Fig. 2). However, a dramatic induction of MAPK phosphorylation and, thus, MAPK activity is observed when the FGF receptor is activated by mutation or ligand in the presence of XFRS2 (Fig. 2). This induction may, in part, be due to the increased MAPK activity maintained by MPF (cyclinB/cdc2). These data show that in oocytes, XFGFR1act can induce a low level of MAPK activity in oocytes expressing FRS2. Oocytes were injected with the indicated RNAs and cultured in the presence or absence of bFGF as noted. Oocytes were later scored for GVBD by internal examination. These results represent three independent experiments. R1/FRS2, XFRS2, R1kd, XFGFR1act; R1, wild-type XFGFR1.

**TABLE I**

| Co-expression of XFRS2 and activated XFGFR1 induces GVBD |
|---------------------------------------------------------|
| Oocytes were either injected with XFGFR1act (R1act), XFGFR1kd (R1kd), XFGFR1 (R1) RNA, or co-injected with XFRS2 (FRS2) RNA. Oocytes were later scored for GVBD by internal examination. These results represent five independent experiments. |

| No. displaying GVBD | No. injected | GVBD |
|---------------------|-------------|------|
| Uninjected          | 0           | 126  |
| R1act               | 0           | 110  |
| R1act/FRS2          | 93          | 123  |
| R1kd                | 0           | 95   |
| R1kd/FRS2           | 0           | 95   |
| R1                  | 0           | 108  |
| R1/FRS2             | 0           | 110  |
| Progesterone        | 65          | 67   |
| FRS2                | 0           | 106  |

**TABLE II**

| Ligand-stimulated wild-type and activated XFGFR1 display similar activities |
|-----------------------------------------------------------------------------|
| Oocytes were injected with the indicated RNAs and cultured in the presence or absence of bFGF as noted. Oocytes were later scored for GVBD by internal examination. These results represent three independent experiments. R1act, XFGFR1act; R1kd, XFGFR1kd; R1, XFGFR1; R1/FRS2, XFRS2. |

| No. displaying GVBD | No. injected | GVBD |
|---------------------|-------------|------|
| Uninjected          | 0           | 45   |
| R1                  | 0           | 45   |
| R1/FRS2             | 0           | 45   |
| R1/FRS2/FGF         | 33          | 41   |
| R1/FGF              | 0           | 44   |
| R1act               | 0           | 40   |
| R1act/FRS2          | 40          | 43   |
| R1act/FRS2/FGF      | 40          | 43   |
| R1kd/FRS2/FGF       | 0           | 40   |

**FIG. 1.** XFRS2 expression mediates XFGFR1act-induced GVBD in oocytes. A, morphology of oocytes injected with the indicated RNA or treatment. B, H1 kinase activity associated with oocytes injected with the indicated RNAs. C, Western analysis of lysates prepared from oocytes expressing the indicated proteins and probed with an XFGFR1 antibody. H1 kinase results are representative of two independent experiments, and Western analyses are representative of three independent experiments. R1act, activated XFGFR1; FRS2, XFRS2; R1kd, kinase-dead XFGFR1; R1, wild-type XFGFR1.
activation but that high levels of MAPK activity are only obtained in the presence of FRS2. Although these results showed that FRS2 is required for GVBD and full MAPK activation in oocytes expressing an activated XFGFR1, the question of whether Mek/MAPK activity was necessary for XFGFR1act-induced maturation was still unanswered.

To determine whether the Mek/MAPK pathway was necessary for XFGFR1act-induced GVBD, we employed a dominant-negative form of kinase suppressor of Ras (DnKSR) (66). DnKSR has been shown to block MAPK activation by preventing the appropriate localization of Mek, an activator of MAPK (67). DnKSR RNA (14 ng/oocyte) was introduced into oocytes. Two hours later, oocytes were injected with XFGFR1act (14 ng) plus FRS2 RNA (7 ng) or Tpr-met RNA (0.5 ng) or treated with progesterone (2 μg/ml). Although XFGFR1act plus FRS2 RNA-injected oocytes displayed GVBD (82%; Table III), the co-expression of DnKSR almost completely blocked maturation (1% GVBD; Table III) as evidenced by the presence of the germinal vesicle (Fig. 3A). DnKSR also reduced the percentage of oocytes displaying GVBD induced by another tyrosine kinase, Tpr-met, from 97 to 3% (Table III). In contrast, expression of DnKSR did not block progesterone-treated oocytes from undergoing GVBD (93%) (Table III, Fig. 3A).

Western analysis of extracts prepared from these oocytes demonstrates that substantial phospho-MAPK can only be observed in extracts from oocytes either injected with XFGFR1act plus FRS2 RNA or Tpr-met RNA or treated with progesterone (Fig. 3B). Expression of DnKSR was able to effectively block MAPK activation from both the FGF1 pathway and the progesterone pathway. Western analysis of the extracts shows that XFGFR1act is expressed at equivalent levels in the presence or absence of DnKSR (Fig. 3C). H1 kinase activity was examined to provide biochemical evidence regarding the induction or inhibition of GVBD in these oocytes (Fig. 3D). Because H1 kinase activity is necessary for both the induction of GVBD during meiosis I and for the metaphase arrest of oocytes during meiosis II, groups of progesterone-treated oocytes were collected at both GVBD and at least 6 h post-GVBD for H1 kinase assays. H1 kinase activity was readily detected in oocytes injected with XFGFR1act plus FRS2 RNA or Tpr-met RNA or treated with progesterone. However, this activity was dramatically reduced when DnKSR RNA was also injected (Fig. 3D). H1 kinase activity was extremely low in the oocytes where GVBD had been blocked by DnKSR expression (XFGFR1act/FRS2- or Tpr-met-expressing oocytes). However, progesterone-treated oocytes displayed abundant H1 kinase activity at GVBD regardless of whether DnKSR was co-expressed. In contrast, H1 kinase activity was nearly absent in DnKSR-expressing oocytes that were treated with progesterone and collected several hours after GVBD, suggesting that initiation and/or maintenance of a metaphase II arrest was blocked (Fig. 3D).

Collectively, these data demonstrate that Mek/MAPK signaling is essential for FGF receptor-induced but not progesterone-induced GVBD in oocytes.

PLCγ associates with the activated FGFRI via phosphorylated tyrosine 766 and becomes tyrosine-phosphorylated and activated, leading to hydrolysis of phosphatidylinositol 4,5-bisphosphate (2). The biological significance of this activation is not completely clear. To test whether PLCγ binding and activation play a role in XFGFR1act-induced cell cycle progression, we generated an equivalent PLCγ binding mutation in the context of the XFGFR1act protein (XFGFR1actY672F). To confirm that this mutation inhibits the association of the XFGFR1 with PLCγ, RNA encoding XFGFR1act (14 ng), FGFR1KD (14 ng), and XFGFR1actY672F (14 ng) was injected into oocytes, and the XFGFR1 was immunoprecipitated using XFGFR1-specific antibodies. The immune complexes were subjected to Western analysis using anti-PLCγ antibodies. PLCγ co-immunoprecipitated with the XFGFR1act, but not the XFGFR1actY672F or the XFGFR1KD, indicating that PLCγ signaling from the XFGFR1actY672F was abrogated (Fig. 4A). Western analysis of the oocyte extracts shows that comparable expression of the various XFGFR1 mutants was obtained (Fig. 4B).

The XFGFR1actY672F mutant was expressed in oocytes either alone or in combination with FRS2 in three separate experiments. Although co-injection of XFGFR1act and FRS2 RNA resulted in GVBD in 78% (56/71) of oocytes, co-expression of XFGFR1actY672F and FRS2 yielded GVBD in 13% (12/91) of oocytes. Although these data demonstrate that PLCγ binding is not essential for GVBD induced by the activated XFGFR1, PLCγ association enhances the signals necessary for cell cycle progression in this system. This observation was biochemically confirmed by performing H1 kinase activity assays on the oocyte extracts. H1 kinase activity was not observed in extracts prepared from oocytes that did not display the white spot on the animal pole but had been injected with RNA encoding XFGFR1actY672F and FRS2 (Fig. 4C). In contrast, H1 kinase activity was observed in the small percentage of oocytes displaying the white spot (Fig. 4C). MAPK phosphorylation was examined by Western analysis using anti-phospho-MAPK antibodies. A low level of MAPK phosphorylation was observed in extracts.

**Table III**

| No. displaying GVBD | No. injected | GVBD % |
|---------------------|-------------|--------|
| Uninjected          | 0           | 52     |
| R1act               | 0           | 55     |
| DnKSR               | 0           | 40     |
| R1act/FRS2          | 68          | 83     |
| R1act/FRS2/DnKSR    | 1           | 87     |
| Progesterone        | 92          | 97     |
| Progesterone/DnKSR  | 84          | 90     |
| Tpr-met            | 39          | 40     |
| Tpr-met/DnKSR       | 1           | 40     |

![Fig. 2. Maximum MAPK activation requires expression of XFGFR1act and FRS2.](Image) Western analysis of extracts prepared from bFGF (200 ng/ml)-treated or untreated oocytes expressing the indicated proteins and probed with anti-Phospho-MAPK antibody (A), anti-ERK2 antibody (B), and anti-XFGFR1 antibody (C). (R1act, activated XFGFR1; FRS2, XFRS2; R1, wild-type XFGFR1). These data are representative of two independent experiments.
from immature oocytes expressing XFGFR1actY672F alone or in conjunction with XFRS2, but a high level of MAPK activity was detected in extracts from those oocytes that underwent GVBD (Fig. 4D). Western analysis showed that the XFGFR1actY672F protein was appropriately expressed in all cases (Fig. 4E).

The docking protein FRS2 is a major downstream effector that links FGF and nerve growth factor receptors with the Ras/MAPK signaling cascade. Recently, it has been demonstrated that FRS2 also plays a pivotal role in FGF-induced recruitment and activation of PI 3-kinase (7). To determine whether PI 3-kinase activity was necessary for XFGFR1act-induced GVBD, XFGFR1act RNA was injected alone or with XFRS2 RNA and either treated with the PI 3-kinase inhibitor LY294002 (100uM) or left untreated. As controls, a group of oocytes was also treated with progesterone (2 μg/ml) in the absence or presence of XFRS2. Western analysis of lysates from oocytes expressing the indicated proteins and probed with anti-XFGFR1 antibody (B) and anti-PLCγ1 antibody (C). C, H1 kinase activity in extracts prepared from oocytes expressing the indicated proteins. Western analysis showed that the PI 3-kinase binding mutant of an activated XFGFR1 and XFRS2 (R1actY672F/FRS2) were separated based upon the presence of a white spot for assay. Western analysis was performed on the oocyte extracts and probed with either anti-phospho-MAPK antibody (D) or anti-XFGFR1 antibody (E). These results are representative of three independent experiments. R1act, activated XFGFR1; FRS2, XFRS2; R1kd, kinase-dead FGF1R; R1actY672F, activated-PLCγ binding mutant XFGFR1.
observed when these oocytes were treated with LY294002 (Fig. 5). These results are consistent with the study using Pleurodeles FGFR1 (65) and suggest that PI 3-kinase activity is essential for XFGFR-induced oocyte maturation but is dispensable for progesterone-induced maturation.

Finally, because the previous experiments were designed to determine the contribution made by FRS2-dependent and -independent positive signaling components to FGFR1-induced oocyte maturation, we thought it necessary to examine an inhibitory component of FGFR1 signaling. One such inhibitor of FGF signaling, termed Sprouty (Spry), was originally identified in *Drosophila* (68). Spry mutations in *Drosophila* lead to excessive tracheal branching (68), which is a similar phenotype to that observed with inappropriate activation of FGF signaling. Genetic evidence indicates that Spry is an inhibitor of the MAPK pathway, but it is unclear where it may function in this pathway.

We first examined the influence that Xenopus Spry may have on XFGFR1/FRS2-induced GVBD in oocytes. One group of oocytes was injected with XFGFR1act plus XFRS2 RNA alone or co-injected with RNA encoding a Myc-tagged version of Xenopus Spry2. Another group of oocytes was either left un.injected or injected with Xspry2-Myc-tagged RNA or treated with the Mek inhibitor U-0126 and subsequently treated with progesterone 4 h later. When Xspry2 was expressed at high levels, it effectively blocked GVBD in the oocytes expressing XFGFR1act and XFRS2 (72% GVBD was reduced to 8%, Table IV). In contrast, the vast majority of oocytes treated with progesterone underwent GVBD regardless of whether they were treated with the Mek inhibitor or expressed Xspry2 (90% GVBD and 93% GVBD, respectively; Table IV). Western analysis demonstrated that Xspry2 substantially reduced the phosphorylation of MAPK in oocytes expressing XFGFR1act and XFRS2, whereas Xspry2 appeared to have no effect on the phosphorylation of MAPK induced by progesterone (Fig. 6A). Control oocytes treated with both progesterone and the Mek inhibitor (U-0126) displayed a low level of phosphorylated MAPK, similar to the levels displayed in the Xspry2/XFGFR1act/XFRS2-expressing oocytes (Fig. 6A). Western analysis with anti-XFGFR1 antibodies or anti-Myc antibodies demonstrated that XFGFR1act and Myc-tagged Xspry2 were appropriately expressed (Fig. 6, B and C, respectively). These results indicate that Xspry2 overexpression is able to dramatically reduce GVBD and MAPK activity induced by XFGFR1 signaling but not that induced by progesterone.

It is still unclear at what point in the FGF-signaling pathway Spry exerts its influence. Xspry2 has been shown to inhibit Ca2+ mobilization independent of the MAPK block (69). There are also studies suggesting that Spry inhibits FGF signaling by blocking Ras activation (70, 71) and other studies suggesting that Spry functions downstream by inhibiting Raf (72, 73). In an effort to define where Xspry2 may function in the Ras/MAPK pathway, Xspry2 was co-expressed with an activated form of Ras or Raf1 (Fig. 7). Oocytes were either left alone or injected with Xspry2 RNA. Four hours later, these oocytes were either treated with progesterone or injected with RNA encoding XFGFR1act plus XFRS2 or H-RasV12 or RafY340D. Oocytes were scored for GVBD the following day. GVBD was observed in the majority of oocytes expressing either the activated XFGFR1 plus XFRS2 or H-RasV12 (75 and 93% GVBD, respectively; Table V), but few displayed GVBD when Xspry2 was co-expressed (5 and 15% GVBD, respectively; Table V). In contrast, oocytes expressing the activated form of Raf1 (RafY340D) exhibited GVBD regardless of whether Xspry2 was also expressed (77 and 71% GVBD, respectively; Table V). The same result was observed for oocytes expressing an activated form of Mek (data not shown).

We next examined whether H1 kinase activity and MAPK activity was also affected by Xspry2 in these oocytes. Western
yses are representative of three independent experiments. Western analyses of extracts prepared from oocytes injected with the indicated RNAs and/or treated with progesterone and probed with anti-phospho-MAPK antibody (A), anti-XFGFR1 antibody (B), anti-Ras antibody (C), anti-Raf antibody (D), and anti-Raf antibody (E). F, H1 kinase activity in extracts prepared from oocytes injected with the indicated RNAs and/or treated with progesterone. Rlact, XFGFR1act; FRS2, XFRS2; H-RasV12, activated Ras; Rafact, activated c-Raf1. H1 kinase results are representative of two independent experiments, and Western analyses are representative of three independent experiments.

FIG. 7. Myc-tagged-Xspry2 inhibits H1 kinase activity and MAPK activity induced by XFGFR1act/XFRS2 and activated Ras, but not activated c-Raf1 or progesterone. Shown is Western analysis of extracts prepared from oocytes injected with the indicated RNAs and/or treated with progesterone and probed with anti-phospho-MAPK antibody (A), anti-Myc antibody (B), anti-XFGFR1 antibody (C), anti-Ras antibody (D), and anti-Raf antibody (E). F, H1 kinase activity in extracts prepared from oocytes injected with the indicated RNAs and/or treated with progesterone. Rlact, XFGFR1act; FRS2, XFRS2; H-RasV12, activated Ras; Rafact, activated c-Raf1. H1 kinase results are representative of two independent experiments, and Western analyses are representative of three independent experiments.

TABLE V
Xspry2 inhibits XFGFR1act and H-RasV12 but not Rafact-induced GVBD

| No. displaying GVBD % | No. injected | GVBD |
|-----------------------|-------------|------|
| Uninjected            | 0           | 0    |
| Rlact/FRS2            | 27          | 36   |
| Rlact/FRS2/Spry       | 2           | 36   |
| H-RasV12              | 41          | 44   |
| H-rasV12/Spry         | 8           | 52   |
| Rafact                | 34          | 44   |
| Rafact/Spry           | 37          | 52   |

PAGE 33202

FRS2 Mediates FGFR1-induced GVBD

The Xenopus oocyte system was used to examine the contributions made by FRS2 to FGFR1 signaling. Here we show that Xenopus FRS-2 is essential for the induction of oocyte maturation by an FGFR1 harboring activating mutations (XFGFR1act) that are associated with skeletal abnormalities in humans.

We also show that ligand-activated exogenously expressed wild-type XFGFR1 also is dependent upon exogenous XFRS2. Using a dominant-negative form of kinase suppressor of Ras (KSR), we show that Mek/MAPK activity is required for GVBD induced by co-expression of XFGFR1act and XFRS2. Collectively, these data suggest that XFRS2 may be limiting in the oocyte, and it is required for appropriate transmission of FGFR1 signals leading to cell cycle progression.

The role of MAPK during oocyte maturation has been an area of great interest. Our data show that H1 kinase activation in response to FGFR signaling absolutely requires Mek/MAPK activation. In contrast, Mek/MAPK activation is not essential for H1 kinase activity initiated by the natural inducer progesterone during meiosis I. Mek/MAPK activity leads to the activation of Rsk, which plays an important role in both GVBD and arrest in meiosis I (40, 74–76). Although it is generally assumed that Mek/MAPK activity is necessary for progesterone-induced GVBD (77), a few studies provide evidence questioning this concept (63, 76, 78). One study showed that inhibition of the MAPK pathway by the specific Mek inhibitor, U-0126, allowed oocytes to enter meiosis I after progesterone treatment but failed to form metaphase I spindles and entered S phase rather than meiosis II (79). The data presented here provide clear evidence that Mek/MAPK is not necessary for the induction of GVBD in meiosis I but is critical for the induction and/or maintenance of H1 kinase activity during meiosis II in Xenopus oocytes.

Activation of the FGFR initiates autophosphorylation at a carboxy-terminal tyrosine residue that leads to binding and activation of PLCγ, which in turn results in activation of the protein kinase C and calcium signaling (2). In vitro functional assays using a PLCγ binding mutant of FGFR1 have failed to show a requirement for this pathway in any FGFR1-induced response. Recently, a mouse harboring a mutation of the tyrosine 766 of the FGFR1 was generated and shown to have alterations in A-P patterning of the vertebral column in a direction opposite to hypomorphic alleles (80). From this study it was suggested that phosphorylation of Tyr-766 may play a role in the negative regulation of FGFR1 activity in vivo (80). In another study, use of the PLCγ inhibitory peptide on oocytes expressing FGFR1 or the stimulation by platelet-derived growth factor (PDGF)-BB of oocytes expressing a PDGF receptor/FGFR1 chimeric protein mutated on the PLCγ binding site prevented GVBD and MAPK phosphorylation (65). In our study, an activated XFGFR1 containing a mutation in the PLCγ binding site (XFGFR1actY672F) displayed a reduced ability to induce cell-cycle progression in oocytes, suggesting that PLCγ may not be required but does augment FGFR1 signaling in this system.

The role of PI 3-kinase in progesterone-induced maturation is unclear. PI 3-kinase is activated in response to both progesterone and insulin (81–84). One report shows that a dominant negative form of PI 3-kinase inhibits progesterone-induced oocyte maturation (81), whereas another indicates that the SH2-containing inositol phosphatase (SHIP) inhibits the induction of oocyte maturation by overexpressed PI 3-kinase and insulin but does not inhibit progesterone-induced maturation (84).
Furthermore, the PI 3-kinase inhibitor wortmannin has been reported to block insulin-induced maturation but not progesterone-induced maturation (82, 83). Most recently, the Xenopus progesterone receptor has been shown to co-precipitate with active PI 3-kinase (85). However, the PI 3-kinase inhibitor, wortmannin, only delayed progesterone-induced maturation while completely blocking the insulin-dependent maturation (85). In our study, oocytes co-expressing XFGFR1act and XFRS2 showed substantial H1 kinase activity, but this activity was blocked when the oocytes were treated with the PI 3-kinase inhibitor LY294002. These data strongly indicate that although PI 3-kinase activity is essential for XFGFR1act/XFRS2-induced oocyte maturation, this activity is not required for GVBD induced by progesterone.

Finally, we examined whether a negative regulatory protein in FGF signaling, Spry, could influence XFGFR1act/XFRS2-induced oocyte maturation. It has been recently shown in Xenopus oocytes that Xspry2 acts downstream of the activated XFGFR1 to inhibit calcium mobilization but does not block Ras/MAPK activity (69). It is worth mentioning that in our studies, high levels of Xspry2 expression were required to inhibit ARVM and MAPK activity induced by XFGFR1act plus XFRS2, and this inhibition may reflect a higher level of expression than in the previous report (69). In either case, Nutt et al. (69) show that Ca$^{2+}$ mobilization induced by an activated FGF1 is effectively blocked by Xspry2 expression, but the mechanism is not known. Activated PLC leads to the release of calcium stores from the endoplasmic reticulum, whereas DAG and Ca$^{2+}$ activate PKC (2). Xspry2 does not inhibit the recruitment of PLC to activated FGF1, a step critical to Ca$^{2+}$ efflux induced by FGF. Thus the mechanism by which Xspry2 inhibits Ca$^{2+}$ release remains to be determined.

Recent studies suggest that Sprotty functions either upstream or downstream of Ras (69–73). In our study, we demonstrated that overexpression of Xspry2 greatly reduced MAPK activation of and GVBD induced by the activated XFGFR1 plus XFRS2 or an activated Ras (H-RasV12) but not an activated Raf1. The present data are in agreement with a recent report indicating that Spry2 inhibits MAPK activation by inhibiting activation of Raf1 (73). Because Spry2, but not Spry1 and 4, inhibits Raf1 activation in transiently transfected 293T cells (73), it will be of interest to determine whether Spry1 and Spry4 have any effect on GVBD and MAPK activation in Xenopus oocytes. It is still formally possible that Xspry2 is a scaffolding protein that mediates and regulates Ras/Raf signaling from FGFR1 and that when this protein is overexpressed it acts as an inhibitory protein perhaps in a manner similar to Kermitt (86) or KSR (68). Regardless, our results suggest that when overexpressed in oocytes, Xspry2 can exert an inhibitory function upstream or parallel to Raf and downstream of Ras. Thus Xspry2 oocytes provide a useful model to dissect the functions and site of action of Spry and perhaps other novel inhibitors of the FGFR and Ras pathways.

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2 K. Mood and I. O. Daar, unpublished results.

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