Fibroblast growth factor receptors (FGFRs) are a family of transmembrane tyrosine kinases involved in signaling via interactions with the family of fibroblast growth factors. Alternative splicing of the juxtamembrane region of FGFR1–3 leads to the inclusion or exclusion of two amino acids, valine and threonine, at the VT site. The presence or absence of VT (VT+ or VT−, respectively) affects the signaling potential of the receptor. The VT+ receptor isoform is required for Erk2 phosphorylation, a component of the mitogen-activated protein kinase signaling pathway. FRS2 is an adaptor protein that links FGFRs to the mitogen-activated protein kinase signaling pathway. FRS2 interacts with a region of the juxtamembrane domain of FGFR1 that includes the alternatively spliced VT site. We investigated the interaction of FRS2 with murine Fgfr1 juxtamembrane domain. We showed the alternatively spliced VT motif, at the juxtamembrane domain of Fgfr1 is required for FRS2 interaction with Fgfr1. Activation of signaling pathways from FRS2 is likely to be regulated by controlling the Fgfr1/FRS2 interaction through alternative splicing of the VT motif of Fgfr1.

Fibroblast growth factor receptors (FGFRs) are a family of four transmembrane receptor tyrosine kinases whose signaling functions are executed via interactions with the fibroblast growth factor family of ligands (FGFs) (1, 2). FGFR activation induces signaling cascades that result in multiple biological responses, including cell proliferation, differentiation, migration, and survival (2–4).

Four unlinked FGFR genes have been cloned in humans (FGFR1–4), mice (Fgfr1–4), chickens, frogs, and newts (5–10), and homologues have also been identified in Caenorhabditis elegans and sea urchins (11–13). A key feature of FGFR1 to -3 is that they are subject to post-transcriptional regulation by alternate splicing, resulting in the expression of multiple receptor isoforms (14). Alternative splicing of the third immunoglobulin-like domain (IgIII) in the extracellular region of the receptor determines ligand specificity (15). IgIII has a common IgIIIa exon that is spliced to either an IgIIb or an IgIIIC variant exon (16–20). The inclusion of the IIIb or IIIc exon determines receptor binding specificity.

A second splicing event involves the use of alternative donor splice sites located 6 bp apart at the 3′-end of exon 10, which leads to the inclusion or exclusion of two amino acids, valine 427 and threonine 428 (FGFR1 numbering) in the intracellular juxtamembrane region (21–23), producing two forms of the receptor, here termed VT+ and VT−. Several studies have implicated the VT motif in the intracellular signaling functions of the receptor. In Xenopus, the Thr of the VT motif of the FGFR1 VT+ isoform is phosphorylated by protein kinase C (PKC). The biological activity of VT+ FGFR1 was reduced by phosphorylation by PKC, suggesting that PKC could negatively regulate FGFR1 VT+ activity (22). Studies in transfected mammalian cells have shown that the VT+, but not the VT− form, could activate the Ras/MAPK signaling cascade, as measured by phosphorylation of Erk2 (23). This finding indicates that a major downstream channel of FGFR signaling is controlled by alternative splicing of the VT motif and that this splicing mechanism could therefore have important implications for FGFR function.

Two adaptor proteins, FGF receptor substrate 2 (FRS2, FRS2e, or SNT1) and FGF receptor substrate 3 (FRS3, FRS2b, or SNT2), have been implicated in downstream signaling from activated FGFRs by means of direct interaction with the receptor (24–26). These proteins share 49% sequence identity and are composed of an N-terminal phosphotyrosine binding (PTB) motif containing an N-terminal myristoylation site and an effector domain, which contains multiple potential sites for tyrosine phosphorylation by activated FGFRs. FRS2 and FRS3 exhibit distinct spatial-temporal patterns of gene expression in vivo (27). Disruption of murine FRS2 is embryonic lethal at stage E7–E7.5 indicating that FRS2 plays an essential role in early development (28).

The effector domain of FRS2 has been shown to bind multiple substrate proteins in an FGFR kinase-dependent manner. Four binding sites for Grb2 (Tyr186, Tyr306, Tyr539, Tyr802) (24) and two for Shp-2 (Tyr436 and Tyr577) (29, 30) have been identified. Shp2 activation and binding to FRS2 are essential for FGFR-induced differentiation of PC12 cells (30).

In addition, protein kinase Ca (PKCa) and protein kinase Cζ (PKCe), members of the atypical PKCs, have been shown to interact with FRS2 following FGF stimulation (31). Activation of PKCa is necessary and sufficient for its association with FRS2, although FRS2 was not a substrate for the PKCs. Activity of the atypical PKCs has been shown to be necessary for mitogenic signaling via the MAPK cascade (32, 33). An active mutant of PKCe activated MAPK and mitogen-activated pro-
tein kinase/extracellular signal-regulated kinase kinase, and a dominant negative PKC impaired their activation. Therefore, FRS2 may activate MAPK by two pathways, through recruitment of Grb2 (28) and atypical PKCs (31).

FRS2 also mediates recruitment of phosphoinositol-3-kinase to FGFRs via interaction of an adaptor protein, Gab1, with FRS2-associated Grb2 (28, 34). Activation of phosphoinositol-3-kinase is involved in signal transduction downstream of most tyrosine kinase receptors and is implicated in the regulation of mitogenesis, migration, and cell survival (35). Taken together, this evidence indicates that the interaction of FRS2 with FGFRs and the concomitant formation of a multiprotein signaling complex is a central element in multiple cellular responses to FGFR signaling.

The juxtamembrane region of FGFR1 and the PTB domain of FRS2 are required and sufficient for interaction and phosphorylation of FRS2 (26, 36–38). The sequence within the juxtamembrane region required for specific interaction with FRS2 was identified as KS1LRRVTVS (amino acids 419–430) (37), which includes the alternatively spliced VT motif.Alanine-scanned mutagenesis of residues Lys419, Ile421, Phe422, (37), which includes the alternatively spliced VT motif. Alanine-scanned mutagenesis of residues Lys419, Ile421, Phe422, Glu423, Arg425, and Val429 of FRS2 significantly diminished the binding of FRS2 to FGFR1 and had an inhibitory effect on MAPK activation. The structure of FRS2 PTB domain in a 1:1 complex with a peptide from the juxtamembrane region of human FGFR1 (amino acids 409–430) has been solved by nuclear magnetic resonance methods (38). In combination with site-directed mutagenesis, residues Leu423 and Val429 of FRS2 were found to be most important for the FRS2/FGFR1 interaction followed by Val414, Leu417, and Arg425, and Val429, supporting Ong et al. (37). Taken together, this evidence suggests that the functional significance of the VT+ and VT− alternatively spliced isoforms of FGFRs might be to control the formation of the FRS2-dependent signaling complex, thereby regulating the signaling processes that are activated by ligand engagement. Here we test this hypothesis by analysis of the interaction of FRS2 with different forms of the juxtamembrane domain of murine Fgfr1.

We show that inclusion of the VT motif is required for binding of FRS2 to Fgfr1 in a kinase-independent manner. We also show that VT+/VT− splicing is regulated independently of splicing in the extracellular domain and that both VT isoforms are co-expressed in the same organs in vivo.

**EXPERIMENTAL PROCEDURES**

**Antibodies**

Goat anti-human IgG Fe HRP (31416) was purchased from Pierce; monoclonal anti-Myc, 9E10 (MM5150R), was from Babco; monoclonal anti-Grb2 (G16720) antibody was from Transduction Laboratories; monoclonal anti-Myc, 9E10 antibody was from Babco; monoclonal anti-phosphotyrosine antibodies 4G10 (05 321) and PY20 (69-137) were from Upstate Biotechnology, Inc. (Lake Placid, NY) and ICN Biomedicals Inc., respectively; monoclonal anti-glutathione S-transferase (GST) (G1160) was from Sigma; and sheep anti-mouse IgG HRP (NA9310) was from Amersham Pharmacia Biotech.

**Construction of Plasmids**

pEF-BOS-ires-Topaz Vector—Splice overlap PCR was used to fuse the encephalomyocarditis virus (EMCV) internal ribosome entry site (ires) (kind gift from P. Mountford) with the Topaz (Packard) (primers: Tpz1, TGACTCGAGGCGGAAATTCGGCC; Tpz2, GTCATACATGTTT-TGGGCAAGCTTATC; Tpz3, GCCACACATGTGAGCTAGAGGGA; Tpz4, GGCTCTAGAATTCCTTACTTGAGCGACTGCTCCGA). The cloned irpes topaz fragment was inserted into pcDNA3 (Invitrogen) using XhoI and XbaI sites, excised from pcDNA3 using BamHI and XbaI, and inserted into pEF-BOS (39).

**Constitutively Active Fgfr1 VT+ (FcFgfr1 VT+)**—Splice overlap PCR was used to fuse the human FGR2 signal sequence with human IgG1Fc (primers: HB1 (CGCGGATCCATAGCTGAGCTCATCATGCTAC-GGGGTCCCTT), ss3 (GATTGGGCGTCGCCCGAGAGGACAGTG-GCC), Fc5 (CGGGGCGGACCGAAAATTCGTAATGACAACCTC), and Fc3 (GGGGATCCATATGTTTACCCCGAGACGGAGGAGG)). The DNA fragment was inserted into the pEF-BOS-ires-Topaz vector using BamHI and EcoRV restriction sites. The region of murine Fgfr1IIIc (VT+) was amplified (primers: mflg5 (TCCAAGCTGAGGTTTCTGGACCTG), and mflg3 (GCCGGTGCCCGGAGGGTCGTTTC)) was inserted into the pEF-BOS-ires-Topaz vector using HindIII and EcoRI sites, replacing FcFgfr1 VT+ with the mutated form.

**Kinase-dead FcFgfr1**—Using the FcFgfr1 VT+ as a template, the truncated receptor was amplified by PCR (primers: HB1 (CGCGGATCCATAGCTGAGCTCATCATGCTAC-GGGGTCCCTT), and mflg3 (GCCGGTGCCCGGAGGGTCGTTTC)) and negative control (CTGAGATCCCGGGGCTAGCGTAC-TCTCGTGCGAGCAGC), VA splice overlap (CAGGTAGCAGTGCAGCTCATT), and mflg3 (39)). The PCR products were inserted into the FcFgfr1 VT+ construct using Ndel and NotI sites, replacing the FcFgfr1 VT+ with the mutated form.

**Truncated FcFgfr1**—The truncated FcFgfr1 VT+ as a template, the truncated receptor was amplified by PCR (primers: HB1 (CGCGGATCCATAGCTGAGCTCATCATGCTAC-GGGGTCCCTT), and mflg3 (GCCGGTGCCCGGAGGGTCGTTTC)) and negative control (CTGAGATCCCGGGGCTAGCGTAC-TCTCGTGCGAGCAGC), VA splice overlap (CAGGTAGCAGTGCAGCTCATT), and mflg3 (39)). The PCR products were inserted into the FcFgfr1 VT+ construct using Ndel and NotI sites, replacing the FcFgfr1 VT+ with the truncated version.

**Amplification of FRS2 cDNA**—Rat PC12 RNA was used in RT-PCR. Qiagen Omniscript reverse transcriptase kit was used according to the manufacturer’s instructions with a poly(dT) primer. Rat FRS2 cDNAs were amplified from the cDNA pool (primers: FRS2 5′/CagCGGATCCTCTGTCATCATCTATAAGAT) and GST–juxt 3′ (CGGCGTACCATGGGTAGCTGTTGTAGCTGT) and negative control (CTGAGATCCCGGGGCTAGCGTAC-TCTCGTGCGAGCAGC), VA splice overlap (CAGGTAGCAGTGCAGCTCATT), and mflg3 (39)). The PCR products were inserted into the pEF-BOS-ires-Topaz vector using BamHI and NotI sites, replacing FcFgfr1 VT+ with the truncated form.

**GST–FRS2**—GST–FRS2 amplification by PCR (primers: GST–FRS2 5′ (CGGGCATCGGGTACATCGGTGTGTA) and GST–FRS2 3′ (CTGAGATCCCGGGGCTAGCGTAC-TCTCGTGCGAGCAGC), VA splice overlap (CAGGTAGCAGTGCAGCTCATT), and mflg3 (39)). The PCR products were inserted into the pEF-BOS-ires-Topaz vector using BamHI and EcoRI sites, replacing hOSM 196 mod3 with FRS2.

**GST–VT+ and GST–VT− Juxtamembrane Fgfr1**—The juxtamembrane regions of VT+ and VT− forms of murine Fgfr1 were amplified from the templates FcFgfr1 VT+ and VT− (primers: GST–juxt 5′ (TGGAGGATCCCTCGTCTCATCTAATTAGAT) and GST–juxt 3′ (CGGCGTACCATGGGTAGCTGTTGTAGCTGT)) and negative control (CTGAGATCCCGGGGCTAGCGTAC-TCTCGTGCGAGCAGC), VA splice overlap (CAGGTAGCAGTGCAGCTCATT), and mflg3 (39)). The PCR products were inserted into the pEF-BOS-ires-Topaz vector using BamHI and EcoRI sites, replacing FRS2 with the juxtamembrane regions.

**Cell Lines**

Human 293T kidney epithelial cells were cultured at 37 °C, 5% CO2 in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 2 mM l-glutamine, 0.1 mg/ml streptomycin, 0.2 units/ml penicillin, 1 mM sodium pyruvate, and 10% fetal calf serum (Labtech International).

**Transient Transfections**

Cells were transiently transfected using CaPO4 precipitation. Cells were grown to 70% confluency in 162-cm2 flask (25 nM of medium. To 155 μl of 2 μM CaCl2, 60 μg of Qiagen prepared DNA were added. Water was added to a final volume of 1.5 ml, and the DNA/CaCl2 mixture was added dropwise to 1.5 ml of 2× HBS (1.6% NaCl (w/v), 1.2% Hepes (w/v), 0.04% Na2HPO4 (w/v), pH 7.12). The DNA/CaPO4 precipitate was incubated on the cells overnight and washed the following morning in media without serum. This was replaced with 25 ml of Ultrafresh media (Bio-Whittaker) supplemented with 0.1 mg/ml strep-
mycin and 0.2 units/ml of penicillin. Recombinant proteins were expressed for 48 h.

Immunoprecipitation— transiently transfected 293T cells were washed twice in 25 ml of cold PBS and then lysed at 4 °C using 2 ml of lysis buffer (10 mM Tris-HCl, pH 8.0, 2.5 mM MgCl2, 5 mM EDTA, pH 8.0, 0.5% Triton X-100 (w/v), 1 mM Na3VO4, 50 mM NaF, and 1 tablet of protease inhibitor mixture (Roche Molecular Biochemicals) per 10 ml of buffer) per 162-cm2 flask of cells. The supernatant was used for immunoprecipitation. To 15 μl of protein A-Sepharose 4 fast flow (Amersham Biosciences, Inc.) 1 ml of 293T cell lysate was added and incubated for 1 h, at 4 °C, with mixing. The protein A-Sepharose was washed three times with 1 ml of cold PBS. Washed protein A-Sepharose resuspended in 500 μl of PBS was put down a sucrose column (400 μl of 20% sucrose, 300 μl of 10% sucrose) to remove precipitated proteins. After a final 1-ml wash in PBS, bound protein was eluted by boiling for 5 min in 15 μl of 2 × SDS sample buffer (125 mM Tris/HCl, pH 6.8, 20% glycerol (w/v), 4% SDS (w/v), 0.1% bromophenol blue (w/v), 10% mercaptoethanol (w/v)) for analysis by SDS-PAGE.

Western Blotting— Each 4–20% SDS-PAGE gel (Invitrogen) was calibrated with 3 μl of high molecular weight rainbow markers (Amer- sham Pharmacia Biotech). Protein was transferred to polyvinylidene difluoride membrane (Millipore Corp.) at 100 V for 1 h. The membrane was blocked overnight at 4 °C in 5% bovine serum albumin (w/v) in TBS-T (10 mM Tris/HCl, pH 7.4, 150 mM NaCl, 0.05% Tween 20 (w/v)). Primary antibodies were diluted in 1% bovine serum albumin (w/v) in TBS-T and incubated with the membrane for 1 h at room temperature. The blot was washed three times for 15 min in TBS-T and probed with the conjugated antibody for 30 min at room temperature, diluted in 1% bovine serum albumin (w/v) in TBS-T. The filter was washed five times in TBS-T, followed by a final wash in TBS (no Tween 20). Enzyme chemiluminescence (Pierce) was used for visualization and exposed to hyperfilm (Amersham Biosciences). Blots were stripped for reprobing with 0.1M glycine-HCl, pH 2.5, for 5 min at room temperature. The wells were washed again before applying the OPD substrate (Dako) used according to the manufacturer’s specifications. The PCR was performed in 30 cycles: 92 °C, 20 s; touch-down 60 to 50 °C, 15 s; 72 °C, 20 s. The PCR product was digested with PstAI (New England Biolabs) and run on an agarose gel with 1-κb ladder (Invitrogen).

RESULTS

Construction of a Constitutively Active Form of Murine Fgfr1 (FcFgfr1)—A constitutively active form of Fgfr1 (FcFgfr1) was generated by replacing the extracellular region of murine Fgfr1 with the Fc region of a human antibody molecule, IgG1. Part of the extracellular domain (last 24 bp) and the whole of the transmembrane and intracellular regions of Fgfr1 were fused to the Fc (Fig. 1). In this configuration, Fgfr1 kinase is activated constitutively via disulfide-mediated dimerization of the Fc extracellular domain. Functional studies were used to test for constitutive activation by assessing receptor dimerization and intracellular tyrosine phosphorylation. The receptor was transiently expressed in 293T cells, the cells were lysed, and the receptor was immunoprecipitated by direct interaction of the Fc of the receptor with protein A-Sepharose. Expression of the receptor was observed using an anti-Fc HRP antibody by Western blot (Fig. 2a). Reprobing the same blot with an anti-phosphotyrosine antibody revealed that the receptor had tyrosine kinase activity (Fig. 2b). Under reducing conditions, the receptor ran as a monomer of ~100 kDa. Under nonreducing conditions, disulfide bonds remained intact, and oligomeric complexes of the receptor were detected (Fig. 2c). Therefore, the Fc fusion receptor was constitutively active in the absence of ligand. Interaction of the receptor with known signaling proteins (PLCγ and Grb2) was confirmed, and the receptor was shown to correctly localize to the plasma membrane of transfected cells (results not shown).

Using site-directed mutagenesis, mutant forms of the constitutively active FcFgfr1 receptor were constructed that block docking sites for intracellular signaling proteins. A "kinase-
dead” receptor was made that has the catalytic base in the phosphotransfer reaction Asp623 mutated to Ala (40), rendering the receptor enzymatically inactive despite receptor dimerization. A Y766F mutant blocks the association and activation of PLCγ/H9253, as previously described (41–43). Mutants were made of the alternatively spliced VT motif, which has been implicated in Ras/MAPK regulation (23). Point mutations of the VT site were constructed, a VT to VA mutation and VT to TT mutation. Finally, a truncated form of the constitutively active receptor was generated in which the entire intracellular region of the receptor was deleted, leaving only the Fc for dimerization and the transmembrane domain for localization at the plasma membrane.

The VT Motif of Fgfr1 Is Required for FRS2 Interaction—A full-length FRS2 cDNA was isolated from PC12 cell RNA by RT-PCR and tagged at the N terminus with Myc and poly-His epitopes for immunodetection. The interaction of FRS2 with FcFgfr1 was studied by comparing FRS2 association with the constitutively active Fgfr1 and the mutant forms described above by transient co-expression in 293T cells and immunoprecipitation (Fig. 3a).

Complex formation. This finding indicates that the identity of the valine residue in the VT motif has a more significant contribution to the FRS2 interaction than the neighboring threonine. In this experimental system, the Thr has little impact on FRS2 recognition. Mutation of Tyr766 in the C-terminal tail had no effect on FRS2 association, and removal of the entire intracellular region of the receptor abolished FRS2 interaction with the receptor.

The ability of FRS2 to associate with the endogenous effector protein Grb2 was examined by reprobing the immunoprecipitates with an anti-Grb2 antibody (Fig. 3a). Grb2 association was found to require both association of FRS2 with FcFgfr1 and an active Fgfr1 kinase; no Grb2 was isolated with the kinase-dead form of Fgfr1. In addition, no Grb2 was found to be associated with the VT+/H11001 form of the receptor, which correlates with the absence of FRS2 association with this form of the receptor. This indicates that, in 293T cells, association of Grb2 with tyrosine-phosphorylated FRS2 is the principle potential connection with the Ras/MAPK pathway. Grb2 has been reported to associate with Fgfr1 via binding the adaptor protein Shc (44) that requires a functional kinase domain (45). Since loss of FRS2 binding to the receptor leads to concomitant abrogation of Grb2 binding, it seems probable that Shc-dependent activation of the MAPK signaling cascade may not be a major pathway in 293T cells.

FRS2 Interacts with FcFgfr1 via the PTB Domain—It has
been previously shown that FRS2 interacts with the Fgfr1 juxtamembrane region through the FRS2 PTB domain (26, 37, 38). The coexpression/immunoprecipitation experiments described above were repeated with a truncated form of FRS2, containing the myristoylation site and PTB domain of FRS2 (Fig. 3b). The same interaction of FRS2 PTB domain with Fgfr1 as wild type FRS2 was observed, confirming that FRS2 interacts with the Fgfr1 VT site through its PTB domain. In these experiments, no association of Grb2 was detected, since the Grb2 recognition epitopes are located in the C-terminal effector domain of FRS2 (24).

Direct Interaction of FRS2 with Fgfr1 VT+. The interaction of FRS2 with Fgfr1 VT+ could either be direct or indirect mediated by additional proteins present in 293T cells. To test this, we examined the interaction of purified Fgfr1 juxtamembrane domain with purified FRS2 in solution. The juxtamembrane regions of Fgfr1 with and without the VT motif and FRS2 fused to GST were expressed and purified from bacteria. Direct interaction of the VT+VT− juxtamembrane proteins with GST-FRS2 was investigated by a modified enzyme-linked immunosorbent assay (Fig. 4). GST-FRS2 bound preferentially to the VT+ form of the Fgfr1 juxtamembrane region but not to the VT− form of the receptor. This results shows that FRS2 can interact directly with the Fgfr1 VT+ form of the juxtamembrane domain, although the need for further co-factors to form a stable high affinity complex between FRS2 and Fgfr1 cannot be eliminated, since the estimated EC50 of the interaction in solution is ~240 nM.

Expression of Fgfr1 VT+ and VT− RNAs in Mouse Tissues—The experiments described above show that alternative splicing of the VT motif determines binding of FRS2 to Fgfr1 (and presumably Fgfr2 and -3, which also exhibit VT splicing (23)). As documented above, the association of FRS2 links Fgfr activation with multiple signaling pathways. Therefore, activation of these pathways could be regulated in part by the expression levels of the Fgfr1 VT+ and VT− isoforms. RT-PCR was used in order to determine relative expression levels of Fgfr1 VT+ and VT− in different mouse tissues. The VT motif is produced by alternative splicing of six nucleotides at the end of exon 10. Exons 8 and 9 of the Fgfr1 are also alternatively spliced to exon 10, giving rise to the IIIb and IIIc splice variants, respectively. The forward primer was selected to distinguish the IIIb (exon 8) and IIIc (exon 9) splice variants, and enzyme digestion was used to detect the VT+ and VT− forms of Fgfr1. In all tissues studied, the major splice variants IIIb and IIIc existed in combination with both the VT+ and VT− forms of the receptor (Fig. 5 and data not shown). It is interesting to note that differential tissue-specific expression of the VT+ and VT− isoforms was not observed in the tissues examined.

DISCUSSION

Here we have shown that alternative splicing of the juxtamembrane region of Fgfr1, leading to the inclusion or omission of the two-amino acid VT motif, controls the ability of the receptor to associate with the signaling adaptor protein FRS2. As a result of this splicing event, signaling functions associated with adaptors, such as Grb2, that associate with activated Fgfrs via FRS2 is abrogated. This finding explains our previous report (23), which showed that the VT− splice variant was unable to activate the Ras/MAPK pathway in transfected cells.

These findings also confirm that the FRS2 PTB domain constitutively interacts with the juxtamembrane region of Fgfr1 (26, 36–38). From single amino acid substitutions of the VT site, it emerges that FRS2 interaction with valine of the VT site is most significant. By contrast, mutation of the threonine residue has little effect on FRS2 binding. This indicates that phosphorylation of the threonine by PKC or other kinases may not play a significant role in FRS2 docking. This is supported by mutagenesis studies and NMR structural data by others (37, 38). The structural data also provide an explanation for the loss of affinity for FRS2 exhibited by the VT− variant; it essentially creates a “frameshift” between residues that are required for binding to the PTB domain of FRS2.

Here we have shown that the loss of FRS2 adaptor binding to Fgfr1 results in concomitant loss of other effectors that bind to FRS2, such as Grb2. Signaling pathways likely to be influenced by VT splicing include activation of MAPK through recruitment of Grb2-Sos to FRS2 (44, 46); recruitment of PKCα and PKCε, which have also been shown to activate MAPK (31–33); and Gab1-dependent recruitment of phosphoinositide 3-kinase (38, 34). However, other signaling channels dependent on receptor kinase activity such as recruitment of PLCγ described here remain intact in the VT− isoform.

No significant differences in levels of expression of the two alternatively spliced Fgfr1 isoforms were detected between different mouse tissues using RT-PCR. The VT+ and VT− Fgfr1 isoforms were co-expressed in all tissues studied. However, differential expression of VT+ and VT− isoforms of Fgfr1 has been identified during mesoderm formation in Xenopus embryos (47), and a more extensive survey in the mouse may...
reveal cell types in which differential expression of the VT isoforms does occur. If the relative levels of mRNA are reflected in expression of receptor protein, this indicates that alternative splicing of the juxtamembrane region represents bifurcation in Fgfr signaling in which different signal outputs may be quantitatively regulated. Combined with the tissue-specific expression of the FRS2 adaptor, this contributes to a mechanism by which different signaling channels may be selectively activated during Fgfr signaling in different cell types.

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Association of the Signaling Adaptor FRS2 with Fibroblast Growth Factor Receptor 1 (Fgfr1) Is Mediated by Alternative Splicing of the Juxtamembrane Domain

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