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Sea Urchin FGFR Muscle-Specific Expression: Posttranscriptional Regulation in Embryos and Adults

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We have shown previously by *in situ* hybridization that a gene encoding a fibroblast growth factor receptor (*SpFGFR*) is transcribed in many cell types during the initial phases of sea urchin embryogenesis (*Strongylocentrotus purpuratus*) (McCoon *et al., J. Biol. Chem. 271,* 20119–20195, 1996). Here we demonstrate by immunostaining with affinity-purified antibody that SpFGFR protein is detectable only in muscle cells of the embryo and appears at a time suggesting that its function is not in commitment to a muscle fate, but instead may be required to support the proliferation, migration, and/or differentiation of myoblasts. Surprisingly, we find that *SpFGFR* transcripts are enriched in embryo nuclei, suggesting that lack of processing and/or cytoplasmic transport in nonmuscle cells is at least part of the posttranscriptional regulatory mechanism. Western blots show that SpFGFR is also specifically expressed in adult lantern muscle, but is not detectable in other smooth muscle-containing tissues, including tube foot and intestine, or in coelomocytes, despite the presence of *SpFGFR* transcripts at similar concentrations in all these tissues. We conclude that in both embryos and adults, muscle-specific SpFGF receptor synthesis is controlled primarily at a posttranscriptional level. We show by RNase protection assays that transcripts encoding the Ig3S variant of the ligand binding domain of the receptor, previously shown to be enriched in embryo endomesoderm fractions, are the predominant, if not exclusive, *SpFGFR* transcripts in lantern muscle. Together, these results suggest that only a minority of *SpFGFR* transcripts are processed, exported, and translated in both adult and embryonic muscle cells and these contain predominantly, if not exclusively, Ig3S ligand binding domain sequences.

Key Words: **posttranscriptional regulation; secondary mesenchyme; nuclear RNA; muscle.**

INTRODUCTION

Recently we described the cloning and developmental expression of a gene, *SpFGFR*, encoding a member of the vertebrate fibroblast growth factor receptor (FGFR) family (McCoon *et al.*, 1996). FGF signaling pathways are known to play central roles in mesoderm patterning and morphogenesis in both vertebrate (Amaya *et al.*, 1991, 1993; Yamaguchi *et al.*, 1994; Deng *et al.*, 1994; Peters *et al.*, 1994; Griffin *et al.*, 1995) and invertebrate (Glazer and Shilo, 1991; DeVore *et al.*, 1995) embryos. *In situ* hybridization and RNase protection assays showed that *SpFGFR* transcripts accumulate during the blastula–gastrula period of development, are distributed in most tissues, and later become enriched in the actively dividing cells of oral ectoderm and endoderm of the pluteus larva (McCoon *et al.*, 1996). This temporal pattern correlates with the period of initial morphogenesis in the sea urchin embryo, but the widespread distribution of transcripts does not lead to predictions of possible cell-type-specific developmental functions. In fact, both the temporal and spatial profiles of RNA accumulation are like those of many genes expressed in the sea urchin embryo, including those whose protein products suggest roles in cell cycling and growth (Kingsley *et al.*, 1993).

To explore further the possible function of SpFGFR, in the experiments reported here we have used an affinity-purified polyclonal antibody against the nonconserved

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C-terminal portion of the receptor to determine receptor distribution in developing sea urchin embryos. Surprisingly, we found that SpFGFR protein is highly enriched in, and probably restricted to, some muscle cells in the embryo and accumulates predominantly, and perhaps exclusively, in lantern muscle of adults. Since *SpFGFR* transcripts accumulate to significant levels in other cell types in both embryos and adults, lack of receptor accumulation in these cells is regulated by a posttranscriptional mechanism. We show here that *SpFGFR* transcripts accumulate to unusually high levels in nuclei of nonmuscle cells of embryos, suggesting that these RNAs fail to be completely processed and/or transported to the cytoplasm. The timing of SpFGFR accumulation in muscle lineages is consistent with a role for this signaling pathway in myoblast migration and/or the dramatic cell shape changes accompanying myogenesis in the sea urchin embryo.

MATERIALS AND METHODS

Embryo culture. Sea urchins were obtained from Marinus, Inc. (Venice, CA). Embryo cultures were prepared as described previously (Angerer and Angerer, 1981).

Production and purification of antibodies. Purified fusion protein representing the C-terminal 112 amino acids of SpFGFR was produced as follows: The nucleotide sequence was synthesized by PCR with primers containing *Bam*HI sites, using an *SpFGFR* cDNA template, and inserted in the *Bam*HI-digested pET15b vector (Novagen, Inc., Madison, WI) for production of His-tagged fusion proteins in *Escherichia coli* strain BL21 following the manufacturer's (Novagen, Inc.) instructions. The His-tagged protein was purified on nickel affinity columns and used as an immunogen to make a polyclonal rabbit antiserum. For affinity purification of antibodies against SpFGFR, a second, glutathione *S*-transferase fusion protein was produced containing the C-terminal 138 amino acids of SpFGFR. The nucleotide sequence was PCR-amplified from an *SpFGFR* cDNA template using *Nde*I- and *Xho*I-containing forward and reverse primers, respectively, and inserted into pGEX-KG (Guan and Dixon, 1991) at the corresponding restriction sites.

Antisera to the purified His-tagged fusion protein were produced by Pocono Rabbit Farms (Canadensis, PA) and Babco (Berkeley, CA). For immunostaining, antibody was affinity purified by binding to and elution with 0.2 M glycine, pH 2.8, from immunoblots (Immobilon; Olmsted, 1987) of fusion proteins purified by SDS–PAGE. For negative controls, preimmune sera were subjected to the same purification procedure.

Immunocytochemistry. Purified antibodies were used to stain whole embryos of selected stages that had been fixed with methanol (4°C, 30 min) and immobilized on poly-L-lysine (Sigma, St. Louis, MO)-coated slides. Binding of affinity-purified anti-SpFGFR antibody was detected with FITC-conjugated goat anti-rabbit secondary antibody (Cappel, Durham, NC) diluted 1/250. Stained embryos were mounted in Gelvatol (Monsanto, Indian Orchard, MA) containing 100 mg/ml DABCO (Sigma). Muscle cells were identified by immunostaining with a polyclonal anti-sea urchin myosin heavy-chain rabbit antibody (diluted 1:200; a kind gift of Dr. Judith Venuti, Columbia), followed by the secondary antibody described above. Confocal images of the anti-SpFGFR-stained embryos were obtained using a Bio-Rad MRC-1000 confocal system and a Nikon Diaphot microscope, generously provided by Dr. Robert Summers, University of Buffalo; the myosin image was obtained with a Zeiss confocal microscope (courtesy of Zeiss, Inc.).

In situ hybridization. *In situ* hybridizations with digoxigenin-labeled riboprobes were carried out as follows. Sense and antisense RNA probes were prepared as described previously (Ransick *et al.*, 1993) and hybridized using procedures similar to those described (Harkey *et al.*, 1992) with the following modifications: Embryos were fixed with Streck's fixative (Streck Laboratories, Inc., Omaha, NE) at 4°C, washed with PBST (phosphate-buffered saline + 0.1% Tween 20), and hybridized at a probe concentration of 0.7 μg/ml at 46°C for 20 h in a solution containing 50% formamide, 0.6 M NaCl, 20 mM Tris–HCl, pH 7.5, 500 μg yeast RNA/ml, 500 μg heparin/ml, 10% polyethylene glycol (M_r 6–8 $\times$ 10^3), and 1 $\times$ Denhardt's solution (0.02% each Ficoll, polyvinylpyrollidone, and bovine serum albumin). Prior to incubation with alkaline phosphatase (AP)-conjugated sheep polyclonal anti-digoxigenin Fab fragments (Boehringer-Mannheim), embryos were treated with 10% heat-inactivated (70°C) sheep serum. AP staining was carried out for 16 h.

Immunoblots. Adult urchin tissues were lysed by boiling in buffer containing 63 mM Tris–HCl, pH 6.8, 10% glycerol, 2% SDS, 10% 2-mercaptoethanol, and 1% BPB and proteins were electrophoresed through a 10% SDS–PAGE gel and blotted to Immobilon (Millipore, Bedford, MA) in buffer containing 25 mM Tris, 192 mM glycine, and 20% methanol. FGFR protein was detected with anti-SpFGFR polyclonal antiserum diluted 1:100, followed by horseradish peroxidase-conjugated anti-rabbit secondary antibody (Cappel, Inc.) diluted 1:5000. Chemiluminescent detection was carried out with a solution of 100 mM Tris–HCl, pH 8.5, 2.25 mM *p*-coumaric acid (Sigma), 12.5 mM luminol (Sigma), and 0.015% hydrogen peroxide for 1 min and then exposed to Kodak X-OMAT film.

Immunoprecipitation of SpFGFR from COS cells. COS-7 cells were plated in 35-mm plates at a density of 7 $\times$ 10^4 per plate and transfected according to manufacturer's instructions the next day using, per plate, 8 μl lipofectamine (Gibco-BRL, Gaithersburg, MD) and 1 μg of a recombinant plasmid containing SpFGFRHA (hemagglutinin-tagged at the C-terminus; YPYDVPDYA) inserted into the expression vector pSVL (Pharmacia). Two days after transfection, cells were labeled with 100 μCi [^{35}S]methionine (1000 Ci/mmol, 10 mCi/ml) in 1 ml methionine-free medium per plate for 4 h. Cells were washed once with phosphate-buffered saline (PBS) and lysed in ice-cold lysis buffer containing 1% Triton X-100, 150 mM NaCl, 10 mM Tris–HCl, pH 7.5, 1 mM phenylmethylsulfonyl fluoride, and 1% aprotinin. After incubation on ice for 30 min with rocking, cells were scraped from plates and centrifuged at 4°C for 10 min at 10,000*g*. Supernatants were transferred to fresh tubes for preimmune clearing: Twenty microliters of preimmune serum was added to the supernatant and incubated for 1 h on ice. Fifty microliters of pansorbin (Calbiochem, San Diego, CA), centrifuged and resuspended in lysis buffer, was added and incubation was continued for 30 min. Samples were centrifuged at 4°C for 10 min at 10,000*g* and supernatants were removed to fresh tubes. Two microliters of anti-SpFGFR C-terminal antiserum was added and incubated for 4 h. Fifty microliters of pansorbin, which had been incubated in 2% BSA and washed in lysis buffer, was added to each tube and incubation proceeded for 30 min. Pansorbin-bearing immune complexes were pelleted for 1 min in a microcentrifuge and washed successively in 1-ml volumes of the following ice-cold wash solutions: (1) 100 mM Tris–HCl, pH 8, 1 M NaCl, 0.1% NP-40; (2) 10 mM Tris–HCl, pH 8, 100 mM NaCl, 1% NP-40, 1 mM

EDTA, 0.3% SDS; and (3) 10 mM Tris–HCl, pH 8, 0.1% NP-40. Washed pellets were solubilized in 1× SDS–PAGE sample buffer and electrophoresed through 7.5% SDS–PAGE gels which were fluorographed using Enhance (Dupont-NEN, Boston, MA).

Isolation of nuclear and cytoplasmic RNA. All steps were carried out at 0–4°C unless otherwise noted. Embryos were collected at either 48 h (gastrula) or 72 h (pluteus) after fertilization and washed by low-speed centrifugation (1000*g*, 3 min) successively in the following solutions: (1) 0.5 M KCl; (2) 0.5 M KCl, 0.5 mM EDTA, pH 8.0; and (3) 1 M glycine, 2 mM EDTA, pH 8.0. The partially dissociated embryos were then washed by sedimentation through a solution containing 0.25 M sucrose, 10 mM Tris–HCl, pH 8.0, and 0.1 mM EDTA at 9600*g* for 5 min. The pellet was resuspended in 5 embryo volumes of 0.32 M sucrose, 10 mM Tris–HCl, pH 8.0, 5 mM $MgCl_2$, 1 mM spermidine, 1 mM EGTA, 1% Triton X-100 and the cells were lysed by 5 strokes with the A pestle of a Dounce homogenizer. Approximately one-third of the homogenate was used to isolate cytoplasmic RNA as follows: Nuclei were removed by sedimentation at 9600*g* for 10 min and the supernatant was adjusted to 0.3 M sodium acetate, pH 6.0, 10 mM EDTA, 1% SDS. RNA was purified by two cycles of phenol–chloroform extraction at room temperature and ethanol precipitation at 0°C. The remainder of the homogenate was subjected to 10 additional strokes in the Dounce homogenizer to ensure that most cells were lysed, and any remaining intact cells were removed by sedimentation at 3800*g* for 3 min. Nuclei in the supernatant were purified of cytoplasmic material by sedimentation through 12 ml of 0.75 M sucrose containing 1% Triton X-100 at 7300*g* for 10 min. The clear nuclear pellet was resuspended in a solution containing 0.32 M sucrose, 10 mM EDTA, 0.3 M sodium acetate, pH 6.0, and SDS was added to 1% final concentration. The solution was passed through an 18-gauge needle several times to reduce DNA viscosity and nucleic acids were purified as described above. Both nuclear and cytoplasmic fractions were treated with 100 μg DNase I/ml at 37°C for 30 min and purified by organic extraction and ethanol precipitation.

RNase protection assays. These assays were performed as previously described (Gagnon *et al.*, 1992) by hybridizing 5.5 μg of cellular RNA to a sequence excess of ^{32}P-labeled antisense riboprobes representing either a portion of the tyrosine kinase (TK) domain (McCoon *et al.*, 1996; +2341 to +2529; $\sim 2 \times 10^8$ dpm/μg), *actin CyIIIa* intron (*CyIIIa*-int; Gagnon *et al.*, 1992; $\sim 4 \times 10^8$ dpm/μg), or *actin CyIIIa* exon (*CyIIIa*-ex; Gagnon *et al.*, 1992; $\sim 2 \times 10^7$ dpm/μg).

RNA blots. Total RNA was isolated from embryos of selected stages as described previously (Hurley *et al.*, 1989) or from adult tissues using the Trizol RNA purification kit (Gibco/BRL), electrophoresed through 0.8 or 1% agarose–formaldehyde denaturing gels, and transferred to Genescreen Plus (Dupont-NEN, Boston, MA). Embryo RNA blots were prehybridized and then hybridized in a solution containing 2× SSC, 50 mM sodium phosphate, pH 6.8, 0.1% sodium pyrophosphate, 5× Denhardt's solution, 1% SDS, 10% dextran sulfate, 20% formamide, and 200 μg calf thymus DNA/ml with 1×10^6 cpm ^{32}P-labeled randomly primed probe per milliliter of hybridization solution. Blots of adult RNA were hybridized similarly with the following modifications: 2× SSC was replaced with 0.9 M NaCl, 5 mM EDTA; polyethylene glycol replaced dextran sulfate; the formamide concentration was increased to 35%; and the salmon sperm DNA concentration was increased to 1 mg/ml. Hybridization temperatures were adjusted to approximately T_m −25°C. In both cases, blots were washed successively in 4× SSC, 2× SSC, 1× SSC, and 0.1× SSC, each containing 1% SDS at a stringency of $\sim T_m$ −10°C.

RESULTS

In embryos, SpFGFR protein is detectable only in muscle cells. To detect SpFGFR, we used affinity-purified, polyclonal anti-SpFGFR antiserum that was raised against the C-terminus of SpFGFR because that is the most divergent portion of tyrosine kinase receptors and is therefore unlikely to cross-react with other receptor peptides. Identical results were obtained with antiserum affinity purified with two different fusion proteins and with embryos from both *Strongylocentrotus purpuratus* and *Lytechinus pictus*. The confocal image shown in Fig. 1B results from assembling a stack of 25 optical 1-μm sections through the center of a pluteus larva stained with anti-SpFGFR antibody. Figure 1A shows an embryo of the same age and in a similar orientation with arrows indicating the corresponding positions of labeled regions in Fig. 1B. A strong signal is observed in the long cytoplasmic processes of cells in the circumesophageal muscles (green arrow) and lower signals in cardiac sphincter muscle (arrowhead) of the pluteus larva and the pyloric and anal sphincters (not shown). Myoblasts lying along the esophagus (white arrows) are also strongly positive. Figure 1C shows the absence of signal in a similarly prepared confocal image of an embryo treated with preimmune serum that had been subjected to the same affinity purification procedure. The position of the esophagus in this embryo, which is oriented as in Fig. 1B, is indicated by the green arrow. Staining very similar to the SpFGFR pattern was observed with an anti-myosin antibody (Fig. 1F) as described previously (Wessel *et al.*, 1990). Supporting the idea that signals are found only in muscle cells during embryogenesis and not in the nerves that innervate them, we have observed that SpFGFR protein is detectable only at the tip of everted archentera in LiCl-induced and spontaneously occurring exogastrulae (data not shown) that are well separated from nerves emanating from the oral ectoderm region.

The earliest stage at which we were able to detect signal was in the late gastrula at 50 h postfertilization, when the only labeled cells are several secondary mesenchyme cells, the first muscle precursors that are beginning to differentiate near the tip of the invaginating archenteron (confocal and DIC images, arrows in Figs. 1E and 1D, respectively). Cells in similar positions, i.e., on either side of the archenteron and slightly below its tip, have been shown to express muscle actin mRNA (Cox *et al.*, 1986) and SUM1, a sea urchin myoD1-related transcription factor (Venuti *et al.*, 1993).

SpFGFR transcripts are found predominantly in nuclei of sea urchin embryos. The muscle-specific expression of SpFGFR peptide contrasts with the ubiquitous accumulation of *SpFGFR* transcripts and indicates that a posttranscriptional regulatory mechanism operates in sea urchin

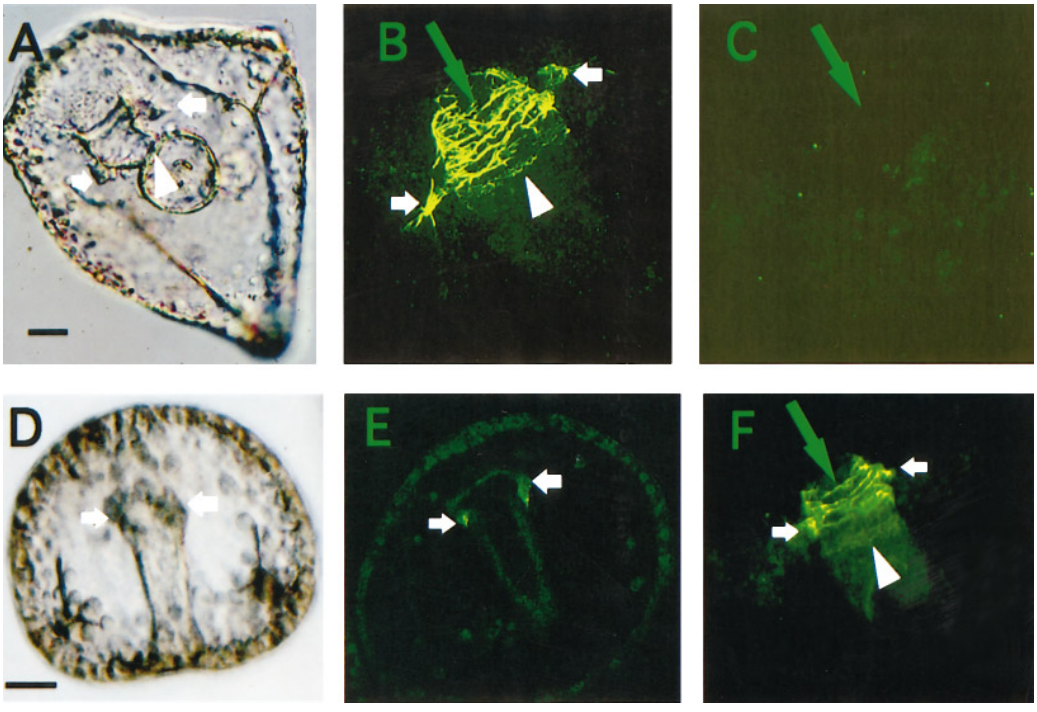

FIG. 1. SpFGFR is muscle-specific in sea urchin embryos. (A) Pluteus larva of the same age and oriented in a similar manner to the immunostained specimen shown in B. (B) Pluteus larva stained with affinity-purified, anti-SpFGFR antibody showing intense staining of the circumesophageal muscle cells. Identical results were obtained with *Strongylocentrotus purpuratus* and *Lytechinus pictus* embryos and with preparations affinity purified using two different fusion proteins. The green arrow points to the esophagus and musculature surrounding it, the white arrows indicate myoblasts, and the arrowhead points to the cardiac sphincter region. (C) Pluteus larva stained with preimmune serum subjected to the same affinity purification procedure. The orientation of this embryo is the same as those in A and B and the arrow points to the esophagus. (D) Micrograph of an embryo of the same stage and orientation as in E. (E) Gastrula showing staining of several secondary mesenchyme cells (arrows) near the tip of the archenteron. (F) Antimuscle myosin staining of a pluteus larva, oriented as in A–C, and showing the pattern previously described (Wessel *et al.,* 1990). Arrow and arrowheads indicate cells and structures as described for B. The images in B, C, E, and F were obtained by confocal microscopy. The bars in A and D represent 20 μm.

embryos. Various levels at which this might be achieved include primary transcript processing, export to the cytoplasm, translation, and mRNA or protein stability. Evidence supporting the idea that this regulation might operate primarily in the nucleus was initially obtained by whole-mount *in situ* hybridizations carried out with *SpFGFR* digoxigenin-labeled probes. *SpFGFR* RNAs are detected in all cell types, as established previously with radioactive probes (McCoon *et al.,* 1996), and much of the signal appears to be nuclear, as illustrated in Fig. 2A, AS. While nuclear accumulation of *SpFGFR* transcripts is apparent at all stages in which signal can be detected, it is most obvious by this assay at the mesenchyme blastula stage, when the distinction between nuclei (arrow) and cytoplasm is clearest. However, because this technique often provides artifactual nuclear signals for known cytoplasmic target mRNAs (e.g., Ransick *et al.,* 1995), we used an independent method to confirm that *SpFGFR* transcripts accumulate in nuclei. Relative concentrations of *SpFGFR* transcripts in nuclear and cytoplasmic RNA were determined by RNase protection assays with an SpFGFR exon probe. As an internal control, a probe for an mRNA located predominantly in the cytoplasm (*actin CyIIIa* exon; *CyIIIa*-ex) was cohybridized with the *SpFGFR* probe in the same samples (Fig. 2B). As expected, most of the *CyIIIa*-ex signal was found in the cytoplasm, whereas the majority of the *SpFGFR* signal was nuclear at both gastrula (G) and pluteus (P) stages. Figure 2C shows a similar experiment carried out with a probe representing a known nucleus-restricted sequence (*actin CyIIIa* intron; *CyIIIa*-int). As expected, *CyIIIa*-int transcript levels are higher in the nuclear RNA population at both stages. Signals for each protected hybrid in Figs. 2B and 2C and the values obtained in each experiment for the internal control probe (*CyIIIa-ex*) were quantitated with a Phosphorimager and the *SpFGFR*/*CyIIIa*-ex ratio was compared to the *CyIIIa*-int/*CyIIIa*-ex ratio (Table 1). The use of the internal control eliminates any errors that might arise in amounts of nuclear or cytoplasmic RNAs added to each reaction or differences in recovery of hybrids among different samples (for example, *CyIIIa*-ex signal differences in Figs. 2B and 2C

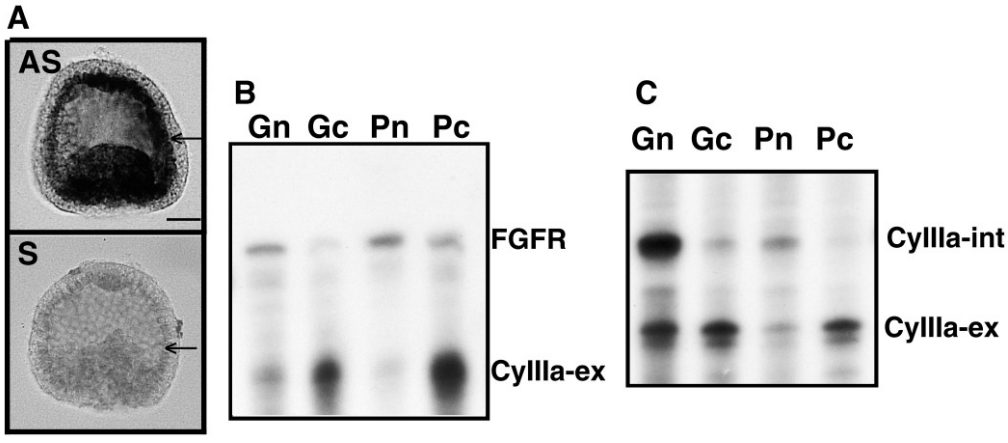

FIG. 2. *SpFGFR* transcripts are highly enriched in nuclear RNA as are intron-containing transcripts. (A) Whole-mount *in situ* hybridization of antisense (AS) and sense (S) *SpFGFR* probes to mesenchyme blastulae, oriented with their vegetal poles down. At this stage, nuclei of presumptive ectoderm (e.g., arrow) lie in a basal position in each cell and are much more highly stained than the adjacent cytoplasm. The bar in the upper panel represents 15 μm. (B) RNase protection assays in which probes representing *SpFGFR* and *CyIIIa* exon sequences (2×10^8 cpm/μg and 2×10^7 cpm/μg, respectively) were combined and hybridized to equal masses of nuclear and cytoplasmic RNAs, purified as described under Materials and Methods. (C) A second assay in which the actin *CyIIIa* intron probe (4×10^8 cpm/μg) replaced the *SpFGFR* probe. Control hybridizations with yeast tRNA and all three probes yielded no protected fragments (not shown). RNAs were isolated from gastrula nuclei (Gn), gastrula cytoplasm (Gc), pluteus nuclei (Pn), and pluteus cytoplasm (Pc). Positions of full-length RNase-resistant fragments are indicated at right in B and C.

indicate there were differences in load and/or recovery). After the *SpFGFR* and *CyIIIa*-int signals are corrected by dividing by the internal control value, it can be seen that the distributions of *SpFGFR* and *CyIIIa*-int transcripts in nuclear and cytoplasmic compartments are within 15% of each other at both gastrula and pluteus stages. We conclude that *SpFGFR* transcripts are highly enriched in nuclei.

SpFGFR is also expressed specifically in adult lantern muscle. We have been unable to detect the target protein in Western blots of whole embryo proteins, undoubtedly because cells stained with anti-SpFGFR antibody constitute only a few percent of the embryo's cells. We therefore searched for SpFGFR in adult muscle tissues which can be isolated in larger quantity and high purity. Figures 3A and 3B show blots of equivalent quantities of total protein from lantern muscle, tube feet, and coelomocytes stained with either immune or preimmune serum, respectively. A very prominent immunoreactive protein, of approximately 120 kDa, was detected in lantern muscle. Signals at this position in other tissues are extremely faint and estimated to be at least several orders of magnitude lower than that in lantern muscle, since the 120-kDa species can be detected using 100-fold less lantern muscle protein (data not shown). The protein faintly detectable with preimmune serum in the lantern muscle lane (Fig. 3B) migrates to a different position, corresponding to a mass of about 105 kDa. The identity of the lower molecular weight (~58 kDa) and less abundant immunoreactive protein in coelomocytes is not known and it is not present in lantern muscle.

The size of SpFGFR inferred from the amino acid sequence is about 107 kDa (McCoon *et al.*, 1996) in the absence of any posttranslational modifications. However, it is likely that, when synthesized in eukaryotic cells, the receptor is N-glycosylated at some or all of 11 potential sites present in its extracellular domain (McCoon *et al.*, 1996). To determine whether N-glycosylation contributes to the larger mass of immunoreactive protein in lantern muscle, full-length *SpFGFR* cDNA [encoding a protein of 972 amino acids plus a 9-amino-acid hemagglutinin (HA) epitope tag] was labeled with [^{35}S]methionine in COS cells and immunoprecipitated with either anti-C-terminal SpFGFR or anti-HA antibody. As shown in Fig. 3C, left lanes, a prominent protein of approximately 125–130 kDa is present in COS cells transfected with *SpFGFR* recombinant plasmid and absent from cells containing vector only. To

TABLE 1

SpFGFR Transcripts Are Highly Enriched in Nuclei of Sea Urchin Embryos

	Gastrula			Pluteus		
	Nuc	Cyto	Nuc/Cyto	Nuc	Cyto	Nuc/Cyto
SpFGFR/*CyIIa*-ex	0.74	0.11	6.7	2.1	0.17	12.3
CyIIIa-int/*CyIIa*-ex	2.3	0.26	8.8	1.4	0.12	11.7

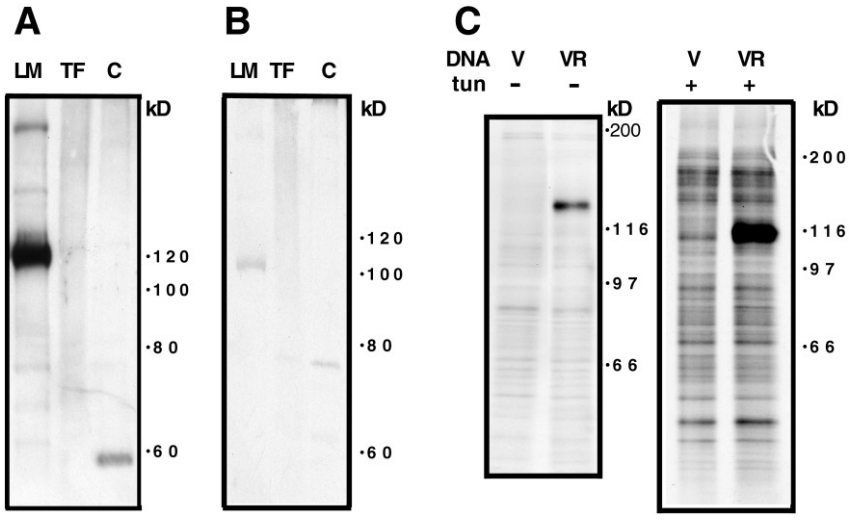

FIG. 3. SpFGFR is an N-glycosylated protein expressed in adult lantern muscle. (A) Western blot of equivalent amounts of total protein from lantern muscle (LM), tube feet (TF), and coelomocytes (C) stained with anti-SpFGFR antibody; (B) replicate Western blot incubated with preimmune serum. No immunoreactive proteins were detected in intestine, but it is not shown here because of some proteolysis in the sample. (C) Recombinant SpFGFR synthesized in COS7 cells is similar in size to the peptide identified in sea urchin lantern muscle and larger than the size inferred from the cDNA sequence, at least partially as a result of N-glycosylation. ^{35}S-labeled proteins from COS7 cells transfected with pSVL vector only (V) or pSVL-SpFGFR/HA recombinant (VR) were immunoprecipitated with anti-SpFGFR antibody (left two lanes). A protein of ~125–130 kDa is present only in the recombinant DNA-transfected sample. A similar experiment in which proteins made in COS cells were labeled in the presence of tunicamycin (tun) and immunoprecipitated with anti-hemagglutinin antibody (right two lanes) shows a reduction in size of the recombinant protein. Similar results were obtained with anti-SpFGFR antibody (data not shown). Molecular weight markers are indicated to the right of each panel.

verify that the greater size of SpFGFR synthesized in COS cells was due, at least in part, to N-glycosylation, transfected cells, labeled with [^{35}S]methionine, were grown for 3 h in medium containing 50 μg tunicamycin/ml. As shown in Fig. 3C, right lanes, the immunoprecipitable protein migrates close to the position of the 116-kDa marker, reflecting a reduction in molecular mass compared to the same protein synthesized in the absence of the drug and indicating that the glycosylation level is reduced under these conditions. The small difference in mass between the receptor synthesized in sea urchin lantern muscle and COS cells undoubtedly reflects species-specific glycosylation differences. We conclude that the anti-C-terminal SpFGFR antibody recognizes recombinant N-glycosylated receptor and a protein of very similar size in adult sea urchin lantern muscle.

SpFGFR transcript analysis. The findings that most embryonic *SpFGFR* transcripts are nuclear and produced in cells that do not express the receptor raise the possibility that the structure of ubiquitous *SpFGFR* transcripts and cytoplasmic muscle *SpFGFR* mRNAs might be different. Blots of RNAs from embryos at selected stages and adult tissues were probed with sequences representing the ligand binding domain, the tyrosine kinase domain, or the 5′ UTR. In each case, two long RNAs, ca. 8.5 and 8.0 kb (see arrows), were detected in total embryo RNAs at both high (T_m −10°C) (Fig. 4A) and low (T_m −35°C, data not shown) stringency. Relative levels of *SpFGFR* transcripts in embryos at different developmental stages are in excellent agreement with previous RNase protection and *in situ* hybridization assays (McCoon *et al.*, 1996): *SpFGFR* transcript concentrations are very low in eggs and cleaving embryos and increase during blastula stage but to only relatively low levels, estimated to be about 10–20 copies/cell. The signals at the mobility of the rRNAs result from nonspecific probe binding, because they are absent in preparations enriched for polyadenylated RNA (Fig. 4B). Again, two transcripts in the 8-kb range can be discerned (see arrows), demonstrating that these long nuclear transcripts are polyadenylated.

It is not clear whether either of the embryonic transcripts detected in Figs. 4A and 4B represents *SpFGFR* mRNA translated in muscle, which is expected to be very rare since it accumulates only in a few muscle cells. Although we previously detected two different open reading frames in the Ig3 region of the ligand binding domain of the receptor, Ig3L and Ig3S, their coding sequences differ by only 102 nt which is too short to be resolved on this gel in the 8-kb range. To search for muscle-specific transcripts, we probed blots of RNAs isolated from lantern muscle, which expresses the receptor, and coelomocytes and intestine, which do not (Fig. 3). As shown in Fig. 4C, *SpFGFR* transcripts

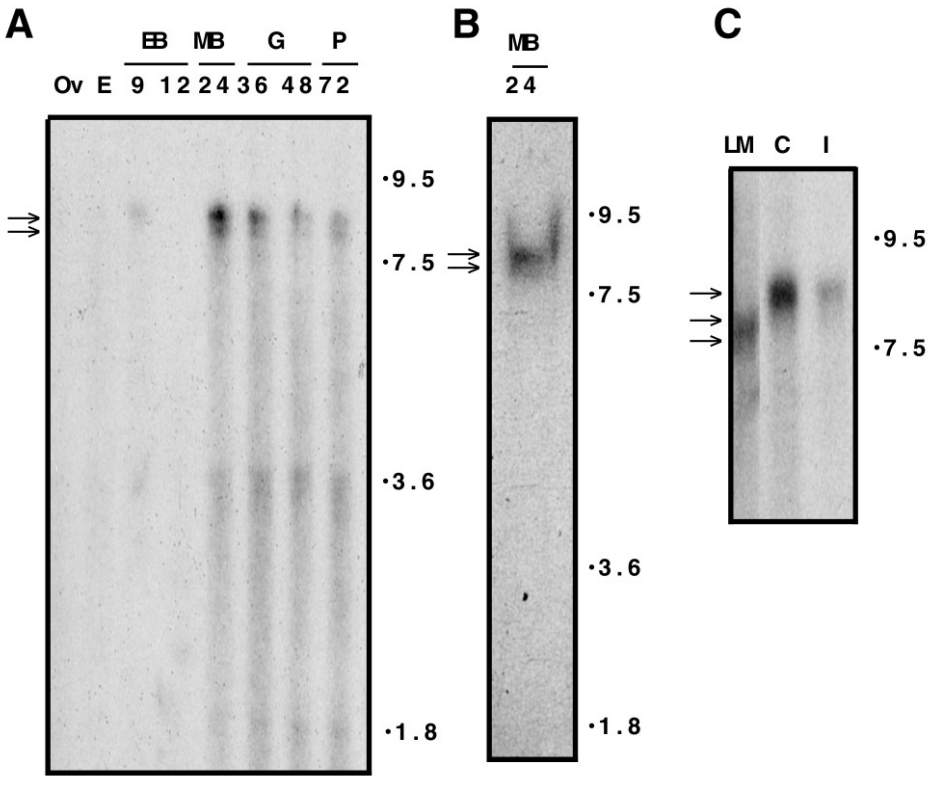

FIG. 4. RNA blot analysis to determine *SpFGFR* transcript length. (A) RNA blots of total RNA isolated from ovary (Ov), eggs (E), or embryos of indicated stages [9–12 h, early blastula (EB); 24 h, mesenchyme blastula (MB); 36–48 h, mid to late gastrula (G); 72 h, pluteus larva (P)] were hybridized to randomly primed probes representing the SpFGFR ligand binding domain. Two transcripts of approximately 8.0 and 8.5 kb are detected. (B) Blot of mesenchyme blastula RNA enriched for polyadenylated transcripts by oligo(dT) chromatography to reduce nonspecific signal at the position of rRNAs (3.6 and 1.8 kb). The probe represents the ligand binding and tyrosine kinase domains and demonstrates that these long *SpFGFR* transcripts are polyadenylated. (C) Blot of RNAs isolated from lantern muscle (LM), coelomocytes (C), and intestine (I). Ethidium bromide stains of rRNAs in the samples in A and C showed that the amounts of RNA loaded were very similar and that the RNA was intact. RNA size markers in kilobases are shown to the right of each panel.

migrating between the 9.5- and 7.5-kb markers are detected in all three tissues, but those in lantern muscle are clearly the shortest and are likely to include mRNAs even shorter than any detected in embryo RNAs. We conclude that, in nonexpressing tissues of embryos and adults, most, if not all, *SpFGFR* transcripts are longer than those in expressing cells. Since most *SpFGFR* transcripts are nuclear, it is likely that their larger size results from incomplete processing which is not yet defined.

Ig3S variant transcripts are highly enriched in lantern muscle that expresses SpFGFR. In embryos, Ig3S transcript isoforms accumulate preferentially compared to Ig3L variants in endomesoderm tissues enriched for muscle cells (McCoon *et al.*, 1996), raising the possibility that only Ig3S RNAs are translated. Since SpFGFR specifically accumulates in adult lantern muscle, we used RNase protection assays to test whether the ratio of Ig3S to Ig3L transcripts was higher in this tissue compared to that in other tissues which do not express the receptor. Figure 5 demonstrates that Ig3S RNA, which encodes a protein more similar to vertebrate FGFR sequences in the ligand binding domain, is the only detectable species in lantern muscle. In contrast, Ig3L RNA is the major species in intestine and in tube feet that lacks a detectable receptor (Fig. 3A), while both forms are present at lower levels in coelomocytes. The lack of receptor protein accumulation in these tissues is not because putative Ig3L protein isoforms lack the C-terminal epitope recognized by anti-SpFGFR (McCoon *et al.*, 1996). We conclude that SpFGFR synthesized in lantern muscle contains the Ig3S version of the ligand binding domain. The Ig3L vs Ig3S difference is insufficient to account for the length differences among SpFGFR transcripts in lantern muscle. Elucidation of additional sequence differences will require further molecular analyses.

The RNase protection assays provide more reliable quantitative measures of target RNA concentration and confirm the RNA blot data demonstrating that production of this receptor is also regulated posttranscriptionally in adult

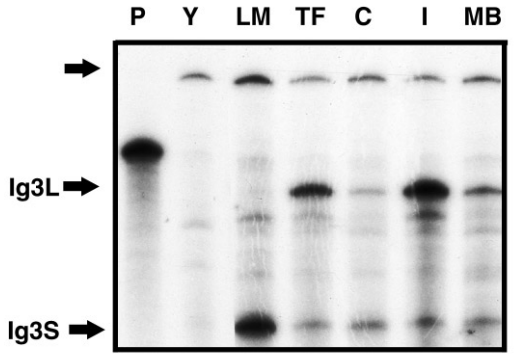

FIG. 5. *SpFGFR* is regulated posttranscriptionally in adult tissues. RNase protection assays were carried out with 10 μg total RNA isolated from lantern muscle (LM), tube feet (TF), coelomocytes (C), intestine (I), or embryos at the mesenchyme blastula stage (MB) and hybridized as described previously (McCoon *et al.*, 1996) with a probe that includes the Ig3L extra exon and flanking sequences present in both Ig3L and Ig3S transcripts. P indicates a lane containing unhybridized probe and Y indicates a negative control lane in which the probe was incubated with yeast tRNA. A radioactive DNA fragment was added after hybridization to assess recovery during processing steps and is indicated by an arrow. Compared to other tissues, the Ig3S/Ig3L transcript ratio is much higher in lantern muscle, the only tissue to accumulate detectable levels of SpFGF receptor.

tissues. Several tissues that do not produce detectable SpFGFR protein contain *SpFGFR* transcripts (Ig3L + Ig3S) at levels similar to those in lantern muscle (Ig3S).

Ig3S-containing transcripts are not restricted to lantern muscle, although their concentration in other tissues is relatively low. We believe it likely that these Ig3S transcript levels, albeit low, would be sufficient to provide detectable protein if translated at the same efficiency as in lantern muscle. The concentration of SpFGFR/total protein is at least two orders of magnitude greater in lantern muscle than in other tissues, a factor that significantly exceeds the Ig3S transcript concentration differences. These observations suggest that some Ig3S encoding transcripts also are not translated.

DISCUSSION

Posttranscriptional regulation of muscle-specific SpFGFR accumulation. The key findings presented in this work are that accumulation of a sea urchin fibroblast growth factor receptor is highly restricted to embryonic muscle cells and adult lantern muscle and that this specificity is achieved, at least in part, by a posttranscriptional regulatory mechanism. An antibody raised against an amino acid sequence from the C-terminal portion of SpFGFR that is not conserved among receptor tyrosine kinases or FGFRs and affinity purified against either of two different fusion proteins recognizes circumesophageal muscles and gut sphincters in embryos. The anti-SpFGFR antibody also recognizes a protein of ~125 kDa specifically in adult lantern muscle that is the same size as the immunoprecipitable protein synthesized in COS cells from transfected *SpFGFR* cDNA recombinant plasmids. Despite the fact that SpFGFR protein is detected only in muscle cells, *SpFGFR* transcripts are present in only a slightly lower concentration in nonmuscle tissues of the adult. Previous studies (McCoon *et al.*, 1996) showed that a similar phenomenon occurs in embryos: Transcripts are present long before detectable SpFGFR accumulates and in cells of ectoderm that never express detectable receptor protein. Furthermore, only Ig3S transcript variants are detectable in lantern muscle which expresses the receptor, providing strong evidence that the SpFGFR contains an Ig3S ligand binding domain, which much more closely resembles the sequence of known functional vertebrate receptors. However, it is unlikely that all Ig3S-containing transcripts are exported to the cytoplasm and translated since, for example, some adult tissues accumulate Ig3S-containing transcripts but lack detectable SpFGFR protein. Further work will be required to define precisely the sequence differences between muscle-specific SpFGFR and nontranslated transcripts.

The finding that *SpFGFR* transcripts are highly enriched in the nuclei of many cell types indicates that nuclear sequestration is a major mechanism underlying SpFGFR posttranscriptional regulation, at least in embryos. It is important to note that, although the level of *SpFGFR* transcripts is only slightly higher than that of many rare messenger RNAs in sea urchin embryos (~3 copies/cell; Galau *et al.*, 1974), they are 10- to 20-fold more abundant than typical nuclear RNA precursors (~0.5–1 copy/cell; Hough *et al.*, 1975). Relatively abundant nuclear transcripts have been reported for some functional cellular mRNAs (e.g., Condie *et al.*, 1990), although these cases are certainly very rare. The only other case of which we are aware in which the major mode of regulation of protein production is confinement of cellular (i.e., nonviral) transcripts to nuclei is the retention of most mature histone mRNAs in sea urchin egg pronuclei until the first cleavage division (Showman *et al.*, 1982; DeLeon *et al.*, 1983).

The vast majority of *SpFGFR* transcripts in both embryos and adults are long (8–8.5 kb), containing at least 5 kb of sequence in addition to the open reading frame. All the cDNA sequences obtained from libraries or by RT-PCR of embryo RNAs are devoid of introns interrupting the open reading frame, suggesting that many of the splicing steps have been completed. The 5′ untranslated sequence is unusually long, containing at least 450 nt that is represented on the predominant *SpFGFR* transcripts as shown by RNase protection and blot assays (McCoon *et al.*, 1996). No differences have been detected by 5′ RACE between 5′ UTR sequences in embryo and lantern muscle SpFGFR transcripts (our unpublished observations); whether processing

in 3′ UTR regions is different in muscle and nonmuscle tissues will require further analyses.

Possible SpFGFR functions. The finding that SpFGFR protein accumulates to detectable levels only in muscle suggests that signaling via this receptor is required during muscle differentiation in sea urchin embryos. Several different kinds of muscle cells differentiate in the sea urchin embryo and each expresses SpFGFR. In a related species, the sand dollar, *Dendraster excentricus* (Burke, 1981), the circumesophageal muscles are smooth muscle cells, the cardiac sphincter is composed of striated myoepithelial cells, while the pyloric and anal sphincters appear similar to surrounding stomach or intestinal cells, except that each contains a single band of thick and thin myofilaments at its basal end. All these muscles are mononucleate and clearly different from terminally differentiated vertebrate striated muscle. In adult sea urchins, the muscles in the lantern, tube feet, and intestine are all of the smooth muscle type (Cobb and Laverack, 1966; Florey and Cahill, 1977), but only lantern muscle expresses the receptor. These observations suggest that SpFGFR expression may be required for some aspect of muscle development other than specification of a smooth muscle cell type.

In embryos, it is unlikely that SpFGFR is required for initial myoblast commitment, which occurs between early and midgastrula stages (Venuti *et al.*, 1993). SpFGFR protein appears first in a few myoblasts (<5) just before these cells migrate from the archenteron and begin to accumulate muscle-specific proteins (SUM1, a MyoD-like muscle-specific transcription factor; Venuti *et al.*, 1991, 1993) and myosin (Wessel *et al.*, 1990). Because the relative sensitivity of detection of these proteins probably varies, we cannot exclude the possibility that a low level of SpFGFR accumulates in early gastrulae 10 h earlier than first detectable in these studies and before these muscle-specific mRNAs appear (Venuti *et al.*, 1993; Rose *et al.*, 1987). However, we favor the view that SpFGFR-mediated signaling functions after cells commit to muscle fate and during migration of the myoblasts and formation of the circumesophageal musculature (Cox *et al.*, 1986; Burke and Alvarez, 1988; Venuti *et al.*, 1993). The continued accumulation of SpFGFR also suggests that it functions after initial specification and through stages beyond myoblast migration.

The timing of SpFGFR appearance in sea urchin myoblasts suggests possible roles in stimulating myoblast migration or proliferation or extension of cytoplasmic processes. These features are strikingly similar to those of myoblasts as well as other migratory cells that respond to FGFR signaling pathways in many other embryos. For example, *FREK* expression in the chick embryo is upregulated in migrating, proliferating myoblasts (Marcelle *et al.*, 1995). Targeted expression of a dominant negative FGFR variant in the mouse lung prevents migration of smooth muscle myoblasts (Peters *et al.*, 1994). A *Drosophila* FGFR, breathless, regulates migration of follicle border cells during oogenesis (Murphy *et al.*, 1995), migration of glial cells during embryogenesis, and trachea formation (Klaembt *et al.*, 1992; Reichman-Fried *et al.*, 1994) as well as extension of long cytoplasmic processes during later stages of tracheole formation (Reichman-Fried and Shilo, 1995) that strongly resemble sea urchin myoblast processes. A second *Drosophila* FGFR, heartless, is required for dorsal mesoderm migration (Gisselbrecht *et al.*, 1996), and a *Caenorhabditis elegans* FGFR receptor regulates migration of sex myoblasts to the gonad (DeVore *et al.*, 1995). Even the requirement for FGF signaling in early *Xenopus* and mouse embryo mesoderm patterning (Slack *et al.*, 1987; Paterno *et al.*, 1989; Amaya *et al.*, 1991, 1993; Saint-Jeannet *et al.*, 1994; Yamaguchi *et al.*, 1994; Deng *et al.*, 1994) may primarily be the regulation of morphogenetic movements of gastrulation necessary to establish proper mesodermal positional information. This hypothesis is strongly supported by recent observations that cells mutant for FGFR fail to migrate through the primitive streak during gastrulation in chimeric mouse embryos (Ciruna *et al.*, 1997). Our observations on SpFGFR expression in myoblasts of sea urchin embryos are consistent with a role in migration of these cells from the archenteron to the coelomic pouches and esophagus. FGFR-mediated signaling probably persists at postmigratory stages of myogenesis, as suggested in mammalian embryos (Partenen *et al.*, 1991; Stark *et al.*, 1991; Wanaka *et al.*, 1991), since SpFGFR accumulates to high levels along cytoplasmic processes in the embryonic circumesophageal musculature, in the constrictions of the differentiating gut, and in well-differentiated adult lantern muscle tissue.

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REFERENCES

Amaya, E., Musci, T. J., and Kirschner, M. W. (1991). Expression of a dominant negative mutant of the FGF receptor disrupts mesoderm formation in *Xenopus* embryos. *Cell* **66,** 257–270.

Amaya, E., Stein, P. A., Musci, T. J., and Kirschner, M. W. (1993). FGF signaling in the early specification of mesoderm in *Xenopus*. *Development* **11,** 477–487.

Angerer, L. M., and Angerer, R. C. (1981). Detection of poly (A)+ RNA in sea urchin eggs and embryos by quantitative *in situ* hybridization. *Nucleic Acids Res.* **9,** 2819–2840.

Burke, R. D. (1981). Structure of the digestive tract of the pluteus larva of *Dendraster excentricus* (Echinodermata:Echinoida). *Zoomorphology* **98,** 209–225.

Burke, R. D., and Alvarez, C. M. (1988). Development of the esophageal muscles in embryos of the sea urchin *Strongylocentrotus purpuratus*. *Cell Tissue Res.* **252,** 411–417.

Ciruna, B. G., Schwartz, L., Harpal, K., Yamaguchi, T. P., and Rossant, J. (1997). Chimeric analysis of *fibroblast growth factor receptor-1 (FGFR1)* function: A role for FGFR1 in morphogenetic movement through the primitive streak. *Development* **124,** 2829–2841.

Cobb, J. L. S., and Laverack, M. S. (1966). The lantern of *Echinus esculentus* (L.). III. The fine structure of the lantern retractor muscle and its innervation. *Proc. R. Soc. London B* **164,** 651–658.

Condie, B. G., Hemmati-Brivanlou, A., and Harland, R. M. (1990). Most of the homeobox-containing Xhox 36 transcripts in early *Xenopus* embryos cannot encode a homeodomain protein. *Mol. Cell. Biol.* **10,** 3376–3385.

Cox, K. H., Angerer, L. M., Lee, J. J., Davidson, E. H., and Angerer, R. C. (1986). Cell lineage-specific programs of expression of multiple actin genes during sea urchin embryogenesis. *J. Mol. Biol.* **188,** 159–172.

DeLeon, D. V., Cox, K. H., Angerer, L. M., and Angerer, R. C. (1983). Most early variant histone mRNA is contained in the pronucleus of sea urchin eggs. *Dev. Biol.* **100,** 197–206.

Deng, C.-X., Wynshaw-Boris, A., Shen, M. M., Daugherty, C., Ornitz, D. M., and Leder, P. (1994). Murine FGFR-1 is required for early postimplantation growth and axial organization. *Genes Dev.* **8,** 3045–3057.

DeVore, D. L., Horvitz, H. R., and Stern, M. J. (1995). An FGF receptor signaling pathway is required for the normal cell migrations of the sex myoblasts in *C. elegans* hermaphrodites. *Cell* **83,** 611–620.

Florey, E., and Cahill, M. A. (1977). Ultrastructure of sea urchin tube feet. Evidence for connective tissue involvement in motor control. *Cell Tissue Res.* **177,** 195–214.

Gagnon, M. L., Angerer, L. M., and Angerer, R. C. (1992). Posttranscriptional regulation of ectoderm-specific gene expression in early sea urchin embryos. *Development* **114,** 457–467.

Galau, G. A., Britten, R. J., and Davidson, E. H. (1974). A measurement of sequence complexity of polysomal mRNA in sea urchin embryos. *Cell* **2,** 9–20.

Gisselbrecht, S., Skeath, J. B., Doe, C. Q., and Michelson, A. M. (1996). *heartless* encodes a fibroblast growth factor receptor (DFR1/DFGF-R2) involved in the directional migration of early mesodermal cells in the *Drosophila* embryo. *Genes Dev.* **10,** 3003–3017.

Glazer, L., and Shilo, B.-A. (1991). The *Drosophila* FGF-R homolog is expressed in the embryonic tracheal system and appears to be required for directed tracheal cell extension. *Genes Dev.* **5,** 697–705.

Griffin, K., Patiant, R., and Holder, N. (1995). Analysis of FGF function in normal and *no tail* zebrafish embryos reveals separate mechanisms for formation of the trunk and the tail. *Development* **121,** 2983–2994.

Guan, K. L., and Dixon, J. E. (1991). Eukaryotic proteins expressed in *Escherichia coli*—An improved thrombin cleavage and purification procedure of fusion proteins with glutathione *S*-transferase. *Anal. Biochem.* **192,** 262–267.

Harkey, M. A., Whiteley, H. R., and Whiteley, A. H. (1992). Differential expression of the msp130 gene among skeletal lineage cells in the sea urchin embryo: A three dimensional *in situ* hybridization analysis. *Mech. Dev.* **37,** 173–184.

Hough, B. R., Smith, M. J., Britten, R. J., and Davidson, E. H. (1975). Sequence complexity of heterogeneous nuclear RNA in sea urchin embryos. *Cell* **5,** 291–299.

Hurley, D., Angerer, L. M., and Angerer, R. C. (1989). Altered expression of spatially regulated embryonic genes in the progeny of separated sea urchin blastomeres. *Development* **106,** 567–579.

Kingsley, P. D., Angerer, L. M., and Angerer, R. C. (1993). Major temporal and spatial patterns of gene expression in the sea urchin embryo. *Dev. Biol.* **155,** 216–234.

Klaembt, C., Glazer, L., and Shilo, B.-Z. (1992). *breathless*, a *Drosophila* FGF receptor homolog, is essential for migration of tracheal and specific midline glial cells. *Genes Dev.* **6,** 1668–1678.

Marcelle, C., Wolf, J., and Bronner-Fraser, M. (1995). The *in vivo* expression of the FGF receptor FREK mRNA in avian myoblasts suggests a role in muscle growth and differentiation. *Dev. Biol.* **172,** 100–114.

McCoon, P. E., Angerer, L. M., and Angerer, R. C. (1996). SpFGFR, a new member of the fibroblast growth factor receptor family, is developmentally regulated during early sea urchin development. *J. Biol. Chem.* **271,** 20119–20125.

Murphy, A. M., Lee, T., Andrews, C. M., Shilo, B-Z., and Montell, D. J. (1995). The *breathless* FGF receptor homolog, a downstream target of *Drosophila* C/EBP in the developmental control of cell migration. *Development* **121,** 2255–2263.

Olmsted, J. B. (1987). Analysis of cytoskeletal structures using blot-purified monospecific antibodies. *Methods Enzymol.* **134,** 457–472.

Partenen, J., Makela, T. P., Eerla, E., Karkonen, J., Hirvonen, H., Claesson-Welse, L., and Alitalo, K. (1991). FGFR4, a novel acidic fibroblast growth factor receptor with a distinct expression pattern. *EMBO J.* **10,** 1347–1354.

Paterno, G. D., Gillespie, L. L., Dixon, M. S., Slack, J. M. W., and Heath, J. K. (1989). Mesoderm-inducing properties of INT-2 and kFGF: Two oncogene-encoded growth factors related to FGF. *Development* **106,** 79–83.

Pawson, T., and Bernstein, A. (1990). Receptor tyrosine kinases: Genetic evidence for their role in *Drosophila* and mouse development. *Trends Genet.* **6,** 350–356.

Peters, K., Werner, S., Liao, X., Wert, S., Whitsett, J., and Williams, L. (1994). Targeted expression of a dominant negative FGF receptor blocks branching morphogenesis and epithelial differentiation of the mouse lung. *EMBO J.* **13,** 3296–3301.

Ransick, A., and Davidson, E. H. (1995). Micromeres are required for normal vegetal plate specification in sea urchin embryos. *Development* **121,** 3215–3222.

Reichman-Fried, M., Dickson, B., Hafen, E., and Shilo, B.-Z. (1994). Elucidation of the role of *breathless*, a *Drosophila* FGF receptor homolog, in tracheal cell migration. *Genes Dev.* **8,** 428–439.

Reichman-Fried, M., and Shilo, B-Z. (1995). Breathless, a *Drosophila* FGF receptor homolog, is required for the onset of tracheal cell migration and tracheole formation. *Mech. Dev.* **52,** 265–273.

Rose, S. J., III, Rosenberg, J., Britten, R. J., and Davidson, E. H. (1987). Expression of myosin heavy chain gene in the sea urchin: Coregulation with muscle actin transcription in early development. *Dev. Biol.* **123,** 115–124.

Saint-Jeannet, J.-P., Thiery, J.-P., and Koteliansky, V. E. (1994). Effect of an inhibitory mutant of the FGF receptor on mesoderm-derived α-smooth muscle actin-expressing cells in *Xenopus* embryo. *Dev. Biol.* **164,** 374–382.

Showman, R. M., Wells, D. E., Anstrom, J., Hursh, D. A., and Raff, R. A. (1982). Message-specific sequestration of maternal histone mRNA in the sea urchin egg. *Proc. Natl. Acad. Sci. USA* **79,** 5944–5947.

Slack, J. M. W., Darlington, B. G., Heath, J. K., and Godsave, S. R. (1987). Mesoderm induction in early *Xenopus* embryos by heparin-binding growth factors. *Nature* **326,** 197–200.

Stark, K. L., McMahon, J. A., and McMahon, A. P. (1991). FGFR-4, a new member of the fibroblast growth factor receptor family, is expressed in the definitive endoderm and skeletal muscle lineages of the mouse. *Development* **113,** 641–651.

Venuti, J. M., Goldberg, L., Chakraborty, T., Olson, E. N., and Klein, W. H. (1991). A myogenic factor from sea urchin embryos capable of programming muscle differentiation in mammalian cells. *Proc. Natl. Acad. Sci. USA* **88,** 6219–6223.

Venuti, J. M., Gan, L., Kozlowski, M. T., and Klein, W. H. (1993). Developmental potential of muscle cell progenitors and the myogenic factor SUM-1 in the sea urchin embryo. *Mech. Dev.* **4,** 3–14.

Wanaka, A., Johnson, E. M., and Johnson, E. M., Jr. (1991). Expression of FGF receptor gene in rat development. *Development* **111,** 455–468.

Wessel, G. M., Zhang, W., and Klein, W. H. (1990). Myosin heavy chain accumulates in dissimilar cell types of the macromere lineage in the sea urchin embryo. *Dev. Biol.* **140,** 447–454.

Yamaguchi, T. P., Harpal, K., Henkemeyer, M., and Rossant, J. (1994). fgfr-1 is required for embryonic growth and mesodermal patterning during mouse gastrulation. *Genes Dev.* **8,** 3032–3044.

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