The mRNAs encoding acidic FGF, basic FGF and FGF receptor are coordinately downregulated during myogenic differentiation

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Summary

Acidic and basic fibroblast growth factors (FGFs) are members of a family of proteins that exert pleiotropic effects in a range of cell types including skeletal myocytes. Previous studies demonstrate that exogenously supplied FGFs stimulate proliferation of myoblasts and inhibit their differentiation in culture, but little information is available concerning endogenous expression of FGFs by skeletal myocytes. In this study acidic and basic FGF mRNAs were found to be expressed in murine and rat skeletal muscle, and expression was demonstrated to vary with the tissue and species examined. Myogenic cell lines were then analyzed to determine if FGFs are expressed in myoblasts, and if so, whether expression is regulated during myogenic differentiation. Murine Sol 8 and rat L6 myoblasts were found to express acidic and basic FGF mRNAs, and the expression of both growth factors was downregulated at the transcriptional level during myogenic differentiation. A decrease in expression of the mouse homologue of the human FGF receptor paralleled the decrease in acidic and basic FGF mRNAs in Sol 8 cells, indicating that the decrease in FGF receptor abundance previously observed during myogenic differentiation is regulated at the mRNA level. The results of this study suggest that a coordinate decrease in endogenously produced acidic and basic FGFs and their cognate receptor may participate in the regulation of myogenic differentiation. Furthermore, the observation that expression of a myogenic determination gene, myogenin, increases as FGF transcripts decline, together with previous data demonstrating suppression of myogenin expression by FGF, suggest a mechanism whereby endogenously produced FGFs may exert their effect on differentiation.

Key words: FGF, skeletal myocyte, FGF receptor, myogenesis.

Introduction

The fibroblast growth factor (FGF) family of heparin binding proteins mediates a wide variety of biological processes (Baird and Bohlen, 1990). Acidic and basic FGF are potent mitogens for skeletal myoblasts, and independent of their effects on cellular proliferation, FGFs are capable of blocking the differentiation of myoblasts displaying surface FGF receptors (Gospodarowicz et al. 1976; Linkhart et al. 1980; Lathrop et al. 1985; Spizz et al. 1986; Clegg et al. 1987). In addition, FGF receptors are permanently lost from myoblasts during terminal differentiation (Olwin and Haushka, 1988). Inhibition of differentiation by FGF, together with receptor loss during differentiation, suggest a potential role for FGF-mediated events in myocyte development. The participation of FGF in myogenesis is further supported by the recent finding that basic FGF suppresses transcription of the myogenic determination genes myogenin (Brunetti and Goldfine, 1990) and MyoD1 (Vaidya et al. 1989).

Although attention has focused on the effects of exogenously supplied FGF on myogenesis, heparin binding fibroblast growth factors are present in skeletal muscle (Kardami et al. 1985; DiMario et al. 1989; Vaca et al. 1989; Morrow et al. 1990), and may participate in the regulation of myogenesis in vivo. A recent study indicates that the abundance of transcripts encoding acidic and basic FGF decreases in muscle during fetal development, suggesting that FGF expression is developmentally regulated (Alterio et al. 1990). However, since endothelial (Schweigerer et al. 1987) and smooth muscle (Winkles et al. 1987) cells are known to synthesize FGFs, the contribution (if any) of myocytes to the synthesis of acidic and basic FGF in skeletal muscle is unknown. A decrease in FGF mRNA abundance during differentiation has been observed in primary cultures containing both myocytic and non-
myocytic cells, but FGF expression could not be confirmed in pure myocyte cultures (Alterio et al. 1990).

The purpose of this study was to determine if skeletal myocytes express acidic and/or basic FGF, and whether the expression of one or both of these genes is regulated during myogenic differentiation. We detected the mRNAs encoding both acidic and basic FGF in mouse and rat skeletal muscle, and demonstrated tissue- and species-specific variations in expression of these growth factors. Expression of FGFs by cultured myogenic cells was then examined. Transcripts encoding both acidic and basic FGF were present in mouse Sol 8 myoblasts, and the expression of these growth factors decreased coordinately with myogenic differentiation. Qualitatively similar results were obtained with rat L6 myocytes. Sol 8 (but not L6) myoblasts were demonstrated to express the mouse homologue of the human Flg FGF receptor, and the abundance of this mRNA decreased during differentiation in parallel with that of acidic and basic FGF. The observed decrease in FGF receptor mRNA in this study, together with the decrease in receptor number observed during myogenesis in mouse cell lines (Olwin and Haushka, 1988), suggests that FGF receptor expression is regulated at the transcriptional level during development. Finally, the decrease in abundance of acidic and basic FGF mRNAs was paralleled by an increase in myogenin expression in both Sol 8 and L6 myocytes, consistent with the observation that FGF inhibits myogenin expression (Brunetti and Goldfine, 1990). These data support the hypothesis that endogenously produced FGFs promote proliferation and suppress differentiation of myoblasts, and that a coordinate decrease in expression of these growth factors (and their cognate receptor) facilitates myogenic differentiation.

Materials and methods

Cell culture

Rat L6 (Yaffe, 1968), and murine Sol 8 (gift of Christian Pinset, Institute Pasteur) (Muelle et al. 1988) myoblasts were maintained in Dulbecco's modified Eagle's medium containing 4.5 g L⁻¹ of glucose (DMEM), 100 U ml⁻¹ penicillin, 100 μg ml⁻¹ streptomycin and 20% fetal calf serum (Sol 8) or 10% calf serum (L6) until confluent. Differentiation was induced by culturing in DMEM containing either 10% horse serum for 3 to 5 days (Sol 8) or 5% horse serum for 5 to 7 days (L6). Progression from myoblasts to multinucleate myotubes was confirmed by light microscopy.

RNA isolation

RNA was extracted from cells and tissues by a modification of the method of Chirgwin et al. (1979). RNA was isolated from Sol 8 and L6 cells at three stages of myogenic differentiation. Subconfluent myoblasts were harvested at 24 to 48 h after plating, confluent cells were harvested within 12 h of reaching confluence, and myotubes were harvested after culturing in differentiation media for the indicated time. RNA was extracted from tissues obtained from 6 to 8 week old mice and from 6 to 12 month old rats. RNA was quantified by spectrophotometry and concentrations were verified by fractionation on agarose-formaldehyde denaturing gels.

RNAase protection analysis

Murine and rat acidic and basic FGF, murine α skeletal actin, and rat myoadenylate deaminase (AMPD) mRNAs were assessed by RNAase protection analysis (Melton et al. 1984). The amount of RNA used in the RNAase protection experiments was quantified in three ways. First, RNA concentration was estimated spectrophotometrically. Second, using the estimated spectrophotometric concentration, equal amounts of the RNAs to be compared were electrophoresed on a denaturing gel and the RNA stained with ethidium bromide. RNA integrity and relative concentration were assessed by examining the ethidium-bromide-stained gel. These data were used to determine the amount of RNA to be hybridized in the RNAase protection analysis. Finally, for the experiments examining relative transcript abundance of acidic and basic FGF in myotubes and myoblasts, aliquots of the RNAs were run on denaturing gels, and northern analysis was performed for FGF receptor and/or myogenin expression. The same blots were then hybridized to tubulin and demonstrated similar RNA contents per lane.

RNAase protection probes were generated by digesting plasmids with appropriate enzymes and then performing in vitro transcription to generate [32P]UTP-labeled antisense transcripts. Murine acidic FGF was detected using a probe complementary to 240 bases of the third exon and portions of the second exon of acidic FGF and was generated from the T7 promoter of the NcoI-digested clone A1 plasmid (gift of Gail Martin) (Hebert et al. 1990). The mouse basic FGF probe was complementary to 313 bases of exon 3, exon 2 and portions of exon 1 of basic FGF and was transcribed from the T3 promoter of the FokI-digested clone L2b plasmid (gift of Gail Martin) (Hebert et al. 1990). The mouse α skeletal actin probe was complementary to 65 bases of the 5' untranslated region of a skeletal actin and was generated from the T3 promoter of the EcoRI-digested BSask plasmid (gift of Margaret Buckingham) (Sasoon et al. 1988).

A probe complementary to 165 bases of the 3' untranslated region of rat acidic FGF was transcribed from the T3 promoter of the FokI-digested plasmid clone 3 (a gift of Stephen Goodrich) (Goodrich et al. 1989). A probe complementary to 477 bases of the coding region of rat basic FGF was generated from the T7 promoter of the NcoI-digested plasmid ROBFGF-S03 (gift of Andrew Baird) (Shimasaki et al. 1989). Rat myoadenylate deaminase (AMPD) mRNA was detected using a probe complementary to 300 bases of the 3' untranslated and coding region of the skeletal muscle AMPD transcript. The probe was transcribed from the T3 promoter of the Syl-digested AMPD1 plasmid (gift of Margaret Buckingham) (Sasoon et al. 1988).

Northern analysis

Equal amounts of total RNA (as assessed first spectrophotometrically and then by ethidium bromide staining after gel electrophoresis) were fractionated on 1% agarose-formaldehyde denaturing gels and transferred to Nytran filters (Schleicher & Schuell Inc.). Probes for RNA hybridization were labeled with [32P]dCTP using a multiprime DNA-labeling system (Amersham). FGF receptor was detected using a 1129 base fragment of the cDNA clone complementary to 477 bases of the coding region of rat basic FGF. The probe was complementary to 65 bases of the 5' untranslated region of a skeletal actin and was generated from the T3 promoter of the EcoRI-digested BSask plasmid (gift of Margare
a 1.3 kb PstI fragment prepared from the chicken α tubulin cDNA (gift of Rick Woychik).

Results

Expression of acidic and basic FGF mRNAs in mouse and rat skeletal muscle

We first examined whether the mRNAs encoding acidic and basic FGF are detectable in skeletal muscle from adult mouse, and then compared their expression to that in heart and brain. RNAase protection analysis was used since FGF mRNA abundance is low in most tissues. The results are shown in Fig. 1. Mouse skeletal muscle expressed both acidic and basic FGF, but transcript abundance in skeletal muscle compared to that in brain and heart varied for the two growth factors. Furthermore, in skeletal muscle the abundance of acidic FGF mRNA was greater than that of basic FGF since the intensity of the protected fragment for acidic FGF exceeds that for basic FGF, although the uridine content of the [32P]UTP-labeled acidic FGF probe is less than that of the basic FGF probe (54 versus 73 uridine bases).

To determine whether the pattern of expression in tissues is conserved across species, rat tissues were examined using RNAase protection analysis (Fig. 2). Both acidic and basic FGF mRNAs were expressed in rat skeletal muscle, with the pattern of transcript abundance different for the two FGFs. Taking into account uridine content (47 for acidic, 95 for basic FGF), the abundance of acidic FGF mRNA was greater than basic FGF in skeletal muscle. Thus the mRNAs encoding both acidic and basic FGF are detectable in skeletal muscle, with the abundance of acidic FGF mRNA exceeding that for basic FGF. Furthermore, the relative distribution of acidic and basic FGF transcripts exhibit tissue- and species-specific variations.

Acidic and basic FGF mRNA abundance is regulated during myogenic differentiation in murine Sol 8 and rat L6 cells in vitro

We examined myogenic cells in vitro to determine if these cells express acidic and basic FGF mRNAs, and whether the expression of these growth factors is regulated during myogenesis. The model system used was Sol 8 cells, a myoblast cell line derived from mouse soleus skeletal muscle (Mulle et al. 1988) that recapitulates myogenic differentiation. Sol 8 myoblasts were cultured and RNA obtained either from subconfluent myoblasts, confluent myoblasts before the appearance of myotubes, or differentiated myotubes. The state of differentiation was assessed using a probe to mouse α skeletal actin. The mRNA encoding α skeletal actin was not detectable in myoblasts but was present in confluent myoblasts and myotubes.

The effect of myogenic differentiation on FGF mRNAs is illustrated in Fig. 3A and B. Acidic and basic FGF mRNAs were expressed in subconfluent Sol 8

![Fig. 1. RNAase protection analysis of FGF expression in mouse tissues. RNA (30 μg) from mouse brain, heart and skeletal muscle was analyzed by RNAase protection to assess acidic and basic FGF mRNA. The 315 base acidic FGF and 388 base basic FGF probes are shown on the left. The protected fragments for acidic FGF (240 bases) or basic FGF (313 bases) are present in tissue and cell RNA but absent in the tRNA controls. In the acidic FGF assay, partially undigested probe is present in the tRNA and sample lanes. Hybridizations were performed in a 50 % formamide hybridization solution at 45°C for >12 h, and samples were fractionated on a 6 % denaturing polyacrylamide gel. Autoradiograph was exposed for 16 h.](image-url)
myoblasts, but unlike mouse skeletal muscle (Fig. 1), the abundance of basic FGF was significantly greater than acidic FGF mRNA (length and uridine content of protected fragment taken into account). At confluence, when biochemical differentiation was apparent (α skeletal actin was expressed) but myotube formation had not yet commenced, acidic FGF mRNA was no longer detectable (Fig. 3A). In the experiment shown for basic FGF, the mRNA abundance was similar in subconfluent and confluent cultures (Fig. 3B). In other experiments, basic FGF mRNA was decreased in confluent compared to subconfluent cultures. The variation in basic FGF mRNA in cells harvested at confluence may be attributable to small differences in the length of time cells were confluent prior to sampling. Regardless of the variability in basic FGF expression, with the formation of myotubes there was a further decrease in acidic and basic FGF mRNA abundance. Thus in Sol 8 cells, both acidic and basic FGF mRNAs decrease in parallel with differentiation.

Since differences in the response to FGF, the expression of FGF receptors, and the expression of myogenic determination genes have been reported for mouse versus rat myogenic cells (Braun et al. 1989; Florini and Magri, 1989; Olwin and Haushka, 1989), we analyzed rat L6 myoblasts for FGF expression. Differentiation was assessed using a probe to rat myoadenylate deaminase (Sabina et al. 1989). Changes in acidic and basic mRNAs during differentiation were qualitatively similar to those for Sol 8 myocytes. Acidic FGF transcript abundance decreased as the myoblasts reached confluence, with a further decrease during myotube formation (Fig. 4A). The decrease in FGF mRNA abundance preceded differentiation as assessed by myoadenylate deaminase expression (AMPD) and the morphologic appearance of myotubes. Basic FGF mRNA (Fig. 4B) followed a pattern qualitatively similar to that observed for acidic FGF, with a decrease at confluence and a further decrease with differentiation. Thus in rat L6 myoblasts, decreases in both acidic and basic FGF mRNAs precede myotube formation and are thus potential candidates for participation in the regulation of myogenic differentiation in this cell line. In addition, similar to that observed in rat skeletal muscle in vivo, the abundance of acidic FGF mRNA in myoblasts is greater than basic FGF (taking into account the length and uridine content of the protected fragments). Although the relative abundance of acidic versus basic FGF mRNA is different in mouse Sol 8 and rat L6 myoblasts, these two transcripts are both downregulated during differentiation in these myogenic cells.
Fig. 3. RNAase protection analysis of FGF expression during the differentiation of murine Sol 8 cells. RNA (20 μg) from subconfluent and confluent Sol 8 myoblasts, and fused myotubes was analyzed for acidic (A) and basic (B) FGF mRNA expression. Murine acidic FGF yields a protected fragment of 240 bases, while basic FGF protects a 313 base fragment. Partially undigested probe is apparent at 315 bases in the acidic FGF lanes, and at 388 bases in the basic FGF lanes. Differentiation was assessed using a probe complementary to α skeletal actin, and is indicated by a 60 base protected fragment. RNAase protection was conducted as in Fig. 2, and autoradiographic exposure was for 16 h (α skeletal actin), 4 days (acidic FGF), or 5 days (basic FGF). Similar results were obtained for acidic FGF expression in two experiments, and for basic FGF expression in three experiments.

Fig. 4. RNAase protection analysis of FGF expression during differentiation of rat L6 cells. RNA (20 μg) from subconfluent and confluent L6 myoblasts, and fused myotubes, was analyzed for acidic (A) and basic (B) FGF mRNA expression. The 165 base protected fragment is specific for rat acidic FGF mRNA (A), and the 477 base fragment for basic FGF. Undigested probe (524 bases) is present in all lanes of the basic FGF RNAase protection analysis. As a marker of differentiation, a probe complementary to AMP deaminase (AMPD) mRNA was included in the hybridization reactions. AMPD mRNA is indicated by a protected fragment of 300 bases. RNAase protection was conducted as in Fig. 1, and autoradiographic exposure was for 16 h. Similar results were obtained for acidic FGF mRNA in five experiments and for basic FGF mRNA in three experiments.
FGF receptor expression during myogenic differentiation

Previous studies demonstrate that FGF receptors are present in mouse myoblasts, but are lost during differentiation of these cell lines (Olwin and Haushka, 1988). We examined whether the homologue of the human Flg FGF receptor (Ruta et al. 1989) is expressed in myoblasts, and whether expression is regulated at the level of transcript abundance during differentiation. FGF receptor mRNA was detectable in subconfluent Sol 8 myoblasts, decreased at confluence, and decreased further with myotube formation (Fig. 5). Thus in Sol 8 myocytes, a decrease in FGF receptor mRNA expression parallels the decrease observed in the expression of acidic and basic FGF mRNAs. Consistent with previous studies indicating the absence of FGF receptors in L6 myoblasts (Olwin and Haushka, 1989), FGF receptor mRNA was not detectable in subconfluent, confluent, or differentiated L6 cells (not shown).

Expression of myogenin during myogenesis in Sol 8 and L6 cells

Previous reports indicate that FGF suppresses the expression of myogenic determination genes such as myogenin (Brunetti and Goldfine, 1990). We examined whether myogenin was regulated in parallel with FGF expression during myogenesis in Sol 8 and L6 myocytes. Montarras et al. 1989 have shown that Sol 8 myoblasts do not express the myogenin gene prior to differentiation. Similarly, Fig. 6 demonstrates that in subconfluent L6 myoblasts, where acidic and basic FGF mRNAs are expressed, there was no detectable myogenin transcript. In confluent cultures (where FGF expression had decreased), myogenin mRNA was detectable in both Sol 8 and L6 cultures. Myogenin expression increased with myotube formation, while FGF expression decreased, thus providing correlative support for a possible role for endogenously produced FGF in the regulation of myogenic differentiation.

Discussion

The effects of exogenously supplied FGF on skeletal myogenesis has been extensively studied (reviewed in Florini and Magri, 1989). In myogenic cell lines capable

Fig. 5. Northern analysis of FGF receptor expression in Sol 8 myocytes. RNA (10 μg) was analyzed using as a probe the partial cDNA encoding Flg, a human FGF receptor. Blots were hybridized at 42°C in a solution containing 5×SSPE, 2×Denhardt's, 0.2% SDS, 0.1 mg/ml denatured salmon sperm DNA, 10% dextran sulfate, 50% denized formamide and 1.5×10⁸ cts min⁻¹ ml⁻¹ of [³²P]dCTP-labeled Flg cDNA and were washed at room temperature in 2×SSC and 0.1% SDS, followed by washes in 0.1×SSC, and 0.1% SDS at 60°C. A 4.2 kb transcript corresponding to the FGF receptor is present at all stages of Sol 8 differentiation. To control for amount of RNA per lane, the blot was hybridized to the chicken α-tubulin cDNA.

Fig. 6. Northern analysis of myogenin expression in Sol 8 and L6 myocytes. RNA (10 μg) was analyzed using a probe to the rat myogenin cDNA. Blots were hybridized at 42°C in a solution containing 50% denized formamide, 5×SSC, 2×Denhardt's, 0.2% SDS, 0.1 mg/ml denatured salmon sperm DNA and 1.0×10⁶ cts min⁻¹ of [³²P]dCTP-labeled myogenin cDNA probe and were washed at room temperature in 2×SSC, 0.1% SDS, and 0.1% NaHPO₄, followed by washes in 0.1×SSC, 0.1% SDS, and 0.1% NaHPO₄ at 50°C. A 1.9 kb transcript corresponding to myogenin was observed. To control for amount of RNA per lane, the blot was hybridized to the chicken α-tubulin cDNA. A repeat experiment yielded similar results.

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Discussion

The effects of exogenously supplied FGF on skeletal myogenesis has been extensively studied (reviewed in Florini and Magri, 1989). In myogenic cell lines capable
of expressing FGF receptors, basic FGF induces proliferation and inhibits differentiation of myoblasts. Since heparin binding fibroblast growth factors are found in skeletal muscle (Kardami et al. 1985; DiMario et al. 1989; Vaca et al. 1989; Morrow et al. 1990), it is possible that the endogenous production of FGF is regulated in such a manner as to influence myocyte differentiation through either autocrine or paracrine mechanisms.

We first examined the expression of acidic and basic FGF mRNAs in skeletal muscle and found acidic FGF mRNA more abundant than basic FGF mRNA in both mouse and rat muscle. In addition, the relative abundance of acidic and basic FGF mRNAs in skeletal muscle compared to other tissues demonstrates distinct tissue- and species-specific variations. Although these data do not identify the cellular source of FGF mRNA, they indicate that both acidic and basic FGF are expressed in skeletal muscle and suggest the participation of FGFs in myogenic differentiation. A recent study (Alterio et al. 1990) indicating that the expression of these growth factors is decreased in neonatal compared to fetal skeletal muscle also supports a role for FGF in skeletal muscle development.

We then addressed the question of whether myocytes express the genes encoding acidic and basic FGF, and whether the expression of one or both of these growth factors is regulated during myogenic differentiation. Our results, in contrast to those of others (Alterio et al. 1990), demonstrate that rat L6 and mouse Sol 8 myoblasts express acidic and basic FGF mRNAs, and that the abundance of both of these mRNAs decreases with myotube formation. In addition, in confluent L6 myoblasts, a decrease in the abundance of acidic and basic FGF mRNAs precedes the expression of myoadenylate deaminase (AMPD) and the formation of myotubes. In Sol 8 myoblasts, a decrease in acidic FGF mRNA was apparent in confluent myoblasts expressing an early marker of biochemical differentiation (a skeletal actin) but basic FGF mRNA abundance was variable at this stage of differentiation. Since differentiation is not synchronous in these cultures, and cells were harvested at a time in development when rapid changes in gene expression occur, the variability in basic FGF mRNA abundance may reflect heterogeneity in the state of differentiation of subpopulations of cells. However, for both cell types the expression of acidic and basic FGF mRNAs decreased as cells progressed from confluent myoblasts to fully differentiated myotubes.

Changes in the endogenous expression of acidic and basic FGF may influence myocyte differentiation through the regulation of expression of myogenic determination genes (Vaidya et al. 1989; Brunetti and Goldfine, 1990). Myogenin expression is decreased in Sol 8 myoblasts compared to myotubes (Montarras et al. 1989), and recent studies demonstrate that the transcription of myogenin is suppressed by exogenously supplied basic FGF (Brunetti and Goldfine, 1990). The potential effect of myogenin on FGF expression is unknown. Our results confirm that myogenin expression increases during differentiation of Sol 8 and L6 myocytes, and is paralleled by decreases in acidic and basic FGF mRNAs. These data provide correlative support for a potential link between the expression of FGFs and myogenin in the regulation of myocyte differentiation. In light of the reciprocal regulatory interactions of the myogenic determination genes, it is possible that myogenin (or MyoD1) in turn regulates FGF expression. Further studies will be needed to address these possibilities.

Previous studies (Olwin and Haushka, 1988) show a loss of fibroblast growth factor receptors during differentiation of mouse MM14 myoblasts and suggest that changes in receptor abundance participate in myogenic differentiation. The level at which receptor downregulation occurs has not been reported. FGF receptors have been purified from several species (Lee et al. 1989; Ruta et al. 1989; Mansukhani et al. 1990) and their genes cloned. We used a probe to the human Flg gene to determine if the mouse homologue of this FGF receptor is regulated at the mRNA level during myogenic differentiation. We found that Sol 8 myoblasts express the mRNA encoding this FGF receptor, and that its abundance decreases with myotube formation. This and previous results (Olwin and Haushka, 1988) suggest that the expression of this receptor may play a role in regulating myocyte development. However, a decrease in expression of the homologue of the Flg FGF receptor alone cannot be sufficient to trigger differentiation since L6 myoblasts express neither FGF receptor mRNA nor protein. It is possible that other FGF receptors (not detected with the Flg probe) exist intracellularly in L6 cells, and that these receptors participate in transduction of the FGF signal. Further information is needed concerning the characterization and transduction pathways of the family of FGF receptors thus far identified.

In summary, acidic and basic fibroblast growth factors are expressed by proliferating Sol 8 and L6 myoblasts in culture, and these growth factors are coordinately downregulated at the mRNA level during differentiation. In addition, in myogenic cells exhibiting FGF receptors (Sol 8), FGF receptor mRNA decreases in parallel with FGF mRNA abundance during differentiation. Although the data are correlative, the results suggest that a decrease in the endogenous production of FGF, together with loss of responsiveness to previously synthesized FGF, may participate in the process of myogenic differentiation. It is possible that the effect of FGF is mediated through the regulation of expression of myogenic determination genes. Future studies are designed to test this hypothesis directly by determining the influence of changes in endogenous FGF expression on myogenic differentiation.

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