Differential Regulation of Urokinase-type Plasminogen Activator Expression by Basic Fibroblast Growth Factor and Serum in Myogenesis

REQUIREMENT OF A COMMON MITOGEN-ACTIVATED PROTEIN KINASE PATHWAY*

(Received for publication, July 1, 1997, and in revised form, October 31, 1997)

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The broad spectrum protease urokinase-type plasminogen activator (uPA) has been implicated in muscle regeneration in vivo as well as in myogenic proliferation and differentiation in vitro. These processes are known to be modulated by basic fibroblast growth factor (FGF-2) and serum. We therefore investigated the mechanism(s) underlying the regulation of uPA expression by these two stimuli in proliferating and differentiating myoblasts. The expression of uPA mRNA and the activity of the uPA gene product were induced by FGF-2 and serum in proliferating myoblasts. uPA induction occurred at the level of transcription and required the uPA-PEA3/AP1 enhancer element, since deletion of this site in the full promoter abrogated induction by FGF-2 and serum. Using L6E9 skeletal myoblasts, devoid of endogenous FGF receptors, which have been engineered to express either FGF receptor-1 (FGFR1) or FGF receptor-4 (FGFR4), we have demonstrated that both receptors, known to be expressed in skeletal muscle cell precursors, were able to mediate uPA induction by FGF-2, whereas serum stimulation was FGFR receptor-independent. The induction of uPA by FGF-2 and serum in FGFR1- and in FGFR4-expressing myoblasts required the mitogen-activated protein kinase pathway, since treatment of cells with a specific inhibitor of the mitogen-activated protein kinase/extracellular signal-regulated kinase-2 kinase, PD98059, blocked uPA promoter induction. Although FGF-2 and serum induced uPA in proliferating myoblasts, their actions on cell-cell contact-induced differentiating myoblasts differed dramatically. FGF-2, but not serum, repressed uPA expression in differentiation-committed myoblasts, and these effects were also shown to occur at the level of uPA transcription. Altogether, these results indicate a dual regulation of the uPA gene by FGF-2 and serum, which ensures uPA expression throughout the whole myogenic process in different myoblastic lineages. The effects of FGF-2 and serum on uPA expression may contribute to the proteolytic activity required during myoblast migration and fusion, as well as in muscle regeneration.

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1 The abbreviations used are: uPA, urokinase-type plasminogen activator; GM, growth medium; ITS, insulin-transferrin-sodium selenite medium; PBS, fetal bovine serum; FGF-2, basic fibroblast growth factor; FGFR, fibroblast growth factor receptor; FGFR1, fibroblast growth factor receptor 1; FGFR4, fibroblast growth factor receptor 4; ERK-2, extracellular signal-regulated kinase-2; MAPK, mitogen-activated protein kinase; MKP-1, mitogen-activated protein kinase phosphatase-1; MCK, muscle creatine kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MAP, mitogen-activated protein; kb, kilobase pair(s); hp, base pair(s); PBS, phosphate-buffered saline; TPA, 12-O-tetradecanoylphorbol-13-acetate.

This paper is available online at http://www.jbc.org
cells, whereas FGFR4 is expressed in the myotomally derived skeletal muscle (17–19).

FGF-2 and serum can stimulate the growth and prevent the differentiation of several myoblast cell lines (20, 21). FGF-2 is also thought to play a role in embryonic myogenesis and muscle regeneration (2, 16, 22, 23). Reports from different laboratories indicate that FGF-2 and serum regulate myoblast growth and differentiation by independent mechanisms. Moreover, serum and FGF-2 provide myogenic cells with distinct differentiation potentials in response to cell-cell contact; while FGF-2 can abrogate cell-cell contact-induced differentiation, serum cannot override this process (24).

The regulation of uPA expression by growth factors in a number of cell types is well documented (reviewed in Ref. 25); however, little is known about its regulation in skeletal muscle cells. It has been recently reported that FGF-2 increases uPA proteolytic activity in L6 rat myoblast cells devoid of FGFRs, which were engineered to overexpress functional FGF receptor-1 (FGFR1) (26). It was shown that phospholipase-C-γ1 was not involved in the regulation of uPA. However, the involved modulatory molecule was not identified, nor the level at which uPA induction occurred. We therefore examined the mechanism(s) underlying FGFR- and serum-mediated uPA induction in myoblast cells and analyzed the differential regulation of uPA gene expression by FGF-2 and serum in the two independent myogenic stages: proliferation and differentiation.

Our results demonstrate that both FGF-2 and serum induce uPA transcriptional activity in proliferating myoblasts. This induction required a PEAA3/AP1 promoter sequence, located at −2.4 kb from the transcription start site, and was mediated by the Ras-MAP kinase intracellular pathway. The induction of uPA by FGF-2 could be mediated not only by FGFR1, but also by FGFR4. Finally, our results indicate that uPA expression during cell-cell contact-induced differentiation is negatively regulated by FGF-2, but in contrast, it is not affected by serum.

EXPERIMENTAL PROCEDURES

Cell Culture—The murine myoblast C2C12 cell line was obtained from the American Type Culture Collection. Rat myoblastic L6E9 cells (27) and derived cell lines expressing FGF receptors have been described (28). All myoblast cell lines were maintained in Dulbecco’s modified Eagle’s medium containing 15% FBS, designated as growth medium (GM). L6E9-R1, L6E9-R4, and L6E9 stably transfected with medium for 12 h and then stimulated with 30 ng/ml FGF-2 or 20% FBS (GM). L6E9-R1, L6E9-R4, and L6E9 stably transfected with modified Eagle’s medium containing 15% FBS, designated as growth serum albumin, 0.1% Tween 20, PBS for 1 h at room temperature. Anti-Erk-2 antibody (Transduction Laboratories) was added at 0.2 μg/ml in 1% bovine serum albumin, 0.1% Tween 20, PBS for 1 h at room temperature, and the membrane was washed twice for 15 min in 0.1% Tween 20, 0.05% Triton X-100, PBS before the addition of a peroxidase-labeled secondary antibody for 45 min. The membrane was washed twice in 0.1% Tween 20, 0.05% Triton X-100, PBS and once with PBS without detersents. The filter was developed using ECL reagents (Amersham).

Plasmids—p-8.2Luc, p-6.6Luc, and p-6.6PESV-AP1Luc murine uPA Promoter luciferase reporter plasmids have been described previously (14). p-35Luc was constructed by digestion of p-6.6Luc with Kpn1 and p-35 Ap1 promoter (30) with further ligation of a murine exonuclease blunt-ended backbone to generate a murine uPA minimal promoter construct. pMCK-Luc, pSG5-3CH134 (MKP-1), and pRhoRasN17 have been described elsewhere (32–35). p3xAP1Luc contains three copies of the collagenase AP1-binding site inserted upstream of the minimal promoter of the thymidine kinase gene.

Transfection Assays—Reporter plasmids were transfected using the liposome-mediated transfection reagent DOTAP (Boehringer Mannheim). 1.5 × 10⁵ cells were seeded overnight on 35-mm dishes. Next day, cells were cotransfected with 2 μg of uPA-luciferase plasmid and 0.5 μg of pRSV CAT or pRL-SV40, as internal controls. After transfection, cells were grown in 0.5% FBS for 12 h before the addition of 30 ng/ml FGF-2 and 20% serum for 8 h, and reporter activities were analyzed. When transfected, cells were cotransfected with 0.5 μg of uPA promoter plasmid and 1 μg of MKP-1 or Ras N17 expression plasmids or empty vector alone, together with 0.5 μg of internal control. Inhibition of MAP kinase/ERK kinase was performed by pretreating transfected cells with 100 μM PD98059 (Calbiochem) for 30 min prior to the addition of stimulator. To analyze the reporter activities in differentiation-induced myoblasts, cells were transfected with either 2 μg of p-6.6Luc or 2 μg of pMCKLuc, together with pRL-SV40 as internal control, and grown in growth medium until near confluence. Medium was then changed to ITS alone, or ITS supplemented with 30 ng/ml FGF-2 or 20% serum, and reporter activity was analyzed 48 h later. All transfections were performed at least three times.

RESULTS

FGF-2 and Serum Induce uPA mRNA and Proteolytic Activity in C2C12 Myoblast Cells—We have previously shown that uPA expression is modulated during myogenesis (8). Since serum and FGFs are known to regulate myogenesis in vitro (20) and FGFs induce mesoderm formation in vivo (15), we examined whether FGF-2 and serum might also be regulators of uPA expression in cultured muscle cells. As shown in Fig. 1A, C2C12 mouse myoblasts expressed a 2.7-kb transcript corresponding to murine uPA mRNA, which was clearly increased when C2C12 cells were cultured in the presence of either FGF-2 or serum, with respect to untreated cells. The UPA mRNA induction by both agents was not due to an unspecific up-regulation of RNA synthesis, since FGF-2 and serum did not significantly modify the levels of GAPDH mRNA. Accordingly, uPA proteolytic activity was also induced by FGF-2 and serum, as assessed by zymography (Fig. 1B). The increase in activity was time-dependent, as the casein degradation band detected at 45 kDa, corresponding to the murine uPA active enzyme, was more intense at 10 h than at 5 h. To gain an insight into the mechanisms leading to increased uPA mRNA expression in mitogen-activated C2C12 cells, we studied the effects of RNA and protein synthesis inhibitors on the UPA transcript level in cells that were stimulated with FGF-2 and serum. As shown in
Fig. 1. Influence of FGF-2 and serum on uPA expression in C2C12 myoblasts. A, analysis of uPA mRNA expression in mitogen-stimulated C2C12 myoblasts. C2C12 cells were grown in GM until approximately 60% density and then switched to 0.5% FBS for 12 h. Next day, cells were treated with 30 ng/ml FGF-2 or 20% serum for 4 h. RNA blot hybridizations were performed using the mouse uPA and GAPDH cDNA probes as indicated. B, zymographic analysis of uPA induction by FGF-2 and serum in C2C12 cells. C2C12 myoblasts were switched to 0.5% FBS for 12 h and subsequently grown in the presence of 30 ng/ml FGF-2 or 20% serum for 5 (lanes 2 and 4) or 10 h (lanes 1, 3, and 5). Cells were harvested at the indicated time points, and 40 μg of protein/lane were analyzed by SDS-PAGE followed by zymography. The photograph was taken after overnight incubation at 4 °C and 4 h at 37 °C. The 45-kDa molecular mass species corresponds to murine uPA, and was calculated according to standard molecular mass markers electrophoresed in an adjacent lane and stained with Coomassie Blue. C, effect of RNA and protein synthesis inhibitors on mitogen-stimulated uPA expression. Cells were treated as in A, except that myoblasts were grown in the presence (+) or absence (−) of actinomycin D (5 μg/ml) or cycloheximide (10 μg/ml), which were added 15 min prior to the addition of FGF-2 and serum.

FGF-2 and Serum Induce uPA Gene Promoter Activity in Myoblasts—We next examined the effect of FGF-2 and serum on the activity of the uPA promoter. To do this, a murine uPA genomic fragment (~6.6 kb to +398 bp), ligated upstream of the firefly luciferase reporter gene, p-6.6Luc (14), was assessed for luciferase activity in C2C12 cells. Comparison of luciferase activities generated by p-6.6Luc, between unstimulated and FGF-2- and serum-stimulated C2C12 cells, showed that the uPA promoter activity was augmented 10- and 14-fold following FGF-2 or serum treatment, respectively (Fig. 2). In contrast, a -35 bp uPA minimal promoter was unaffected by either agent. These results demonstrate that the uPA promoter contains serum- and FGF-2-responsive sequences that might account, at least in part, for the mitogen-mediated induction of uPA in proliferating myoblasts.

Induction of uPA Gene Expression by FGFR4; FGF-2 and Serum Use Different Cell-surface Signaling Molecules for uPA Induction—Because FGFR4 is expressed in vivo in skeletal muscle precursors, we examined whether, similar to FGFR1 (26), it is able to mediate FGF-dependent uPA induction in myoblasts. To this end, L6E9 rat myoblasts that are devoid of FGFRs and engineered to individually express functional FGFR1 or FGFR4 were utilized (28). Moreover, this experimental system could also be used to evaluate whether FGFRs are required for uPA induction by serum. As shown in Fig. 3A, parental L6E9 cells expressed similar levels of uPA mRNA in the presence or absence of FGF-2. By contrast, FGF-2 significantly induced uPA expression in L6E9 cells expressing FGFR1 (L6E9-R1 cells) and in FGFR4-expressing cells (L6E9-R4 cells). The level of induction correlated well with the reported levels of FGF-binding sites in each cell line (3-fold more FGFR1 than FGFR4 sites; Ref. 28). Thus, the induction of uPA by FGF-2 was dependent upon the presence of FGFRs. By contrast, serum could induce the expression of uPA mRNA in parental L6E9 cells, indicating that serum induction occurred independently of FGFRs.

To examine whether the induction of uPA mRNA by FGF-2
and serum was mediated via a transcriptional activation mechanism, we studied the ability of each of these stimuli to induce uPA-promoter activity. Parental L6E9 cells, as well as L6E9-R1 and L6E9-R4 cells, were transfected with the uPA promoter-luciferase plasmid (p-6.6Luc), and promoter activity was examined following stimulation with FGF-2 or serum. As shown in Fig. 3B, FGF-2 induced luciferase activity only in L6E9-R1 and L6E9-R4 cells (5- and 2-fold, respectively), but not in parental L6E9 cells. Again, the level of luciferase expression was in accordance with the number of FGF-binding sites for each receptor. Similar to FGF-2, serum induced uPA promoter activity, but in contrast to FGF-2, such induction was also observed in parental L6E9 cells. Taken together, these results suggest that the stimulation of uPA by serum can either bypass the FGFR requirement or may operate through different mechanisms.

**FGF-2- and Serum-induced Phosphorylation of ERK-2 Mediates uPA Gene Induction in C2C12 Cells—**Tyrosine phosphorylation of cellular substrates is a common mechanism involved in FGF-2- and serum-induced signal transduction. Activation of the MAP kinase cascade by FGF-2 has been suggested in fibroblastic and neuronal cell lines, as well as in the MM14 mouse myoblast cell line (14, 18). Since the signaling pathways responsible for the induction of uPA in muscle cells remain unknown, the ability of FGF-2 and serum to induce MAP kinase phosphorylation leading to uPA induction was evaluated in C2C12 cells. As shown in Fig. 4A, FGF-2 and serum induced a rapid phosphorylation of MAP kinase (ERK-2) in C2C12 myoblast cells as assessed by Western blotting and immunodetection analysis using an anti-ERK-2 antibody (Fig. 4A). In addition, MAPK phosphorylation is typically accompanied by a slight shift in its electrophoretic mobility, which was in fact observed upon FGF-2 and serum stimulation in C2C12 cells (28). To confirm that the ERK signaling pathway was directly involved in the induction of uPA by FGF-2 and serum, we examined the ability of inhibitors of this pathway to block the induction of uPA promoter activity. Two inhibitors were used: MKP-1, a protein phosphatase known to inactivate ERK as well as JNK and p38 pathways (33, 36, 37), and PD98059, a specific inhibitor of MAPK kinase/ERK kinase (38). As shown in Fig. 4B, both molecules abrogated FGF-2- and serum-induced uPA promoter activity. The phorbol ester TPA (100 ng/ml), which preferentially activates the ERK pathway (39, 40), also induced uPA promoter activity in C2C12 cells, and this induction could be blocked by both MKP-1 and PD98059 inhibitors (Fig. 4B). Moreover, overexpression of wild-type ERK-2 further increased uPA promoter induction by TPA, FGF-2 and
serum in muscle cells (data not shown). Taken together, these results confirmed that the ERK pathway was directly involved in the transcriptional activation of the uPA gene by serum and FGF-2 in myogenic cells.

One mechanism by which the FGF-2 and serum signals may be relayed to the MAPK cascade is via activation of p21 Ras. To analyze the putative involvement of Ras in mediating uPA induction by FGF-2, a dominant negative form of Ha-Ras, Ras N17 (34), was overexpressed in cotransfection experiments with p-6.6Luc in C2C12 cells, with or without FGF-2 treatment (Fig. 5). The uPA promoter activity induced by FGF-2 was strongly suppressed by the dominant negative form of Ras. Analogously, serum induction of uPA promoter activity was also down-regulated by Ras N17, although to a lesser extent. Altogether, these results suggested that FGF-2 and serum preferentially activate a pathway involving Ras and MAPK in myoblast cells leading to uPA induction.

Involvement of the PEA3/AP1 Enhancer in uPA Promoter Activation by FGF-2 and Serum—It is known that the PEA3/AP1 enhancer element, located at -2.4 kb from the mRNA start site of the mouse uPA gene, plays an important role in the induction of uPA by different extracellular stimuli in numerous cell types. However, the function of the uPA enhancer had not yet been investigated in myogenic cells. To evaluate whether the PEA3/AP1 site was involved in the FGF-2 and/or serum inducibility of the uPA promoter, C2C12 cells were transfected with a plasmid lacking the mentioned PEA3/AP1 element, p-6.6(ΔPEA3/AP1)Luc. Comparison of luciferase activities produced by wild type and mutated uPA promoter plasmids after FGF-2 stimulation showed that a deletion eliminating the PEA3/AP1 site almost abrogated induction by FGF-2 in C2C12 cells (Fig. 6). These results suggested that the PEA3/AP1 site was responsible for uPA induction by FGF-2 in C2C12 cells. Similarly, we investigated the involvement of the PEA3/AP1 enhancer element in the serum induction of the uPA gene. Deletion of the PEA3/AP1 site produced a strong decrease of uPA promoter activity in C2C12 cells. But in contrast to FGF-2 induction, p-6.6(ΔPEA3/AP1)Luc still retained a 40% inducibility by serum. This suggested that sites other than the PEA3/AP1 also play a role in the induction of the uPA gene by serum. In agreement with these results, a thymidine kinase promoter-driven luciferase gene containing three copies of the AP1 binding site of the collagenase gene, p3xAP1Luc, was strongly induced by FGF-2 and serum in C2C12 cells. No significant differences (p > 0.05) were observed between -6.6 and -8.2 kb uPA promoter constructs in mediating FGF-2 and serum induction.

FGF-2, but Not Serum, Inhibits uPA Expression in Differentiation-committed Myoblasts—We have reported elsewhere that uPA is expressed both in proliferating myoblasts and in differentiating myotubes, with an increase occurring prior to the onset of fusion and differentiation in C2C12 cells (8). Once we had demonstrated that FGF-2 and serum could induce uPA in proliferating myogenic cells, we analyzed the effects of these mitogens on uPA expression in post-mitotic differentiating myoblasts. Thus, we compared the ability of FGF-2 and serum to modulate uPA expression during myoblast fusion and differentiation. C2C12 cells were plated at high density in differentiating promoting conditions (ITS medium), in the presence or absence of FGF-2 and serum, and uPA induction was analyzed at the mRNA and promoter levels. As shown in Fig. 7A, under these conditions, uPA mRNA expression was down-regulated in the presence of FGF-2 with respect to cells grown in ITS alone. The uPA mRNA decrease occurred concomitantly with the down-regulation of muscle-specific gene expression and the inhibition of myoblast fusion, as demonstrated by the dramatic decrease of myogenin mRNA expression (Fig. 7A) and the absence of long plurinucleated myotubes (Fig. 7B) in the presence of FGF-2. In contrast, these inhibitory effects caused by FGF-2 were not observed when confluent C2C12 cells were grown in ITS supplemented with serum as shown in Fig. 7A. uPA mRNA levels remained similar when cells were grown in ITS alone, or ITS supplemented with serum. Moreover, myogenin mRNA was not down-regulated during differentiation, nor was myoblast fusion altered in the presence of serum. The divergent effects exerted by FGF-2 and serum on uPA mRNA expression during myogenic differentiation correlated with the effects caused by both agents on uPA promoter activities, as assessed by transient transfection assays of uPA-promoter luciferase plasmids in C2C12 cells, grown in differentiation-promoting medium, in the absence or presence of FGF-2 and serum (Fig. 7C). p-6.6Luc activity was 50% lower in differentiation-committed C2C12 cells grown for 48 h in the presence of FGF-2 than in control cells grown in differentiating medium alone. In contrast, luciferase activity of p-6.6Luc was similar in differentiating cells grown in the presence or absence of serum. These results were in agreement with the effects caused by FGF-2 and serum on the promoter activity of a muscle-specific gene promoter; while FGF-2 was able to abrogate the muscle creatine kinase (MCK) promoter activity, detected when myoblasts were grown in the presence of the growth factor, the effects on
Magnification factors, no additions, 30 ng/ml FGF-2, and serum treatments are indicated. A, Northern blot analysis of 10 μg of total RNA from C2C12 cells grown in ITS, in the absence or presence of 30 ng/ml FGF-2 and 20% serum. B, low power views of the representative fields show the effects of the two mitogens on myotube formation: C, transciptional activities of uPA and MCK promoters in differentiating myoblasts. p-6.6Luc and pMCKLuc plasmids were transiently transfected into C2C12 cells, and, after transfection, cells were grown in GM until near confluence. The medium was then switched to ITS in the absence or presence of 30 ng/ml FGF-2 and 20% serum. Luciferase activity was determined 48 h later. Results are expressed as percentage of luciferase activity obtained with each reporter construct in ITS medium alone and represent the mean of three independent experiments. D, transcriptional activities of uPA and MCK promoters in differentiating L6E9 and FGFR-L6E9-derived cell lines. Cell transfections with reporter plasmids and measurements of luciferase activities were performed as described in C.

**DISCUSSION**

In this study, the mechanism underlying uPA induction by FGF-2 and serum in skeletal myoblasts was dissected. We have shown that uPA can be induced in C2C12 myoblasts by both FGF-2 and serum at the level of gene transcription. In particular, we found that a cis-element composed of a PEA3/AP1 site, which is conserved in murine, porcine, and human uPA promoters, and known to mediate the response to numerous extracellular stimuli (25), is required for the induction by both FGF-2 and serum, since deletion of this site abrogated mitogen stimulation of uPA.

By using L6E9 myoblasts engineered to overexpress FGFR1 and FGFR4, we have observed that, in contrast to the parental cells whose uPA mRNA levels were unaffected by FGF-2, these FGFR-expressing cell lines have gained the ability to respond to FGF-2, as assessed by increased uPA mRNA synthesis. These results clearly showed that a specific lack of FGFR expression distinguished FGF-inducible from FGF-uninducible uPA expression. By contrast, the induction of uPA by serum was FGFR receptor-independent, as parental L6E9 cells, devoid of any FGFR, were as responsive to serum as were FGFR-expressing cells.

Despite the different requirement of cell surface receptors by these two mitogens, we have observed that both serum and FGF-2 lead to uPA gene induction via a common pathway involving MAP kinase. Accordingly, MKP-1, a MAPK phosphatase known to dephosphorylate and inactivate MAPK in vivo (33), as well as PD98059, a compound known to specifically inactivate MAPK/ERK kinase (38), were capable of abrogating the serum- and FGF-2-induced up-regulation of the uPA gene promoter in C2C12 and in FGFR1- and FGFR4-expressing L6E9 cells, respectively. FGF-2 activates the Grb-2/Sos, Ras, Raf-1, MAPK pathway in NIH3T3 fibroblasts, which predominantly express FGFR1 (14). In contrast, MAPK was not activated by FGF-2 in MM14 myoblast cells (42), which also express FGFR1 and require FGF-2 for growth. Differential activation of MAPK between MM14, C2C12, and FGFR-expressing L6E9 myoblasts might be due to either a different number of expressed FGFR receptors or to distinct responses elicited by FGF-2 in different myoblast cell lines. In summary, our results indicate that FGF-2 (via FGFR1 or FGFR4) and serum signaling convey at the mitogen-activated protein kinase pathway to induce uPA gene transcription in myoblasts.

The convergence of signals induced by two types of mitogens, such as FGF-2 and serum, on common intracellular molecules is not surprising. Jak-STAT and Ras signaling pathways meet at MAPK to stimulate c-Fos transcription (43). In addition, the MAP kinase cascade, used by different tyrosine kinase receptors, can be activated by G-protein-coupled receptors, which are thought to play a role in serum signaling (31, 44).

We have recently reported that uPA expression is required during C2C12 myoblast fusion and differentiation, since the inhibition of uPA activity was able to block both processes (8). Therefore, we examined whether FGF-2 and serum could also modulate uPA expression during cell-cell contact-induced dif-
fereferentiation. FGF-2 was able to down-regulate uPA promoter activity and mRNA levels in C2C12, L6E9-R1, and L6E9-R4 differentiating myoblasts, demonstrating the ability of each individual receptor in mediating the expression of uPA expression by FGF-2 during differentiation. By contrast, in the presence of serum, uPA mRNA level and promoter activity remained unchanged in differentiating C2C12 cells. Moreover, a direct correlation could be established between the levels of uPA and the ability of muscle cells to fuse and differentiate, since FGF-2, but not serum, was able to down-regulate uPA expression concomitantly with the inhibition of myogenic fusion and differentiation. Altogether, these results indicated that, although FGF-2 and serum play a similar role in the induction of uPA in proliferating myoblasts, their actions on uPA expression are quite distinct in cell-cell contact-induced differentiation-committed C2C12 cells. In agreement with this, lysophosphatidic acid, a bioactive phospholipid contained in serum, and FGF-2 were also shown to provide differentiation-committed myogenic cells with distinct differentiation potentials (24).

Since uPA is expressed in different myoblast cell types, endowed with different myogenic potentials, we hypothesized that uPA may play a distinct role in the different myogenic stages: proliferation, fusion, and differentiation. uPA regulation by FGFR1 or FGFR4 might respond to myoblast proliferation and migration requirements as well as to the maintenance of the nondifferentiating state. In contrast, regulation of uPA by serum seems to act in all myogenic stages, including fusion and differentiation. The regulation of uPA expression by either FGFR1 or FGFR4 might be of potential physiological relevance as these two receptors are expressed in muscle cell precursors in vivo. In situ hybridization studies have shown FGFR1 expression in the mesenchyme of developing mouse limb bud (9.5–10.5 days post coitum) and FGFR4 transcripts have been found in the myotome region of mouse 9.5-day embryonic somites and in 14.5-day myotomal derived skeletal muscle (17, 19). In addition, FGFR1 and FGFR4 expression has been found in isolated satellite cells from adult mouse muscle. These observations suggest a possible involvement of these two receptors in the repair and regeneration functions mediated by satellite cells in adult skeletal muscle, a process in which the involvement of uPA has been demonstrated (9). We hypothesize that the dual regulation of uPA by serum and FGF-2 in myoblasts shown in the present study may account, at least in part, for the expression of uPA proteolytic activity required throughout the whole myogenic process in vitro (8) and muscle regeneration in vivo (9).

Acknowledgments—We are grateful to Dr. Y. Nagamine for uPA-luciferase constructs, Drs. N. Tonks and A. Bennett for pSG5–3CH134, Dr. J. DiDonato for pSR-xras N17, Dr. H. Weintraub for myogenin cDNA, and Dr. K. Walsh for MCK-luciferase. We also thank Alberto Martin for help in the preparation of the manuscript.

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