Differentiation of $C_2C_{12}$ myoblasts is critically regulated by FAK signaling

Carolina F. M. Z. Clemente, Marcus A. F. Corat, Sara T. O. Saad, and Kleber G. Franchini

Department of Internal Medicine, State University of Campinas School of Medicine, Campinas, Sao Paulo, Brazil

Submitted 28 May 2004; accepted in final form 10 May 2005

FAK is critically regulated by FAK signaling. Am J Physiol Integr Comp Physiol 289: R862–R870, 2005. First published May 12, 2005; doi:10.1152/ajpregu.00348.2004.—This study examined whether focal adhesion kinase (FAK) plays a role in the differentiation of $C_2C_{12}$ myoblasts into myotubes. Differentiation of $C_2C_{12}$ myoblasts induced by switch to differentiation culture medium was accompanied by a transient reduction of FAK phosphorylation at Tyr-397 (to $\sim50\%$, at 1 and 2 h), followed by an increase thereafter (to 240% up to 5 days), although FAK protein expression remained unchanged. FAK and phosphorylated FAK were found at the edge of lamellipodia in proliferating cells, whereas the later increase in FAK phosphorylation in differentiating cells was accompanied by its preferential location at the tip of well-organized actin stress fibers. Hyperexpression of FAK autophosphorylation site (Tyr-397) mutant (MT-FAK) reduced FAK phosphorylation at Tyr-397 in proliferating cells and was accompanied by reduction of cyclin D1 and increase of myogenin expression. These cells failed to progress to myotubes in differentiation medium. In contrast, hyperexpression of a wild-type FAK construction (WT-FAK) increased baseline and abolished the transient reduction of FAK phosphorylation at Tyr-397 in serum-starved $C_2C_{12}$ cells. Cells transfected with WT-FAK failed to reduce cyclin D1 and to increase myogenin expression, as well as to progress to terminal differentiation in differentiation medium. These data indicate that FAK signaling plays a critical role in the control of cell cycle as well as in the progression of $C_2C_{12}$ cells to terminal differentiation. Transient inhibition of FAK phosphorylation at Tyr-397 contributes to trigger the myogenic genetic program, but its later activation is also central to terminal differentiation into myotubes.

DIFFERENTIATION OF SKELETAL MUSCLE occurs via a multistep process characterized by a cell cycle withdrawal, expression of muscle specific genes, fusion into multinucleated cells, and assembly of the contractile apparatus (14, 20, 26). This complex phenomenon is orchestrated and coordinately regulated by the activity of primary and secondary myogenic regulatory factors (MRFs) (14, 18, 27). The primary MRFs Myf5 and MyoD are required for the formation, propagation, and survival of myoblasts, whereas the secondary MRFs myogenin and MRF4 act later in the program, likely as differentiation factors.

It is yet unclear how the myogenic regulatory factors themselves are activated during myogenesis, but it is well established that a set of environmental signals, including soluble factors and cell-cell and cell-extracellular matrix interaction may influence myoblast decision to differentiate (3, 15, 16). The ability of myoblasts to differentiate in vitro is prevented by a number of specific mitogens such as basic fibroblast growth factor (FGF-2) and transforming growth factor (TGF)-β (1, 2, 6, 7), which, in contrast, stimulate myoblast proliferation. The critical role of signaling through adhesion sites on myoblast proliferation and differentiation is indicated by the influence of diverse isoforms of integrins in the initial signaling and in the later morphogenic processes such as cell fusion and maintenance of myotube integrity (15). However, the downstream mechanisms that mediate the effects of integrins complex remain unclear.

Integrins mediate signal transduction across the plasma membrane by activating several intracellular molecules, including focal adhesion kinase (FAK), which plays a prominent role in integrin signaling (2, 13). Available data support a requirement for FAK activation to baseline myoblast proliferation (22). Accordingly, integrin-dependent phosphorylation of FAK has been shown to induce cyclin D1, a crucial step in the decision of cells to proliferate, while disruption of FAK signaling induces cell cycle arrest and apoptosis (19, 24, 28). In addition, FAK is phosphorylated and, presumably, activated via an integrin-dependent mechanism in differentiating $C_2C_{12}$ cells (10, 16). However, a clear demonstration that FAK plays a role in terminal differentiation of myoblasts is still lacking.

In this study we investigated the role of FAK in myogenesis by evaluating the influence of changes in FAK signaling on the expression of cyclin D1, myogenin, and morphological changes in differentiating $C_2C_{12}$ murine myoblasts. By interfering in FAK signaling through overexpression of a FAK mutant at Tyr-397 or a wild-type FAK, we showed that FAK signaling is critical to maintain $C_2C_{12}$ cells in proliferation, but it is also central to terminal differentiation of these cells into myotubes.

MATERIAL AND METHODS

Reagents. Antibodies anti-FAK-A-17, anti-myogenin, and anti-c-Myc-9E10 were from Santa Cruz Biotechnology. Anti-phospho-FAK-Tyr-397 was from Biosource, anti-cyclinD1-CC11-100 and Protein G plus were from Oncogene, and anti-rabbit biotined secondary antibody was from Amersham, Trizol reagent, SuperScript II enzyme, and Lipofectamine were from Invitrogen, and the creatine phosphokinase assay kit was from Roche Diagnostics. Dulbecco’s modified Eagle’s minimal medium (DMEM) and penicillin-streptomycin were from Nutricell, fetal bovine and horse sera were from GIBCO, and 35-mm plastic culture dishes were from Corning.

Cell culture. The skeletal muscle cell line $C_2C_{12}$ from adult mouse leg (ATCC) was grown in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin under a humidified atmosphere of 5% CO2 in air and maintained at low confluence. To induce differentiation, we changed culture medium to 2% horse serum instead of 10% fetal bovine serum when cells reached 70% confluence. Cell differentiation was assessed through creatine kinase activity and by analysis of cell fusion index calculated as the ratio of the
number of nuclei in differentiated cells and the total number of nuclei in culture dishes, according to a method previously described (22).

Expression plasmids and transient transfection experiments. Constructions of murine FAK pRc/CMV-FAK wt [wild-type (WT)-FAK] and pRc/CMV-FAK397 [mutant (MT)-FAK] were obtained from Dr. Steven K. Hanks (Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN) and were described previously (4). WT-FAK and MT-FAK constructs were subcloned into pRc/CMV cytomegalovirus promoter-driven eukaryotic expression vector containing c-Myc epitope tag (Invitrogen). WT-FAK or MT-FAK was transfected into C2C12 cells with the use of Lipofectamine reagent. Initial transfection was performed 24 h after plating of proliferating C2C12 cells. Transfection was repeated 48 h of Lipofectamine reagent. Initial transfection was performed 24 h after the initial transfection. Briefly, 35-mm cell dishes were incubated with 2 μg of plasmids, 2 μl of Lipofectamine, and 0.85 ml of DMEM (without antibiotics) for 2 h. After this period, 2 ml of medium were added to a final concentration of 10% fetal bovine serum after the first transfection or 2% horse serum after the second and third transfections.

Western blotting analysis. Equal amounts (30 μg/lane) of whole cell protein extracts were resolved in SDS-PAGE. Resolved proteins were electrophoretically transferred to nitrocellulose membranes. Membranes were blocked with 5% milk solution and incubated overnight at 4°C with primary polyclonal antibodies against FAK, FAK-pY397, myogenin, or cyclin D1. Subsequently, the membranes were incubated with 2 μCi [125I]-labeled protein A (30 μCi/μg) in 1% milk solution for 6 h at room temperature and were detected by autoradiography using preflashed Kodak XAR film (Eastman Kodak, Rochester, NY) with Cronex Lightning Plus intensifying screens (Du Pont, Wilmington, DE) at ~80°C for 24 h. Band intensities were quantified using optical densitometry.

Immunoprecipitation. Equal amounts (2.5 mg) of whole cell protein extracts were incubated overnight at 4°C with 15 μl of anti-c-Myc monoclonal antibody. Protein G plus (Oncogene) was added and incubated for 2 h at 4°C. Subsequently, the samples were centrifuged (20 min, 11,000 rpm, 4°C), washed and boiled with Laemmli buffer, and resolved using SDS-PAGE.

RT-PCR analysis. Whole cell RNA was extracted using Trizol reagent, and 5-μg RNA aliquots were converted to cDNA with the use of SuperScript II enzyme, according to the manufacturer’s instructions. Amplifications for myogenin and β-actin were performed using the primers 5′-GGAATTCCGAAATATGATGA-3′ and 5′-CTTACAGAAGAGG-3′, and 5′-TCTTCAATGACTGATGTTG-3′ and 5′-CTTTCCCTAATGCTGACGACGAA-3′, respectively.

Analysis of creatine kinase activity. Cells were washed with PBS, lysed by incubation with PBS containing 0.1% Triton-X 100 plus protease inhibitors (10 mM sodium orthovanadate, 2 mM PMSF, 0.2 mg/ml aprotinin) for 15 min at 4°C and passaged through an ultrafine needle. The samples were centrifuged (15 min, 14,000 rpm, 4°C), and the supernatant was collected. Creatine kinase activity was determined using the UV kinetic method, with a creatine phosphokinase assay kit. The creatine kinase activity was expressed as units divided by protein concentration (U/mg).

Confocal microscopy. Cells were washed with PBS and fixed with 4% paraformaldehyde plus 4% sucrose for 15 min at room temperature. Subsequently, the cells were washed with PBS and blocked with 3% milk plus 0.6% Triton-X 100 for 1 h at room temperature. Cells were then washed with PBS and incubated with 1% milk solution containing the primary antibody anti-FAK or anti-FAK-pY397 overnight at 4°C. After this, the cells were washed and incubated with 1% milk added anti-rabbit biotined secondary antibody by 2 h at room temperature. Subsequently, the cells were washed and incubated with streptavidin-Cy2 and rhodamine-conjugated phallolidin for 45 min at room temperature and were thereafter mounted with Vectashield onto the slides. The slides were analyzed using confocal microscope Zeiss LSM 510.

Hematoxylin and eosin staining. Cells were fixed with methanol at −20°C. Subsequently, the cells were incubated with Harris hematox-
liferating C2C12 cells with Myc-tagged WT or MT-FAK constructs driven by cytomegalovirus promoter. Previous study (17) has shown that overexpression of MT-FAK construct not only reduces FAK activity but also reduces tyrosine phosphorylation of focal-adhesion-associated proteins, indicating the critical role of this site for FAK signaling. Preliminary experiments indicated that maximum expression of WT-FAK or MT-FAK was reached after 48 h of transfection. Thus we retransfected cells 48 h after the initial transfection to induce sustained interference on FAK signal along the experimental

Fig. 1. Focal adhesion kinase (FAK) phosphorylation at Tyr-397 during myogenic differentiation. A: representative blots and densitometric readings showing the average values (5 experiments) of percent changes in the amount of FAK, detected with anti-pFAK antibody, in C2C12 cells in serum-starved medium (up to 5 days) compared with proliferating cells. B: control experiments performed with withdrawal and replacement of proliferating medium. Immunoblots (IB) are representative of 3 different experiments. C, extracts from cells cultured in proliferating medium; W, extracts from washed cells obtained 2 h after the washing procedure. *P < 0.05 compared with proliferating cells (C).

Fig. 2. Distribution of FAK in C2C12 cells. C2C12 cells were fixed, double-labeled with Cy2-conjugated anti-FAK antibody and rhodamine-conjugated phalloidin, and viewed under a laser confocal microscope. A and B: proliferating cells. FAK was concentrated at the perinuclear region and cellular periphery (B). C and D: cells serum-starved for 24 h. FAK was located at the tip of actin stress fibers (D). Areas of FAK/phalloidin colocalization appear as yellow.
The ability of cells to express the exogenous WT- or MT-FAK was tested by immunoprecipitation with anti-c-Myc monoclonal antibody. As shown in Fig. 4, in cells transfected with WT-FAK or MT-FAK, FAK was detected in the immunoprecipitates of anti-c-Myc antibody, but, as expected, no FAK was recovered from the immunoprecipitates of cells transfected with empty plasmid DNA. To demonstrate the influence of WT- or MT-FAK transfection in FAK phosphorylation at Tyr-397, we blotted cell homogenates with anti-pFAK antibody. As shown in Fig. 5A, WT-FAK transfection increased FAK protein expression and phosphorylation at Tyr-397, whereas MT-FAK transfection increased the amount of FAK protein expression but reduced the amount of FAK detected with antibody against phosphorylated FAK in proliferating cells. Transfection with WT-FAK suppressed the transient reduction of FAK phosphorylation after cells were switched to differentiation medium (Fig. 5B). Similar to what was found in nontransfected cells, FAK phosphorylation at Tyr-397 was increased later in differentiating cells. In contrast, transfection with MT-FAK markedly attenuated the rise of FAK phosphorylation at Tyr-397 in C2C12 cells cultured in differentiation medium (Fig. 5B). The influence of transfection with WT-FAK and MT-FAK on FAK immunostaining in C2C12 cells was assessed in cells cultured in differentiation medium. As shown in Fig. 5, C and D, no major change could be detected in FAK immunostaining in cells transfected with WT-FAK.
Fig. 5. Effects of WT-FAK and MT-FAK overexpression on FAK phosphorylation at Tyr-397. To assess the effect of WT-FAK or MT-FAK transfection on FAK phosphorylation at Tyr-397, we probed cellular extracts from proliferating and differentiating cells with the phosphospecific anti-pFAK antibody. A: immunoblots of antibodies against FAK (anti-FAK) and phosphorylated FAK (anti-pFAK) of extracts obtained from proliferating cells transfected with EP, WT-FAK, or MT-FAK. B: representative examples and average values of immunoblots of anti-FAK and anti-pFAK antibodies (4 experiments) obtained from extracts of proliferating (C) and differentiating (harvested at 2 h and 1, 3, and 5 days after culture medium was switched to differentiation medium). C: cells transfected with WT-FAK cultured in differentiation medium for 4 days, double-labeled with Cy2-conjugated anti-FAK antibody and rhodamine-conjugated phalloidin. D: same cells as in C showing anti-FAK immunostaining. E: cells transfected with MT-FAK cultured in differentiation medium for 4 days, double-labeled with Cy2-conjugated anti-FAK antibody and rhodamine-conjugated phalloidin. F: same cells as in E showing anti-FAK immunostaining. *P < 0.05 compared with proliferating cells (C).
WT-FAK. In contrast, in cells transfected with MT-FAK anti-FAK immunostaining could no longer be detected at the edge of cell filaments (Fig. 5, E and F).

Effects of WT-FAK and MT-FAK overexpression in myogenic differentiation. We next investigated whether changes in FAK Tyr-397 phosphorylation with WT-FAK or MT-FAK might influence the expression of the cell cycle regulator cyclin D1 and the secondary myogenic regulatory factor and marker myogenin. As shown in Fig. 6, A and B, cyclin D1 is highly expressed in proliferating C2C12 cells transfected with empty plasmid. However, after 1 day on differentiation medium, cyclin D1 was markedly reduced and remained low throughout the rest of the experimental period. Transfection with WT-FAK did not change the baseline expression of cyclin D1 in proliferating cells compared with that in cells transfected with empty plasmid (Fig. 6B) but inhibited the reduction in cyclin D1 induced by differentiation medium (Fig. 6A). In contrast, transfection with MT-FAK markedly reduced the baseline expression of cyclin D1 in proliferating C2C12 cells (Fig. 6B) as well as in cells growing in differentiation medium (Fig. 6A).

On the other hand, myogenin could not be detected by anti-myogenin antibody in proliferating C2C12 myoblasts transfected with empty plasmid (Fig. 6, C and D). After 1 day on differentiation medium, myogenin was already detectable and reached the maximal expression levels by the fifth day. Transfection with WT-FAK did not change the baseline expression of myogenin and suppressed the increase of myogenin in cells maintained in differentiation medium (Fig. 6, C and D). In contrast, transfection with MT-FAK induced C2C12 cells to express myogenin even in proliferation medium (Fig. 6, C and D). Switching the culture medium to differentiation medium did not affect the high levels of myogenin protein expression of MT-FAK-transfected C2C12 cells (Fig. 6C).

Results of RT-PCR analysis with specific primers directed to myogenin in samples of total RNA from proliferating and serum-starved cells confirmed the protein data. As shown in Fig. 7, myogenin mRNA was not detected in proliferating cells transfected with empty plasmid, but the switch to differentiation medium induced its expression, with the higher levels observed at the third day and thereafter. In cells transfected with WT-FAK, myogenin was not detected in proliferating cells and remained remarkably low after culture medium was switched to differentiation medium. In contrast, in cells transfected with MT-FAK, myogenin expression was detected at high levels in proliferating cells and remained elevated after cells were changed to differentiation medium.

To assess the differentiation status of C2C12 cells, we first examined the activity of creatine kinase in cells transfected with empty plasmid, WT-FAK, or MT-FAK. As indicated in Fig. 8A, cells transfected with empty plasmid showed a progressive increase in creatine kinase activity after the switch to differentiation medium. In cells transfected with WT-FAK, the change in culture medium to horse serum was not accompanied by increases in creatine kinase activity. On the other hand, transfection with MT-FAK attenuated the increase in creatine kinase activity in C2C12 cells in differentiation medium. Next, we analyzed the phenotypic changes of WT-FAK- or MT-FAK-transfected C2C12 cells cultured in differentiation medium. Figure 8B shows a representative example of proliferating cells transfected with empty plasmid. As shown in Fig. 8C,
cells transfected with empty plasmid were able to form myotubes by the fifth day after culture medium was changed to differentiation medium. However, cells transfected with WT-FAK or MT-FAK failed to differentiate into myotubes after they were placed in differentiation medium (Fig. 8, D and E). Cell fusion was severely inhibited in cells transfected with WT-FAK or MT-FAK. The fusion index of C2C12 cells transfected with empty plasmid was 67 ± 3.2%, whereas the fusion indexes of cells transfected with MT-FAK or WT-FAK were 4 ± 1.2 and 6 ± 1.2%, respectively.

DISCUSSION

The cellular lineage of murine myoblast C2C12 is a well-established model of myogenic differentiation in vitro (14, 18, 27). Upon mitogen withdrawal, specific transcription factors
are activated, leading to the induction of myogenin and other myogenic factors. Myogenin expression marks the commitment of myoblasts to the differentiation pathway. Irreversible cell cycle arrest is triggered by an increase in p21 and expression of terminal differentiation markers that include sarcomeric proteins and muscle creatine kinase. Finally, fusion of the differentiated myocytes leads to the formation of multinucleated myotubes and functional muscle. Although many soluble factors (1, 2, 6, 7) have been implicated in myogenesis, available data point to a critical role of cell-substrate interaction, via integrin signaling, to myoblasts differentiation. Previous observations have shown that FAK, a major mediator of integrin signaling, is required to baseline proliferation (22) and that terminal differentiation of myoblasts into myotubes are accompanied by FAK activation (10, 16). Our present data extended these previous observations to show that differentiation of C2C12 myoblasts into myotubes is critically dependent on both a transient reduction and a latter sustained increase of FAK phosphorylation at Tyr-397. These assumptions are supported by our demonstration in this study that 1) myoblast differentiation induced by the switch from culture medium containing high levels of serum growth factors to low-serum culture conditions leads to a transient reduction, followed by a later increase of FAK phosphorylation at Tyr-397, in parallel with terminal differentiation and myoblast fusion into myotubes; 2) disruption of FAK signaling in C2C12 cells by transfection with a FAK mutant reduced cyclin D1 expression and induced the expression of myogenin but failed to differentiate into myotubes; and 3) overexpression of the wild-type FAK, which suppressed the transient reduction of FAK phosphorylation at Tyr-397, impaired the reduction of cyclin D1 expression and the induction of myogenin expression as well as the terminal differentiation triggered by the cell culture switch to differentiation medium. Thus our present data are compatible with the notion that the transient reduction of FAK phosphorylation at Tyr-397 has a permissive role in the expression of myogenic genetic program, but myoblast fusion into myotubes is critically dependent on the later phosphorylation of FAK.

The major findings of this study are the demonstrations that the suppression of cyclin D1, induction of myogenin, and, presumably, induction of the myogenic genetic program, require a transient diminution of FAK signaling. Because FAK signaling is required for baseline myoblast proliferation (22) as well as the activity of cyclin-dependent kinases and cyclins in proliferating cells (19, 24, 28), one may argue that the transient reduction of FAK signaling also triggers cell cycle exit after C2C12 cells are switched to differentiation medium. In support of this suggestion, disruption of FAK signaling by diverse experimental approaches has been shown to cause cell cycle arrest, possibly by reducing the activity of ERK1/2, cyclin D1, and/or p21 (9, 12, 28, 29). Our present data confirm this assumption by showing that transfection with MT-FAK markedly reduced the cyclin D1 expression in proliferating myoblasts, whereas transfection with WT-FAK impaired the suppression of cyclin D1 induced by the switch to differentiation medium. These data indicate that increases in FAK signaling inhibit the withdraw from the cell cycle, allowing the cells to remain proliferating. Accordingly, a previous study (22) has shown that myoblasts transfected with wild-type CD2-FAK fusion construction remain proliferative with an increased ratio of G2 to G1 cells compared with untransfected cells and do not initiate fusion and terminal differentiation. Whether cell cycle exit and the expression of myogenic factors are mediated by common downstream signaling mechanisms was not explored in the present study. However, data from a previous study (5) indicate that cell cycle exit and the expression of specific myogenic factors induced by cell-cell contact via N-cadherin activation are regulated by divergent downstream signaling pathways. Thus this might also be the case for stimuli triggered by cell-substrate contact and FAK signaling.

The transient reduction of FAK phosphorylation at Tyr-397 after the culture medium was switched to differentiation medium might be related to the abrupt reduction of mitogens and growth factors caused by the replacement of growth by differentiation culture medium. Accordingly, previous studies demonstrated that activation of growth factor receptors can trigger autophosphorylation of FAK at Tyr-397 (8, 13). Thus the level of FAK phosphorylation at Tyr-397 in proliferating C2C12 cells might be dependent on a complex cooperation of signaling mechanisms mediated by growth factor receptors and adhesion, via integrins.

Our present results also extended previous observations indicating that FAK is activated in differentiating C2C12 cells to show that FAK phosphorylation at Tyr-397 is required for fusion of myoblasts into myotubes. This is supported by our demonstration that disruption of FAK signaling caused by overexpression of MT-FAK attenuated the rise of creatine kinase activity and myoblast fusion into myotubes in C2C12 cells cultured in differentiation medium despite the fact that such cells expressed considerable amount of myogenin and therefore were presumably committed to terminal differentiation. Muscle regulatory transcription factors have been shown to be necessary to commit and successfully differentiate myoblasts, but they are not sufficient to induced terminal differentiation (27). Accordingly, we showed an impairment of myotube formation despite the high expression of myogenin in cells transfected with MT-FAK, indicating that terminal differentiation might be dissociated from myogenin expression. Interestingly, although creatine kinase activity of MT-FAK transfected cells was reduced compared with cells transfected with empty plasmid, it still increased significantly by the fourth and fifth days in differentiation medium, indicating that FAK phosphorylation, and presumably activation, is important to later events of differentiation that lead to cell fusion. Thus it is apparent from these data that signals conveyed by adhesion via FAK signaling mechanisms are essential for the completion of normal myogenesis. It is plausible to assume a model in which transient FAK suppression induces cell cycle arrest and triggers the activation of myogenin expression, but the proper localization and later phosphorylation of FAK at focal adhesion contacts would coordinate signals involved in cell fusion and terminal differentiation of C2C12 cells into myotubes.

The mechanisms involved in FAK phosphorylation at Tyr-397 in differentiating cells are not clear. Our data indicate that most FAK detected with anti-phosphospecific FAK antibody is localized in large cell adhesion spots, suggesting that FAK phosphorylation at Tyr-397 in differentiating C2C12 cells may be related to adhesion and integrin-mediated mechanism. This idea is strengthened by our finding in this study indicating that FAK influence on cell differentiation is dependent on phosphorylation of Tyr-397, which has been shown to be dependent
on signaling mechanisms mediated by the engagement of integrins (17). Moreover, we have shown that overexpression of MT-FAK reduced considerably the location of FAK at the filaments of cells cultured in differentiation medium. Accordingly, a critical role of Tyr-397 autophosphorylation to FAK activation and clustering has been shown at focal adhesion sites (11, 21) and in cardiac myocytes in response to cyclic stretch (25). At least one previous study (16) has shown that disruption of myotube contact with extracellular matrix reduces FAK phosphorylation and, in contrast, that replacement of extracellular matrix is associated with FAK phosphorylation in C2C12 cells.

In summary, in the present study we have shown a complex and crucial role of FAK in myogenesis. Our data are compatible with a model in which the commitment of C2C12 cells to differentiation is dependent on a transient reduction of FAK signaling but, in contrast, terminal differentiation, with formation of myotubes, is critically dependent on FAK phosphorylation at Tyr-397 and presumably its activation. Further studies are necessary to dissect out the signaling mechanisms involved in FAK-mediated activation of myogenic factors and the regulation of phenotypic changes represented by cell fusion and terminal myotube formation.

GRANTS

This study was sponsored by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (Proc. 01/11698-1) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (Proc. 521098/97-1).

REFERENCES


