Control of Myoblast Proliferation with a Synthetic Ligand*

Marsha L. Whitney‡§§, Kevin G. Otto¶, C. Anthony Blauǁ, Hans Reinecke§, and Charles E. Murry‡§§*

From the Departments of Bioengineering and Pathology and the Division of Hematology, University of Washington, Seattle, Washington 98195-7335

Skeletal myoblast grafts can form contractile tissue to replace scar and repair injured myocardium. Although potentially therapeutic, generating reproducible and sufficiently large grafts remains a challenge. To control myoblast proliferation in situ, we created a chimeric receptor composed of a modified FK506-binding protein (F36V) fused with the fibroblast growth factor receptor-1 cytoplasmic domain. Mouse MM14 myoblasts were transfected with this construct and treated with AP20187, a dimeric F36V ligand, to induce receptor dimerization. Transfected myoblasts proliferated in response to dimerizer (comparable with basic fibroblast growth factor (bFGF) treatment), whereas the dimerizer had no effect on non-transfected cells. Similar to bFGF treatment, dimerizer treatment blocked myotube formation and myosin heavy chain expression and stimulated mitogen-activated protein (MAP) kinase phosphorylation in transfected cells. Non-transfected cells differentiated normally and showed no MAP kinase phosphorylation with dimerizer treatment. Furthermore, myoblasts treated with dimerizer for 30 days in culture reduced MAP kinase phosphorylation, withdrew from the cell cycle, and differentiated normally upon drug withdrawal, demonstrating reversibility of the effect. Thus, forced dimerization of the fibroblast growth factor receptor-1 cytoplasmic domain reproduces critical aspects of bFGF signaling in myoblasts. We hypothesize that in vivo administration of AP20187 following myoblast grafting may allow control over graft size and ultimately improve cardiac function.

Basic fibroblast growth factor (bFGF)1 induces proliferation of skeletal myoblasts and prevents differentiation and fusion into multinucleated myotubes (8). Thus its administration may be envisioned as a method for inducing grafted myoblast proliferation. However, bFGF can also stimulate proliferation of nongraft cells such as tissue fibroblasts, which may lead to fibrosis. A method for inducing proliferation selectively in the grafted cells would facilitate myoblast expansion without these undesired effects.

The myoblast proliferative response to bFGF is mediated through dimerization of fibroblast growth factor receptor-1 (FGFR-1, Refs. 9 and 10) and subsequent activation of intracellular signaling molecules. Although the exact mechanism by which this signal is transduced is not completely understood, it has been demonstrated that activation of the MAP kinase pathway is necessary for cell cycle progression (11) in MM14 cells. However, FGFR-1 dimerization leads to the activation of a number of other signaling molecules including phospholipase Cγ (PLCγ) and Src, which may interact with proteins of the MAPK pathway to affect mitogenesis as well as chemotaxis and cell survival (12–14).

Recently, an alternative approach to activating receptor signaling has been developed in which the intracellular domain of a receptor is fused with a drug binding domain, followed by expression of the chimeric receptor in the target cell type (15). Treatment of the transfected cells with a bivalent drug results in forced dimerization of the receptor signaling domains in the absence of growth factor. This technique has been applied successfully to induce expansion of genetically modified hematopoietic stem cells expressing chimeric cytokine receptors in vitro (16–18) and in vivo (19) following bone marrow transplantation.

To generate a cell line in which FGFR-1 signaling occurs independently of bFGF binding, we transfected mouse MM14 skeletal myoblasts with a retroviral vector encoding a chimeric receptor consisting of a modified FK506-binding protein (FKBP) domain, F36V (20), fused with the cytoplasmic domain of FGFR-1. The F36V domain contains a binding site for a bivalent synthetic ligand, AP20187 (ARIAD Pharmaceuticals, Cambridge, MA). In the present study, we demonstrate that addition of AP20187 to MM14 cells stably expressing the fusion receptor stimulates myoblast proliferation, blocks differentiation into myotubes, and mimics bFGF signaling by activating the MAP kinase pathway.

* These studies were supported by National Institutes of Health Grants K08HL03094, P01HL03174, R01HL61553, and R24HL64387-01. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ Supported by a Cardiovascular Pathology Training Grant (National Institutes of Health, HL07312).

‡ To whom correspondence should be addressed: Dept. of Pathology, University of Washington, Seattle, WA 98195-7335. Tel.: 206-616-8685; Fax: 206-543-3644; E-mail: murray@u.washington.edu.

1 The abbreviations used are: bFGF, basic fibroblast growth factor; FGFR-1, fibroblast growth factor receptor-1; MAP, mitogen-activated protein; FKBP, FK506-binding protein; GFP, green fluorescent protein; FACS, fluorescence-activated cell sorting; BrdUrd, bromodeoxyuridine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate-buffered saline; ERK, extracellular signal-regulated kinase; PLCγ, phospholipase Cγ; PDGF, platelet-derived growth factor; MAPK, MAP kinase.
Molecular Control of Myoblast Proliferation

**EXPERIMENTAL PROCEDURES**

**Materials—**bFGF was kindly donated by Scios Inc. (Sunnyvale, CA). AP20187 was provided by ARIAD Pharmaceuticals Inc.

**F36Vfgfr-1 Plasmid Construction—**The FGR-1 cytoplasmic domain was amplified by polymerase chain reaction from a rat FGR-1 cDNA template (B. J. Ballermann and co-workers, Ref. 21; S540048) using Taq polymerase (Promega) and primers flanking the cytoplasmic domain (sense: 5’-GGGGGCGGCGACAGGAGAAAACCCAGAG-3’ and antisense: 5’-GGGGGTTGTACGGCGCCCTTTGTGAAGA-3’) to which SalI restriction sites were added (underlined). Following amplification, the fragment was digested with SalI and ligated in-frame into the pBjF36V cloning vector (22) at the C-terminal end of the F36V domain. This created a fusion gene containing the C-terminal myristylation domain, the modified FGFR-1 domain (F36V), the FGR-1 domain, and a hemagglutinin epitope tag at the C-terminus. The resulting F36Vfgfr-1 region was excised by a SalI/ BamHI double digest and subsequently ligated into a bicistronic expression vector (23) containing the murine stem cell virus long terminal repeat with an enhanced green fluorescent protein (GFP) expression unit upstream of an internal ribosomal entry sequence (IRES) and the F36Vfgfr-1 receptor. Expression is driven by the murine stem cell virus long terminal repeat (LTR), M, myristylation sequence; F36V, modified FG-506-binding protein; fgfr-1, fibroblast growth factor receptor-1 cytoplasmic domain; HA, hemagglutinin epitope tag.

**RESULTS**

**Chimeric Receptor Construction and Expression—**Mouse MM14 myoblasts were stably transfected with a retrovirus encoding a chimeric receptor composed of a modified FK506-binding protein domain (F36V) fused with the cytoplasmic domain of FGFR-1 (Fig. 1). The receptor construct also includes a hemagglutinin epitope tag as well as an N-terminal myristylation site to target the protein to the plasma membrane. Cells expressing this construct were identified by FACS based on the reporter protein eGFP. Subsequent studies were performed using a polyclonal population of GFP-positive myoblasts with non-transfected wild type MM14 cells used as controls.

**Dimerizer Treatment induces Proliferation in Transfected Myoblasts in the Absence of bFGF—**In the absence of bFGF, wild type MM14 cells cease proliferating and differentiate into...
multinucleated myotubes expressing structural proteins such as myosin heavy chain. Wild type and F36Vfgfr-1-transfected MM14 cells were cultured in 24-well plates for 48 h in the presence of 6 ng/ml bFGF or a bivalent F36V ligand, AP20187. Following the treatment period, the relative number of cells/well was assessed by MTT assay (Fig. 2). Cell number was normalized to the bFGF-treated control wells. In the absence of bFGF, wild type cells ceased proliferating, resulting in a reduced cell number in comparison to bFGF-treated cells (24.2 ± 1.6 versus 100 ± 8.6%). Transfected MM14 samples also had significantly lower cell numbers in the absence of bFGF (44.8 ± 5.1 versus 120.3 ± 13.8%). Treatment of transfected cells with 10 or 100 nM AP20187, however, resulted in myoblast proliferation rates comparable with bFGF-treated controls (108.6 ± 7.5% and 122.0 ± 11.6%, respectively). The mean number of cells/well in AP20187-treated transfected cells was not significantly different from bFGF-treated transfected cells (p > 0.05) at either concentration. Treatment with 10 or 100 nM AP20187 did not stimulate proliferation in non-transfected control cells (26.5 ± 0.9% and 31.9 ± 1.6%, respectively). Thus, dimerization of the FGFR-1 cytoplasmic domain by AP20187 stimulated myoblast proliferation to the same extent as direct addition of bFGF. Interestingly, transfected cells appeared to proliferate at somewhat higher levels than non-transfected cells regardless of treatment (see “Discussion”). This difference was not significant between wild type and transfected cells treated with bFGF (p > 0.05); however, the number of cells in untreated transfected wells was significantly higher than in untreated wild type cells (p < 0.005).

**Fig. 2. Stimulation of cell proliferation by AP20187.** MM14 myoblasts were treated with bFGF (6 ng/ml) or AP20187 for 48 h prior to incubation with MTT. MTT conversion was determined by spectrophotometry (A₅₇₀ nm) as a measure of cell number and normalized to bFGF treatment. Myoblasts expressing the FGFR-1 chimeric receptor proliferated in response to dimerizer treatment, similar to the bFGF response. Wild type myoblasts did not proliferate in response to dimerizer. Data are the mean ± S.D. from three replicate wells and are representative of four independent experiments.

**Dimerizer Treatment Inhibits Differentiation of Transfected Myoblasts in the Absence of bFGF—**Upon withdrawal or depletion of bFGF from the culture medium, wild type MM14 cells exit the cell cycle and begin to differentiate. Differentiation is characterized by expression of muscle-specific proteins (such as sarcomeric myosin heavy chains) followed by myoblast fusion to form multinucleated myotubes. To determine whether AP20187 treatment can prevent myoblast differentiation in the absence of bFGF, wild type and F36Vfgfr-1-transfected MM14 cells were cultured for 48 h in differentiation medium (low serum, high insulin, and high calcium compared with growth medium) containing either 6 ng/ml bFGF or 100 nM AP20187. Untreated samples were included as controls. Cells were sub-

**Fig. 3. Inhibition of myoblast differentiation by AP20187.** Cells were treated for 48 h as indicated and fixed, and immunofluorescence was performed to evaluate expression of sarcomeric myosin heavy chain. In the presence of bFGF, both wild type (A) and transfected (D) myoblasts were proliferating mononuclear cells that did not express myosin heavy chain. When untreated, both wild type (B) and transfected (E) cells fused to form multinucleated myotubes that expressed myosin heavy chain. Treatment with the synthetic dimerizer AP20187 blocked formation of the myotubes and expression of myosin heavy chain in transfected cells (F), similar to treatment with bFGF. Dimerizer treatment had no effect on wild type cells (C). Red (rhodamine), myosin heavy chain immunofluorescence; blue, Hoechst 33342 nuclear staining.
sequently fixed, and immunocytochemical staining was performed to detect sarcomeric myosin heavy chain expression (Fig. 3). As expected, wild type cells treated with bFGF remained mononucleated and did not express myosin heavy chain (Fig. 3A), whereas bFGF withdrawal resulted in formation of myosin-positive multinucleated myotubes (Fig. 3B). Similarly, cells expressing the FGFR fusion protein remained mononucleated and myosin-negative after bFGF treatment (Fig. 3D) and differentiated normally after growth factor withdrawal (Fig. 3E). In contrast, treatment of the transfected cells with AP20187 completely prevented formation of myotubes and also blocked myosin heavy chain expression (Fig. 3F). AP20187 had no effect on control non-transfected cells (Fig. 3C). Thus, AP20187 appeared to block differentiation by inducing dimerization of the FGFR signaling domains in a manner similar to bFGF treatment.

**Activation of the MAP Kinase Pathway in Transfected Myoblasts Treated with AP20187**—Signaling studies were performed with whole cell lysates from MM14 cells to determine whether the observed bFGF-like effects of dimerizer treatment were caused by the activation of similar pathways. Wild type and F36Vfgfr-1-transfected cells were cultured in the presence of 6 ng/ml bFGF, 100 nM AP20187, or were left untreated. Western blotting of cell lysates was performed with an antibody against the phosphorylated forms of ERK 1/2. ERK1 and ERK2 were phosphorylated in both wild type and transfected cells treated with bFGF. Note the enhanced phosphorylation in transfected cells treated with AP20187. AP20187 treatment did not induce ERK phosphorylation in wild type MM14 cells, whereas transfected cells treated with AP20187 showed intense phosphorylation of ERK 1 and 2.

Dimerizer-induced proliferation is reversible. Transfected MM14 cells were cultured in growth medium supplemented with 100 nM AP20187 (without bFGF) for 30 days and then switched to differentiation medium for 48 h in the presence (B) or absence (A) of AP20187. Following withdrawal of the dimerizer, myoblasts differentiated normally as evidenced by myotube formation and sarcomeric myosin heavy chain expression. Red (rhodamine), myosin heavy chain immunofluorescence; blue, Hoechst 33342 nuclear staining. To determine whether ERK activity is down-regulated in 30-day AP20187-treated cells, cell lysates were prepared and analyzed by Western blotting. In the absence of AP20187, ERK phosphorylation was reduced in comparison with cells that received continued dimerizer treatment (C).

---

**Fig. 4.** Dimerizer-induced activation of the MAP kinase cascade. Wild type (WT) and F36Vfgfr-1-transfected (TF) cells were cultured in the presence of 6 ng/ml bFGF or 100 nM AP20187 or were left untreated. Western blotting of cell lysates was performed with an antibody against the phosphorylated form of MAP kinases ERK 1/2. ERK1 and ERK2 were phosphorylated in both wild type and transfected cells treated with bFGF. Note the enhanced phosphorylation in both wild type and transfected cells treated with bFGF. Western blots probed with an antibody against the phosphorylated forms of ERK 1/2 demonstrated that ERK phosphorylation was reduced following dimerizer withdrawal in comparison with cells that received continued dimerizer treatment (C).

**Fig. 5.** Dimerizer-induced proliferation is reversible. Transfected MM14 cells were cultured in growth medium supplemented with 100 nM AP20187 (without bFGF) for 30 days and then switched to differentiation medium for 48 h in the presence (B) or absence (A) of AP20187. Following withdrawal of the dimerizer, myoblasts differentiated normally as evidenced by myotube formation and sarcomeric myosin heavy chain expression. Red (rhodamine), myosin heavy chain immunofluorescence; blue, Hoechst 33342 nuclear staining. To determine whether ERK activity is down-regulated in 30-day AP20187-treated cells, cell lysates were prepared and analyzed by Western blotting. In the absence of AP20187, ERK phosphorylation was reduced in comparison with cells that received continued dimerizer treatment (C).
DISCUSSION

Recently, several studies have demonstrated that skeletal muscle stem cells, or satellite cells, can be isolated from adult tissue, expanded in culture, and reimplanted into the hearts of the donors to generate contractile muscle to replace scarring following myocardial infarction (2–5, 25). These successes have led to initiation of the first clinical trials of this technique (26). Despite its promise, myoblast grafting requires the harvesting and expansion of large numbers of cells, and graft size can vary enormously even when the injection procedure and cell number are kept consistent (27). These technical limitations may be overcome by developing a means of specifically and reversibly stimulating grafted skeletal muscle cell proliferation in vivo.

In the present study, we demonstrate that dimerization of the FGFR-1 cytoplasmic domain with a bivalent synthetic ligand, AP20187, can mimic critical aspects of bFGF-induced FGFR-1 signaling in myoblasts. Previous studies have demonstrated that bFGF is required for cell cycle progression and repression of differentiation in MM14 myoblasts (8). In the absence of bFGF, AP20187 stimulated proliferation and prevented differentiation in MM14 cells expressing the F36/Vgfr-1 chimera in a manner that was indistinguishable from bFGF treatment. Administration of AP20187 also activated the MAPK pathway in MM14 cells expressing the chimeric receptor, thereby reproducing key events involved in bFGF-mediated signal transduction. Importantly, cells treated with dimerizer for 30 days withdrew from the cell cycle and differentiated normally upon withdrawal of the dimerizer, demonstrating that the effect was reversible. Thus, the data presented offer proof of the concept that a genetically modified population of myoblasts can be expanded specifically and reversibly in response to a small synthetic molecule.

It is interesting to compare our results with those recently published by Kudla et al. (28). These investigators studied MM14 cells expressing a chimeric receptor composed of the PDGF-βR extracellular domain and the FGFR-1 intracellular domain. In response to PDGF-BB treatment, the MM14 cells (which express no intrinsic PDGF receptors) phosphorylated the chimeric cytoplasmic domains and activated the MAPK pathway. Similar to our results, activation of the chimeric receptor blocked myoblast differentiation. In contrast to our findings, however, activation of this chimeric receptor was insufficient to induce cell proliferation. It is not clear why we observed cell proliferation when they did not. Both systems employed forced dimerization of the FGFR-1 cytoplasmic domain in MM14 cells, and both resulted in MAPK activation, although our system used a small molecule to dimerize the receptors whereas the other system employed a relatively large dimeric protein ligand and included the extracellular domain of the PDGF receptor. It is possible that the extracellular domain of the PDGF receptor induced unfavorable allosteric changes in the FGFR-1 cytoplasmic domain. Alternatively, the spacing of the receptor dimers induced by PDGF binding may be different from that induced by AP20187 binding. Such differences may influence coupling of the receptor to additional downstream signaling pathways, e.g. PLCγ.

We observed that treating transfected cells with dimerizer resulted in greater levels of MAPK phosphorylation than was seen when wild type cells were bFGF-treated. This result may be explained by differences in the level of endogenous FGFR-1 expression, as only ~700 molecules are expressed on MM14 cells (29), compared with the chimeric receptor in which expression is driven by a retroviral promoter. We were surprised to note, however, that MM14 cells expressing the chimeric receptor demonstrated more robust MAPK activation than did wild type cells when both were treated with bFGF. Similarly, we observed that transfected myoblasts proliferated at a higher rate than non-transfected cells either basally or after bFGF treatment. Because the chimeric receptor cannot respond to bFGF, these data suggest that the modified receptor may have some constitutive activity. This hypothesis is in accordance with the work of Kudla et al. (30), who showed that overexpressing FGFR-1 in the absence of ligand could activate MAPK in MM14 cells. The level of activity in our cells was not sufficient, however, to interfere with myoblast differentiation, as the transfected cells differentiated normally upon withdrawal of AP20187 or bFGF, and MAPK phosphorylation was downregulated in the absence of bFGF or AP20187 in both cell types. Another important distinction between bFGF and dimerizer treatment is that the dimerizer can only activate FGFR-1-mediated signal transduction, whereas bFGF can also bind to and activate isofoms of FGFR-2, -3, and -4 (31), thereby stimulating multiple receptor-induced signaling pathways within the same cell. Although FGFR-1 is the predominant FGFR subtype expressed in MM14 cells, this is an important consideration in comparing the effects of bFGF with dimerizer treatment (9, 10) in cells that express multiple FGFR isoforms.

Long term (30 days) treatment with dimerizer did not interfere with the ability of myoblasts to withdraw from the cell cycle or differentiate into myotubes. There were a few notable differences, however, between naive transfected cells and the long term-treated cells. As shown in Fig. 6, comparable fractions of naive and long term-treated cells were in S phase after bFGF or dimerizer treatment. On the other hand, when cell number was used to measure proliferation, the long term-treated cells showed increased cell accumulation compared with naive cells (data not shown). This suggests that the long term dimerizer treatment may select for a population of cells with shorter cell cycle times or greater survival rates. Additionally, we were surprised to note that bFGF treatment of the long term-treated cells actually resulted in reduced phosphorylation of ERK (n = 3 experiments; data not shown), despite the fact that the cells proliferated well in response to bFGF. Incubation with the MAPK/ERK kinase (MEK) inhibitor U0126 significantly reduced proliferation of these cells when either bFGF or dimerizer was used to drive proliferation. Thus, it appears that the long term-treated cells require the MAP
kinase pathway for proliferation, but steady-state levels of ERK phosphorylation do not reflect the pathway’s activity following bFGF treatment. Taken together, the results presented support the hypothesis that dimerizer-mediated proliferation acts through induction of MAPK signaling, similar to bFGF treatment. The results also strongly suggest, however, that subtle differences in signaling dynamics exist between the two receptor pathways, which may be amplified following long term signaling through the chimeric receptor in the absence of bFGF. Importantly, transfected MM14 cells appeared phenotypically normal with respect to differentiation, and uncontrolled proliferation following dimerizer treatment was not observed.

Using a small molecule to expand genetically modified myoblasts offers several advantages over conventional grafting approaches, including the possibility of using fewer cells for injection, better control over the graft size, and the potential for increasing cell-cell communication with host myocardium by reducing the intervening scar area. Use of a promoter active in myoblasts but not fibroblasts, e.g., the desmin promoter, may permit selective expansion of myoblasts from impure primary muscle cultures. Furthermore, the drug has proven non-toxic in vivo, e.g., the desmin promoter, may

Acknowledgments—We thank Dr. Stephen Hauschka for generously providing the MM14 myoblasts and Dr. Tim Clackson (ARIAD Pharmaceuticals; www.arian.com/regulation kits) for providing AP20187. We also thank Kathy Allen for expert technical assistance with FACS analysis.

REFERENCES

1. Ozawa, C. R., Springer, M. L., and Blau, H. M. (2000) Annu. Rev. Pharmacol. Toxicol. 40, 295–317
2. Atkins, B. Z., Lewis, C. W., Kraus, W. E., Hutcherson, K. A., Glower, D. D., and Taylor, D. A. (1999) Ann. Thorac. Surg. 67, 124–129
3. Chiu, R. C., Zihaitis, A., and Kao, R. L. (1995) Ann. Thorac. Surg. 60, 12–18
4. Murry, C. E., Wiseman, R. W., Schwartz, S. M., and Hauschka, S. D. (1996) J. Clin. Invest. 98, 2512–2523
5. Taylor, D. A., Atkins, B. Z.,Hungspreugs, P., Jones, T. R., Reedy, M. C., Hutcherson, K. A., Glower, D. D., and Kraus, W. E. (1998) Nat. Med. 4, 929–933
6. Morgan, J. E., Hoffman, E. P., and Partridge, T. A. (1990) J. Cell Biol. 111, 2437–2449
7. Mendell, J. R., Kissel, J. T., Amato, A. A., King, W., Signore, L., Prior, T. W., Sahenk, Z., Benson, S., McAndrew, P. E., and Rice, R. (1995) N. Engl. J. Med. 333, 832–838
8. Clegg, C. H., Linkhart, T. A., Olwin, B. B., and Hauschka, S. D. (1987) J. Cell Biol. 105, 949–956
9. Olwin, B. B., and Hauschka, S. D. (1986) Biochemistry 25, 3487–3492
10. Templeton, T. J., and Hauschka, S. D. (1992) Dev. Biol. 154, 169–181
11. Jones, N. C., Fedorov, Y. V., Rosenthal, R. S., and Olwin, B. B. (2001) J. Cell. Physiol. 186, 104–115
12. Klint, P., and Claesson-Welsh, L. (1999) Front. Biosci. 4, D165–D177
13. Mohammadi, M., Dikic, I., Seroklin, A., Burgess, W. H., Jaya, M., and Schlessinger, J. (1996) Mol. Cell. Biol. 16, 977–989
14. Boily, B., Vercoutter-Edouart, A. S., Hondermarck, H., Nurcombe, V., and Le Bourhis, X. (2000) Cytokine Growth Factor Rev. 11, 295–302
15. Spencer, D. M., Wandeliss, T. J., Schreibler, S. L., and Crabtree, G. R. (1993) Science 262, 1019–1024
16. Richard, R. E., Wood, B., Zeng, H., Jin, L., Papayannopoulou, T., and Blau, C. A. (2000) Blood 95, 430–436
17. Jin, L., Sritiratnarakul, N., Emery, D. W., Richard, R. E., Kaushansky, K., Papayannopoulou, T., and Blau, C. A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8093–8097
18. Jin, L., Asano, H., and Blau, C. A. (1998) Blood 91, 890–897
19. Jin, L., Zeng, H., Chien, S., Otto, K. G., Richard, R. E., Emery, D. W., and Blau, C. A. (2000) Nat. Genet. 26, 64–66
20. Clackson, T., Yang, W., Rozamus, L. W., Hatada, M., Amara, J. F., Rollins, C. T., Stevenson, L. F., Magari, S. R., Wood, S. A., Courage, N. L., Lu, X., Cerasoli, F., Gilman, M., and Hult, D. A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 10437–10442
21. Kim, E. G., Kwon, H. M., Burrow, C. R., and Ballermann, B. J. (1993) J. Cell Biol. 124, 959–973
22. Blau, C. A., Peterson, K. R., Drachman, J. G., and Spencer, D. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 3076–3081
23. Cheng, L., Du, C., Murray, D., Tong, X., Zhang, Y. A., Chen, B. P., and Hawley, R. G. (1997) Gene Ther. 4, 1013–1022
24. Linkhart, T. A., Clegg, C. H., and Hauschka, S. D. (1981) Dev. Biol. 86, 19–30
25. Reinecke, H., MacDonald, G. H., Hauschka, S. D., and Murry, C. E. (2000) J. Cell Biol. 149, 731–740
26. Menasche, P., Hagege, A. A., Sorscin, M., Pouzet, B., Desnos, M., Duboc, D., Schwartz, K., Vilquin, J. T., and Marolleau, J. P. (2001) Lancet 357, 279–280
27. Reinecke, H., and Murry, C. E. (2000) Cardiovasc. Pathol. 9, 337–344
28. Kudla, A. J., Jones, N. C., Rosenthal, R. S., Arthur, K., Clase, K. L., and Olwin, B. B. (1998) J. Cell Biol. 142, 241–250
29. Olwin, B. B., and Hauschka, S. D. (1988) J. Cell Biol. 107, 761–769
30. Kudla, A. J., John, M. L., Bowen-Pope, D. F., Rainish, B., and Olwin, B. B. (1995) Mol. Cell. Biol. 15, 3238–3246
31. Ornitz, D. M., Xu, J., Calvis, S., McEwen, D. G., MacArthur, C. A., Coulter, F., Gao, G., and Goldfarb, M. (1996) J. Biol. Chem. 271, 15292–15297
32. Amara, J. F., Clackson, T., Rivera, V. M., Gao, T., Keenan, T., Natesan, S., Pollock, R., Yang, W., Courage, N. L., Hult, D. A., and Gilman, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10618–10623
