Basic Fibroblast and Platelet-Derived Growth Factors as Modulators of Actin and α-Actinin in Chick Myocardiocytes During Development (43925)

C. VÉLEZ, A. E. ARÁNEGA, J. C. PRADOS, C. MELGUIZO, L. ALVAREZ, AND A. ARÁNEGA[1]

Basic Cardiovascular Research Section, Department of Morphological Sciences, School of Medicine, University of Granada, E-18071 Granada, Spain

Abstract. **To test whether cardiac muscle is a target for regulation by peptide growth factors, we analyzed the effects of two growth factors on actin and α-actinin expression at the subcellular level. Sodium dodecyl sulfate-gel electrophoresis, immunoblotting, and fluorescense-activated cell sorter analysis were used to quantify the effects of basic fibroblast growth factor and platelet-derived growth factor on cultures of chick myocardiocytes during development. Cytoplasmic and cytoskeletal concentrations of actin and α-actinin were dependent on the stage of embryonic development and on the type of growth factor added to the culture. The most significant finding was the increase in actin and α-actinin expression in the cytoplasmic compartment after treatment with basic fibroblast growth factor of chick heart cells at Hamburger and Hamilton's stage 19. At stage 39, basic fibroblast growth factor induced less marked changes in the accumulation of actin and α-actinin. Platelet-derived growth factor decreased α-actinin expression slightly in the cytoskeletal compartment in more mature stages of heart development. Our findings support the hypothesis that basic fibroblast growth factor plays a role in cardiomyocyte differentiation during the early stages of development.** [P.S.E.B.M. 1995, Vol 210]

Two factors have enhanced our understanding of the processes that regulate cell growth and differentiation: the discovery of cellular oncogenes which encode mitogenic peptides and their receptors, and the elucidation of the effects of growth factors, which induce signals that are transmitted from the surface of the cell membrane to the nucleus (1). Growth factors are soluble polypeptides (molecular weight 5,000–35,000) with a range of multifunctional activities, including both stimulation and inhibition of cell proliferation (2). These factors act by binding to specific receptors on the cell surface and triggering a cascade of intracellular events such as hypertrophy or hyperplasia, or by blocking differentiation, and are thus closely related with phenomena of cell transformation (3).

Highly vascularized tissues, including heart muscle, contain large amounts of heparin-linked factors (4). Two different types of mitogens can bind heparin with physiological ionic strength: fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF). These growth factors are involved in embryogenesis, angiogenesis, and several pathological situations such as atherogenesis, hypertrophy, and healing infarction (5).

Basic fibroblast growth factor (bFGF) is a potent mitogen for a wide range of cells. Although originally found in cells of mesodermal origin, recent biochemical and immunohistochemical findings have detected bFGF in most tissues, but not in serum (6). This growth factor has been identified in heart tissues from mammals and other vertebrates, as well as in rat and

[1] To whom requests for reprints should be addressed at Sección de Investigación Básica Cardiovascular, Departamento de Ciencias Morfológicas, Facultad de Medicina, Avenida de Madrid s/n, E-18071 Granada, Spain.

Received July 29, 1994. [P.S.E.B.M. 1995, Vol 210]
Accepted May 5, 1995.

0037-9727/95/2101-0057$10.50/0

chick heart cells (7–9). The widespread distribution of bFGF in the developing rat heart suggests that this growth factor plays an important role in heart cytodifferentiation and morphogenesis (7). *In vitro* studies have shown that bFGF exerts a regulatory effect on cell proliferation and differentiation; this factor stimulates DNA synthesis in rat ventricular myocardiocytes (9). In addition, a functional role has been attributed to this peptide in the fully differentiated myocardium; this finding was supported by the appearance of augmented expression during myocardial ischemia and infarction, and in response to loading (5).

Platelet-derived growth factor is an active mitogen that acts through a receptor present on different target cells, including muscle cells. Results obtained *in vitro* have shown PDGF to be a potent growth factor for cells of mesenchymal origin (10). The biochemical mechanism by which PDGF regulates the growth of cells is not known. In the developing rat, this factor is thought to be involved in myoblast proliferation and differentiation (11), and PDGF-like mitogenic activity was reported in cultured chicken embryonic limb buds (12). This factor has been implicated as a possible modulator of embryonic differentiation in a variety of systems (13). Potts *et al.* (14) identified PDGF α receptor during chicken development, whereas another study found no measurable PDGF receptor in adult rat muscle (10). Together, these findings indicate that PDGF plays a role in tissue muscle function only during embryonic growth or during muscle regeneration (15).

The present study focuses on actin and α-actinin, two fundamental components for the correct functioning of the myocardial contractile apparatus (16, 17). Actin comprises up to 20% of the total protein content in muscle tissue and contributes to the structure and consistency of the cytoplasm; this protein also forms part of the thin muscular filaments, providing the mechanical and chemical foundation for muscle contraction (18). α-Actinin is a major component of the Z line in skeletal muscle (19). *In vitro* studies have shown that α-actinin is linked to the ends of actin filaments and is specifically located in muscle cell Z lines, suggesting that this protein bundles actin filaments within the Z line (20).

The present study was designed to investigate the effect of bFGF and PDGF on the expression and subcellular compartmentalization of actin and α-actinin in cultured chick embryo myocardiocytes in different stages of development. Our purpose was to trace the modifications induced by these growth factor at different stages of cardiomyocyte differentiation.

Material and Methods

Chick Embryo Heart Cell Culture. Chick myocardiocytes (CMC) were isolated and cultured according to the method described by Cole *et al.* (21) as modified in our laboratory (16). Hearts were ablated from the embryos after 3.5, 6.5, and 13–13.5 days of incubation, when the embryos had reached stage 19, 29, and 39 of Hamburger and Hamilton (HH19, HH29, HH39) (22). The hearts were removed and chopped into pieces measuring approximately 2–4 mm square. The pieces were placed in trypsin (0.05%) (Sigma Chemical Co. St. Louis, MO) in phosphate-buffered saline (137 mmol NaCl, 2.7 mmol KCl, 1.5 mmol KH_2PO_4, 8 mmol Na_2HPO_4, pH 7.3) for 15–20 min, at a concentration of 1 ml trypsin per 100 mg tissue suspension, after which the supernatant was discarded (23). The suspension was kept at 36.5°C for 30 min, and immediately thereafter 2 ml of Dulbecco's modified Eagle's medium (DMEM) (Flow Laboratories, Irvine, CA) supplemented with 20% fetal bovine serum (Flow Laboratories) was added. The tissues were disaggregated by repeatedly taking up and expelling the suspension through a pasteur pipet. The cells were seeded at a rate of 1×10^6 cells/ml in 25 cm^2 culture flasks (Greiner, Nürtingen, Germany) pretreated with collagen (6 $\mu g/cm^2$) (Sigma). After incubation for 4 days in an incubator with a saturated atmosphere of 95% O_2 and 5% CO_2, the cells had formed a thick monolayer. Contamination by nonmuscle cells was avoided by adding 10 μ*M* cytosine-β-D-arabinofuranoside (Sigma) on Day 1 and 3 of culture (24).

Treatment of Chick Embryo Myocardial Cells with bFGF and PDGF. On Day 4 of culture, when the monolayer had formed, the medium was decanted from each flask and replaced with 10 ml of fresh medium containing one of the growth factors. Three different treatments were studied: Group 1 (control) received DMEM supplemented with 10% fetal bovine serum (FBS); Group 2 received DMEM, 10% FBS, and 10 ng/ml bFGF (Sigma), Group 3 received DMEM, 10% FBS, and 3.64 ng/ml PDGF (Sigma).

Processing CMC Extracts for Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis. The CMC were processed according to methods established by Lewis *et al.* (25, 26). The medium was decanted after 48 hr, and the cells washed three times with 2 ml of 40 m*M* NaCl at 4°C. To 8×10^5 cells from each sample we added 1 ml of buffer F (2 m*M* Tris [BioRad, Richmond, CA], 0.2 m*M* $CaCl_2$ [Merck, Darmstadt, Germany], 0.2 m*M* ATP [Sigma], and 0.2 m*M* 2-mercaptoethanol [BioRad, Richmond, CA], pH 8.0) containing 0.1% Triton X-100 (Sigma). Cells were harvested immediately with a curved pasteur pipet and homogenized in a glass-glass manual homogenizer set in crushed ice (4°C) for 30 sec at 60 strokes/min. The suspensions were centrifuged for 5 min at 13000*g* in a microcentrifuge (BHG-Hermle, Gosheim, Germany) at 4°C.

Pellet (Triton X-100-insoluble) fractions from cen-

trifuged CMC extracts were washed five times in buffer F (500 μl) and centrifuged at 13000*g* for 5 min at 4°C. The supernatants were discarded and the residues were resuspended in 500 μl of diluted Laemmli's sample buffer (27) without bromophenol blue or 2-mercaptoethanol. All steps in the process were done at 4°C to avoid the action of cellular proteases (28).

Protein content in the homogenate and cell subfractions was determined in 100-μl aliquots by the Bradford technique (29). Appropriate volumes of Laemmli's sample buffer were added to each fraction to a final concentration of 1 μg protein/μl. The samples were placed in a boiling water bath for 3 min and centrifuged at 13000*g* for 5 min at room temperature.

SDS-PAGE and Immunoblotting; Densitometric Study of CMC Fractions. The Triton X-100- and SDS-soluble fractions (15 μl, 1 μg protein/μl) were separated by 12% SDS-PAGE (Mini Protean II cell, BioRad) at 60 mA for 1 hr at room temperature, stained with Coomassie brilliant blue R-250, and destained with the methods described by Laemmli *et al.* (27). The gels were dried on filter paper (Whatman, Maisdtone, United Kingdom) with a BioRad model 583 drier. All reagents were from BioRad.

The protein bands (stained with Coomassie brilliant blue) in the different lanes were evaluated by densitometry (Beckman Appraise, Brea, CA) at 540 nm with an integrating computer, using a slit width of 4 mm.

After electrophoretic separation, the polypeptides were transferred electrophoretically onto nitrocellulose paper (30). The blots were treated with a blocking solution (20 m*M* Tris, 0.9% NaCl, 10% nonfat milk), then reacted with a 1:200 dilution of anti-α-actinin monoclonal antibody (mAb) (clone no. BM-75.2, Sigma) and anti-L sarcomeric actin mAb (clone No. A-2172, Sigma). To visualize specific antigen-antibody reactions, we used a commercial enzyme immunoassay kit and protocol (BioRad Immunoblot AGR-HRP Assay Kit). The protein bands were evaluated by densitometry, under the same conditions as described above.

Preparation of Cells for Fluorescence-activated Cell Sorting. After 48 hr of treatment, cells were harvested with PBS-EDTA (0.02%) and transferred to universal screw cap tubes containing sterile PBS, then washed and centrifuged at 225*g* for 5 min, discarding the supernatant. The washing and centrifugation steps were repeated once or twice. The cells were permeabilized with methanol for 10 min, then washed three times in PBS and once in distilled water. After incubation at a concentration of 1×10^6 cells/ml for 30 min at 4°C with the anti-L sarcomeric actin (dilution 1:200) and anti-α-actinin (dilution 1:200) mAbs, the cells were washed twice in cold PBS and reincubated with fluorescein isothiocyanate (FITC)-conjugated antimouse IgG at a dilution of 1:50 for fluorescence-activated cell sorter (FACS) analysis.

Results

Chick Heart Cell Cultures. The primary cultures of chick heart cells grew well after isolation and seeding. After 48–72 hr, the cells were clearly confluent, and after 4 days a uniform monolayer had formed. Individual cells showed spontaneous beating at room temperature under light microscope, and in monolayers, specific zones showed synchronous beating. Nonmuscular cells made up less than 5% of the entire population.

CMC Treated with Basic Fibroblast Growth Factor. Table I and II, and Figure 1 and 2 show the results of the densitometric quantitation of actin and α-actinin protein from immunoblotting assays after 48 hr of treatment with growth factors. Treatment with a growth factor for 48 hr had different effects on actin levels (Table I). Actin expression at HH19 was 15.80% according to immunoblot results in the cytoplasmic (CP) subfraction, and 12% in the cytoskeletal (CS) subfraction. In the group treated with bFGF in this stage, α-actin expression increased by 21% in the cytoplasm ($P < 0.001$) and by 16% in the cytoskeleton ($P < 0.01$). In control cultures at HH29, actin expression was 31.08% in the CP compartment and 24.5% in the CS subfraction. Exposure to bFGF increased this pro-

Table I. Effect of Treatment for 48 hr with Growth Factors on Actin Protein Levels in Cultured Chick Myocardiocytes at Hamburger and Hamilton's Stages 19, 29, and 39

Fraction	Stage	Control	bFGF	PDGF
Cytoplasmic	19 HH	15.80 ± 0.14	19.18 ± 0.06[a] *mf* 21%	14.01 ± 0.11[b] *mf* −9%
	29 HH	32.37 ± 0.12	37.00 ± 0.15[b] *mf* 14%	30.02 ± 0.13[c] *mf* −7%
	39 HH	33.05 ± 0.11	35.95 ± 0.09[b] *mf* 9%	30.5 ± 0.06[c] *mf* −7%
Cytoskeletal	19 HH	12.27 ± 0.15	14.00 ± 0.09[b] *mf* 16%	11.12 ± 0.06[c] *mf* −7%
	29 HH	24.52 ± 0.24	26.95 ± 0.11[b] *mf* 10%	23.71 ± 0.09 *mf* −3%
	39 HH	23.57 ± 0.11	26.07 ± 0.07[b] *mf* 11%	23.03 ± 0.06 *mf* −2%

Note. All data are means ± SEM of three independent determinations. Data are percentage values of protein content calculated automatically from densitometric readings of the bands obtained by immunoblotting. bFGF, basic fibroblast growth factor; PDGF, platelet-derived growth factor; *mf*, relative percentage modification with respect to controls, calculated as protein concentration after treatment × 100/protein concentration of untreated CMC. Significance of the differences was checked with Student's *t* test. A significant difference was said to exist when [a]$P < 0.001$, [b]$P < 0.01$ or [c]$P < 0.05$.

Table II. Effect of Treatment for 48 hr with Growth Factors on α-Actinin Protein Levels in Cultured Chick Myocardiocytes at Hamburger and Hamilton's Stages 19, 29, and 39

Fraction	Stage	Control	bFGF	PDGF
Cytoplasmic	19 HH	9.95 ± 0.07	13.52 ± 0.05[a] *mf* 36%	8.95 ± 0.10[b] *mf* −10%
	29 HH	17.93 ± 0.05	22.40 ± 0.04[a] *mf* 25%	17.02 ± 0.09[c] *mf* −5%
	39 HH	19.27 ± 0.12	21.57 ± 0.17[b] *mf* 12%	18.30 ± 0.09[c] *mf* −5%
Cytoskeletal	19 HH	9.62 ± 0.10	9.9 ± 0.06[a] *mf* 29%	8.92 ± 0.09[c] *mf* −7%
	29 HH	16.70 ± 0.08	19.02 ± 0.09[b] *mf* 14%	14.35 ± 0.04[b] *mf* −14%
	39 HH	16.95 ± 0.09	18.63 ± 0.09[b] *mf* 10%	14.40 ± 0.12[b] *mf* −15%

Note. All data are means ± SEM of three independent determinations. Data are percent values of protein content calculated automatically from densitometric readings of the bands obtained by immunoblotting. Abbreviations are as in the footnote to Table I. Significance of the differences was checked with Student's *t* test. A significant difference was said to exist when $^{a}P < 0.001$, $^{b}P < 0.01$ or $^{c}P < 0.05$.

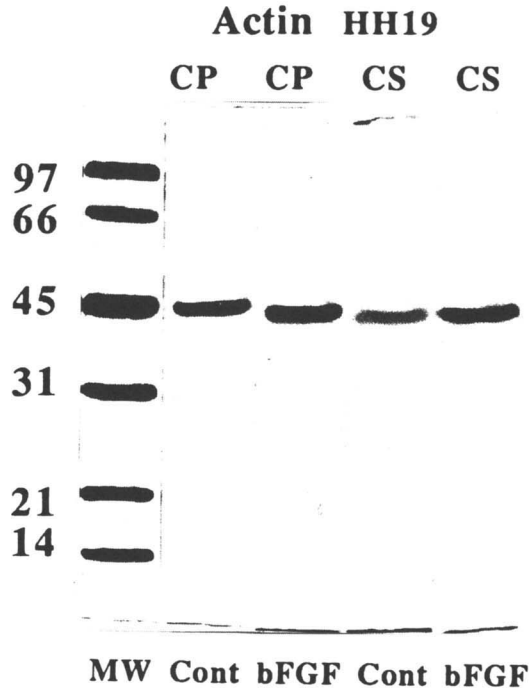

Figure 1. A representative selection of immunoblotting bands used in the densitometric analyses of actin protein in cultured chick myocardial cells treated with basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) for 48 hr at Hamburger and Hamilton's (HH) stage 19. MW, molecular weight control; Cont, control cultures; CP, Triton X-100-soluble cytoplasmic fraction; CS, SDS-soluble cytoskeletal fraction.

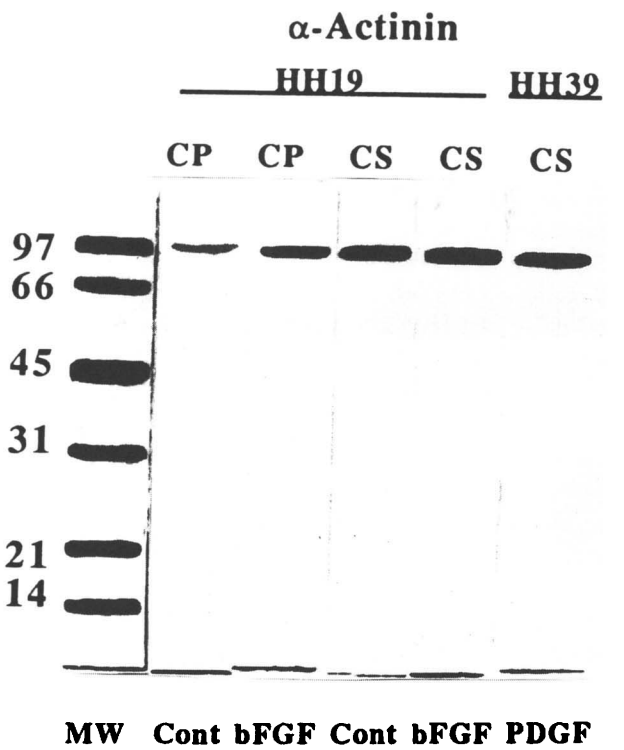

Figure 2. A representative selection of immunoblotting bands used in the densitometric analyses of α-actinin protein in cultured chickmyocardial cells treated with basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) for 48 hr at Hamburger and Hamilton's (HH) stage 19 and 39. MW, molecular weight control; Cont, control cultures; CP, Triton X-100-soluble cytoplasmic fraction; CS, SDS-soluble cytoskeletal fraction.

tein by 14% in the CP subfraction and by 10% in the CS (both $P < 0.01$), in comparison with control cultures. After 48 hr of treatment with bFGF at HH39, densitometric analyses of the bands representing actin showed increases in protein content of 9% in the CP subfraction and 11% in the cytoskeleton (both $P < 0.01$).

In untreated control cultures, α-actinin expression at HH19 was 9.95% in the CP subfraction, and 9.62% in the CS subfraction (Table II). Treatment with bFGF in this stage increased α-actinin expression by 36% in the cytoplasm, and by 29% in the cytoskeleton (both $P < 0.001$). In control cultures at HH29, α-actinin expression was 17.93% in the CP compartment and 16.7% in the CS subfraction. After 48 hr of exposure to bFGF, the concentration of this protein increased by 25% in the CP subfraction ($P < 0.001$) and by 14% in the cytoskeleton ($P < 0.01$), in comparison with control cultures. Treatment with bFGF at HH39 led to increases in α-actinin content of 12% in the CP subfraction and 10% in the cytoskeleton (both $P < 0.01$).

CMC Treated with Platelet-Derived Growth Factor. Exposure to PDGF at HH19 reduced the concentration of actin in cultured myocardiocytes. This contractile protein decreased by 9% in the cytoplasm ($P <$

0.01), and by 7% in the cytoskeleton ($P < 0.05$) (Table I). At HH29, the levels of actin decreased by 7% ($P < 0.01$) and 3% (nonsignificant) in the CP and CS subfractions respectively. In cultures treated at HH39, the concentration of actin decreased by 7% ($P < 0.05$) in the cytoplasm, and showed a nonsignificant decrease in the cytoskeleton.

Treatment with PDGF at HH19 decreased α-actinin expression by 10% in the cytoplasm ($P < 0.01$) and by 7% in the cytoskeleton ($P < 0.05$) (Table II). After 48 hr of exposure to PDGF, the concentration of this protein decreased by 5% in the cytoplasmic fraction ($P < 0.05$) and by 14% in the cytoskeleton ($P < 0.01$), in comparison with control cultures (HH29). At HH39 densitometric analyses of the bands representing α-actinin showed a 5% reduction in the CP fraction ($P < 0.05$) and a 15% decrease in the cytoskeleton ($P < 0.01$).

FACS Analysis. Quantitative data on the changes in expression of the actin and α-actinin in CMC after treatment with bFGF and PDGF were provided by FACS analysis (Fig. 3). Mean fluoresence measured with actin increased significantly in early stages of development with bGF (320.74 ± 3.4 in HH19 and 618.28 ± 3.8 HH29); mean fluorescense produced by α-actinin also increased (to 250.81 ± 3.7 in HH19 and 400.50 ± 4.5 in HH29). Treatment with PDGF slightly decreased mean fluorescense with both proteins.

Discussion

The biochemical mechanisms that regulate cell growth and differentiation are unknown. The use of growth factors in conjunction with tissue and cells culture has made it possible to study biochemical changes associated with differentiation. In this study, we examine how bFGF and PDGF modulate the expression of actin and α-actinin proteins in cultured chick myocardial cells during development, and focus on alterations in these proteins at the subcellular level.

In our cell cultures, the specific response to bFGF was dependent on the stage of development of myocardiocytes. We found a progressive deceleration in the rate of increase in these proteins after treatment with this growth factor, with greater effects in less differentiated than in more differentiated stages of development. These findings are in accordance with Consigli and Joseph-Silverstein (31), who observed a progressive decrease in bFGF expression in the myocardium of the developing chick heart, and with the observations of Abraham *et al.* (32), who found weaker levels of bFGF mRNA expression than in many adult tissues. Less differentiated stages responded by significantly increasing the concentration of actin and α-actinin. Lathrop *et al.* (33) suggested that, during embryonic development, this growth factor induces differentiation of mesodermal tissues and represses terminal muscle cell differentiation and the expression of muscle-specific proteins. These authors found that FGF decreased the synthesis of creatine phosphokinase (CPK) in the muscle cell line BC_3-H1. bFGF might promote differentiation of early cardiomyocytes, but may also antagonize differentiation in a cell line selected for continuous growth in a culture medium.

Adequate expression of α-actinin is fundamental for the correct development of the contractile apparatus of myocardiocytes (19, 34). In previous studies, we reported a significant increase in α-actinin expression throughout heart development in the chick embryo, with greater increases occurring in the cytoplasmic compartment in early stages. The CP/CS ratio for α-actinin changes as the heart matures, due to the steady accumulation of this protein in the cytoskeletal compartment as cardiac differentiation progresses (34). In our study, bFGF led to greater increases in α-actinin expression in early than in late stages, fundamentally in the cytoplasmic compartment. As development progressed, the rate of increase in α-actinin

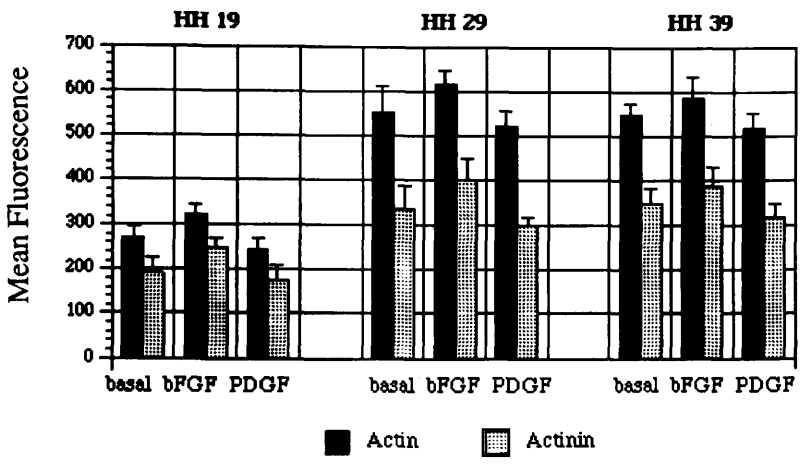

Figure 3. Fluorescence-activated cell sorting analysis. Modifications in mean fluorescence in chick myocardial cells in Hamburger and Hamilton's stages 19, 29, and 39, incubated with anti-actin and anti-α-actinin monoclonal antibodies after 48 hr of treatment of cell cultures with bFGF or PDGF. Basal, levels of actin and α-actinin in untreated myocardiocyte cultures; bFGF, treated with 10 ng/ml basic fibroblast growth factor; PDGF, treated with 3.64 ng/ml platelet-derived growth factor. All data are means ± SEM of three measurements (Student's *t* test).

became similar in both subfractions. Overproduction of α-actinin is known to cause some myopathies (35), and this protein may play a role either in the organization of microfilaments or in their interactions with the membrane to keep it polarized (36). These relationships lend support to the notion that changes in the compartmentalization of α-actinin at the subcellular level may affect the normal functioning of the contractile apparatus.

In cultures of neonatal rat cardiac myocytes, Parker *et al.* (37) found that bFGF had the paradoxical effect of stimulating transcription of skeletal α-actin. This factor modified the levels of actin in cultured myocardiocytes at all stages of development investigated in the present study. The changes in actin after treatment were similar to those in α-actinin, although less marked in the cytoplasmic compartment. The parallel increase in protein in both subcellular fractions after exposure suggests an equilibrium between the rate of synthesis (cytoplasm) and the rate of integration into the cytoskeleton. These findings are in agreement with those of Joseph-Silverstein *et al.* (8), who found that bFGF levels in the chick embryo decreased as muscle cell differentiation progressed, suggesting that bFGF plays a regulatory role in embryonic muscle development. During the prenatal period, bFGF may be an indispensable factor in the measurable increase in protein accumulation in fetal myocardiocytes. The presence of fewer receptors for this growth factor in mature heart tissue (38) may account for the weaker effects of growth factors on actin and α-actinin concentration in more advanced stages of embryogenesis in the present study.

Kardami and Fandrich (4) reported that cultures of chick skeletal myoblasts did not respond to PDGF. In contrast, in our cultures of CMC, this growth factor slightly reduced actin and α-actinin concentrations at all stages of development analyzed. Actin decreased in the cytoplasmic fraction more than in the cytoskeletal fraction, whereas α-actinin decreased mainly in the cytoskeletal subfraction, especially in more advanced stages. Yablonka-Reuveni *et al.* (39) showed that in adult mouse skeletal muscle the presence of PDGF was associated with a decrease in the number of myosin-positive cells, and that myoblasts from different developmental and physiological stages may respond differently to PDGF. Our findings are in consonance with those of Herman and Pledger (40), who found that treatment with PDGF altered vinculin distribution and disrupted actin filaments in BALB/c-3T3 fibroblast cells. Moreover, Sarzani *et al.* (41) found that PDGF receptors decreased with age in rat myocardiocytes. These findings suggest that more mature cells lack the receptors or the mechanisms necessary for an induced signal to be transmitted to the cytoplasm. In our myocardial cell cultures, PDGF lowered the concentration of actin and α-actinin proteins, suggesting that the cells reverted to a less differentiated stage of development (16, 17).

Our data provide evidence that the modulating effects of these growth factors on the levels of actin and α-actinin depend on the stage of development analyzed. Because the loss of receptor for bFGF from the cell surface correlates with the degree of terminal differentiation (8), our findings support the hypothesis that bFGF plays a role in cardiomyocyte differentiation during the early stages of development.

This study was supported by the Comisión Interministerial de Ciencias y Tecnología through Grant MAT90-0695-C02-02 and by the Fondo de Investigaciones Sanitarias de la Seguridad Social (FISS) through Project 90/0849. We thank Karen Shashok for revising the English language of the manuscript.

1. Ueno H, Perryman MB, Roberts R, Schneider MD. Differentiation of cardiac myocytes after mitogen withdrawal exhibits three sequential states of the ventricular growth response. J Cell Biol **107**:1911–1918, 1988.
2. Sporn MB, Roberts AB. Peptide growth factors are multifunctional. Nature **332**:217–219, 1988.
3. Weinberg JB, Mason SN. Relationship of acivicin-induced monocytoid differentiation of human myeloid leukemia cells to acivicin-induced modulation of growth factor, cytokine, and protooncogene messenger RNA expression. Cancer Res **51**:1202–1209, 1991.
4. Kardami E, Fandrich RR. Heparin-binding mitogen(s) in the heart: In search of origin and function. In: Kedes HK, Stockdale FE, Eds. Cellular and Molecular Biology of Muscle Development. New York: Alan R. Liss, pp 315–325, 1989.
5. Parker TG. Molecular biology of cardiac growth and hypertrophy. Herz **18**:245–255, 1993.
6. Emoto N, Isozaki O, Arai M, Murakami H, Shizume K, Baird T, Demura H. Identification and characterization of basic fibroblast growth factor in porcine thyroids. Endocrinology **128**:58–64, 1991.
7. Spirito P, Fu Y-M, Yu Z-X, Epstein SE, Casscells W. Immunohistochemical localization of basic and acidic fibroblast growth factors in the developing rat heart. Circulation **84**:322–332, 1991.
8. Joseph-Silverstein J, Consigli SA, Lyser KM, Pault C. Basic fibroblast growth factor in the chick embryo: Immunolocalization to striated muscle cells and their precursors. J Cell Biol **108**:2459–2466, 1989.
9. Kardami E, Fandrich R. Basic fibroblast GF in atria and ventricles of the vertebrate heart. J Cell Biol **109**:1865–1875, 1989.
10. Westermark B, Siegbahn A, Hieldin CH, Claesson WL. B-type receptor for platelet-derived growth factor mediates a chemotactic response by means of ligand-induced activation of the receptor protein-tyrosine kinase. Proc Natl Acad Sci USA **87**:128–132, 1990.
11. Jin P, Rahm M, Claesson-Welsh L, Heldin C-H, Serjersen T. Expression of PDGF A-chain and β-receptor genes during rat myoblast differentiation. J Cell Biol **110**:1665–1672, 1990.
12. Bell KM. The preliminary characterization of mitogens secreted by embryonic chick wing bud tissues in vitro. J Embryol Exp Morphol **93**:257–265, 1986.
13. Mercola M, Wang C, Kelly J, Brownlee C, Jackson-Grunsby L, Stiles C, Bowen-Pope D. Selective expression of PDGF A and

its receptor during early mouse embryogenesis. Devel Biol **138**:114–122, 1990.
14. Potts JD, Carrington JL. Selective expression of the chicken platelet-derived growth factor α (PDGFα) receptor during limb bud development. Devel Dynamics **198**:14–21, 1993.
15. Tidball JG, Spencer MJ. PDGF stimulation induces phosphorylation of talin and cytoskeletal reorganization in skeletal muscle. J Cell Biol **123**:627–635, 1993.
16. Vélez C, Aránega AE, Muros MA, Gonzalez FJ, Prados-Salazar JC, Alvarez L, Aránega A. Quantification and distribution of troponin T in cultures of chick embryo myocardiocytes. Acta Anat **145**:269–276, 1992.
17. Prados J, Melguizo C, Aránega AE, Fernández JE, Alvarez L, Aránega A. Production of a new monoclonal antibody recognizing α-actinin: Analysis of the changes in subcellular expression in the developing chick heart. Cell Mol Biol **39**:93–103, 1993.
18. Eisenberg E, Hill TL. Muscle contraction and free energy transduction in biological systems. Science **227**:999–1006, 1985.
19. Bush WA, Stromer MH, Goll DE, Suzuki A. Ca^{2+}-specific removal of Z lines from rabbit skeletal muscle. J Cell Biol **52**:367–381, 1972.
20. Li J, Lemanski LF. Immunofluorescent studies for α-actinin in cultured cardiomyopathic hamster heart cells. Anat Rec **228**:46–52, 1990.
21. Cole RJ, Paul J. The effects of erythropoietin on haem synthesis in mouse yolk sac and cultured foetal liver cells. J Embryol Exp Morphol **15**:246–260, 1966.
22. Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. J Morphol **84**:49–92, 1951.
23. Jim J-C, Matsumura F, Yamashiro-Matsumura S. Tropomyosin-enriched and alpha-actinin-enriched microfilaments isolated from chicken embryo fibroblasts by monoclonal antibodies. J Cell Biol **98**:116–127, 1984.
24. Claycomb WC, Moses RL. Culture of atrial and ventricular cardiac muscle cells from the adult squirrel monkey *Saimiri sciureus*. Exp Cell Res **161**:95–100, 1985.
25. Lewis W, Gonzalez B. Actin isoform synthesis by cultured cardiac myocytes. Effects of doxorubicin. Lab Invest **56**:295–301, 1987.
26. Lewis W, Perillo NL, Gonzalez B. α-Actin synthesis changes in cultured cardiac myocytes: Relationship to anthracycline structure. J Lab Clin Med **112**:43–51, 1988.
27. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**:680–685, 1970.
28. Goll DE, Dayton WR, Singh I, Robson RM. Studies of the α-actinin/actin interaction in the Z-disk by using calpain. J Biol Chem **266**:8501–8510, 1991.
29. Bradford MM. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72**:248–254, 1976.
30. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci **76**:4250–4354, 1979.
31. Consigli SA, Joseph-Silverstein J. Immunolocalization of basic fibroblast growth factor during chicken cardiac development. J Cell Physiol **146**:379–385, 1991.
32. Abraham JA, Mergia A, Whang JL, Tumolo A, Friedman J, Hjerrild KA, Gospodarowicz D, Fiddes JC. Nucleotide sequence of a bovine clone encoding the angiogenic protein basic fibroblast growth factor. Science **233**:545–548, 1986.
33. Lathrop B, Olson E, Glaser L. Control by fibroblast growth factor of differentiation in the BC_3H1 muscle cell line. J Cell Biol **100**:1540–1547, 1985.
34. Vélez C, Aránega AE, Muros MA, Melguizo C, Gonzalez FJ, Alvarez L, Aránega A. Changes in subcellular accumulation of contractile proteins in myocardiocyte cultures. Effects of fibric acid derivatives. J Cardiovasc Pharm **21**:40–46, 1993.
35. Paulus W, Peiffer J, Becker I, Roggendorf W, Schumm F. Adult-onset rod disease with abundant intranuclear rods. J Neurol **235**:343–347, 1988.
36. Dos Santos Mermelstein C, Moura Neto V, Chagas C. Alpha-actinin in the electric organ of *Electrophorus electricu* L. CR Acad Sci [III] **307**(13):717–721, 1988.
37. Parker TG, Parker SE, Schneider MD. Peptide growth factors can provoke "fetal" contractile gene expression in rat cardiac myocytes. J Clin Invest **85**:507–514, 1990.
38. Olwin BB, Hauschka SD. Fibroblast growth factor receptor levels decrease during chick embryogenesis. J Cell Biol **119**:503–509, 1990.
39. Yablonka-Reuveni Z, Balestreri TM, Bowen-Pope DF. Regulation of proliferation and differentiation of myoblasts derived from adult mouse skeletal muscle by specific isoforms of PDGF. J Cell Biol **111**:1623–1629, 1990.
40. Herman B, Pledger WJ. Platelet-derived growth factor-induced alterations in vinculin and actin distribution in Balb/c-3T3 cells. J Cell Biol **100**:1031–1040, 1985.
41. Sarzani R, Arnaldi G, Takasaki I, Brecher P, Chobanian AV. Effects of hypertension and aging on platelet-derived growth factor and platelet-derived growth factor receptor expression in rat aorta and heart. Hypertension **18**(III):93–99, 1991.