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Differential regulation of skeletal α-actin transcription in cardiac muscle by two fibroblast growth factors

(differentiation/gene regulation/heart/heparin-binding growth factors/type β transforming growth factors)

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ABSTRACT **In cardiac muscle, acidic and basic fibroblast growth factors (aFGF and bFGF) regulate at least five genes in common (including α and β myosin heavy chains, atrial natriuretic factor, and the sarcoplasmic reticulum calcium ATPase), provoking a generalized "fetal" phenotype similar to events in pressure-overload hypertrophy; however, aFGF and bFGF differentially control the striated α-actins. bFGF stimulates and aFGF inhibits skeletal α-actin transcripts associated with the embryonic heart, whereas cardiac α-actin mRNA is inhibited by aFGF but not bFGF. To elucidate mechanisms for these selective and discordant actions of aFGF and bFGF on cardiac muscle, chicken skeletal and cardiac α-actin promoter-driven reporter genes were introduced into neonatal rat cardiac myocytes by electroporation. Skeletal α-actin transcription was selectively stimulated by bFGF, whereas the cardiac α-actin promoter was unaffected. In contrast, aFGF suppressed both transfected α-actin genes. The differential regulation of skeletal α-actin transcription was equivalent with either purified or recombinant FGFs and was observed with 5′ flanking sequences from either nucleotide −202 or −2000 to nucleotide −11. Positive and negative modulation of α-actin transcription by growth factors corresponded accurately to the endogenous genes in all permutations studied. These investigations provide a model for reciprocal control of gene transcription by aFGF vs. bFGF.**

Initially identified through their ability to evoke mitotic or anchorage-independent growth, respectively, fibroblast growth factor (FGF) and transforming growth factor type β (TGFβ) are multifunctional agents that also regulate cellular differentiation in a lineage- and stage-specific context (1–3). Acidic FGF (aFGF) and basic FGF (bFGF), archetypes for a multigene family of heparin-binding peptides, share substantial sequence identity, compete for receptor binding, and possess similar biological actions (1). The impact of peptide growth factors on differentiation may be understood best in skeletal muscle. First, aFGF and bFGF are potent mitogens for skeletal myoblasts (4), which prevent the expression of muscle-specific proteins even under conditions that do not provoke mitotic growth (4, 5). Second, aFGF and bFGF individually cause the formation of muscle primordia in *Xenopus* animal pole cells, comparable to induction by vegetal pole cells (6). Mesoderm-inducing activity is potentiated by TGFβ1 (6), which, like the heparin-binding growth factors, inhibits differentiation in cells committed to the skeletal muscle lineage (7, 8). Third, neither bFGF nor TGFβ1 has been shown to modulate muscle-specific genes in postmitotic skeletal myocytes (4, 7, 8), though down-regulation of growth factor receptors may be the consequence and not the cause of terminal differentiation (9–11). Conversely, aFGF and bFGF each promote differentiation in neurons (12).

In cardiac muscle we demonstrated that aFGF, bFGF, and TGFβ1 selectively provoke a "fetal" program of contractile protein genes that differs from the more straightforward block or augmentation of a differentiated state in other lineages (13) and resembles the recapitulation of embryonic properties during cardiac adaptation to load (3, 14, 15). aFGF, bFGF, and TGFβ1 exert mutually concordant, reciprocal effects on β (embryonic) and α (adult) myosin heavy chain (MHC) gene expression in neonatal rat cardiac myocytes. bFGF and TGFβ1 also increase skeletal α-actin and vascular smooth muscle α-actin gene expression, as found in fetal cardiac muscle (16), but fail to influence cardiac α-actin mRNA abundance. Unlike bFGF, aFGF down-regulates both skeletal and cardiac α-actin transcripts. Furthermore, aFGF, but not bFGF, was a potent mitogen for cardiac myocytes under the conditions tested. Given mutually consistent activities of bFGF and aFGF in other lineages, these differential effects were unexpected. The discordant responses of cardiac myocytes to peptide growth factors and, in particular, counter-regulation of skeletal α-actin transcript abundance by aFGF vs. bFGF (inhibition and stimulation, respectively) provide intriguing opportunities to study mechanisms for the divergent control of cell phenotype by these related trophic signals.

Our specific objectives in the present investigation were: (*i*) to ascertain whether peptide growth factors regulate an even more complex ensemble of cardiac-specific genes than described previously and, if so, whether the additional actions of aFGF and bFGF were concordant; (*ii*) to identify regions of the skeletal and cardiac α-actin genes sufficient for their tissue-specific expression in cardiac muscle; (*iii*) to determine whether counter-regulation of skeletal α-actin by aFGF vs. bFGF reflects differential control of its transcription; and (*iv*) to establish with certainty that dichotomous effects on cardiac expression of skeletal α-actin are intrinsic to aFGF and bFGF by using recombinant growth factors.

MATERIALS AND METHODS

Promoter Construct. 5′ Flanking DNA sequences from the chicken striated α-actin genes were inserted into pSV0CAT, directing transcription of the gene for chloramphenicol acetyltransferase (CAT). Three skeletal α-actin promoters were used (17, 18), which provide comparable activity in chicken primary myoblast cultures: SkA2.0CAT, positions −2000 to

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Abbreviations: FGF, fibroblast growth factor; aFGF and bFGF, acidic and basic FGF; ANF, atrial natriuretic factor; CAT, chloramphenicol acetyltransferase; MHC, myosin heavy chain; TGFβ1, transforming growth factor type β1; SV40, simian virus 40.

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−11 relative to the transcription initiation site; SkA202CAT, −202 to −11; and SkA421CAT, −421 to +24. The cardiac α-actin construct CaA318CAT (−318 to +18; ref. 19) likewise contains upstream regions sufficient for tissue-specific transcription in skeletal muscle (20). pSV2CAT, containing the simian virus 40 (SV40) early promoter enhancer, was used as a positive control.

Cell Culture and Transfection. Cardiac myocytes isolated from the ventricles of 2-day-old Sprague–Dawley rats were pooled in medium supplemented with 10% (vol/vol) fetal bovine serum (13, 21) and were partially depleted of mesenchymal cells by two 30-min cycles of differential adhesiveness. Cardiac myocytes (7×10^6 cells) were resuspended in 700 μl of 140 mM NaCl/15 mM Hepes, pH 7.2, with 20 μg of plasmid DNA in 0.4-cm cuvettes. Cells were subjected to a 240-V 960-μF pulse in a Bio-Rad electroporation unit (22). Cell lysis was ≈50%, and surviving cells were plated at 10^5 cells per cm^2 on polystyrene dishes (no. 3803; Becton Dickinson) in medium containing 10% fetal bovine serum. After 24 hr, cells were maintained for 48 hr in serum-free medium supplemented with 1 nM LiCl, 1 nM Na_2SeO_4, 1.0 nM thyroxine, and 1 μg of insulin, 5 μg of transferrin, and 25 μg of ascorbic acid per ml (13). Transfected cells then were cultured for 48 hr in the serum-free medium, in the presence of aFGF (25 ng/ml), bFGF (25 ng/ml), TGFβ1 (1 ng/ml), or vehicle alone as the control. Secondary cultures of cardiac fibroblasts obtained from the first cycle of differential adhesiveness (13) were dissociated in 0.1% trypsin and then subjected to electroporation and culture precisely as for cardiac myocytes.

Bovine brain aFGF and bFGF were obtained from R&D Systems (Minneapolis). Recombinant human bFGF and bovine aFGF were provided, respectively, by J. Abraham (California Biotechnology, Mountain View, CA) and K. Thomas (Merck Sharp & Dohme). TGFβ1 (R&D Systems) was the homodimer from porcine platelets.

Analysis of CAT Expression. To study growth factor control of α-actin promoters, at least three independent batches of plasmid DNA were purified by two rounds of ultracentrifugation through $CsCl_2$ and transfected into three to seven independent cultures. Transfected cardiac myocytes were harvested after 5 days. Acetylated [^{14}C]chloramphenicol was resolved by TLC and quantitated by liquid scintillation counting. CAT activity in cells treated with growth factors was normalized to activity of each promoter in simultaneous cultures treated with vehicle alone. The use of ostensibly constitutive reporter genes was confounded by the existence of serum-responsive sequences in a variety of viral control elements (e.g., ref. 23). Results were compared by the unpaired two-tailed *t* test and Scheffe's multiple comparison test for analysis of variance; a significance level of $P < 0.05$ was used.

RNA Blot Hybridization. For Northern analysis, neonatal rat cardiac myocytes subjected to serum withdrawal were incubated with growth factors or vehicle for 24 hr (13). Total cellular RNA, size-fractionated by formaldehyde/agarose gel electrophoresis, was transferred to nylon membranes (15 μg per lane). Slow cardiac sarcoplasmic reticulum Ca^{2+}-ATPase mRNA and atrial natriuretic factor (ANF) hybridization probes were the reverse complement of 3′ untranslated nucleotides 1534–1563 (25) and 494–515 (26), respectively, end-labeled to a specific activity of $4–6 \times 10^8$ cpm/μg. Hybridization and washing conditions are given in ref. 13. Single bands of the appropriate size were identified by the Ca^{2+}-ATPase and ANF probes (24, 25).

RESULTS

aFGF and bFGF Regulate at Least Five Cardiac Genes in Common, Yet Differentially Control the Striated α-Actins. Cardiac myocyte cultures, which modulate actin and MHC genes in response to peptide growth factors (13), were tested by RNA blot hybridization for control of the ANF and sarcoplasmic reticulum Ca^{2+}-ATPase genes (Fig. 1), which are up- and down-regulated, respectively, during experimental hypertrophy or end-stage heart failure (3, 13, 24–27). aFGF, bFGF, and TGFβ1 individually inhibited the expression of the Ca^{2+}-ATPase gene >60% (expression relative to vehicle-treated control cells: aFGF, 0.23; bFGF, 0.39; TGFβ1, 0.25). Conversely, each peptide augmented ANF mRNA abundance 2–4 fold (aFGF, 5.2; bFGF, 3.9; TGFβ1, 2.5). Thus, all three growth factors evoked antithetical changes in Ca^{2+}-ATPase and ANF steady-state mRNA levels, as with acute load or myocardial disease. Taken together with α-actin and MHC genes, shown for comparison in Fig. 1, these results extend to five the cardiac genes that respond similarly to three growth factors in a manner resembling pressure-overload hypertrophy *in vivo*. Of seven genes examined, only skeletal and cardiac α-actin were differentially regulated by aFGF vs. bFGF. As none of the growth factors increased total RNA or cell number in cardiac fibroblast cultures (13), there is no indication that these responses are confounded by differential growth of the residual nonmuscle cells.

Exogenous Skeletal and Cardiac α-Actin Promoters Are Efficiently and Specifically Expressed in Neonatal Cardiac Myocytes. To ascertain whether proximal 5′ flanking sequences might suffice for tissue-specific α-actin gene transcription in cardiac muscle, neonatal cardiac myocytes were transfected with α-skeletal and α-cardiac actin promoter–CAT constructs (Fig. 2 *Left*). In cardiac myocytes, pSV2CAT resulted in a mean of 37.2 pmol of acetylated chloramphenicol per 7×10^6 cells (conversion rate = 4.2%). The chicken skeletal α-actin promoter (−202 to −11) was expressed at a level 0.96 ± 0.105 relative to pSV2CAT. The chicken cardiac α-actin promoter (−318 to +18) also was highly expressed (0.68 ± 0.19 relative to pSV2CAT), whereas pSV0CAT was not expressed above background (conversion < 0.03%; 0.004 relative to pSV2CAT). These findings concur with coexpression of the endogenous skeletal and cardiac α-actin genes at the stage of cardiac maturation modeled here (13, 21). Neither a 2-fold (−421 to +24) nor a 10-fold (−2000 to −11) larger promoter fragment was expressed at levels significantly different from the proximal element. A repre-

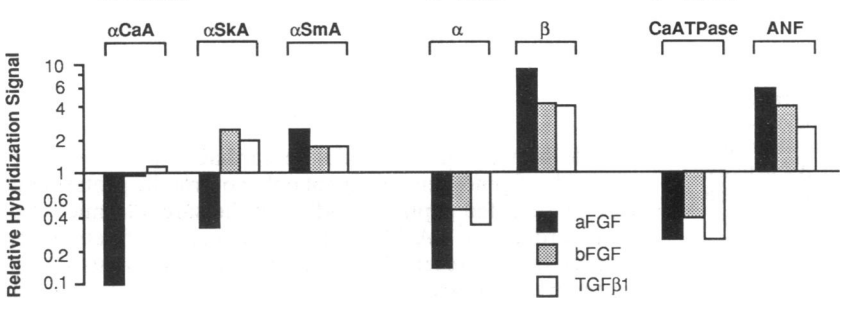

FIG. 1. Cardiac gene expression modulated by peptide growth factors. RNA blot hybridization, analyzed by scanning densitometry, is shown relative to expression in control cells. ANF and sarcoplasmic reticulum Ca^{2+}-ATPase gene expression were up- and down-regulated, respectively, by all three growth factors. Results for actin and MHC genes (14) are shown for comparison. Of seven genes investigated, aFGF and bFGF produced discordant effects only on skeletal (αSkA) and cardiac (αCaA) α-actin. ■, aFGF; □, bFGF; □, TGFβ1. αSmA, smooth muscle α-actin.

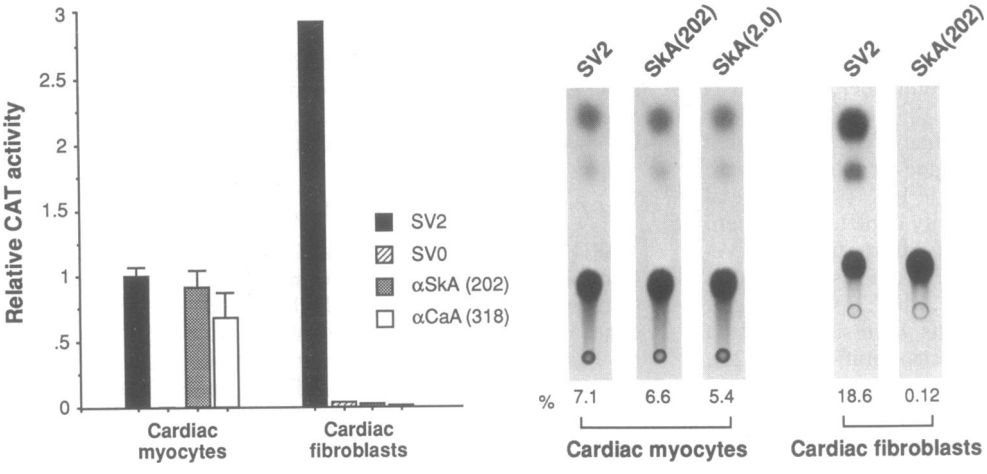

FIG. 2. Coexpression of the skeletal and cardiac α-actin promoters in neonatal cardiac myocytes. (*Left*) Cardiac myocytes and cardiac fibroblasts were transiently transfected with skeletal [−202 to −11: αSkA(202)] or cardiac [−318 to +18: αCaA(318)] α-actin–CAT chimeric genes, pSV2CAT (SV2) or pSV0CAT (SV0). CAT activity is shown relative to pSV2CAT activity in the cardiac myocytes. (*Right*) Comparable activity of the −2000 to −11 skeletal α-actin [SkA(2.0)] and SkA(202) promoters in neonatal cardiac muscle cells and inactivity of SkA(202) in cardiac fibroblasts. The percentage of chloramphenicol acetylated is shown below each lane.

sentative autoradiogram comparing the −2000 to −11 and −202 to −11 promoters is shown in Fig. 2 *Right*. In cardiac fibroblasts, pSV2CAT produced 3-fold higher activity than in cardiac myocytes, yet the skeletal and cardiac α-actin promoters were each expressed at levels no greater than pSV0CAT (Fig. 2 *Left*).

Antithetical Control of Skeletal α-Actin Transcription in Cardiac Muscle by aFGF vs. bFGF. To investigate whether the exogenous skeletal and cardiac α-actin promoters contain sequences sufficient for growth factor modulation in cardiac myocytes, transfected cells in serum-free medium were treated with aFGF, bFGF, or TGFβ1. Skeletal α-actin promoter activity (Fig. 3*A*) was increased by both bFGF (3.33 ± 0.40, $P = 0.001$) and TGFβ1 (2.44 ± 0.36, $P = 0.007$) relative to vehicle-treated cells. In agreement with its divergent effect on endogenous skeletal α-actin mRNA, aFGF inhibited the skeletal α-actin promoter (0.349 ± 0.07, $P = 0.0001$). The dichotomy between aFGF and bFGF effects was significant, with $P = 0.0001$.

Unlike skeletal α-actin, cardiac α-actin transcription (Fig. 3*B*) did not vary significantly from the vehicle control in cells treated with bFGF (1.07 ± 0.198) or TGFβ1 (0.963 ± 0.011), yet cardiac as well as skeletal α-actin transcription was down-regulated by aFGF (0.421 ± 0.05, $P = 0.0003$). This second disparity between aFGF and bFGF also was significant, with $P = 0.03$. None of the growth factors significantly altered expression of pSV2CAT in cardiac muscle cells (Fig. 3*C*). The lack of SV40 response and selectivity for skeletal vs. cardiac α-actin promoters indicate that dichotomous effects

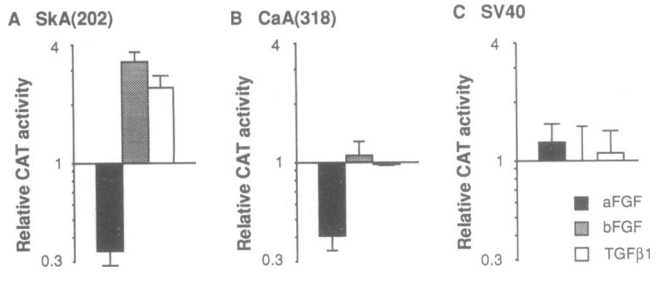

FIG. 3. Differential regulation of skeletal α-actin transcription in cardiac muscle by purified aFGF vs. bFGF. Cardiac myocytes were transiently transfected, cultured in serum-free medium for 48 hr, and then treated for 48 hr with the growth factors shown. CAT activity quantitated by liquid scintillation counting was normalized to the expression of each promoter, respectively, in vehicle-treated cells. (*A*) Skeletal α-actin promoter (−202 to −11). (*B*) Cardiac α-actin promoter (−318 to +18). (*C*) pSV2CAT. ■, aFGF; □, bFGF; □, TGFβ1.

of bFGF and aFGF are not merely due to nonspecific changes in total transcription or spurious consequences of the vector.

aFGF and bFGF Have Intrinsically Dichotomous Effects on Skeletal α-Actin Transcription in Cardiac Muscle. To prove that antithetical effects of purified aFGF and bFGF on skeletal α-actin expression did not result from contaminants, conceivably including other heparin-binding growth factors, cardiac myocytes transfected with SkA(202) were treated with recombinant aFGF or bFGF (Fig. 4). Recombinant bFGF stimulated skeletal α-actin transcription 2.95 ± 0.822-fold, whereas recombinant aFGF decreased expression to 0.419 ± 0.036 of the control. The disparity between recombinant aFGF and bFGF effects was significant, with $P = 0.02$. Quantitatively, these divergent effects precisely concur with those of the respective purified peptides.

To test the relative importance of proximal vs. distal elements to positive and negative control of skeletal α-actin transcription by FGFs, cardiac myocytes also were transfected with a construct containing 2.0 kb of chicken skeletal α-actin 5′ flanking sequences. Basal activities of the −202 to −11 and −2000 to −11 skeletal α-actin inserts were similar (Fig. 2 *Right*). Whereas recombinant bFGF stimulated transcription of the 2.0-kb promoter, aFGF inhibited its expression (1.553 ± 0.47 vs. 0.581 ± 0.42; Fig. 4). The divergence between recombinant aFGF and bFGF effects was significant, with $P = 0.0001$. Thus, <200 nucleotides of 5′ flanking DNA suffice for counterregulation of skeletal α-actin transcription by recombinant aFGF vs. bFGF. No greater degree of control was seen with a 10-fold larger upstream segment of the gene.

Comparing activity of the transfected skeletal and cardiac α-actin promoters to the mRNA abundance (Fig. 1) indicates that growth factor regulation of the exogenous reporter genes corresponds with accuracy to steady-state expression of the endogenous genes in all eight permutations of purified or recombinant growth factors tested thus far ($r^2 = 0.952$; Fig. 4*B*). Thus, both positive and negative control of the striated α-actins by growth factors may be predominantly transcriptional and are dependent upon proximal 5′ flanking sequences.

DISCUSSION

Peptide growth factors modulate cardiac expression of an ensemble of genes, encoding not only contractile proteins but also the secreted peptide, ANF, and the sarcoplasmic reticulum cardiac Ca^{2+}-ATPase. These concerted effects in the absence of load resemble nominally adaptive events during cardiac hypertrophy in rodents and heart failure in man (3, 14,

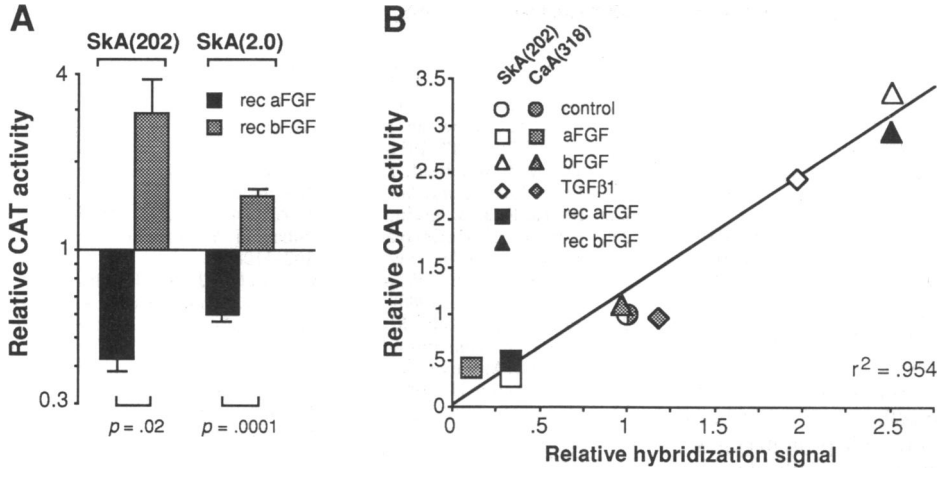

FIG. 4. Antithetical control of skeletal α-actin transcription in cardiac muscle by recombinant aFGF vs. bFGF. (*A*) Cardiac myocytes were transiently transfected with the −202 to −11 [SkA(202)] or −2000 to −11 [SkA(2.0)] skeletal α-actin promoter. CAT activity was normalized to expression of each promoter, respectively, in vehicle-treated cells. ■, Recombinant (rec) aFGF; ■, recombinant bFGF. (*B*) Regulation of skeletal and cardiac α-actin steady-state mRNA abundance in cardiac muscle by aFGF, bFGF, TGFβ1, and recombinant FGFs (data from Fig. 1 on the abscissa) is highly correlated with control of the exogenous promoters (data from Figs. 3 and 4*A* on the ordinate).

15, 24–27). For all seven genes, responses to bFGF vs. TGFβ1 were concordant. Differential regulation of both skeletal and cardiac α-actin by aFGF and bFGF stands in contrast to their shared effects on five other cardiac genes and establishes a model for FGF control of tissue-specific gene expression with specificity and discrimination unlike that reported to date in other systems. First, cardiac α-actin transcription was unchanged by bFGF, yet was suppressed by aFGF. Second, transcription of the skeletal α-actin promoter was counter-regulated—that is, stimulated by bFGF but inhibited by aFGF. For both positive and negative modulation, growth factors thus control sarcomeric α-actin gene expression at least in part through transcriptional mechanisms, and steady-state mRNA levels change no more than transcription does. Counter-examples exist, including insulin induction of glycerophosphate dehydrogenase (28) and lipoproteinase lipase (29) in adipocytes, which are purely posttranscriptional. Concomitant increases in mRNA stability as well as transcription may be required to explain the induction of skeletal α-actin in cardiac myocytes by α_1-adrenergic agonists (30). Recombinant aFGF and bFGF produced dichotomous effects on skeletal α-actin transcription (with each of two promoter constructs) equivalent to the purified growth factors, verifying that the differential actions are intrinsic to these peptides and not the consequence of contaminants during purification, such as the other heparin-binding growth factors (1, 3). Opposite effects on gene transcription by two FGFs have not, to our knowledge, been reported in other cell lineages (*cf.* 1, 3–6, 8).

Potential mechanisms for bifunctional effects of aFGF and bFGF are suggested by the likelihood of multiple FGF receptors in the genome (31–33). Like platelet-derived growth factor receptor isoforms, which distinguish among related ligands (34), the FGF receptor encoded by *flg* (a *fms*-like gene) binds both aFGF and bFGF, perhaps with different affinity (31, 32). A model for disparate control of the three α-actin genes during cardiac myogenesis might involve altered FGF receptor density, isoform distribution, or activity in addition to abundance within the heart of aFGF and bFGF themselves (3, 35). Bifunctional control by aFGF vs. bFGF could also provide a basis for discrepancies observed in skeletal α-actin expression in cardiac muscle: after serum stimulation during the transition from hyperplasia to hypertrophy (21); after hemodynamic load, in younger vs. older rats (36); disparities relative to βMHC after overload (15); and inconsistent expression (37), compared with ANF (26) or the Ca^{2+}-ATPase (27), in diseased human ventricular muscle. Autocrine or paracrine mechanisms coupling short-term contractile responses to long-term trophic effects have been postulated in vascular smooth muscle as well (38).

Growth factor control of both cardiac and skeletal α-actin transcription in cardiac muscle was mediated by proximal 5′ flanking sequences containing the elements necessary for efficient tissue-specific expression. The proximal −202 to −11 segment of the chicken α-skeletal promoter conferred expression equivalent to a fragment 2- or 10-fold larger, as for maximal expression of the chicken promoter in primary chicken skeletal myoblasts (17) or rat L8 cells (39). A proximal element of the human α-skeletal promoter also suffices for maximal expression in L8 cells (40). Although efficient expression in C2C12 cells requires more distal elements out to position −1300 (40), exogenous chicken (41) and human (40) skeletal α-actin promoters are transcribed even in proliferating C2C12 cells and do not correspond to the endogenous genes. In rat cardiac myocytes, the −202 to −11 skeletal α-actin promoter also suffices for differential regulation by bFGF and aFGF, with no additional contribution shown by DNA elements further upstream. This contrasts with the role of elements distal to −450 for norepinephrine stimulation of the human skeletal α-actin promoter in cardiac muscle (N. Bishopric, personal communication).

Differing cis-acting sequences might direct α-actin transcription in cardiac vs. skeletal muscle, as demonstrated for the muscle creatine kinase intragenic enhancer (42) and cardiac troponin T promoter (43). Substantial complexity already exists in the set of known DNA–protein interactions and even in the topography of binding shown for the α-actin genes. Mobility-shift and footprinting assays reveal that at least 8 nuclear protein complexes in skeletal muscle bind to the cardiac α-actin promoter (44) and at least 12 to the skeletal α-actin promoter (K.-L.C. and R.J.S., unpublished data). The precision of muscle-specific gene transcription and its modulation during development or adaptation are postulated to involve interactions among muscle-specific transcription factors, ubiquitous factors, and negative regulatory proteins at multiple neighboring or overlapping sites (20, 44).

Therefore, bipolar effects of bFGF and aFGF on skeletal α-actin transcription in cardiac muscle could be mediated by dissimilar sets of positive and negative cis-acting sequences or could reflect differential trans-activation and trans-repression of particular elements. In both skeletal and cardiac myocytes, the downstream CCAAT box-associated repeat (CBAR) is critical for tissue-specific transcription of skeletal α-actin (refs. 20, 40, and 44; unpublished results), and all constructs containing this element of the corresponding human genes are sensitive to trans-repression by adenovirus E1A proteins (45). The likelihood that the downstream CBAR is also a locus for control by growth factors has been predicted by its similarity to the serum-response element of c-*fos* (46, 47), apparent identity between an actin promoter-binding protein and the serum response factor for c-*fos*

induction (47), cross-binding of other factors (46), and point mutations of the downstream CBAR that abolish both its muscle-specific activity and its up-regulation in fibroblasts by serum (46). The *fos* serum-response element mediates not only rapid induction of c-*fos* by serum and phorbol esters but also its subsequent trans-repression (48). Thus, the skeletal α-actin downstream CBAR is a plausible site for both trans-activation by bFGF and trans-repression by aFGF.

In skeletal muscle, growth factors are likely to control differentiation through signalling mechanisms involving oncogene-encoded proteins such as ras, myc, and fos (3, 49–51). However, fibroblast and transforming growth factors (52), like ras and fos themselves (51), alter muscle-specific gene transcription in large part through a block to expression or activity of myc-like determination proteins that can impart the myogenic phenotype to nonmuscle cells (51, 53). Cardiac and skeletal muscle share numerous contractile protein genes such as each of the α-actins in common, especially during fetal life (3, 14–16, 26). By contrast, *in situ* and Northern hybridization indicate that MyoD1 (54), myogenin (54), myf-5 (55), and MRF4 (56) are not expressed in cardiac muscle. As a consequence, it is thought unlikely that the known MyoD-like proteins are involved in transcriptional control in ventricular or atrial muscle cells or growth factor control of cardiac plasticity.

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