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fos/jun Repression of Cardiac-Specific Transcription in Quiescent and Growth-Stimulated Myocytes Is Targeted at a Tissue-Specific *cis* Element

KEVIN McBRIDE, LYNDA ROBITAILLE, STÉPHANE TREMBLAY, STEFANIA ARGENTIN, AND MONA NEMER*

Institut de recherches cliniques de Montréal, 110 avenue des Pins Ouest, Montréal, Québec H2W 1R7, and Département de pharmacologie, Université de Montréal, Montréal, Québec H3C 3J7, Canada

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Unlike that of skeletal muscle cells in which growth and differentiation appear mutually exclusive, growth stimulation of cardiac cells is characterized by transient expression of early response nuclear proto-oncogenes as well as induction of several cardiac-specific markers. This observation led to the speculation that these proto-oncogenes, particularly c-*fos* and c-*jun*, might act as positive regulators of cardiac transcription. We have examined the role of c-*jun* and c-*fos* in basal and growth-stimulated cardiac transcription, using the cardiac-specific atrial natriuretic factor (ANF) gene as a marker. The results indicate that c-*jun* and c-*fos* are negative regulators of ANF transcription. Inducers of *jun* and *fos* activity, such as mitogens and growth factors, inhibited endogenous ANF transcripts. In transient cotransfection assays, *jun* and *fos* were able to *trans*-repress the ANF promoter in both quiescent and α_1-adrenergic stimulated myocytes. This repression was specific to myocyte cultures and was not observed in nonmuscle cells. Deletion analysis indicated that repression does not require typical AP-1-binding sites (tetradecanoyl phorbol acetate response elements) or serum response elements but is targeted at a cardiac-specific element within the ANF promoter. Various Fos-related proteins, including Fra-1, Fos B, and v-Fos, were able to *trans*-repress ANF transcription. In addition, C-terminal c-*fos* mutants which no longer repress transcription of such early growth response genes as c-*fos* and EGR-1 retained the ability to repress ANF transcription. Repression by c-*jun* occurs via the N-terminal activation domain and does not require the DNA-binding domain, suggesting that proto-oncogene repression involves interaction with one or more limiting cardiac-specific coactivators.

Nuclear proto-oncogenes play a crucial role in cell growth and proliferation and may also be involved in development and cellular differentiation. For example, in transgenic mice, high levels of c-*fos* interfere with normal bone development (48), while overexpression of c-*myc* interferes with maturation of lymphoid cells (32) and has pronounced effects on cardiac myogenesis (28). In addition, investigations of cell lines have pointed to a role of these oncogenes in cellular differentiation, although the molecular mechanisms involved are not yet clear (14, 18, 33, 39, 45, 52, 67). For example, in skeletal muscle cell lines in which proliferation and differentiation appear mutually exclusive, serum, peptide growth factors, and transforming oncogenes *ras*, *fos*, and *jun* inhibit myogenic differentiation. In this case, differentiation may be blocked as a result of the repression of a subset of muscle-specific helix-loop-helix (HLH) transcription factors and/or direct interference with their ability to transactivate muscle genes (7, 10, 19, 22, 33, 34, 43, 63, 70). In contrast with undifferentiated skeletal myoblasts, proliferating cardiac muscle cells express tissue-specific markers and growth stimulation of terminally differentiated cardiomyocytes is associated with the induction of several embryonic cardiac genes. Some of these include the heart-specific isoforms of actin (66), myosin (6, 35), and the atrial natriuretic factor (ANF) gene, which is expressed predominantly in the heart from the earliest stage of cardiac embryogenesis (71).

ANF is a 28-amino-acid peptide hormone with potent biological properties, including natriuresis, diuresis, and hypotension; it is so far the major secretory product of cardiac myocytes (24). The ANF gene is constitutively expressed in cardiac atria where its mRNA accounts for 1 to 3% of total atrial mRNA; however, in heart ventricles, its expression is positively correlated with growth stimulation both in proliferating and nonproliferating myocytes (24, 71). Indeed, terminally differentiated cardiomyocytes lose their ability to proliferate and respond to growth stimulation by increasing their size and not their number. This trophic response, known as hypertrophy, occurs in response to various stimuli including exercise, hormones, increased body volume, or blood pressure; hypertrophy of cardiac cells can also be induced in vitro in cardiocytes maintained in primary cultures by hormones, including adrenergic agonists (58). Both in vivo and in vitro cardiac hypertrophy is accompanied by genetic reprogramming and, in particular, by the reexpression of several embryonic genes including ANF (26, 27, 65). Furthermore, transient induction of several immediate-early growth response genes, particularly proto-oncogenes c-*myc*, c-*jun*, and c-*fos* (29, 56, 62), is observed in response to growth stimulation of terminally differentiated cardiac cells. Since Jun and Fos proteins act as transcriptional activators that mediate the effects of several growth factors and mitogens (2), it has been speculated that these nuclear proto-oncogenes may be responsible for the reactivation of embryonic cardiac genes in terminally differentiated nonproliferating cardiomyocytes (20, 27, 37, 44, 59).

* Corresponding author.

In order to assess the role of the *fos* and *jun* proto-oncogenes on cardiac transcription in quiescent and stimulated cardiomyocytes, we measured the effects of serum and mitogens on ANF mRNA levels and directly tested the interaction of the Fos and Jun proteins with the cardiac-specific ANF promoter in primary cardiocyte cultures. These cultures offer a unique model to study the role of serum, growth factors, and nuclear proto-oncogenes on tissue-specific gene expression in nonproliferating cells. Both ANF transcripts and ANF promoter activity were induced in α_1-adrenergic agonist-stimulated myocytes. However, this induction was dissociated from the *fos* and *jun* oncogenes and some of their inducers such as serum and tetradecanoyl phorbol acetate (TPA), which actually repressed ANF mRNA levels and inhibited the ANF promoter. Transcriptional repression of the ANF promoter by *fos* did not require either TPA or serum response elements (SRE) and may represent a novel mechanism of *fos*-mediated repression through interference with tissue-specific elements.

MATERIALS AND METHODS

Cell cultures and transfections. Primary cardiocyte cultures were prepared from 3- to 4-day-old Sprague-Dawley rats and kept in serum-free medium as previously described (5). L and HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. Transfections were carried out by using the calcium phosphate precipitation technique 24 h after plating. Rous sarcoma virus (RSV)-luciferase or RSV-human growth hormone (hGH) (2 μg per dish) was included as internal control to normalize for transfection efficiencies. Cells were harvested, and the cell media were collected 36 to 48 h after transfection. Luciferase activity was assayed with an LKB luminometer, and hGH was measured in cell media by radioimmunoassay as previously described (5). The amount of reporter plasmid was kept at 3 μg per dish, and the total amount of DNA used was kept constant in all dishes (usually 12 μg) by addition of pBR322 or RSV-neo plasmids. The results reported were obtained from at least four independent experiments, each carried out in duplicate with at least two different DNA preparations for each plasmid.

For α_1-adrenergic stimulation, 10^{-4} M phenylephrine (PE) was added to the cells for 36 to 48 h. Epidermal growth factor (EGF) (10 ng/ml) and TPA (100 ng/ml) treatments were as indicated for various times. Except for PE treatment that resulted in changes in the spontaneous beating of the cardiocytes and produced a significant enlargement of myocytes, there was no significant change in cell morphology or cell number at the end of the treatments. RNA extractions and Northern (RNA) blot hybridization were done as described by Argentin et al. (5).

Plasmids. The hGH plasmids containing various rat ANF promoter fragments have been described previously by Argentin et al. (5). ANF-luciferase constructs were obtained by subcloning the rANF promoter (4) in the pXP-2 vector (16). 5′ deletions were generated by using appropriate restriction enzyme sites or by polymerase chain reaction (PCR) amplification. All PCR-generated mutants were confirmed by sequencing. The heterologous ANF promoter constructs were obtained by inserting a 500-bp (−700 to −135 bp) *Hin*dIII fragment in the *Hin*dIII site upstream of the −81-bp minimal thymidine kinase (TK) promoter or by using synthetic double-strand DNA corresponding to sequences −380 to −345 of the rat ANF promoter (4) inserted in the *Bam*HI site of the TK81-luciferase plasmid. The oncogene expression vectors used are mouse c-*fos* pSV*fos* (53), *fra*-1, *fos* B, c-*jun*, *jun* B, and c-*jun* mutants, and c-jun/GHF-1 chimera, all linked to the RSV long terminal repeat (LTR) (3, 11), kindly provided by M. Karin. pFBJ-2 (v-*fos* [15]), VMM and VMM′ (38), and *fos*SerA and *fos*SerC (42) were kindly provided by I. Verma. The EGR-1 (23) and Pan-1 (40) cDNAs were under control of the cytomegalovirus promoter and were the generous gift of V. Sukhatme and C. Nelson, respectively.

DNA-binding assays. Nuclear extracts were prepared from cardiac myocyte cultures according to the procedure of Schreiber et al. (54). The sequence of oligonucleotides used in gel retardation assays is shown in Fig. 5a. Binding reactions for the TPA response element (TRE) were carried out as described by Smeal et al. (60). Binding with the serum response element (SRE or CArG) probes was done according to the method of Santoro and Walsh (49).

RESULTS

Proto-oncogenes *fos* and *jun* inhibit basal and growth-stimulated ANF promoter activity. In order to evaluate the role of c-*jun* and c-*fos* in basal and growth-stimulated cardiac transcription, we tested their effect on transcriptional activity of the ANF promoter in primary cardiocyte cultures. Cardiocytes derived from neonate rat hearts, which are maintained in monolayer cultures, do not undergo significant cell division and proliferation (58). They express cardiac-specific markers, like ANF, at high levels, and they mimic the hormonal regulation of ANF transcription that is observed in vivo (5). These cells display morphological parameters typical of cardiac muscle cells, such as spontaneous beating and the expression of various growth factor receptors, including the α_1-adrenergic receptor. Stimulation of this receptor with various α_1-adrenergic agonists, such as PE, leads to an increase in the myocyte size and an increase in total RNA and protein content in the absence of any DNA synthesis (58), similar to in vivo hypertrophy. It is also accompanied by genetic reprogramming involving the upregulation of several contractile protein genes and the transient expression of various proto-oncogenes including c-*myc*, c-*fos*, and c-*jun* (62). Figure 1a shows that α_1-adrenergic stimulation of the myocyte cultures led to a significant (two- to threefold) increase in ANF mRNA after 48 h of treatment; longer treatments cause further development of cell hypertrophy and lead to greater (up to fivefold) increases in ANF transcripts. At least part of this induction occurs at the transcriptional level since the ANF promoter was induced two- to threefold in PE-stimulated cells (Fig. 1b). The induction of the ANF promoter is not due to a general increase in the transcriptional activity of the hypertrophied cells because other promoters like the RSV and TK were not similarly induced (Fig. 1b and data not shown). It is apparent that ANF promoter activity is very high in cardiac cells and comparable to that of the RSV promoter. In noncardiac cells (HeLa or L cells), activity of the ANF promoter is only 1 to 3% of RSV (data not shown). Thus, the ANF promoter represents a good model for a cardiac-specific growth-inducible promoter. In agreement with previous reports showing transiently increased levels of c-*fos* and c-*jun* mRNA in PE-stimulated cells, nuclear extracts prepared

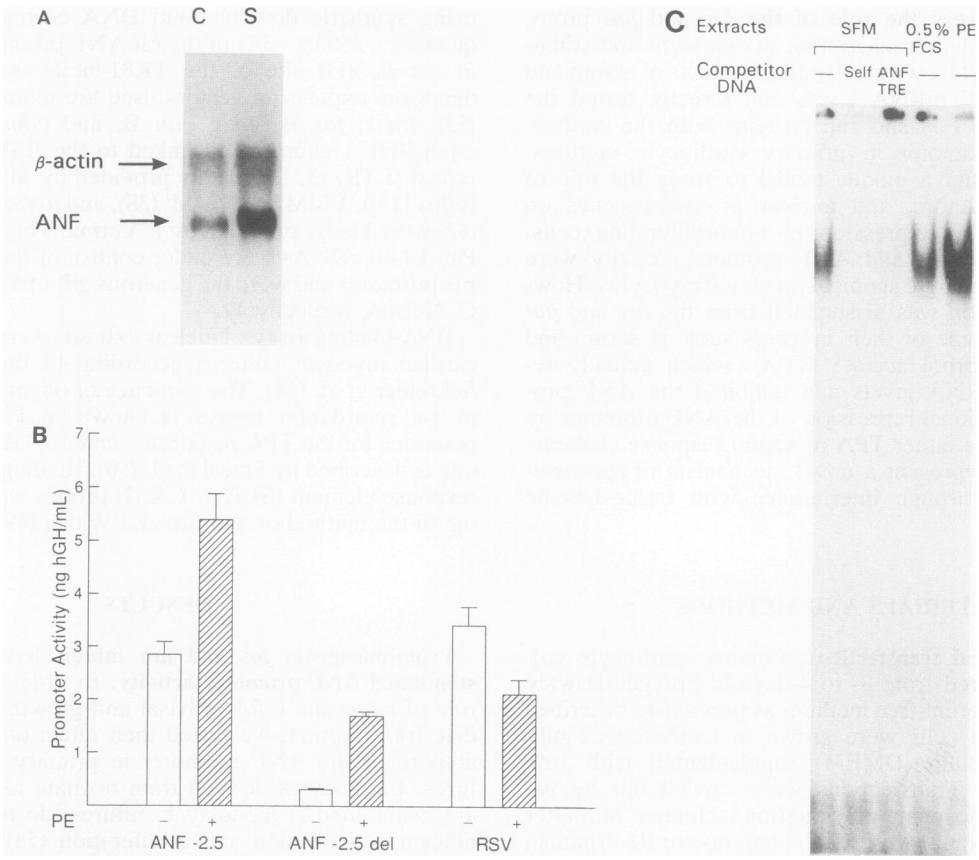


FIG. 1. Induction of ANF transcription and AP-1 activity by PE. Cardiomyocytes were prepared and plated as described in Materials and Methods. (A) Twenty-four hours after plating, 60-mm-diameter dishes were incubated in the absence (C) or presence (S) of 10^{-4} M PE. After a further 48 h, total RNA was extracted for Northern blot hybridization by using specific β-actin and ANF cDNA probes. The ratio of ANF/β-actin mRNA was 2.5-fold higher in PE-treated cells, as calculated from densitometric scanning of the autoradiograph. (B) Cardiac cells (35-mm-diameter dishes) were transfected as indicated with the promoter constructs linked to a human growth hormone reporter gene (ANF-2.5, intact 2.5-kbp ANF promoter; ANF-2.5 del, the 2.5-kbp promoter with an internal deletion between −135 and 640 bp); cells were incubated with (+) or without (−) 10^{-4} M PE for 48 h. In all cases RSV-luciferase was used as an internal control. Similar inductions were obtained when the ANF promoter was linked to luciferase. (C) Nuclear extracts were prepared from control (serum-free medium; SFM) and PE-treated cardiomyocytes (60-mm-diameter dishes), as described in Materials and Methods. For electrophoretic mobility shift assay, about 3 μg of extract was incubated with the double-stranded collagenase TRE oligonucleotide in the presence of 4 μg of poly[d(I-C)]. Samples were run on 5% polyacrylamide gels. Competitions were done with 100-fold excess of unlabelled probe. A 0.5% fetal calf serum (lane labeled 0.5% FCS) treatment (48 h) is shown for comparison with the synthetic, serum-free medium (lanes labeled SFM).

from PE-treated cells showed a marked increase in AP-1 binding (Fig. 1c). The increase in AP-1 activity is not due to a general increase in protein synthesis since similar increases in binding were not observed with other DNA probes, like the SRE (Fig. 5c) or the ANF cardiac-specific (MS) element (discussed later).

Thus, we initially tested whether components of the AP-1 complex directly affect ANF promoter activity. Cotransfection of c-*fos* and c-*jun* expression vectors with various ANF promoter constructs containing at least 700 bp of upstream sequences systematically led to repression of ANF promoter activity in atrial (Fig. 2a) and ventricular myocytes (Fig. 2b). This repression was specific to Jun and Fos proteins and was not observed with other transcription factors including EGR-1, another early growth response protein (64), and Pit-1/GHF-1, a pituitary-specific transactivator (9, 25), or several HLH factors (Fig. 2a and b and 6c; data not shown). Repression of the ANF promoter was specific to muscle cells and was not observed in cell lines such as fibroblast L cells (Fig. 2c) or HeLa cells (data not shown). Proto-oncogenes *fos* and *jun* had a similar inhibitory effect on ANF promoter activity both in quiescent and PE-stimulated cardiomyocytes (Fig. 2d). *fos* and *jun* repression of the ANF promoter was dose dependent (Fig. 2e). The greatest repression was observed with the c-*fos* expression vector, which decreased ANF promoter activity 16-fold in atrial myocytes and 5-fold in ventricular myocytes. At 1.5 μg of *fos* expression vector, transcription from the ANF promoter was already inhibited by 60%. In contrast, 3 μg of *jun* B and 15 μg of c-*jun* were required to attain a similar inhibitory effect. When transfected together, *jun* and *fos* inhibitory effects were additive; in ventricles, cotransfection with 2 μg of either c-*fos* and c-*jun* or c-*fos* and *jun* B expression vectors led to a maximal sevenfold repression (data not shown). Interestingly, at low concentrations of c-*jun* expression vector (between 100 ng and 1.5 μg of DNA), a slight (up to twofold in some experiments) but consistent increase in ANF promoter activity was observed. Such increase was never observed

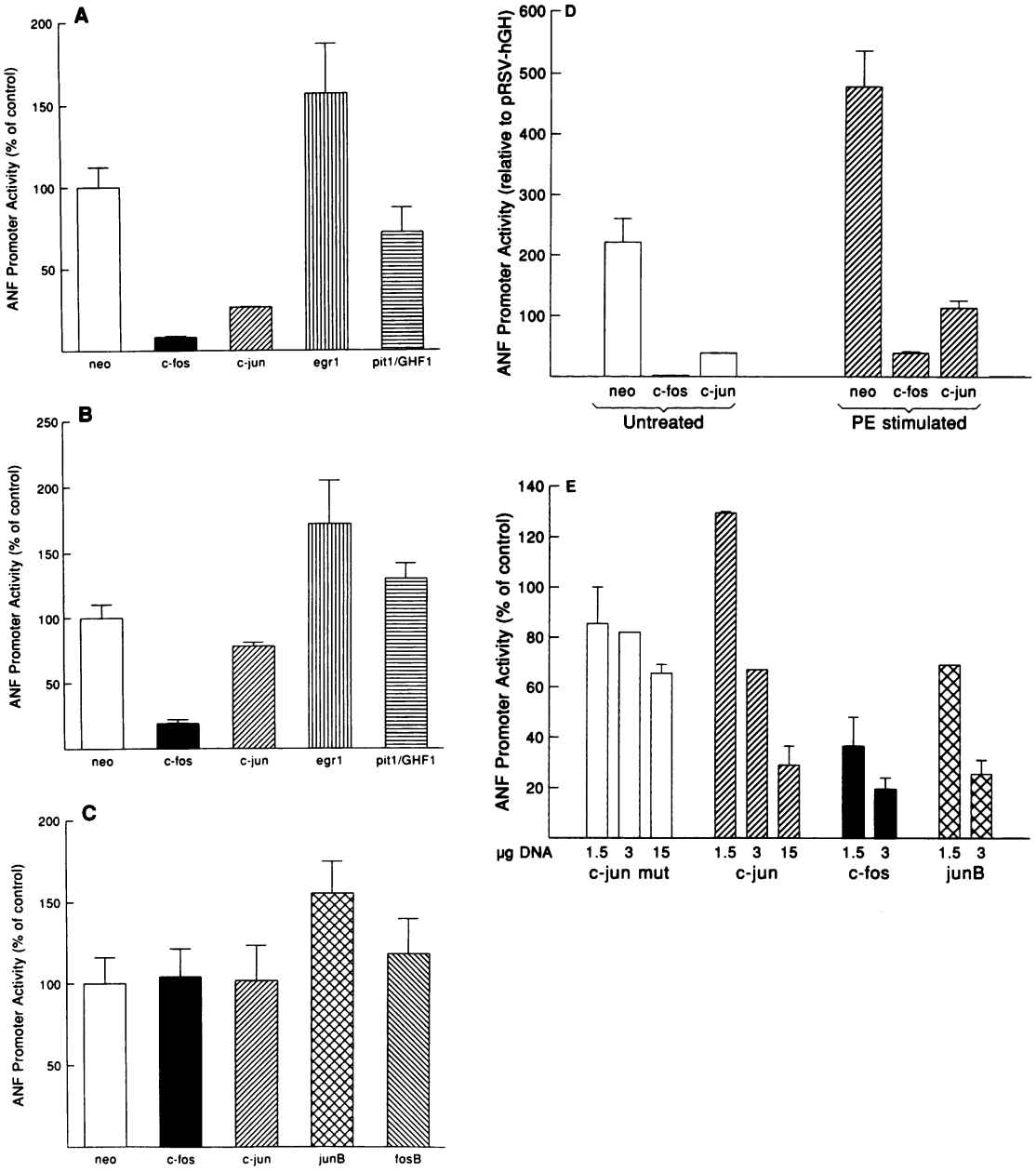
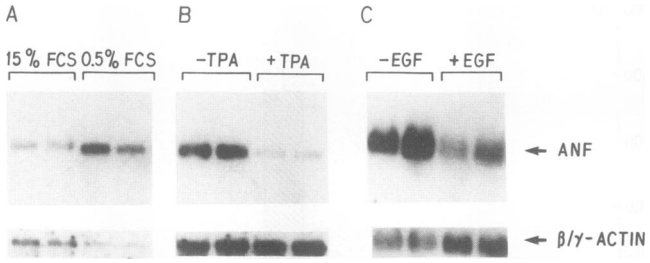


FIG. 2. *jun* and *fos* repression of ANF promoter activity. Atrial (A) or ventricular (B) cardiomyocytes were cotransfected with a 1.0-kbp ANF promoter construct (3 µg) linked to a human growth hormone reporter and either c-*fos* or c-*jun* (3 µg) expression vectors. RSV-luciferase (2 µg) was used as an internal control. After 36 to 48 h, cells were harvested and extracts were assayed for growth hormone and luciferase activity. (C) Specificity of c-*jun* and c-*fos* repression of ANF promoter activity. Fibroblasts L cells were transfected as described above to examine the effect of oncogenes in nonmuscle cells. (D) Cardiomyocytes were cotransfected with the ANF expression vector described for panel A and either c-*fos* or c-*jun* vectors and then treated with (PE-stimulated) or without (untreated) 10^{-4} M PE. Similar results were obtained with ANF promoter fragments up to −3 kbp linked to either hGH or luciferase reporters. (E) Cardiac cells were cotransfected with 3 µg of the 1.0-kbp ANF-hGH and increasing amounts of oncogene vectors as indicated. The total amount of DNA per dish was kept constant by addition of pBR322 DNA. neo, neomycin; mut, mutant; pit1/GHF1 and eyr1 expression vectors are described in Materials and Methods.

with the c-*fos* vector whether at low (100 to 500 ng) or high (>1 µg) DNA input. This may reflect transient derepression, perhaps due to initial titration by c-*jun* of endogenous c-*fos*.

Inducers of AP-1 activity repress endogenous ANF gene expression. In view of the unexpected effect of c-*fos* and c-*jun* on ANF promoter activity, we also examined the inhibitory effect of the Fos and Jun proteins on the in vivo expression of the endogenous ANF gene. In particular, we tested the effect of inducers of AP-1 activity on endogenous ANF mRNA levels in the cardiocyte cultures. Serum and the tumor-promoting phorbol esters such as TPA are well known



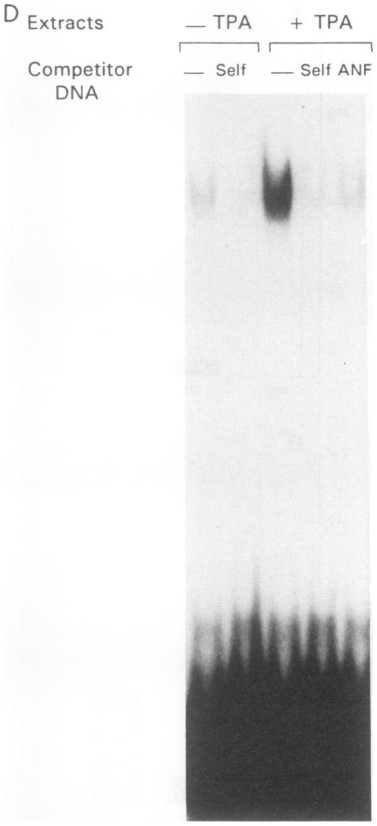


FIG. 3. ANF transcription is repressed by inducers of AP-1 activity. Cardiac cells were plated in 15% fetal calf serum (FCS). After 18 h, the cells were washed and kept for 3 days in 0.5% fetal calf serum (A) or serum-free medium (B and C). Agents were added as indicated for the last 12 to 18 h of incubation. Representative Northern blots from atrial (A) and ventricular (B and C) cultures are shown. An equal amount of total RNA was used (1 μg for atria and 6 μg for ventricles). (D) AP-1 activity in cardiocytes. Cardiocytes (60-mm-diameter dishes) were prepared as described in Materials and Methods and treated with (+) or without (−) TPA (100 ng/ml) for 18 h in serum-free medium. Binding reactions and electrophoretic mobility shift assays were performed with ^{32}P-labeled collagenase TRE as probe. For competitions, 100-fold molar excess of unlabeled TRE duplex (Self) or ANF-TRE (ANF) was used. The sequence of the oligonucleotides used is shown in Fig. 5a.

inducers of *jun* and *fos* synthesis and of AP-1 activity (1, 50). As shown in Fig. 3, stimulation of cardiocytes (maintained in low or serum-free medium) with 15% serum (Fig. 3a) or TPA (Fig. 3b) for 8 to 12 h led to a three- to fourfold decrease of endogenous ANF mRNA levels. We also tested the effect of EGF on ANF mRNA levels. It has previously been shown that EGF stimulates c-*fos* transcription (46) and induces several members of the *jun* family (47). Cardiac cells were kept for 3 days in serum- and mitogen-free medium (5) and then stimulated with 10 ng of EGF per ml for 8 to 12 h. This treatment again led to a marked fourfold decrease in ANF mRNA levels (Fig. 3c). Inhibition of ANF transcripts by TPA, EGF, and serum was accompanied by a similar three- to fourfold decrease in ANF peptide secretion as measured by radioimmunoassay in culture media (data not shown). None of the treatments had any visible effect on cell number or morphology. In order to verify that AP-1 activity was positively modulated by these stimuli in the terminally differentiated cardiac cells, nuclear extracts were prepared from untreated and treated myocytes and tested for AP-1-binding activity by using the TRE from the collagenase promoter (1). Stimulation of cardiac cells with TPA (Fig. 3d), EGF (data not shown), or serum (Fig. 7) led to a marked induction of AP-1 binding. The mobility of the AP-1 complex in the cardiac extracts was indistinguishable from that obtained with HeLa- or L-cell extracts maintained in 15% serum (not shown). These results clearly show that serum, EGF, and TPA which induce Fos and Jun proteins repress cardiac ANF transcription. Given their effects on both transfected and endogenous ANF promoter activity, it appears that Jun and Fos proteins are negative regulators of ANF transcription in cardiac cells. These results may appear to conflict with the PE-induced increase in AP-1 binding (Fig. 1c) and the positive effect of PE on ANF promoter activity (Fig. 1b). They certainly suggest a dissociation between the transcriptional responses to PE and to the oncogenes. Therefore, the localization of ANF promoter sequences conferring responsiveness to PE and Fos and Jun proteins was undertaken.

c-*fos* inhibition is not mediated by AP-1 or SRE. The specificity of *jun* and *fos* repression of the ANF promoter to cardiac cells suggested that repression might involve interaction with a tissue-specific element. Because sequences between −130 and −700 bp are required for cardiac specificity of the ANF promoter (5), we first tested whether these sequences were necessary for oncogene repression. An internal deletion of this region which reduces promoter activity to 20% of control was no longer responsive to TPA treatment nor repressed by c-*fos* transfection (Fig. 4). This deletion is, however, still positively regulated by PE (Fig. 1b). This result suggests a dissociation of ANF transcriptional induction following PE stimulation from Fos and Jun proteins. Similarly, while the 5′ deletion at −640 bp was still repressed by *fos*, deletion of sequences up to −135 bp abolished the inhibitory response (Fig. 4). Thus, these experiments map the region required for oncogene repression to a 500-bp fragment of the ANF promoter which is necessary for cardiac-specific expression. Sequence analysis of this region reveals (Fig. 5a) sequence homologies to TPA (−450 bp) and serum response (−400 bp) elements. In addition, functional studies have located two cardiac-specific elements around −370 and −190 bp which bind nuclear proteins that are not related to the HLH family of transcription factors (3a). Since the SRE site was shown to be involved in c-*fos* inhibition of its own promoter (57), we tested the role of the ANF promoter region containing this and the TRE-like elements both in in vitro binding and in transfection experiments. An oligonucleotide corresponding

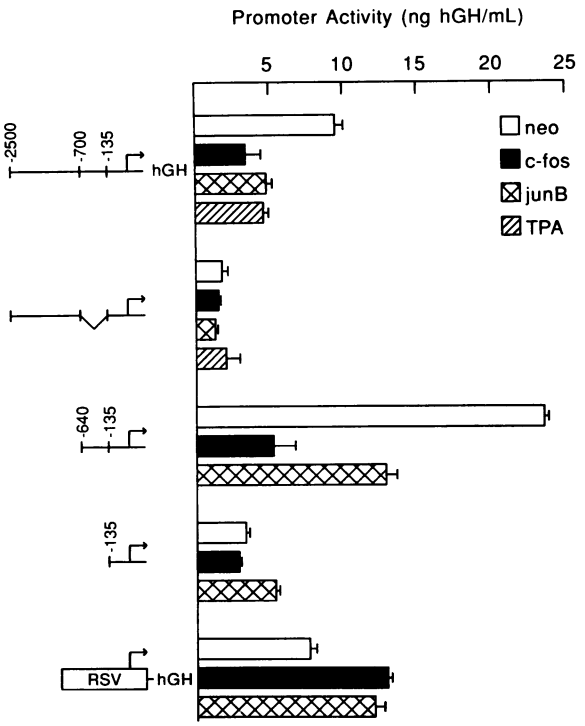


FIG. 4. Effects of TPA and c-*fos* on the activity of the proximal ANF promoter. Cardiomyocytes were cotransfected with ANF promoter hGH constructs and either c-*fos* or RSV-neomycin (neo; control) vectors. RSV-luciferase was used as an internal control. TPA (100 ng/ml) was added for 18 h to cells transfected with ANF-hGH expression vectors. Approximately 48 h after being plated, cells were harvested and extracts were assayed for growth hormone and luciferase activity.

to sequences −422 to −460 bp of the rat ANF promoter (4) and containing an AP-1 consensus (referred to thereafter as ANF-TRE [Fig. 5a]) was used for gel shift assays. Binding of nuclear extracts from HeLa (data not shown) or cardiocyte cells (Fig. 5b) to the ANF-TRE produced two complexes that were inhibited by the collagenase TRE. The ANF-TRE was also able to inhibit binding over the collagenase TRE (Fig. 1c). Similar increases in binding activity over the ANF-TRE were observed as with the collagenase TRE when cardiocyte extracts prepared from TPA- or serum-stimulated cells were used (Fig. 5b). Thus, the ANF promoter contains an element capable of binding to members of the AP-1 complex. Similar in vitro binding experiments were carried out by using a 30-bp synthetic probe containing the SRE/CArG consensus (termed ANF-CArG). Cardiac nuclear extracts showed strong specific binding to this sequence (Fig. 5c). The binding pattern was identical to that obtained with the skeletal actin CArG element (49) and the top complex corresponding to binding of serum response factor(s) (SRF) was inhibited by the *fos* SRE (Fig. 5c). Interestingly, treatment of cardiac cells with TPA, serum, EGF, or PE had no significant effect on binding to this element (Fig. 5c).

In order to assay the functional role of the ANF-TRE and CArG element, successive 5′ deletions were obtained and cotransfected with the c-*fos* expression vector. As shown in Fig. 6a, a TRE deletion construct was still repressed by c-*fos*, and a further 5′ deletion, which removed the SRE/CArG element, was also repressed. Repression by c-*fos* was lost when sequences up to −135 bp were deleted. Because the 40-bp deletion between ANF-180 and ANF-135 which reduces basal promoter activity to 15% of control (only in cardiac cells) was no longer repressed by c-*fos*, we asked whether c-*fos* repression of the ANF promoter was due to interference with the cardiac-specific (MS) element. When a synthetic oligonucleotide containing the core motif shown in Fig. 5a was polymerized in front of the ANF −135 fragment, repression by c-*fos* was recovered (Fig. 6a). Similarly, the 500-bp ANF fragment and a synthetic MS element were able to confer both cardiac-specific activation and *fos* repression to the herpes simplex virus TK promoter (Fig. 6b). Transcriptional activation and *fos* inhibition of these heterologous promoter constructs was restricted to cardiac cells and was not observed in other cells like L cells (Fig. 6c). Again, interference with the positive effect of either the 500-bp fragment or the MSE was specific to *fos* and *jun* and was not observed with other transactivators (Fig. 6c and data not shown). Thus, these experiments localized the ANF element which is necessary and sufficient for c-*fos* repression to a *cis*-acting cardiac-specific element. Mobility shift assays were then carried out to examine the effect of increased Fos and Jun proteins on the interaction of the MSE with proteins present in cardiocyte nuclear extracts. None of the experimental conditions which led to increased AP-1 activity and decreased ANF mRNA levels as did serum or TPA treatment had any qualitative or quantitative effect on protein-DNA binding over this element (Fig. 7). Similarly, PE treatment, which induces AP-1 activity but also increases ANF mRNA levels and ANF promoter activity (Fig. 1), had no effect on the MSE binding to cardiac nuclear factors (data not shown). These findings suggested that the inhibitory effect of the proto-oncogenes is not at the level of DNA binding but that it may involve interference with formation of a higher-order active complex over the myocyte-specific element (MSE).

The C terminus of c-*fos* is not required for repression. In order to elucidate the mechanism of repression, we tested the ability of other Fos proteins to repress the ANF promoter. Two Fos-related proteins, Fra-1 (13) and Fos B (72), and the viral Fos protein (v-Fos) (15) were tested. As shown in Fig. 8a, all Fos-related proteins were able to repress ANF transcription in atrial myocytes, although in general to a lower extent than c-Fos. v-Fos, which shares the first 332 amino acids with c-Fos but diverges considerably at the C-terminal region, was able to repress the ANF promoter although it is unable to repress the c-*fos* promoter (50). A recombinant v-Fos protein in which the C terminal of v-*fos* was replaced by the corresponding c-Fos region (v/c Fos) repressed the ANF promoter to the same extent as that observed with the v-Fos protein. Furthermore, two C-terminal phosphorylation-deficient mutants of c-*fos*, *fos*-SerA and *fos*SerC, which no longer repress transcription from the c-*fos* promoter (42), retain the ability to transrepress the ANF promoter. These results suggest that, in contrast to repression of the c-*fos* promoter in proliferating cells, the C-terminal domain of the c-Fos protein is not crucial for repression of cardiac transcription in the nondividing myocytes.

Repression by c-*jun* requires the N-terminal activation domain. Similarly, we tested the inhibitory effect of various c-*jun* mutants on the ANF promoter (Fig. 8b). Deletion mutants lacking the leucine-zipper region (ΔLZ) or the proline-glutamine-rich region (Δ194-223) were still able to *trans*-repress. In contrast, removal of the activation domain

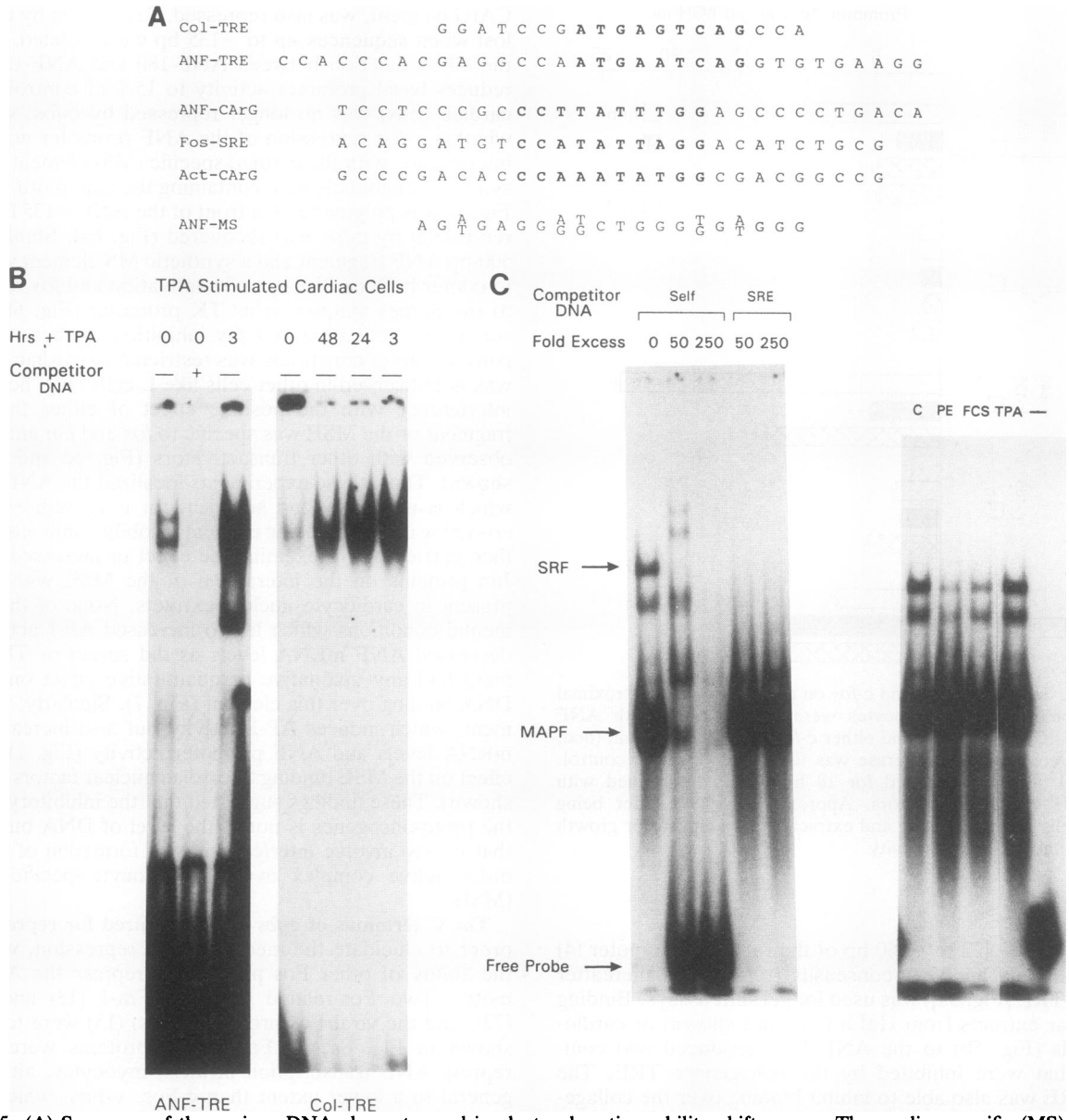


FIG. 5. (A) Sequence of the various DNA elements used in electrophoretic mobility shift assays. The cardiac-specific (MS) sequence represents the consensus derived from the −370 and −190 bp ANF elements. The ANF-TRE is centered around −490 bp, and the ANF-SRE is found around −400 bp. (B) AP-1 activity in nuclear extracts of cardiomyocytes following TPA treatment. Cardiac cells in serum-free medium were treated with (+) and without (−) TPA (100 μg/ml) for the times indicated. Nuclear extracts were analyzed by gel shift mobility assays using ^{32}P-labeled ANF and collagenase TRE (Col-TRE) probes. Competitor DNA for the ANF-TRE was 100-fold excess of unlabeled ANF-TRE. (C) Binding of nuclear factors to the serum response element (ANF-SRE; see Fig. 5A). Nuclear extracts were prepared from untreated cardiomyocytes (C) and from PE-, fetal calf serum (FCS)-, or TPA-stimulated cardiomyocytes approximately 48 h after plating, and they were used in the gel shift assay with ^{32}P-labeled ANF-SRE as probe. Approximately 3 μg of protein was electrophoresed on a 5% polyacrylamide gel in the presence of 0.5 μg of poly[d(I-C)]. Competition experiments were done as indicated with a 50- or 250-fold excess of either unlabeled ANF-SRE duplex (Self) or an excess of unlabeled c-*fos* SRE duplex (SRE). The designation of the muscle actin promoter factor (MAPF) is putative and is based on previous work by Santoro and Walsh (49). Complexes presumed to contain the serum response factor (SRF) and free probe are indicated. A control lane (−) with probe alone is also shown.

(Δ1-87 and Δ6-194) completely abrogated the *jun* inhibitory effect. These results clearly pointed to a requirement for the N-terminal activation domain for *jun* interference with cardiac transcription. To further examine the role of the amino terminus, we used a chimeric Jun-GHF-1 protein containing the DNA-binding region of the pituitary transcription factor GHF-1 fused to the N-terminal activation domain of c-*jun* (3). Cotransfection of ANF promoter constructs with this vector caused a similar decrease in transcriptional activity as that observed with wild-type c-*jun*. Thus, the activation domain of c-*jun* is sufficient for *trans*-repression perhaps through interaction with one or more limiting cardiac coactivators.

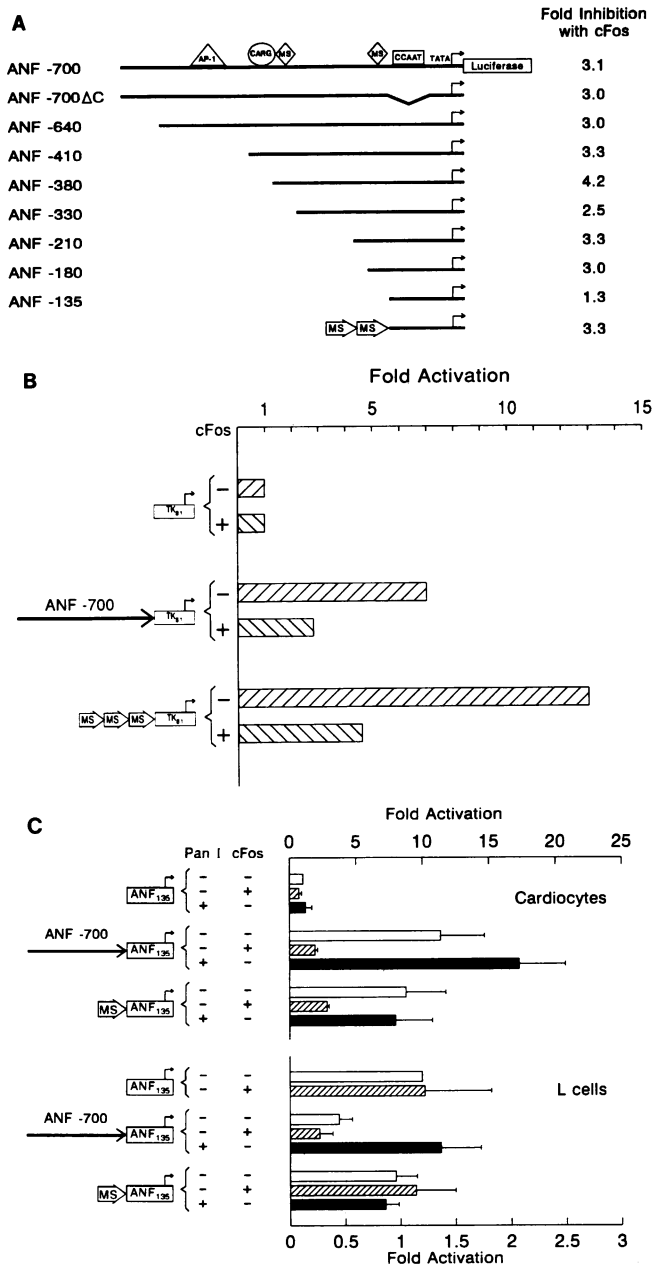


FIG. 6. *fos* interference with a tissue-specific activating element in the ANF promoter. (A) Ventricular cardiomyocytes were cotransfected with one of various ANF promoter deletions (which were linked to a luciferase reporter gene) and 5 μg of c-*fos* expression vector. RSV-hGH was used as an internal control. Results are the means from three experiments performed in duplicate and are expressed as fold inhibition relative to the activity of the corresponding ANF promoter cotransfected with RSV-neomycin. The MS oligonucleotide corresponds to the −370 site. Similar results were obtained for the −190 site. (B) ANF promoter fragments were linked to the herpes simplex virus TK promoter extending to position −81 bp. All promoter constructs were coupled to a luciferase reporter, and the internal control was RSV-hGH. Constructs containing one, two, or three copies of MSE were repressed in both atrial and ventricular cells. The results shown were obtained in ventricular myocytes; greater inhibition was obtained when selected constructs were tested in atrial cells (the MS-ANF construct was repressed four- to fivefold). (C) Effect of the 500-bp fragment and the MSE on basal and *fos*-induced repression of the minimal ANF promoter in cardiocytes and fibroblasts. Note the different scales used for the two cell types. Transfection with an expression vector for an unrelated transcription factor, Pan-1 (a ubiquitous HLH protein), was used as the control. The ANF constructs used in this figure were linked to the hGH gene and RSV-luciferase was used as an internal control. The data represent the means (± standard errors [error bars]) of six different determinations.

DISCUSSION

In skeletal muscle cell lines in which differentiation and proliferation are mutually exclusive, oncogenes *jun* and *fos* repress muscle-specific gene expression. This repression is due in part to interference with the transcriptional activity of the muscle-specific transcription factors myogenin and MyoD (7, 19, 34). In this work, we have investigated the role of Fos and Jun proteins in cardiac muscle transcription by using quiescent and growth-stimulated cardiac cells. Unlike in skeletal muscle, growth stimulation of cardiac cells is associated with coinduction of several proto-oncogenes and tissue-specific markers which led to the speculation that, in contrast to skeletal muscle, Fos and Jun proteins may be positive regulators of cardiac genes. Our results, with the cardiac ANF gene as marker, do not support this hypothesis and actually reveal that *jun* and *fos* act as negative modulators of cardiac-specific transcription. This is evidenced by the inhibitory effect of serum, EGF, and TPA on endogenous myocardial ANF transcripts and, in parallel, by repression of ANF promoter activity by Jun and Fos proteins in cotransfection assays. Transcriptional repression appears targeted at a cardiac-specific *cis*-acting element which is distinct from the binding site of myogenin and MyoD proteins. The mechanism underlying transcriptional interference with this tissue-specific activator appears to be distinct from other mechanisms of repression involving the Fos proteins; indeed, *fos* repression of ANF does not require DNA sequences known to be targets of *fos* repression like TPA, serum or steroid hormone-responsive elements or the C-terminal domain of c-*fos* which is required for *fos* promoter autorepression. The lack of any detectable change in protein-DNA binding over the tissue-specific element and the finding that the N-terminal activation domain of c-*jun* is sufficient for repression suggest that proto-oncogene interference may involve interaction with limiting tissue-specific coactivators.

***jun* and *fos* repression of cardiac transcription: dissociation of *fos* and *jun* from genetic reprogramming in cardiac hypertrophy.** Work from several laboratories has established a role for nuclear proto-oncogenes in growth and differentiation of proliferating cells (reviewed in reference 2). Cardiac muscle cells offer a unique model to define the role of proto-oncogenes in nondividing cells. These cells respond to growth stimulation via two distinct pathways: during embryonic development, cardiomyocytes undergo proliferative growth. However, during the first week of postnatal development, the mitotic index of the heart declines precipitously and cardiac muscle cells lose their ability to proliferate (12). Growth stimulation or increased work load on the heart due to mechanical or pressure overload or to hormonal and trophic factors like steroids, thyroid hormone, peptide growth factors, and adrenergic agents lead to the enlargement of a fixed number of myocytes, which results in cardiac hypertrophy and ultimately cardiac dysfunction. Several cardiac genes undergo developmental switching, and, in many cases, th se genes that are expressed at high levels

during embryonic development and down-regulated in postnatal life are reexpressed in cardiac hypertrophy. However, the mechanism which modulates cardiac gene expression in hypertrophic growth remains elusive. Circumstantial evidence based on the observation that c-*myc* and c-*fos* expression is induced by all hypertrophic stimuli tested so far has led to speculations on a role for these proto-oncogenes in the genetic reprogramming of the hypertrophic heart. Since ANF is the best-documented cardiac-specific gene that is also induced by all hypertrophic stimuli (reviewed in reference 24), we have used it to directly test the role of Fos and Jun proteins in transcriptional activity of the nondividing cardiac myocytes.

Cotransfection of the ANF promoter with *jun* or *fos* expression vectors led to transcriptional repression. The inhibitory effect was the same whether cells were quiescent or stimulated with the hypertrophic α_1-adrenergic agonist PE. Inducers of *jun* and *fos* synthesis and AP-1 activity (serum, TPA, or EGF) had an inhibitory effect on endogenous ANF transcripts and on ANF promoter activity. These results uncouple Jun and Fos proteins from the reactivation of a growth-responsive cardiac gene in at least one model of cardiac hypertrophy. Thus, although stimulation of cardiac myocytes with the α_1-adrenergic agonist PE produces cell hypertrophy and increased AP-1 activity, induction of ANF transcription in this model must result from activation of a *jun-fos* independent pathway. This is clearly the case since overexpression of Jun and Fos proteins neither potentiates nor blocks the effect of PE. Furthermore, *jun-fos* inhibition and PE stimulation can be dissociated at the level of promoter elements. Interestingly, promoter constructs (for example, ANF-135 and ANF-2.5 del) that are no longer repressed by the oncogenes are superactivated by PE (Fig. 1b and data not shown). Thus, rather than stimulating cardiac transcription during hypertrophic growth, Jun and Fos proteins may actually function in a negative feedback manner to limit activation of embryonic genes that may be detrimental to the adult heart. Our results cannot however rule out a different involvement of proto-oncogenes in genetic reprogramming in other models of hypertrophy or during normal embryonic development. In this respect, it is interesting to note that induction of c-*fos* or c-*jun* by systolic wall stress is down-regulated in chronic hypertrophy (56); furthermore, immunocytochemical studies using c-*fos* antibodies failed to detect c-*fos* peptides in cardiac muscle cells at any stage of embryonic development although c-*fos* was present during postnatal cardiac development exclusively in the smooth muscle cells of the coronary artery (61). In two other studies, c-*fos* mRNA could not be detected in the embryonic heart at day 12, 15, or 18 (30, 51), a developmental stage at which the ANF gene is expressed at nearly maximal levels in both atria and ventricles (71). In agreement with our data, others have recently reported that the human ANF promoter is also repressed by c-*fos* in quiescent myocytes (31). Whether *jun* and *fos* have different effects on other cardiac genes is not known at this stage. However, it is interesting that the EGR-1 gene, which encodes an early growth response zinc finger protein which is developmentally expressed in the heart (64) and is induced in PE-stimulated cardiac myocyte cultures (26), is also transcriptionally repressed by c-*fos* (21).

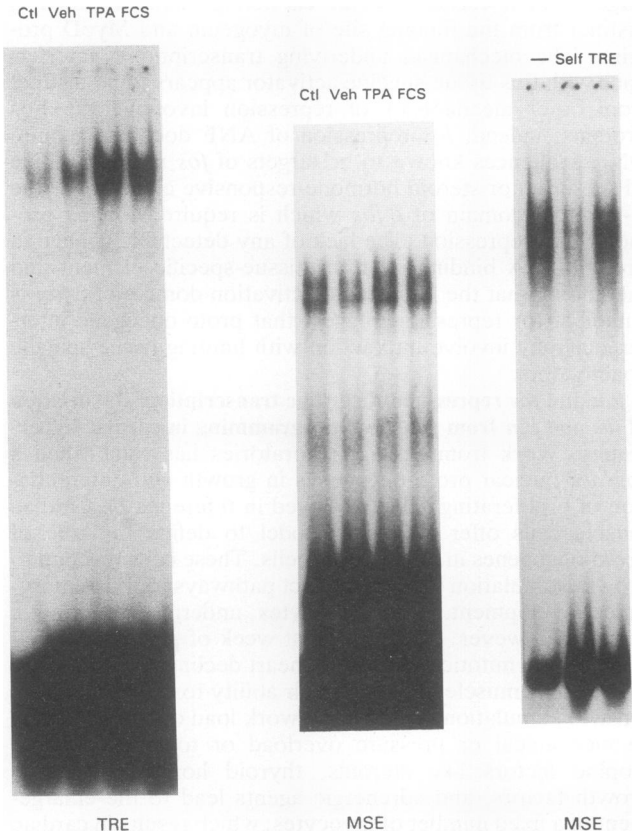


FIG. 7. Effect of TPA and serum on binding of nuclear proteins to the collagenase TRE and to the ANF MSE. The TRE gel retardation assay was performed as described in Materials and Methods. Similar binding and gel conditions were used for detection of binding to the MSE, with the exception that binding reactions (20 min) and electrophoresis were carried out at room temperature. Lanes: Ctl, control nuclear extracts from untreated cardiomyocytes; Veh, cells treated with vehicle alone (0.1% dimethyl sulfoxide); FCS, cells treated with fetal calf serum. The same extract preparations were used for both TRE and MSE. Binding over the MSE was effectively inhibited by competition with a 50-fold excess of cold MSE (Self) but not with excess collagenase TRE. Lane – shows control binding with no added competitor DNA.

Inhibitory effects of mitogens and Jun and Fos proteins on a tissue-specific marker in nondividing cells. As discussed earlier, cardiac myocytes differ from skeletal muscle cells in that growth and differentiation are not mutually exclusive. For example, the ANF gene is expressed at a similarly high level in the proliferating embryonic atrial myocytes and in the adult differentiated atria; in ventricular cells, expression of the ANF gene is positively correlated with growth stimulation whether in proliferating (8, 71) or nondividing myocytes (41, 65). The inhibitory effects of *fos* on ANF transcription are observed both in atrial and ventricular myocytes. In fact, the magnitude of *fos* repression is higher in atrial (over 10-fold) than in ventricular myocytes (usually around 3- to 5-fold), which were prepared in parallel from the same hearts consistent with the higher level of endogenous and transfected ANF transcription in atrial versus ventricular myocytes. Thus, irrespective of their role in genetic reprogramming during hypertrophic growth, *fos* and *jun* exert an inhibitory effect on an early marker of cardiac cell differentiation in nondividing myocytes. Similarly, mitogen stimulation of these cells results in suppression of the ANF gene in the absence of any concomitant increase in cell numbers. These results are in contrast to other studies of muscle (cited in the introduction) and

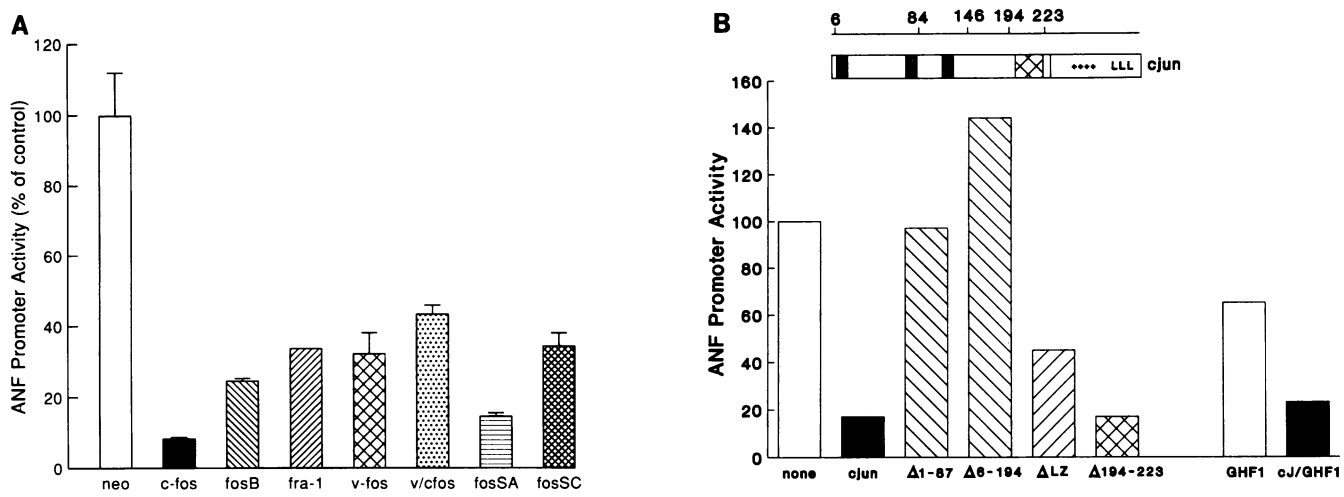


FIG. 8. (A) The C terminus of c-*fos* is not involved in *fos*-mediated repression of ANF transcription. Atriomyocytes were cotransfected with a 1.0-kb ANF promoter-hGH construct and various *fos* family members or mutants, as shown. The v-*fos*–c-*fos* recombinant encoded v-*fos* but with its C terminus replaced by that of c-*fos* (15). The *fos*SA (*fos*SerA) and *fos*SC (*fos*SerC) mutants represent serine-to-alanine mutations in the C terminal of c-*fos*, which have been described previously (42). The results are from two independent experiments, each carried out in duplicate. Similar results (with lower-fold repression) were also obtained with ventricular cells. neo, neomycin. (B) The N-terminal activation domain of c-*jun* is necessary for repression. The structure of the c-*jun* protein is shown at the top of the figure. The *jun*-GHF-1 (cJ/GHF1) recombinant contains the first 194 amino acids of c-*jun* fused to the DNA-binding region of GHF-1. The results are the averages of three different experiments in atrial cells. ΔLZ indicates deletion of the leucine zipper domain.

nonmuscle (17) cell lines in which the inhibitory effect of *fos* on tissue-specific markers was concomitant with changes in cell growth. It is particularly noteworthy that despite marked differences in growth and differentiation of skeletal and cardiac muscle cells, mitogen stimulation and Jun and Fos proteins repress the expression of muscle-specific genes in both muscle cell types. The present study thus offers a unique opportunity to dissociate the effects of oncogenes in differentiation from their role in cell growth and proliferation.

Mechanism of proto-oncogene repression. In addition to their well documented role as transcriptional activators, Jun and Fos proteins can also act as negative regulators. c-Fos has been shown to feedback repress its own promoter (50) and to inhibit transcription of EGR-1, another early growth response gene (21). In both cases, repression is mediated by SREs (21, 57) and requires the C-terminal domain of c-*fos* (21, 42, 68). *fos* has also been shown to inhibit glucocorticoid receptor-dependent transcription via a region in the N terminus of c-*fos* that is poorly conserved among *fos* family members (36). The c-Jun protein is also able to repress steroid receptor-dependent transcription via protein-protein interaction that requires the DNA-binding domain of c-*jun* (55) and results in the inhibition of DNA binding (69).

Cardiac cells express various steroid receptors, and ANF transcription is positively regulated by glucocorticoids (5). However, oncogene repression of ANF promoter activity is not due to antagonism of steroid-dependent transcription since repression is seen in the presence or absence of glucocorticoids and since promoter fragments which are no longer responsive to glucocorticoids (5) are repressed by *jun* and *fos*. The involvement of an SRE in *fos*-mediated repression of ANF transcription was also ruled out. Like many muscle-specific promoters, the ANF promoter contains an SRE/CArG motif (Fig. 5a), which strongly binds proteins present in cardiac nuclear extracts. Although the role of this and other CArG elements in muscle-specific transcription is not yet clear, its presence is not required for *fos* repression as judged from deletion analysis (Fig. 6a).

It is noteworthy that *fos* and *jun* inhibition of ANF transcription is specific to cardiac muscle cells, suggesting the requirement for tissue-specific components. This is further confirmed by the observation that a promoter fragment (ANF-135) which lacks tissue specificity and shows similar activity in cardiac and noncardiac cells is no longer repressed by c-*fos* (Fig. 6a and c). Conversely, a heterologous promoter containing a 500-bp ANF fragment in front of the TK promoter which shows cardiac-specific activation is also repressed by c-*fos* (Fig. 6b). Using a combination of in vitro studies, we have identified an MS element within this 500-bp region (3a). c-*fos* is able to interfere with MS-dependent cardiac activation whether the MS element is placed upstream of a minimal ANF (Fig. 6a and c) or TK promoter (Fig. 6b), suggesting that oncogene repression is targeted at this tissue-specific element. However, the oncogenes do not seem to directly interfere with binding over this element since nuclear extracts prepared from serum, TPA, or EGF-induced cells which have markedly increased levels of AP-1 proteins do not reveal any quantitative or qualitative change in MS binding (Fig. 7). Furthermore, binding over the MSE is not inhibited by Col-TRE or *fos*-SRE oligonucleotides (Fig. 7 and data not shown). *jun* and *fos* could interfere with transcriptional activation perhaps at a post-DNA-binding step through posttranslational modifications or by competing for a cardiac-specific coactivator.

Recently, two independent studies have shown that c-*jun* may interfere with muscle-specific transcription by suppressing MyoD and myogenin-dependent activation of the muscle creatine kinase enhancer (7, 34). The exact mechanism by which this *trans*-repression occurs appears controversial, as one study suggested a direct physical interaction between the c-*jun* leucine zipper domain and the HLH

region of MyoD, which would effectively interfere with binding of MyoD over its site (7). The other study mapped repression to the amino terminus of c-*jun* (34) and suggested that repression may involve interaction between the N terminus of *jun* and a muscle-specific coregulator. The ANF-MSE has no sequence homology to the binding sites of the MyoD family of transcription factors and interacts with nuclear proteins that appear to be distinct from the HLH proteins. However, our results suggest a requirement for the N-terminal activation domain of c-*jun* and are consistent with the possibility that proto-oncogene interference may be due to interaction and titration of one or more limiting muscle-specific coactivators.

Several members of the *fos* family also repress cardiac-specific transcription although c-*fos* appears to be the most potent. Interestingly, repression of cardiac transcription is independent of the C terminus of *fos*, which is crucial for repression of the c-*fos* and EGR-1 promoters. Indeed, repression is also observed with v-*fos* and two C-terminal *fos* mutants which are defective in *trans*-repression of the c-*fos* promoter (42). *fos* can also suppress myogenin (34) and MyoD (33) transactivation of the muscle creatine kinase enhancer, and this property is shared by v-*fos*. Transformation of myoblasts with c- or v-*fos* blocks muscle differentiation and inhibits endogenous MyoD transcripts (33). Together, these studies suggest different mechanisms for *fos* inhibition of early growth response genes and *fos* interference with muscle-specific transcription. Thus, it may be possible to dissociate the *trans*-repressor properties of c-*fos* during cell growth and proliferation from its regulatory functions on cell differentiation.

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REFERENCES

1. **Angel, P., M. Imagawa, R. Chiu, B. Stein, R. J. Imbra, H. J. Rahmsdorf, C. Jonat, P. Herrlich, and M. Karin.** 1987. Phorbol ester-inducible genes contain a common cis element recognized by a TPA-modulated trans-acting factor. Cell **49:**729–739.
2. **Angel, P., and M. Karin.** 1991. The role of jun, fos and the AP-1 complex in cell proliferation and transformation. Biochim. Biophys. Acta **107:**129–157.
3. **Angel, P., T. Smeal, J. Meek, and M. Darin.** 1989. Jun and v-jun contain multiple regions that participate in transcriptional activation in an interdependent manner. New Biol. **1:**35–43.

3a. **Ardati, A., S. Tremblay, and M. Nemer.** Unpublished data.

4. **Argentin, S., M. Nemer, J. Drouin, G. K. Scott, B. P. Kennedy, and P. L. Davies.** 1985. The gene for rat atrial natriuretic factor. J. Biol. Chem. **260:**4568–4571.
5. **Argentin. S., Y. L. Sun, I. Lihrmann, T. J. Schmidt, J. Drouin, and M. Nemer.** 1991. Distal cis-acting promoter sequences mediate glucocorticoid stimulation of cardiac atrial natriuretic factor gene transcription. J. Biol. Chem. **266:**23315–23322.
6. **Barton, P. J. R., and M. E. Buckingham.** 1985. The myosin alkali light chain proteins and their genes. Biochem. J. **231:**249–261.
7. **Bengal, E., L. Ransone, R. Scharfmann, V. J. Dwarki, S. J. Tapscott, H. Weintraub, and I. M. Verma.** 1992. Functional antagonism between c-jun and MyoD proteins: a direct physical association. Cell **68:**507–519.
8. **Bloch, K. D., J. G. Seidman, J. D. Naftilan, J. T. Fallan, and C. E. Seidman.** 1986. Neonatal atria and ventricles secrete atrial natriuretic factor via tissue-specific secretory pathways. Cell **47:**695–702.
9. **Bodner, M., J.-L. Castrillo, L. E. Theill, T. Deerinck, M. Ellisman, and M. Karin.** 1988. The pituitary-specific transcription factor GHF-1 is a homeobox-containing protein. Cell **55:**505–518.
10. **Brennan, T. J., D. G. Edmondson, L. Li, and E. N. Olson.** 1991. Transforming growth factor beta represses the actions of myogenin through a mechanism independent of DNA binding. Proc. Natl. Acad. Sci. USA **88:**3822–3826.
11. **Chiu, R., P. Angel, and M. Karin.** 1989. Jun-B differs in its biological properties from, and is a negative regulator of, c-jun. Cell **59:**979–986.
12. **Claycomb, W. C.** 1975. Deoxyribonucleic acid synthesis and nuclear and cytoplasmic deoxyribonucleic acid polymerase activity. J. Biol. Chem. **250:**3229–3235.
13. **Cohen, D. R., and T. Curran.** 1988. Fra-1: a serum-inducible, cellular immediate-early gene that encodes a *fos*-related antigen. Mol. Cell. Biol. **8:**2063–2069.
14. **Coppola, J. A., and M. D. Cole.** 1986. Constitutive c-myc oncogene expression blocks mouse erythroleukaemia cell differentiation but not commitment. Nature (London) **320:**760–763.
15. **Curran, T., G. Peters, C. Van Beveren, N. M. Teich, and I. M. Verma.** 1982. FBJ murine osteosarcoma virus: identification and molecular cloning of biologically active proviral DNA. J. Virol. **44:**674–682.
16. **De Wet, J. R., K. V. Wood, M. DeLuca, D. R. Helinski, and S. Subramani.** 1987. Firefly luciferase gene: structure and expression in mammalian cells. Mol. Cell. Biol. **7:**725–737.
17. **Distel, R. J., H. S. Ro, B. S. Rosen, D. L. Groves, and B. M. Spiegelman.** 1987. Nucleoprotein complexes that regulate gene expression in adipocyte differentiation: direct participation of c-fos. Cell **49:**835–844.
18. **Dmitrovsky, E., W. M. Kuehl, G. F. Hollis, I. R. Kirsch, T. P. Bender, and S. Segal.** 1986. Expression of a transfected human c-myc oncogene inhibits differentiation of a mouse erythroleukaemia cell line. Nature (London) **322:**748–750.
19. **Edmondson, D. G., T. J. Brennan, and E. N. Olson.** 1991. Mitogenic repression of myogenin autoregulation. J. Biol. Chem. **266:**21343–21346.
20. **Gammage, M. D., and J. A. Franklyn.** 1991. Role of proto-oncogenes in the control of myocardial cell growth and function. Clin. Sci. **80:**405–411.
21. **Gius, D., X. Cao, F. J. Rauscher III, D. R. Cohen, T. Curran, and V. P. Sukhatme.** 1990. Transcriptional activation and repression by *fos* are independent functions: the C terminus represses immediate-early gene expression via CArG elements. Mol. Cell. Biol. **10:**4243–4255.
22. **Grossi, M., A. Calconi, and F. Tato.** 1991. v-jun oncogene prevents terminal differentiation and suppresses muscle-specific gene expression in ASV-17-infected muscle cells. Oncogene **6:**1767–1773.
23. **Gupta, M. P., M. Gupta, R. Zak, and V. P. Sukhatme.** 1991. Erg-1, a serum-inducible zinc finger protein, regulates transcription of the rat cardiac alpha-myosin heavy chain gene. J. Biol. Chem. **266:**12813–12816.
24. **Gutkowska, J., and M. Nemer.** 1989. Structure, expression, and function of atrial natriuretic factor in extraatrial tissues. Endocr. Rev. **10:**519–536.
25. **Ingraham, H. A., R. Chen, H. J. Mangalam, H. P. Elsholtz, S. E. Flynn, C. R. Lin, D. M. Simmons, L. Swanson, and M. G. Rosenfeld.** 1988. A tissue-specific transcription factor containing

a homeodomain specifies a pituitary phenotype. Cell **55**:519–529.
26. **Iwaki, K., V. P. Sukhatme, H. E. Shubeita, and K. R. Chien.** 1990. Alpha- and beta-adrenergic stimulation induces distinct patterns of immediate early gene expression in neonatal rat myocardial cells. J. Biol. Chem. **265**:13809–13817.
27. **Izumo, S., B. Nadal-Ginard, and V. Mahdavi.** 1988. Proto-oncogene induction and reprogramming of cardiac gene expression produced by pressure overload. Proc. Natl. Acad. Sci. USA **85**:339–343.
28. **Jackson, T., M. F. Allard, C. M. Sreenan, L. K. Doss, S. P. Bishop, and J. L. Swain.** 1990. The c-*myc* proto-oncogene regulates cardiac development in transgenic mice. Mol. Cell. Biol. **10**:3709–3716.
29. **Komuro, I., T. Kaida, Y. Shibazaki, M. Kurabayashi, Y. Katoh, E. Hoh, F. Takaku, and Y. Yazaki.** 1990. Stretching cardiac myocytes stimulates protooncogene expression. J. Biol. Chem. **265**:3595–3598.
30. **Komuro, I., M. Kurabayashi, F. Takaku, and Y. Yazaki.** 1988. Expression of cellular oncogenes in the myocardium during the developmental stage and pressure-overloaded hypertrophy of the rat heart. Circ. Res. **62**:1075–1079.
31. **Kovacic-Milivojevic, B., and D. G. Gardner.** 1992. Divergent regulation of the human atrial natriuretic peptide gene by c-*jun* and c-*fos*. Mol. Cell. Biol. **12**:292–301.
32. **Langdon, W. Y., A. W. Harris, S. Cory, and J. M. Adams.** 1986. The c-myc oncogene perturbs B lymphocyte development in e-mu-myc transgenic mice. Cell **47**:11–18.
33. **Lassar, A. B., M. J. Thayer, R. W. Overell, and H. Weintraub.** 1989. Transformation by activated ras or fos prevents myogenesis by inhibiting expression of myoD1. Cell **58**:659–667.
34. **Li, L., J. C. Chambard, M. Karin, and E. N. Olson.** 1992. Fos and jun repress transcriptional activation by myogenin and MyoD: the amino terminus of jun can mediate repression. Genes Dev. **6**:676–689.
35. **Lompré, A.-M., B. Nadal-Ginard, and V. Mahdavi.** 1984. Expression of the cardiac ventricular alpha- and beta-myosin heavy chain genes is developmentally and hormonally regulated. J. Biol. Chem. **259**:6437–6446.
36. **Lucibello, F. C., E. P. Slater, K. U. Jooss, M. Beato, and R. Müller.** 1990. Mutual transrepression of fos and the glucocorticoid receptor: involvement of a functional domain in fos which is absent in fosB. EMBO J. **9**:2827–2834.
37. **Matiuck, N. V., and J. L. Swain.** 1992. Proto-oncogenes and cardiac development. Trends Cardiovasc. Med. **2**:61–65.
38. **Meijlink, F., T. Curran, A. D. Miller, and I. M. Verma.** 1985. Removal of a 67-base-pair sequence in the noncoding region of protooncogene fos converts it to a transforming gene. Proc. Natl. Acad. Sci. USA **82**:4987–4991.
39. **Müller, R., and E. F. Wagner.** 1984. Differentiation of F9 teratocarcinoma stem cells after transfer of c-fos proto-oncogenes. Nature (London) **311**:438–442.
40. **Nelson, C., L.-P. Shen, A. Meister, E. Fodor, and W. J. Rutter.** 1990. Pan: a transcriptional regulator that binds chymotrypsin, insulin, and AP-4 enhancer motifs. Genes Dev. **4**:1035–1043.
41. **Nemer, M., J. P. Lavigne, J. Drouin, G. Thibault, M. Gannon, and T. Antakly.** 1986. Expression of atrial natriuretic factor gene in heart ventricular tissue. Peptides **7**:1147–1152.
42. **Ofir, R., V. J. Dwarki, D. Rashid, and I. M. Verma.** 1990. Phosphorylation of the C terminus of fos protein is required for transcriptional transrepression of the c-fos promoter. Nature (London) **348**:80–82.
43. **Olson, E. N., G. Spizz, and M. A. Tainsky.** 1987. The oncogenic forms of N-ras or H-ras prevent skeletal myoblast differentiation. Mol. Cell. Biol. **7**:2104–2111.
44. **Parker, T. G., and M. D. Schneider.** 1991. Growth factors, proto-oncogenes, and plasticity of the cardiac phenotype. Annu. Rev. Physiol. **53**:179–200.
45. **Prochownik, E. V., and J. Kikowska.** 1986. Deregulated expression of c-myc by murine erythroleukaemia cells prevents differentiation. Nature (London) **322**:848–850.
46. **Prywes, R., and R. G. Roeder.** 1986. Inducible binding of a factor to the c-fos enhancer. Cell **47**:777–784.
47. **Quantin, B., and R. Breathnach.** 1988. Epidermal growth factor stimulates transcription of the c-jun proto-oncogene in rat fibroblasts. Nature (London) **334**:538–539.
48. **Rüther, U., C. Garber, D. Komitowski, R. Müller, and E. F. Wagner.** 1987. Deregulated c-fos expression interferes with normal bone development in transgenic mice. Nature (London) **325**:412–416.
49. **Santoro, I. M., and K. Walsh.** 1991. Natural and synthetic DNA elements with the CArG motif differ in expression and protein-binding properties. Mol. Cell. Biol. **11**:6296–6305.
50. **Sassone-Corsi, P., J. C. Sisson, and I. M. Verma.** 1988. Transcriptional autoregulation of the proto-oncogene fos. Nature (London) **334**:314–334.
51. **Schneider, M. D., P. A. Payne, H. Ueno, M. B. Perryman, and R. Roberts.** 1986. Dissociated expression of c-*myc* and a *fos*-related competence gene during cardiac myogenesis. Mol. Cell. Biol. **6**:4140–4143.
52. **Schneider, M. D., M. B. Perryman, P. A. Payne, G. Spizz, R. Roberts, and E. N. Olson.** 1987. Autonomous expression of c-*myc* in BC3H1 cells partially inhibits but does not prevent myogenic differentiation. Mol. Cell. Biol. **7**:1973–1977.
53. **Schönthal, A., P. Herrlich, H. J. Rahmsdorf, and H. Ponta.** 1988. Requirement for fos gene expression in the transcriptional activation of collagenase by other oncogenes and phorbol esters. Cell **54**:325–334.
54. **Schreiber, E., P. Matthias, M. M. Müller, and W. Schaffner.** 1989. Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells. Nucleic Acids Res. **17**:6419.
55. **Schüle, R., P. Rangarajan, S. Kliewer, L. J. Ransone, J. Bolado, N. Yang, I. M. Verma, and R. M. Evans.** 1990. Functional antagonism between oncoprotein c-jun and the glucocorticoid receptor. Cell **62**:1217–1226.
56. **Schunkert, H., L. Jahn, S. Izumo, C. S. Apstein, and B. H. Lorell.** 1991. Localization and regulation of c-fos and c-jun protooncogene induction by systolic wall stress in normal and hypertrophied rat hearts. Proc. Natl. Acad. Sci. USA **88**:11480–11484.
57. **Shaw, P. E., S. Frasch, and A. Nordheim.** 1989. Repression of c-fos transcription is mediated through p67SRF bound to the SRE. EMBO J. **8**:2567–2574.
58. **Simpson, P.** 1985. Stimulation of hypertrophy of cultured neonatal rat heart cells through an alpha1-adrenergic receptor and induction of beating through an alpha1- and beta1-adrenergic receptor interaction: evidence for independent regulation of growth and beating. Circ. Res. **56**:884–894.
59. **Simpson, P. C.** 1988. Proto-oncogenes and cardiac hypertrophy. Annu. Rev. Physiol. **51**:189–202.
60. **Smeal, T., P. Angel, J. Meek, and M. Karin.** 1989. Different requirements for formation of jun:jun and jun:fos complexes. Genes Dev. **3**:2091–2100.
61. **Snoeckx, L. H. E. H., F. Contard, J. L. Samuile, F. Marotte, and L. Rappaport.** 1991. Expression and cellular distribution of heat-shock and nuclear oncogene proteins in rat hearts. Am. J. Physiol. **261**:H1443–H1451.
62. **Starksen, N. F., P. C. Simpson, N. Bishopric, S. R. Coughlin, W. M. F. Lee, J. A. Escobedo, and L. T. Williams.** 1986. Cardiac myocyte hypertrophy is associated with c-myc protooncogene expression. Proc. Natl. Acad. Sci. USA **83**:8348–8350.
63. **Su, H., T. J. Bos, F. S. Monteclaro, and P. K. Vogt.** 1991. Jun inhibits myogenic differentiation. Oncogene **6**:1759–1766.
64. **Sukhatme, V. P., X. Cao, L. C. Chang, C. H. Tsai-Morris, D. Stamenkobich, P. C. P. Ferreira, D. R. Cohen, S. A. Edwards, T. B. Shows, T. Curran, M. M. Le Beau, and E. D. Adamson.** 1988. A zinc finger-encoding gene coregulated with c-fos during growth and differentiation and after cellular depolarization. Cell **53**:37–43.
65. **Thibault, G., M. Nemer, J. Drouin, J. P. Lavigne, J. Ding, C. Charbonneau, R. Garcia, J. Genest, G. Jasmin, M. J. Sole, and M. Cantin.** 1989. Ventricles as a major site of atrial natriuretic factor synthesis and release in cardiomyopathic hamsters with

heart failure. Circ. Res. **65**:71–82.
66. **Vandekerckhove, J., G. Bugaisky, and M. Buckingham.** 1986. Simultaneous expression of skeletal muscle and heart actin proteins in various striated muscle tissues and cells. J. Biol. Chem. **261**:1838–1842.
67. **Webster, K. A., G. E. O. Muscat, and L. Kedes.** 1988. Adenovirus E1A products suppress myogenic differentiation and inhibit transcription from muscle-specific promoters. Nature (London) **332**:553–557.
68. **Wilson, T., and R. Treisman.** 1988. Fos C-terminal mutations block down-regulation of c-fos transcription following serum stimulation. EMBO J. **7**:4193–4202.
69. **Yang-Yen, H. S., J. C. Chambard, Y. L. Sun, T. Smeal, T. J. Schmidt, J. Drouin, and M. Karin.** 1990. Transcriptional interference between c-jun and glucocorticoid receptor due to mutual inhibition of DNA-binding activity. Cell **62**:1205–1215.
70. **Yutzey, K. E., S. J. Rhodes, and S. F. Konieczny.** 1990. Differential *trans* activation associated with the muscle regulatory factors MyoD1, myogenin, and MRF4. Mol. Cell. Biol. **10**:3934–3944.
71. **Zeller, R., K. D. Bloch, B. S. Williams, R. J. Arceci, and C. E. Seidman.** 1987. Localized expression of the atrial natriuretic factor gene during cardiac embryogenesis. Genes Dev. **1**:693–698.
72. **Zerial, M., L. Toschi, R.-P. Ryseck, M. Schuermann, R. Müller, and R. Bravo.** 1989. The product of a novel growth factor activated gene, fos B, interacts with jun proteins enhancing their DNA binding activity. EMBO J. **8**:805–813.