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Phosphorylation of serum response factor, a factor that binds to the serum response element of the c-*FOS* enhancer

(transcription/growth factor/gene regulation/phosphatase)

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ABSTRACT **Serum and growth factor regulation of c-*FOS* protooncogene transcription is mediated by the serum response element. A factor, serum response factor, binding to this element has already been identified. We demonstrate that serum response factor is phosphorylated *in vivo* on serine residues and that phosphatase treatment of this factor *in vitro* abolishes its DNA-binding activity. These results show phosphorylation of serum response factor to be required for its DNA-binding activity. The importance of serum response factor phosphorylation for the regulation of c-*FOS* expression is discussed.**

Transcription of the c-*FOS* protooncogene is induced rapidly, within 5 min, by serum and by several distinct growth factors (refs. 1–3; for review, see ref. 4). This induction can occur in the presence of protein synthesis inhibitors, suggesting that posttranslational modifications are involved in the transcriptional regulation (2, 3, 5, 6). The c-*FOS* enhancer contains a sequence element, termed serum response element (SRE), that mediates the transcriptional response to serum, epidermal growth factor (EGF), the tumor promoter phorbol 12-myristate 13-acetate, and insulin (7–12). A nuclear factor that binds to SRE is probably involved in regulating the promoter. Such a factor, termed serum response factor (SRF), has been identified (8, 13, 14) and purified to apparent homogeneity (15–17).

In support of the idea that SRF is involved in c-*FOS* regulation, we previously showed that EGF treatment of A431 human epidermal carcinoma cells resulted in increased SRF binding activity in nuclear extracts derived from these cells (14). This activity occurred within 15 min after EGF addition and paralleled the EGF-induced increase in the c-*FOS* transcription rate, which was also maximal at 15 min. However, beyond 15 min, SRF binding activity remained constant, whereas the c-*FOS* transcription rate decreased rapidly, reaching basal levels by 2 hr. These results suggested a model in which two activities of SRF must be distinguished: (*i*) a DNA-binding activity and (*ii*) a transcriptional-initiation stimulatory activity. Thus, it appeared that initially both activities were activated in A431 cells but that subsequently the transcription-activation function was selectively lost. In other cell types (HeLa and NIH 3T3) EGF and serum increased c-*FOS* transcription, but without a parallel increase in SRF DNA-binding activity (ref. 9; R.P. and R.G.R., unpublished data). According to the model suggested, the DNA-binding activity has been constitutively activated in these cell types, and only the transcription function is regulated.

In our analysis of SRF we sought to characterize modifications that might account for the changes in each of the SRF activities. A common regulatory modification is protein phosphorylation (18), and we designed experiments to test whether SRF is phosphorylated *in vivo* and to determine the functional significance of this modification.

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METHODS

Plasmids and Oligonucleotides. The fosCAT plasmids used are as described (ref. 9; T. M. Fisch, R.P., C. Simon, and R.G.R., unpublished manuscript). Plasmid pFC53 was deleted of all c-*FOS* sequences upstream of position −53. Plasmid pFC53X contained oligonucleotide XGL cloned at position −53. Oligonucleotide XGL was filled in with Klenow fragment of *Escherichia coli* DNA polymerase I and synthetic *Xho* I linker DNA was added at position −53 to facilitate cloning. The XGL oligonucleotide resembles that described as Act.L (17). The sequence is CAATCCCTCC-CCCCTTATGATGCCCATATATGGGCATCTTCTGCAG-CA. The oligonucleotide was double stranded, except for the first 12 nucleotides, which were single stranded to facilitate coupling to cyanogen bromide-activated Sepharose 4B (19). The XGL oligonucleotide contains a high-affinity SRF binding site (17). Oligonucleotide TF2 contains four point mutations in the SRE and does not bind SRF (9).

Phosphorylation and Rapid Purification of SRF. HeLa or A431 cells (two confluent 15-cm plates) were washed three times with TBS buffer (50 mM Tris·HCl, pH 7.5/150 mM NaCl) and incubated for 3 hr at 37°C in 4 ml of Dulbecco's modified Eagle's phosphate-free medium containing 10% dialyzed fetal calf serum and 2.5 mCi (1 Ci = 37 GBq) of ^{32}P-orthophosphate (New England Nuclear). The cells were then treated with or without EGF (100 ng/ml) (Collaborative Research, Waltham, MA) for 15 min at 37°C. Nuclear extracts were prepared by a modification (14) of the procedure of Dignam *et al.* (20), except that BC300 buffer (300 mM KCl/20% (vol/vol) glycerol/0.2 mM EDTA/0.5 mM dithiothreitol/0.5 mM phenylmethylsulfonyl fluoride/20 mM Tris·HCl, pH 7.9) was used instead of the standard nuclear extraction buffer. (All the BC buffers are the same except for the indicated mM concentrations of KCl—e.g., BC100 contains 100 mM KCl.)

Nonspecific double-stranded DNA (dsDNA; sonicated herring sperm DNA), the XGL oligonucleotide, and the TF2 oligonucleotide were each separately coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia) (15), and each of the coupled matrices were used as a 50% suspension (vol/vol) in BC300/0.05% Nonidet P-40. Nonidet P-40 (0.05%, final concentration) was added to 0.5 ml of nuclear extract followed by the nonspecific dsDNA matrix. The mixture was incubated for 1 hr at 4°C with continual rotation on a

Abbreviations: SRE, serum response element; SRF, serum response factor; EGF, epidermal growth factor; SDS, sodium dodecyl sulfate; dsDNA, double-stranded DNA; XGL oligonucleotide, oligonucleotide containing a high-affinity SRF-binding site; TF2 oligonucleotide, oligonucleotide containing a mutated SRE site that does not bind SRF.

Labquake apparatus. The matrix was then pelleted by centrifugation at 13,000 × *g* for 1 min, and the supernatant was mixed with either 100 μl of XGL oligonucleotide–Sepharose or TF2 oligonucleotide–Sepharose. After incubating the mixtures for 1 hr at 4°C, the matrices were pelleted and washed five times with BC500/0.05% Nonidet P-40. Protein was eluted from the matrices by boiling with 50 μl of SDS-sample buffer [67 mM Tris·HCl, pH 6.8/2% SDS/10% (vol/vol) glycerol/5 mM EDTA/0.33% 2-mercaptoethanol/0.1% bromophenol blue] and electrophoresed on an SDS/8% polyacrylamide gel as described (21).

Phospho Amino Acid and Tryptic Phosphopeptide Analysis. SRF, labeled with ^{32}P-orthophosphate and electrophoresed as described above, was visualized by autoradiography, excised from the dried gel, and rehydrated by repeated washing in 10% (vol/vol) methanol. The protein in the band was subjected to performic acid oxidation for tryptic phosphopeptide analysis but not for phospho amino acid analysis. The protein in the gel slice was then digested to completion with L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin, and the phosphopeptides were recovered from the supernatant. For phospho amino acid analysis the peptides were subjected to acid hydrolysis (22) and analyzed by two-dimensional thin-layer electrophoresis at pH 1.9 in the first dimension and pH 3.5 in the second dimension (23). For phosphopeptide analysis the peptides were separated by thin-layer electrophoresis at 1000 V for 17 min in the first dimension, and the second-dimension separation was by chromatography in 1-butanol/pyridine/acetic acid/water (75:60:15:60) for 5 hr (24). The phospho amino acids and phosphopeptides were visualized by exposure to Kodak XAR film at −70°C with an intensifying screen for 1 week.

Phosphatase Treatment. SRF, partially purified through phosphocellulose and dsDNA–Sepharose 4B columns or purified further through a specific oligonucleotide column (15), was treated with potato acid phosphatase (Boehringer Mannheim). SRF (1 μl) was incubated with 0.1 μg of phosphatase in 18 μl of gel shift-binding buffer [5% (vol/vol) glycerol/50 mM KCl/bovine serum albumin at 100 μg/ml/0.05% Nonidet P-40/sonicated herring sperm DNA at 100 μg/ml/1 mM dithiothreitol/20 mM Tris·HCl, pH 7.5] for 20 min at 30°C. ^{32}P-labeled XGL oligonucleotide (2 μl, 0.5 ng/μl) was then added for 20 min at 30°C, and the material was loaded on a 4% polyacrylamide gel as described (14). The protein–DNA complex seen with XGL is the same as that seen with other SRE-containing DNAs (ref. 17; R.P. and R.G.R., unpublished data).

SRF, treated with or without phosphatase as described above, was electrophoresed on an SDS/8% polyacrylamide gel and transferred to nitrocellulose as described (25). The filter was blocked with 6% nonfat dry milk (Carnation) in phosphate-buffered saline (PBS), washed with PBS, and incubated overnight at 4°C with a 1:500 dilution of anti-peptide 64B rabbit serum in Dulbecco's modified Eagle's medium with 10% fetal calf serum. The binding was then visualized using an alkaline phosphatase-conjugated anti-rabbit IgG serum and histochemical staining as described by the manufacturer (Promega Biotec, Madison, WI).

Antisera. Peptide P64B (Thr-Ser-Pro-Leu-Phe-Pro-Ile-Thr-Asn-Tyr-Val-Ala-Asn-Cys) was synthesized from the amino acid sequence of a cyanogen bromide fragment of purified SRF where the C-terminal cysteine was added for coupling (R.P., J.A.C., and R.G.R., unpublished data). Peptide P64B was coupled to ovalbumin using the heterobifunctional agent SPDP (Pharmacia) as described by the manufacturer, and 200 μg of ovalbumin/peptide P64B was injected s.c. into a rabbit. The rabbit dose was boosted after 4 weeks with 200 μg of ovalbumin/peptide P64B, and the rabbit was bled 2 weeks later. The serum was purified with a P64B-affinity column with the peptide coupled to thiopropyl–Sepharose 6B (Pharmacia) as described by the manufacturer.

The antiserum was used in a gel mobility-shift assay by incubating 1 μl of serum with 17 μl of gel shift-binding buffer with the SRF-containing protein fraction for 20 min at room temperature. ^{32}P-labeled XGL oligonucleotide was then added (2 μl, 0.5 ng/μl) and after 20 min at room temperature the material was electrophoresed on a 4% polyacrylamide gel as described (14). For some experiments the antiserum was incubated for 15 min at room temperature with 0.5 μg of specific or nonspecific synthetic peptides in 6 μl of gel shift-binding buffer; SRF-containing fractions were then added in gel shift-binding buffer as described above. The nonspecific peptide P64A (Thr-Ser-Pro-Leu-Phe-Pro-Ile-Thr-Leu-Tyr-Val-Ala-Asn-Cys) differed from P64B in one amino acid.

RESULTS

Phosphorylation of SRF *in Vivo*. To determine whether SRF is phosphorylated *in vivo* we labeled HeLa and A431 cells with ^{32}P-orthophosphate for 3 hr, then treated the cells with or without EGF for 15 min, and subsequently purified SRF by a rapid technique (Fig. 1*A*). EGF treatment induces transcription of c-*FOS* maximally in both HeLa and A431 cells after treatment for 15 min (ref. 14; R.P. and R.G.R., unpublished data). Nuclear extracts were prepared from the labeled cells and then mixed in 1.5-ml tubes with nonspecific dsDNA (sonicated herring sperm DNA) coupled to cyanogen bromide-activated Sepharose 4B. At the salt concentration of the nuclear extract, 0.3 M KCl, SRF does not bind to nonspecific DNA sites (15, 17) and remained in the supernatant when the DNA–Sepharose was pelleted, whereas nonspecific DNA binding factors were removed with the matrix.

One aliquot of the supernatant was mixed with a Sepharose-bound oligonucleotide (XGL) that contains a *Xenopus* γ-actin-derived high-affinity SRF-binding site (17). Although SRF elutes from the c-*FOS* SRE site at ≈0.5 M KCl, SRF elutes from the XGL site at >1 M KCl (17). By use of this high-affinity binding site, SRF will bind to the XGL oligonucleotide matrix at a salt concentration, 0.3 M KCl, at which SRF would not bind to the c-*FOS* SRE site. As a negative control, an aliquot of the supernatant was also incubated with a Sepharose-bound nonspecific oligonucleotide (TF2) that spans the c-*FOS* SRE but contains four point mutations (9) that eliminate SRF binding (data not shown). After incubation, the immobilized oligonucleotides were pelleted, washed extensively with buffer containing 0.5 M KCl, and resuspended in SDS-sample buffer for electrophoresis.

With this procedure a 64-kDa phosphoprotein, identical in size to purified SRF (15–17), was bound to the specific (XGL) but not to the nonspecific (TF2) oligonucleotide matrices (Fig. 1*B*). After EGF treatment the level of phosphorylation was unaltered in HeLa cells but increased about 2-fold in A431 cells (Fig. 1*B*). This was expected because the phosphoprotein was isolated on the basis of its DNA-binding activity, which is induced in A431 cells but not in HeLa cells (9, 14). Although a greater increase was expected for A431 cells (14), the EGF-induced increase in DNA-binding activity has been somewhat variable (3- to 10-fold), and the phosphoprotein isolation technique is not clearly quantitative.

Phospho Amino Acid Analysis of SRF. Although the phosphorylation level was not altered in HeLa cells in response to EGF, qualitative changes in phosphorylation at individual sites might have gone undetected by this analysis. To assess this possibility, we first excised the labeled proteins from the SDS/polyacrylamide gel and processed them for phospho amino acid analysis. Phosphorylation of SRF was entirely on serine residues in SRF from untreated or EGF-treated A431 (Fig. 2*A*) or HeLa cells (data not shown).

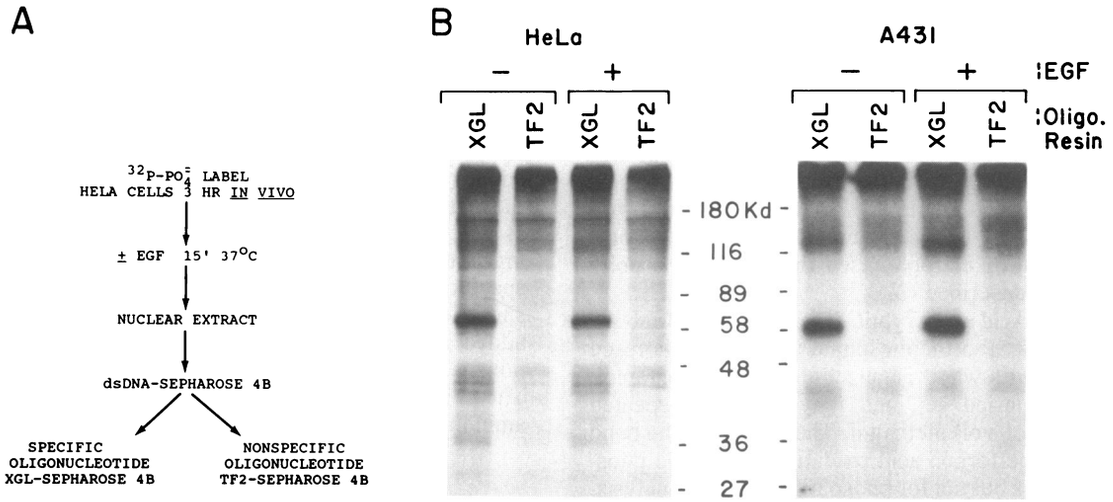

FIG. 1. Phosphorylation of SRF *in vivo*. SRF was rapidly purified from ^{32}P-labeled HeLa or A431 cells as diagrammed (*A*) and described. The purified material was electrophoresed on an SDS/8% polyacrylamide gel (*B*). Specific (XGL) or nonspecific (TF2) oligonucleotides were used in the purification, and the cells were treated with (+) or without (−) EGF for 15 min.

To determine the number of phosphorylation sites on SRF in HeLa cells, the gel-derived phosphoproteins were treated with trypsin, and the resulting peptides were analyzed by two-dimensional electrophoresis and chromatography. The small amount of radioactivity made this analysis difficult; however, in several experiments seven distinct phosphopeptides were consistently found, suggesting that at least seven phosphorylation sites exist (although more could exist because some peptides might be multiply phosphorylated). Significantly, no difference was seen with phosphorylated SRF isolated from EGF-treated or untreated cells. A typical experimental analysis of SRF from untreated HeLa cells is shown in Fig. 2*B*.

Phosphatase Treatment of SRF. To assess the function of SRF phosphorylation we treated a crude SRF preparation with potato acid phosphatase and assayed for DNA-binding activity. These experiments showed convincingly that phosphorylation of SRF is prerequisite to its DNA-binding activity. Phosphatase treatment nearly abolished the DNA-binding activity, an effect inhibited by the addition of the phosphatase inhibitor 10 mM sodium phosphate (Fig. 3*A*). This effect required incubation of SRF with phosphatase before addition of ^{32}P-labeled DNA containing an SRF-binding site. When the SRF–DNA complex was first formed, phosphatase treatment did not affect the amount of binding seen in a gel mobility-shift assay (Fig. 3*B*). This result is probably not due to inhibition of phosphatase activity by the added DNA because nonspecific DNA was present in the incubation buffer. Two explanations for the lack of any phosphatase effect once the SRF–DNA complex is formed are that (*i*) once the complex is formed, phosphorylation is unnecessary for stable binding, or (*ii*) upon complex formation a structural change blocks accessibility of the phosphatase to key phosphates.

We questioned whether our phosphatase preparation might contain protease activity, even though a protease would probably not be inhibited by sodium phosphate. Therefore we directly measured the amount of SRF protein present after phosphatase treatment by an immunoblot analysis with a rabbit anti-SRF peptide serum. The 14-amino acid peptide (P64B) was synthesized according to the amino acid sequence derived from a cyanogen bromide fragment of SRF (R.P., J.A.C., and R.G.R., unpublished data). The antiserum reacted with purified SRF on an immunoblot, whereas preimmune serum did not react (Fig. 4*A*). Furthermore, when the antiserum was incubated with SRF and then specific ^{32}P-

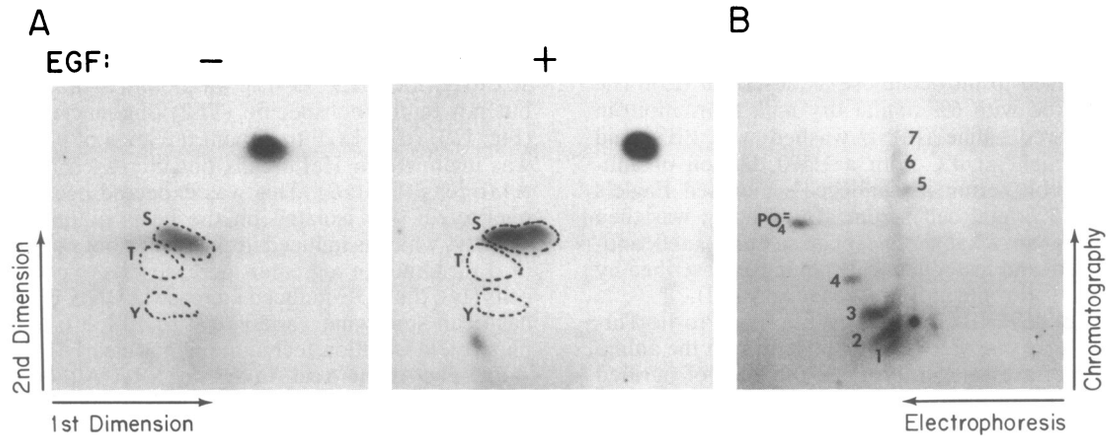

FIG. 2. Phospho amino acid and phosphopeptide analysis of phosphorylated SRF. Phosphorylated SRF was excised from the bands in Fig. 1. Phospho amino acid analysis was done as described, and the dimensions of chromatography are indicated. (*A*) Phosphorylated SRF was taken from A431 cells treated with (+) or without (−) EGF. The positions of migration of phosphoserine (S), phosphothreonine (T), and phosphotyrosine (Y) are indicated. (*B*) Tryptic phosphopeptide analysis of phosphorylated SRF from untreated HeLa cells was done as described. The sample was spotted at the center for electrophoresis in the first dimension. The direction of electrophoresis toward the cathode is indicated; the numbers indicate distinct phosphopeptides.

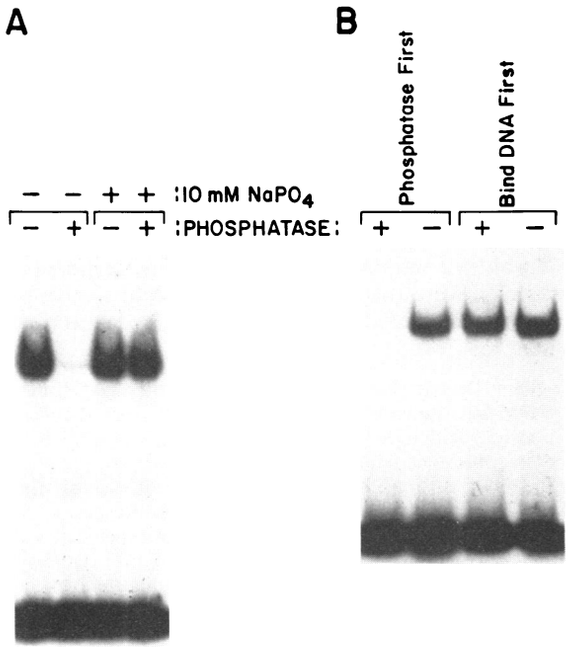

FIG. 3. Phosphatase treatment of SRF. (*A*) Partially purified SRF (from a dsDNA column) was treated with (+) or without (−) potato acid phosphatase as described. Sodium phosphate was added where indicated as a phosphatase inhibitor. After incubation, DNA-binding activity was measured in a gel mobility-shift assay. Upper (retarded) band, specific SRF–DNA complex; lower band, free DNA. (*B*) The SRF-containing fraction was treated either with phosphatase first and then assayed for DNA binding or with specific DNA first followed by phosphatase treatment as described.

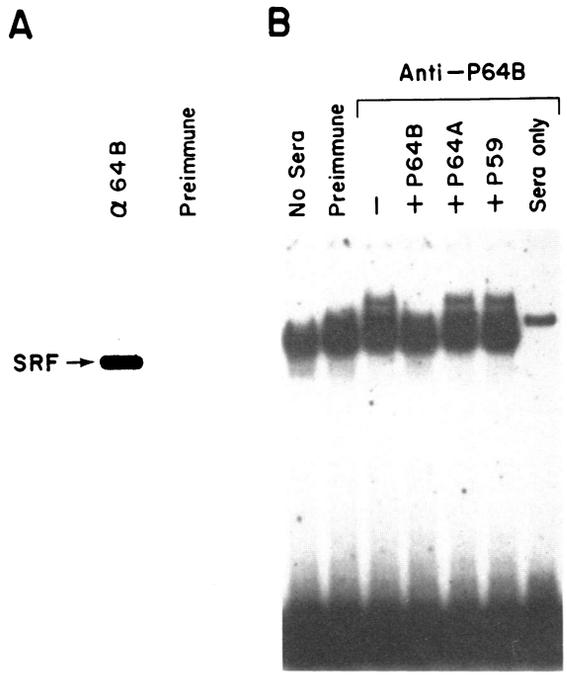

FIG. 4. Specificity of anti-SRF peptide serum. (*A*) DNA-affinity-purified SRF (10 ng) (15) was electrophoresed on an SDS/8% polyacrylamide gel, transferred to nitrocellulose, and incubated either with anti-P64B or preimmune rabbit serum. The binding was visualized with alkaline phosphatase-conjugated second antibody as described. (*B*) DNA-affinity-purified SRF (0.25 ng) was assayed for DNA-binding activity in a gel mobility-shift assay. SRF was pretreated with preimmune or anti-P64B sera as indicated. Where indicated, anti-P64B serum was incubated with 0.5 μg of peptides P64B, P64A, or P59. Peptide P64A differs from P64B in one amino acid, and peptide P59 is a nonspecific 11-amino acid peptide. The more slowly migrating band represents the antibody–SRF–DNA complex. Incubation with anti-P64B serum only (without SRF; last lane on right) resulted in a nonspecific protein–DNA complex.

DNA was added, a specific antibody–SRF–DNA complex was formed, which migrated slightly slower than the SRF–DNA complex in a gel mobility-shift assay. This effect was antagonized by preincubation of the antiserum with the cognate peptide (P64B) but not by preincubation with a peptide containing a single amino acid change (P64A) or a nonspecific peptide (P59) (Fig. 4*B*).

We treated two different SRF-containing fractions (either from a dsDNA–Sepharose 4B column or from an oligonucleotide–Sepharose 4B column) (15) with or without phosphatase and assayed them in parallel for DNA-binding activity and for protein content by immunoblotting. Although DNA-binding activity was greatly reduced (Fig. 5*A*), no change in

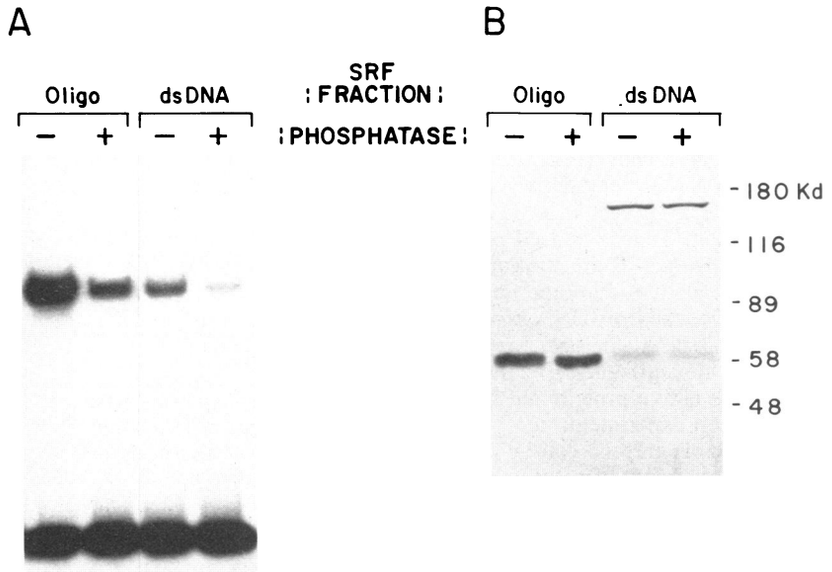

FIG. 5. Immunoblot of phosphatase-treated SRF. (*A*) SRF-containing protein fractions [2 μl from a dsDNA column or a specific oligonucleotide column (15)] were treated with (+) or without (−) phosphatase, and SRF DNA-binding activity was measured in a gel mobility-shift assay. (*B*) SRF-containing fractions treated with phosphatase exactly as shown in *A* were electrophoresed on an SDS/8% polyacrylamide gel, transferred to nitrocellulose, and assayed for SRF with rabbit anti-SRF peptide serum (anti-P64B) as described.

the amount or size of SRF was detected in immunoblots after phosphatase treatment (Fig. 5*B*). Thus, we believe that protease contamination does not account for the loss in DNA-binding activity.

DISCUSSION

Induction of transcription of a number of genes occurs in the presence of protein synthesis inhibitors, suggesting that posttranslational modifications are necessary for the transcriptional regulation (2, 3, 26, 27). The simplest model is one in which the nuclear factors that bind to specific promoter/enhancer sequence elements not only regulate gene expression but are also the proteins that are posttranslationally modified. Because protein phosphorylation is a common regulatory modification (18), such phosphorylation is a likely candidate for the modifying mechanism of transcription factors. Furthermore, use of protein kinase inhibitors has suggested that phosphorylation is necessary for serum induction of the c-*MYC* and c-*FOS* genes (28); there exists a variant of PC12 pheochromocytoma cells deficient in both cyclic AMP-dependent protein kinase and the ability to activate c-*FOS* expression in response to nerve growth factor (29).

We analyzed the growth factor-inducible regulation of the c-*FOS* protooncogene that is mediated by the SRE sequence element. We demonstrated here that SRF, the nuclear factor that binds to SRE, is phosphorylated *in vivo*. In addition, phosphatase treatment abolished SRF-binding activity *in vitro*, demonstrating that phosphorylation is necessary for the DNA-binding activity of SRF. In similar studies of a heat shock regulatory element-binding factor, treatment with calf intestinal phosphatase increased the mobility of the protein–DNA complex in a gel mobility-shift assay (30). This shift suggested that the heat shock factor is phosphorylated but that the phosphorylation is not necessary for DNA binding (30). Although the heat shock factor phosphorylation might be related to transcriptional activation, this relationship was not shown, and the possibility that the altered mobility was caused by a contaminating protease activity was not eliminated. This possibility for SRF was eliminated by using an SRF-reactive antibody in our experiment to show directly (in an immunoblot analysis) that phosphatase treatment changed neither SRF amount nor SRF size. Thus, although SRF undergoes a phosphorylation important for DNA binding, this factor may differ from other transcription factors that may require phosphorylation for a transcriptional activation function but not for DNA binding *per se*.

We had previously observed that SRF binding in nuclear extracts is induced by EGF in A431 cells (14). Because phosphorylation is essential for DNA-binding activity, we propose that the increased binding activity seen in A431 cells is due to increased phosphorylation. That increased phosphorylation occurs *in vivo* begs for direct demonstration—a demonstration not accomplished to date in our experiments because our isolation technique depends on DNA-binding activity. Specific antibodies would be ideal tools for this purpose, but the available antipeptide antibodies effective in immunoblots react poorly with the native protein and have precluded such immunoprecipitation experiments.

Because SRF binding is not inducible in HeLa cells, c-*FOS* induction via the SRE must also involve a different modification, which modulates the ability to activate transcription rather than DNA-binding activity. This type of modification is presumed to occur during induction in A431 cells as well. We analyzed the phosphorylation sites *in vivo* (in HeLa cells) before and after EGF treatment. In both cases phosphorylation is on serine, and the phosphopeptide pattern does not seem to change. Thus, the change in transcriptional activity is apparently not from phosphorylation of SRF. However, a cryptic cellular phosphatase activity might have removed certain SRF phosphorylations during the preparation of nuclear extracts or the isolation of ^{32}P-labeled SRF. Nevertheless, lack of any detectable change in the phosphorylation pattern suggests either that another covalent modification is involved or that another posttranslationally regulated factor interacts with SRF to mediate transcriptional regulation. The transcriptional regulation of c-*FOS* must be reproduced *in vitro* to verify that SRF itself has an altered activity rather than the altered activity stemming from other components of the system. Were this accomplished, structural changes in SRF could be related more directly to its transcriptional function.

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1. Greenberg, M. E. & Ziff, E. B. (1984) *Nature (London)* **311,** 433–438.
2. Kruijer, W., Cooper, J. A., Hunter, T. & Verma, M. (1984) *Nature (London)* **312,** 711–716.
3. Muller, R., Bravo, R., Burckhardt, J. & Curran, T. (1984) *Nature (London)* **312,** 716–720.
4. Curran, T. (1988) in *Oncogene Handbook*, eds. Reddy, E. P., Skalka, A. M. & Curran, T. (Elsevier, Amsterdam), in press.
5. Cochran, B. H., Zullo, J., Verma, I. M. & Stiles, C. D. (1984) *Science* **226,** 1080–1082.
6. Greenberg, M. E., Hermanowski, A. L. & Ziff, E. B. (1986) *Mol. Cell. Biol.* **6,** 1050–1057.
7. Treisman, R. (1985) *Cell* **42,** 889–902.
8. Treisman, R. (1986) *Cell* **46,** 567–574.
9. Fisch, T. M., Prywes, R. & Roeder, R. G. (1987) *Mol. Cell. Biol.* **7,** 3490–3502.
10. Gilman, M. Z. (1988) *Genes Dev.* **2,** 394–402.
11. Greenberg, M. E., Siegried, Z. & Ziff, E. B. (1987) *Mol. Cell. Biol.* **7,** 1217–1225.
12. Stumpo, D. J., Stewart, T. N., Gilman, M. Z. & Blackshear, P. J. (1988) *J. Biol. Chem.* **263,** 1611–1614.
13. Gilman, M. G., Wilson, R. N. & Weinberg, R. A. (1986) *Mol. Cell. Biol.* **6,** 4305–4316.
14. Prywes, R. & Roeder, R. G. (1986) *Cell* **47,** 777–784.
15. Prywes, R. & Roeder, R. G. (1987) *Mol. Cell. Biol.* **7,** 3482–3489.
16. Schroter, H., Shaw, P. E. & Nordheim, A. (1987) *Nucleic Acids Res.* **15,** 10145–10158.
17. Treisman, R. (1987) *EMBO J.* **6,** 2711–2717.
18. Cohen, P. (1982) *Nature (London)* **296,** 613–620.
19. Wu, C., Wilson, S., Walker, B., Dawid, I., Paisley, T., Zimarino, V. & Ueda, H. (1987) *Science* **238,** 1247–1253.
20. Dignam, J. D., Lebowitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11,** 1475–1489.
21. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
22. Feldman, R. A., Hanafusa, T. & Hanafusa, H. (1980) *Cell* **22,** 757–765.
23. Hunter, T. & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1311–1315.
24. Neil, J. C., Ghysdael, J., Vogt, P. K. & Smart, J. E. (1981) *Nature (London)* **291,** 675–677.
25. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **79,** 4350–4354.
26. Kelly, K., Cochran, B. H., Stiles, C. & Leder, P. (1983) *Cell* **35,** 603–610.
27. Sen, R. & Baltimore, D. (1986) *Cell* **47,** 921–928.
28. Zinn, K., Keller, A., Whittemore, L.-A. & Maniatis, T. (1988) *Science* **240,** 210–213.
29. Montminy, M. R., Sevarino, K. A., Wagner, J. A., Mandel, G. & Goodman, R. H. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 6682–6686.
30. Sorger, P. K., Lewis, M. J. & Pelham, H. R. B. (1987) *Nature (London)* **329,** 81–84.