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# Isolation and Characterization of SRF Accessory Proteins

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# Isolation and characterization of SRF accessory proteins

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## SUMMARY

Many genes which are regulated by growth factors contain a common regulatory element, the serum response element (SRE). Activation of transcription by the SRE involves a ternary complex formed between a ubiquitous factor, serum response factor (SRF), and a second protein, p62/TCF. We used a yeast genetic screen to isolate cDNAs encoding a protein, SAP-1, with the DNA binding properties of p62/TCF. The SAP-1 sequence contains three regions of homology to the previously uncharacterized Elk-1 protein, which also acts as an SRF accessory protein. Only two of these regions are required for cooperative interactions with SRF in the ternary complex. The third contains several conserved sites for the MAP kinases, whose activity is regulated in response to growth factor stimulation. We discuss the potential role of these proteins in regulation of the c-fos SRE.

## 1. INTRODUCTION

When susceptible cells are stimulated with growth factors or mitogens, the transcription of a large number of genes is transiently stimulated without the need for prior protein synthesis (see Almendral *et al.* (1988), and references cited therein). This set of cellular so-called 'immediate-early' genes includes the c-fos and c-jun proto-oncogenes, which encode components of the AP1 transcription factor complex (Curran & Franza 1988). Stimulation of fibroblasts with serum or purified growth factors increases the rate of c-fos transcription by up to 50-fold within 5 min of stimulation; however, this activation is transient and transcription rates return to prestimulation levels within 30 min. This results in the transient accumulation of large amounts of unstable c-fos mRNA and protein (reviewed by Rivera & Greenberg 1990; Treisman 1990). Immediate-early gene products, by analogy with the immediate-early transcriptional regulators encoded by viruses, initiate a cascade of regulated gene expression that determines the ultimate response of the cell to the growth factor stimulus.

Gene transfer studies have shown that serum inducible transcriptional activation of the c-fos gene is dependent on the serum response element (SRE), a short sequence of dyad symmetry that is located 300 basepairs (b.p.) to the 5′ side of the site of transcription initiation. SRE sequences are also found in the promoters of other cellular immediate-early genes such as those encoding cytoskeletal actins and two zinc finger proteins, krox20 and zif268 (reviewed by Rivera & Greenberg 1990; Treisman 1990). Many different growth factors activate the SRE, which is linked to both protein kinase C (PKC)-dependent and PKC-independent signalling pathways (for review see Treisman 1990; also Gilman 1988; Fujii *et al.* 1989; Qureshi *et al.* 1991; Alexandropoulos *et al.* 1992). The SRE is also a target for signals generated by activated forms of intracellular signalling molecules such as ras (Stacey *et al.* 1987; Sassone *et al.* 1989; Fukumoto *et al.* 1990; Gauthier-Rouviere *et al.* 1990) and raf (Kaibuchi *et al.* 1989; Jamal & Ziff 1990; Siegfried & Ziff 1990). Recent biochemical and genetic studies have shown that ras and raf lie on a signalling pathway that links signals from activated growth factor receptors to a conserved kinase cascade that in turn activates cellular MAP kinases and RSKs (see Howe *et al.* 1992; Leevers & Marshall 1992; Pelech & Sanghera 1992; Thomas *et al.* 1992; Wood *et al.* 1992). Together, these data support the idea that at least one route by which signals arrive at the SRE is via the MAP kinase pathway.

The SRE contains a binding site for the ubiquitous transcription factor SRF (serum response factor; Norman *et al.* 1988). SRF binding sites are also found in many muscle-specific promoters where they act as constitutive promoter elements (Taylor *et al.* 1989; Walsh 1989; Sartorelli *et al.* 1990). In both types of promoter, mutations that reduce or abolish SRF binding have corresponding effects on promoter activity, consistent with the idea that SRF binding is required for the transcriptional response (reviewed by Treisman 1990). The gene encoding SRF has been cloned: it encodes a protein of 508 amino acids that binds DNA as a dimer and activates transcription *in vitro* (Norman *et al.* 1988). SRF is related to the yeast

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regulatory factor MCM1, which is also involved in extracellular signal-regulated and cell type-specific transcription (Marsh *et al.* 1991). The two proteins are 70% identical within their DNA binding/dimerization domains (Norman *et al.* 1988); however, owing to sequence differences between the N terminal basic part of these domains, their DNA binding specificities are related but distinct (Wynne & Treisman 1992).

Both SRF and MCM1 proteins form ternary complexes with accessory proteins that in their absence bind DNA weakly or not at all (Bender & Sprague 1987; Keleher *et al.* 1988; Errede & Ammerer 1989; Shaw *et al.* 1989). In both cases accessory factor recruitment is mediated by specific sequences within the C terminal part of the DNA binding domain (Mueller & Nordheim 1991; Primig *et al.* 1991; Shaw 1992). At the c-fos SRE, SRF forms a ternary complex with the accessory protein p62/TCF, which in the presence of SRF can bind to an Ets motif located next to the SRF binding site (Shaw *et al.* 1989; Shaw 1992). Two lines of evidence suggest that the formation of the ternary complex is important for regulation. First, in some cells disruptions of the Ets motif causes alterations in the signalling properties of the SRE (Shaw *et al.* 1989; Graham & Gilman 1991; Malik *et al.* 1991). Second, genomic footprinting studies indicate that *in vivo* the SRE is bound by proteins whose interaction with DNA is similar to the SRF/TCF ternary complex (Herrera *et al.* 1989). However, it is not immediately obvious how these observations can be reconciled with the fact that an Ets motif is not conserved at the same position relative to the SRF binding site all SREs (see Treisman 1990).

To gain insight into the role of the ternary complex in SRE function, we developed a yeast genetic screen for the isolation of cDNAs encoding proteins that interact with SRF. The structure and DNA binding properties of these clones shows that the interaction with TCF is indeed conserved at many SREs, and suggests a mechanism by which the ternary complex might serve to link activation of transcription to signals from the cell surface.

## 2. METHODS

Details of all methods used for the yeast genetic screen and the analysis of ternary complex formation *in vitro* are published (Dalton & Treisman 1992; Treisman *et al.* 1992). Methods for functional analysis *in vivo* of the constituents of the ternary complex and the complex itself will be described elsewhere (Marais *et al.* 1993; Hill *et al.* 1993).

## 3. RESULTS AND DISCUSSION

### (*a*) *Strategy for isolation of cDNAs*

Our strategy, which is illustrated in figure 1, exploits the observation that a transcriptional activator domain can function even when it is recruited indirectly to a promoter by protein–protein interaction rather than by direct DNA binding. For example, a fusion protein comprising the yeast GAL80 protein joined to an acidic activator domain allows GAL80 to function as an activator owing to its recruitment to GAL4 operators via protein-protein interaction with the DNA binding GAL4 protein (Ma & Ptashne 1988). It was demonstrated by Fields that these findings could be exploited to identify clones for proteins that interact with a particular target by use of yeast activator domain-tagged cDNA expression libraries (Fields & Song 1989). The indicator yeast strain, S62L, carries a cyc1-lacZ reporter gene under the control of a derivative of the c-fos SRE that can bind SRF and form a ternary complex with p62/TCF, but which cannot be bound by the endogenous yeast activator MCM1. These cells also contain a low copy number plasmid carrying the SRF cDNA under the control of a galactose-inducible promoter, thereby allowing control over the level of expression of SRF *in vivo*. The indicator strain is transformed with a cDNA library in which each cDNA is tagged with an N-terminal transcriptional activation domain from the HSV trans-activator protein VP16, and whose activity is also controlled by a galactose-inducible promoter. Activation of the reporter gene, which requires

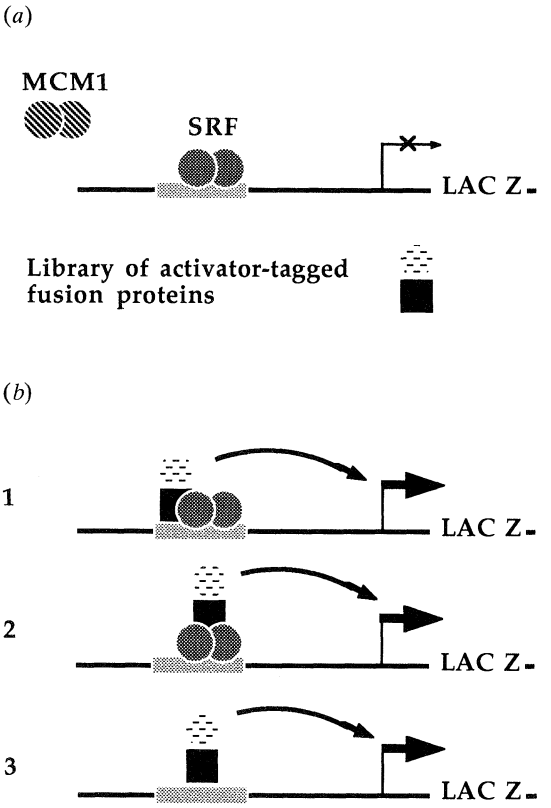


Figure 1. Strategy for isolation of cDNA clones. (*a*) Specification of the indicator strain, in which SRF, but not MCM1, can bind the SRE (shaded box). In these cells the reporter gene is silent as SRF does not activate transcription in yeast. The VP16-cDNA fusion proteins, with the acidic activation domain indicated by dashes and the cDNA encoded sequences as a black box. (*b*) Three expected scenarios for transcriptional activation of the reporter gene: 1, interaction of the fusion protein with both SRF and DNA; 2, interaction of the fusion protein with SRF alone; and 3, interaction of the fusion protein directly with the promoter DNA independently of SRF.




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a promoter-bound activator region, can only occur if the cDNA encodes a protein that can interact with either SRE-bound SRF (figure 1, mechanisms 1 and 2), or the SRE alone (figure 1, mechanism 3).

To perform the screen we used the protocol shown in figure 2. Cells were transformed with the plasmid cDNA library and plated out on nylon filters on selective glucose medium for 32–38 h, to allow cell growth without expression of SRF or the VP16 fusion proteins. The filter is transferred to a galactose plate to induce expression of the SRF and VP16-tagged cDNAs. The activity of the reporter gene is assessed by a simple lacZ assay in which colonies are lysed on the filters (Breeden & Nasmyth 1987). Any colonies in which the reporter gene is active will have a blue phenotype in this colony colour assay. These colonies are grown up, rescreened, and the cDNA plasmids rescued by transformation into into *E. coli*.

### (*b*) *Screening for SRE binding proteins in yeast*

A prerequisite for the success of our approach is that the SRF can be produced in yeast and binds the SRE without activation of the reporter gene. We therefore conducted the control experiments summarized in table 1. Cells were transformed with plasmids carrying galactose-inducible genes encoding either full length SRF (SRF[1–508]), or an SRF-VP16 fusion protein comprising the first 412 residues of SRF joined to the VP16 activator domain (SRF[1–412]/VP16; table 1). Although SRF activates transcription in mammalian cells it fails to activate transcription efficiently in yeast (table 1, lines 1 and 2). However, it is clear that SRF is produced and can bind DNA, because the SRF-VP16 fusion protein activates the reporter gene about one hundred fold over background and turns the colonies blue (table 1, compare lines 2 and 3). Thus although SRF is competent to bind the SRE in yeast, it possesses negligible intrinsic transcriptional activation activity under our assay conditions.

We constructed a library of approximately $10^7$ individual clones of randomly primed HeLa cell cDNA, inserted C terminal to the VP16 activation domain in a vector that allows galactose-inducible expression of the resulting fusion protein. In our first screen of approximately 500 000 colonies, 42 different plasmids were recovered. All but two of these could activate the reporter gene upon transformation into yeast lacking SRF, and must therefore encode SRF-independent activator proteins: indeed, partial DNA sequence analysis showed that one of these plasmids contains a new isolate of the SRF cDNA. In contrast, two plasmids could not activate the reporter gene upon retransformation into a strain lacking SRF. Partial sequence analysis showed that these plasmids contained independent cDNAs derived from the same cellular mRNA. We refer to the protein encoded by these cDNAs as SAP-1 (SRF Accessory Protein 1).

To confirm these findings we quantitated reporter gene activity in different indicator yeast strains transformed with different plasmids using quantitative liquid culture β-galactosidase assays. In cells that contain an intact SRE, expression of VP16/SAP-1

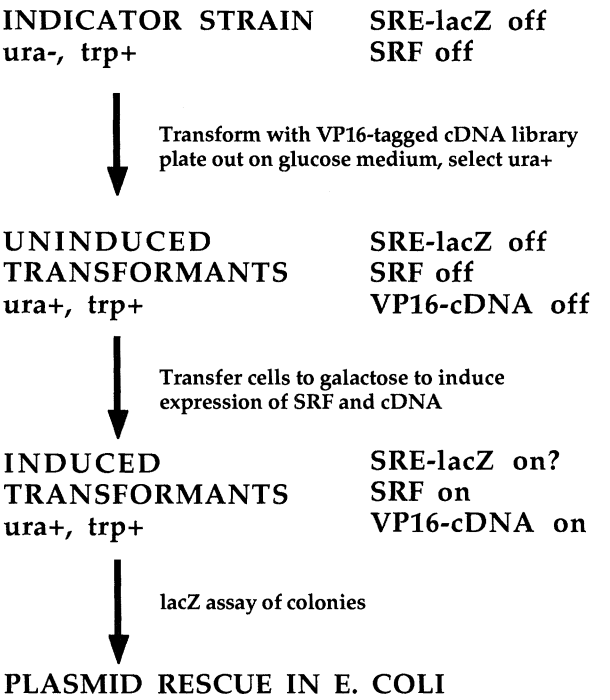


Figure 2. Flow chart for the yeast genetic screen. Status of expression of the reporter gene and the SRF (trp+ marker) and VP16-cDNA (ura+ marker) expression plasmids are indicated at the right. For discussion see text.

together with SRF leads to a 37-fold increase in β-galactosidase activity compared with cells expressing SRF alone, representing a 96-fold increase above background (S62L cells; table 1, column 1). This activation requires DNA bound SRF, since it is not observed in a strain which carries a reporter gene under the control of a mutant SRE that cannot bind

Table 1. *Reporter gene activities in different yeast strains transformed with SRF and SAP-1 expression plasmids*

(In strain S62L, the reporter gene is controlled by a derviative of the c-fos SRE in which core SRF binding site, TCCATATTAGGA, is replaced by the sequence CCCATATATGGG; this SRE cannot bind the yeast SRF-related protein MCM1 (Wynne & Treisman 1992). In strain S62L*, otherwise identical, the reporter gene is controlled by the sequence CCGATATATCGG, which cannot bind SRF (Treisman 1987). The different activator plasmids are indicated at the left, and β-galactosidase activities in the different strains under galactose induction conditions at the right (OD420 per milligram protein per minute).

| | lacZ activity | |
|---|---|---|
| activator plasmid | SRE | SRE* |
| 1 none | 27 | 14 |
| 2 SRF(1–508) | 70 | 43 |
| 3 SRF(1–412)VP16 | 2176 | 143 |
| 4 VP16 SAP1(1–310) | 293 | 96 |
| 5 SRF(1–508) + VP16 SAP1(1–310) | 2594 | 22 |
| 6 SRF(1–508) + VP16 SAP1(1–453) | 2002 | 108 |
| 7 SRF(1–508) + VP16 SAP1(1–170) | 1733 | 86 |
| 8 SRF(1–508) + VP16 SAP1(1–141) | 181 | 143 |
| 9 SRF(1–508) + VP16 SAP1(8–310) | 78 | 47 |




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SRF (S62L* cells; table 1, compare columns 1 and 2). As expected from the colony colour assays, expression of VP16/SAP-1 in S62L cells led to minimal activation of reporter gene activity.

We used a similar strategy to investigate the interaction of SAP-1 with the SRE in mammalian cells *in vivo*. The VP16/SAP-1 fusion protein, expressed by a suitable plasmid will activate the SRE, but only if the SRE contains an intact SRF binding site (data not shown). We also expressed the SAP-1 reading frame by *in vitro* translation and tested its ability to bind the c-fos SRE *in vitro*: in this assay, binding was only detectable in the presence of SRF, and the methylation interference pattern is identical to that found in the ternary complex between SRF and HeLa cell p62/TCF (data not shown). Taken together, these data indicate that SAP-1 has the DNA binding properties of p62/TCF.

### (*c*) *Structure of SAP-1*

We determined the complete sequence of the SAP-1 open reading frame following isolation of further cDNA clones using the polymerase chain reaction (PCR). Two forms of SAP-1 cDNA were isolated which encode isoforms of SAP-1 which differ at their C termini, SAP-1a and SAP-1b (molecular masses 49.7 kDa and 44.7 kDa respectively; figure 3). The SAP-1a protein sequence contains three regions of homology to Elk-1, a previously characterized Ets-related gene of unknown function (Rao *et al.* 1989) (figure 3, regions A–C; see figure 4). This observation strongly suggests that Elk-1 may also act as an SRF accessory protein, and both we and others have subsequently confirmed this prediction (Hipskind *et al.* 1991; Treisman *et al.* 1992). The mRNAs encoding both proteins are ubiquitously expressed.

Homology region A (70/91 identities) comprises an Ets domain, a conserved sequence motif associated with sequence specific DNA binding (Karim *et al.* 1990). Recently, McKnight and colleagues described another Ets-domain protein, GABPα, which also forms a high-affinity DNA binding complex only in association with another polypeptide, GABPβ (LaMarco *et al.* 1991; Thompson *et al.* 1991). It may be that many Ets domain proteins bind DNA only as complexes with other factors although it is too early to conclude that this reflects a particular suitability of the Ets domain for interactions of this type.

Homology region B (the 'B box'), residues 136–156, (14/21 identities) is a hydrophilic region located C terminal to the Ets domain but not at a fixed distance (44 and 55 residues in SAP-1 and Elk-1 respectively). This region is required for cooperative interaction with SRF in the ternary complex.

Homology region C (the 'C box'), encompasses residues 353–402. This region is less well defined and requires gapping of the sequences to achieve the best alignment; moreover this homology is less extensive in SAP-1b. It remains unclear whether there exists a variant of Elk-1 analogous to SAP-1b. The C box region is of interest because it contains several conserved SP and TP motifs, which form the core of potential sites for phosphorylation by the MAP kinases (Pelech & Sanghera 1992). As MAP kinase activity is stimulated by many of the same agents that activate c-fos transcription, the presence of these sequences at the C terminus of SRF accessory proteins suggests a mechanism whereby signals from the cell surface could result in phosphorylation and consequent regulation of the function of the ternary complex with SRF. Homology region C is truncated in SAP-1b, however, and this may affect its function.

### (*d*) *The SAP-1–SRF interaction domain*

We exploited the yeast colony colour assay to map the region of the SAP-1 protein required for interaction with SRF and the SRE. We subcloned pools of nested N and C terminal deletion derivatives of SAP-1 into the VP16 expression plasmid, transformed them into S62L cells containing SRF[1–508] and analysed the transformants using the colony colour assay. Because the deletion process was moderately synchro-

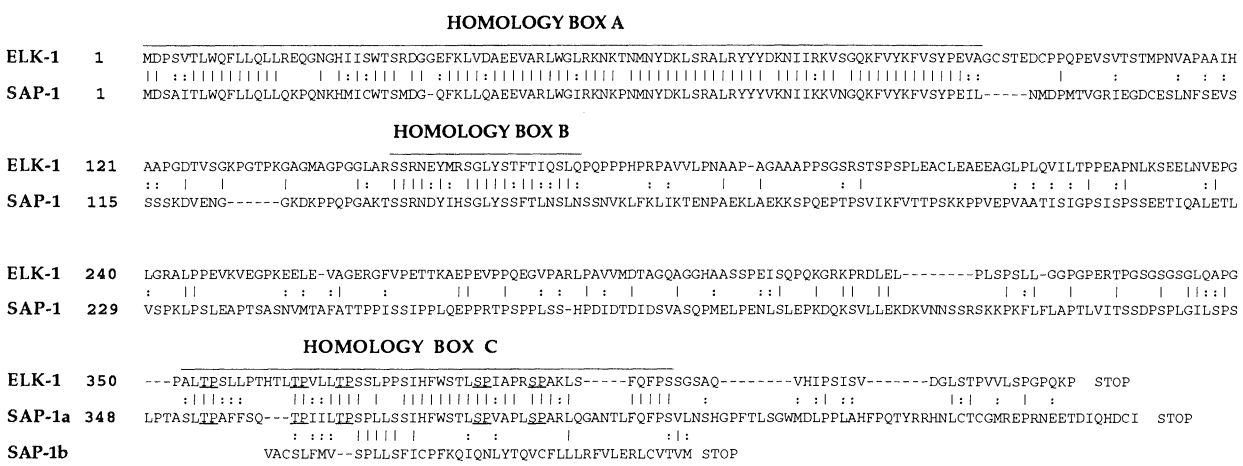


Figure 3. Comparison of the SAP-1 and Elk-1 protein sequences. The predicted sequences of the SAP-1a and SAP-1b polypeptides are shown aligned with that of Elk-1. Identities are shown by vertical lines, and conservative changes by colons. The three homology boxes are indicated, with the conserved SP and TP motifs characteristic of MAP kinase sites underlined.

nous a clear transition in the proportion of white (non-binding) colonies was observed as more sequences were removed. The SAP-1 plasmids were recovered from blue and white colonies chosen from an appropriate pool, and sequenced to determine the deletion endpoints. All N terminal deletion derivatives that retain the Ets domain gave blue colonies: a deletion in which the VP16 activator domain is joined at SAP-1 codon 8 gave no activation over background in liquid culture β-galactosidase assays (see table 1). In the colony colour assay, C terminal deletions that impinge on the B box gave white colonies: a deletion to residue 170, which includes the B box, was blue, while a deletion to residue 141 which truncates the B box, was white. These data, which were confirmed by liquid culture β-galactosidase assays (table 1), suggest that homology regions A and B are required for SAP-1 ternary complex formation with SRF.

To analyse ternary complex formation in more detail we performed ternary complex formation studies *in vitro*, using deletion derivatives of SAP-1[1–310] produced by cell free translation. Equal amounts of each polypeptide, as determined by $^{35}S$ methionine incorporation, were tested for binding to the c-fos SRE by gel mobility-shift assay in the presence or absence of SRF[133–265], a fragment of SRF which containsthe DNA binding domain. We first examined SAP-1 binding in the presence of SRF[133–265]. Consistent with the data from yeast, SAP-1 C terminal deletions that retain homology region B bind cooperatively with SRF[133–265], generating ternary complexes with mobilities that depend on the C terminal derivative used (figure 4). In contrast, C terminal deletions that disrupt or remove homology region B do not bind cooperatively with SRF[133–265], again consistent with the involvement of the B box region in ternary complex formation. Moreover, in this case we could detect autonomous binding of the truncated SAP-1 protein to the SRE suggesting that the B box also acts to inhibit DNA binding in the absence of SRF.

Because neither the length nor the sequence of the polypeptide between these the Ets domain and the B box is conserved between Elk-1 and SAP-1, we tested the effect of deletions and insertions in this region. Derivatives of SAP-1[1–310] were constructed in which amino acids 94–108 are either deleted or duplicated: both mutants efficiently formed ternary complexes cooperatively with SRF (figure 4). Interestingly, a similar experiment can be performed at the DNA level by testing ternary complex formation on probes in which the DNA sequences contacted by the two proteins in the ternary complex are moved apart or inverted. In these experiments, using the Elk-1 protein, we found that substantial variation in binding site spacing can be tolerated with relatively little effect upon the efficiency of ternary complex formation (Treisman *et al.* 1992). Moreover, in each case cooperative interaction of Elk-1 with SRF requires homology B sequences, irrespective the relative position and orientation of the DNA sequences contacted by their DNA binding domains.

Taken together, these results suggest a model for ternary complex formation in which the Ets domain contacts DNA while the B box contacts SRF, the sequence between them acting as a flexible tether region. This may provide a partial explanation for the apparent lack of conservation of the Ets motif among SREs from different cellular immediate-early genes. Even in the *Xenopus laevis* c-fos SRE, the spacing between the SRF site and the Ets motif is increased by three basepairs relative to that in the human c-fos SRE (Mohun *et al.* 1989). It is therefore likely that Elk-1 and SAP-1 can be recruited to many SREs that contain Ets binding consensus sequences near the SRF site.

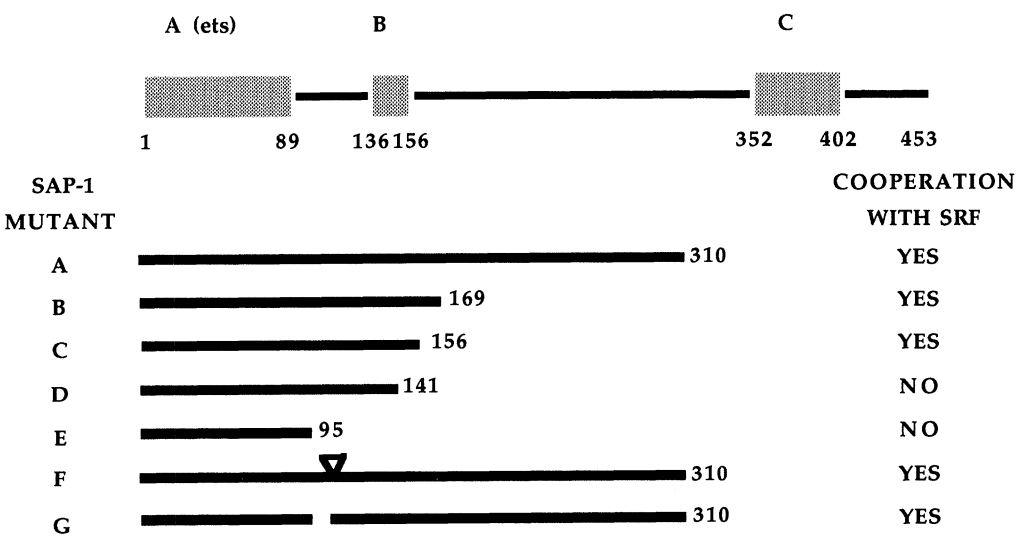


Figure 4. Summary of *in vitro* ternary complex formation assays. Various deletion and insertion derivatives of SAP-1 were produced by *in vitro* translation of appropriate cRNAs and tested for binding to the human c-fos SRE in combination with SRF[133–265], a fragment of SRF containing the DNA binding domain (Norman *et al.* 1988). The structures of the various mutants used are shown relative to the SAP-1a reading frame, in which the homologies to Elk-1 (Rao *et al.* 1989) are indicated as shaded boxes.




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### (*e*) *Function of the C box region*

In experiments which we shall present in detail elswhere, we have shown that the C terminal region of the Elk-1 protein contains multiple sites for phosphorylation by MAP kinase *in vitro*, and a growth factor-regulated kinase *in vivo*: these modifications do not appear to affect the efficiency of ternary complex formation but rather activate a transcriptional activation function associated with this region of the protein (Marais *et al.* 1993). However, in spite of the conservation of several of the sites involved in the C box region of the related protein SAP-1, we have not as yet detected an activation function in this molecule (unpublished data). Further work will be necessary to establish whether the conservation of sequence between Elk-1 and SAP-1 does indeed reflect a common function.

These results show that a major control point of SRE activity is subsequent to DNA binding, and that Elk-1 is a major target for signals at the SRE. A simple model for the regulated activity of the Elk-1 protein would be that following growth factor stimulation and activation of the MAP kinase pathway, Elk-1 would be phosphorylated and activate transcription, the requirement for multiple phosphorylations effectively setting a threshold level of kinase activity below which efficient transcriptional activation cannot occur. Downregulation could be achieved either by removal of phosphates by a nonregulated phosphatase, or by the interaction of Elk-1 with other unidentified factors.

The means by which phosphorylation can activate the transcriptional activation function of Elk-1 is not clear. It is unlikely that activation merely reflects generation of an activation region of globally acidic character, since mutation of a single phosphacceptor site is sufficient to abolish the transcriptional activation function. We speculate that phosphorylation may regulate the ability of the C terminal region to interact with other proteins, perhaps those involved in basal transcription, such as TFIIB or the TFIID complex. According to this view, phosphorylation might unmask an interaction surface, promote interactions, or both. It will be interesting to analyse the effects of C terminal mutations and phosphorylations on the interactions of Elk-1 with other components of the transcription machinery.

### (*f*) *Implications for c-fos SRE function*

The c-fos gene is activated by many agents that activate the MAP kinase pathway, and these stimuli also cause phosphorylation of the Elk-1 protein and potentiate its activity as a transcriptional activator. Moreover, in two different cell lines the ability of a growth factor to activate the MAP kinase pathway correlated with its ability to induce c-fos gene expression and its ability to activate Elk-1.

Does this mean that proteins that binding the Ets motif, such as Elk-1, present the sole target for signals arriving at the c-fos SRE? The results of previous studies of c-fos gene regulation have shown that this cannot be the case. The c-fos SRE is the target of signals elicited by many different growth factors and mitogens, mediated by both PKC-dependent and PKC independent pathways (Gilman 1988). However, in Balb/C 3T3 cells, mutation of the Ets motif blocks only the PKC-dependent component of signals elicited by whole serum, whereas mutation of the SRF binding site completely blocks the response to serum (Berkowitz *et al.* 1989; Graham & Gilman 1991). PKC-independent signals must presumably arrive at the SRE via factors distinct from those that bind the Ets motif: these might include either SRF itself or complexes formed between SRF and other transcription factors. Such factors might include proteins that bind an AP1/ATF site which is conserved next to the SRF site in several immediate-early promoters (see Treisman 1990). Thus, at the c-fos SRE different signalling pathways probably converge at the SRE via different transcription factors (see figure 5). This would allow more potential flexibility in the response of the SRE to growth factor stimulation than could be achieved by its linkage to a single signalling pathway. Our future experiments will aim to understand how such different signals modulate the activity of different transcription factors that functionally cooperate with SRF.

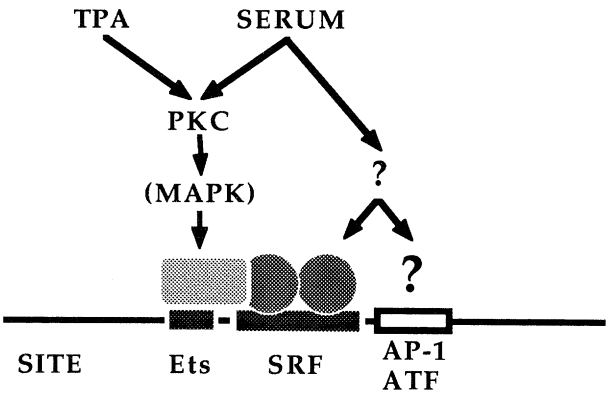


Figure 5. Transcription factor interactions and signalling pathways at the c-fos SRE. SRF is shown as a pair of circles, and ternary complex factors as a rectangle. For discussion see text.

## REFERENCES

Alexandropoulos, K., Qureshi, S. A., Rim, M., Sukhatme, V.P. & Foster, D.A. 1992 v-Fps-responsiveness in the Egr-1 promoter is mediated by serum response elements. *Nucl. Acids Res.* **20**, 2355–2359.

Almendral, J.M., Sommer, D., Macdonald, B.H., Burckhardt, J., Perera, J. & Bravo, R. 1988 Complexity of the early genetic response to growth factors in mouse fibroblasts. *Molec. cell. Biol.* **8**, 2140–2148.

Bender, A. & Sprague, G.J. 1987 MAT alpha 1 protein, a yeast transcription activator, binds synergistically with a second protein to a set of cell-type-specific genes. *Cell* **50**, 681–691.

Berkowitz, L.A., Riabowol, K.T. & Gilman, M.Z. 1989 Multiple sequence elements of a single functional class are required for cyclic AMP responsiveness of the mouse c-fos promoter. *Molec. cell. Biol.* **9**, 4272–4281.

Breeden, L. & Nasmyth, K. 1987 Cell cycle control of the




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

yeast HO gene: cis- and trans-acting regulators. *Cell* **48**, 389–397.

Curran, T. & Franza, B.J. 1988 Fos and Jun: the AP-1 connection. *Cell* **55**, 395–397.

Dalton, S. & Treisman, R. 1992 Characterization of SAP-1, a protein recruited by serum response factor to the c-fos serum response element. *Cell* **68**, 597–612.

Errede, B. & Ammerer, G. 1989 STE12, a protein involved in cell-type-specific transcription and signal transduction in yeast, is part of protein-DNA complexes. *Genes Dev.* **3**, 1349–1361.

Fields, S. & Song, O. 1989 A novel genetic system to detect protein-protein interactions. *Nature, Lond.* **340**, 245–246.

Fujii, M., Shalloway, D. & Verma, I.M. 1989 Gene regulation by tyrosine kinases: src protein activates various promoters, including c-fos. *Molec. cell. Biol.* **9**, 2493–2499.

Fukumoto, Y., Kaibuchi, K., Oku, N., Hori, Y. & Takai, Y. 1990 Activation of the c-fos serum-response element by the activated c-Ha-ras protein in a manner independent of protein kinase C and cAMP-dependent protein kinase. *J. biol. Chem.* **265**, 774–780.

Gauthier-Rouviere, C., Fernandez, A. & Lamb, N.J. 1990 ras-induced c-fos expression and proliferation in living rat fibroblasts involves C-kinase activation and the serum response element pathway. *EMBO J.* **9**, 171–180.

Gilman, M.Z. 1988 The c-fos serum response element responds to protein kinase C-dependent and -independent signals but not to cyclic AMP. *Genes Dev.* **2**, 394–402.

Graham, R. & Gilman, M. 1991 Distinct protein targets for signals acting at the c-fos serum response element. *Science, Wash.* **251**, 189–192.

Herrera, R.E., Shaw, P.E. & Nordheim, A. 1989 Occupation of the c-fos serum response element in vivo by a multi-protein complex is unaltered by growth factor induction. *Nature, Lond.* **340**, 68–70.

Hill, C., Marais, R., Wynne, J., John, S., Dalton, S. & Treisman, R. 1993 Functional analysis of a growth factor responsive transcription factor complex. *Cell* **73,** 381–393.

Hipskind, R.A., Rao, V.N., Mueller, C.G., Reddy, E.S. & Nordheim, A. 1991 Ets-related protein Elk-1 is homologous to the c-fos regulatory factor p62TCF. *Nature, Lond.* **354**, 531–534.

Howe, L.R., Leevers, S.J., Gomez, N., Nakielny, S., Cohen, P. & Marshall, C.J. 1992 Activation of the MAP kinase pathway by the protein kinase Raf. *Cell* **71**, 335–342.

Jamal, S. & Ziff, E. 1990 Transactivation of c-fos and beta-actin genes by raf as a step in early response to transmembrane signals. *Nature, Lond.* **344**, 463–466.

Kaibuchi, K., Fukumoto, Y., Oku, N. *et al.* 1989 Activation of the serum response element and 12-O-tetradecanoylphorbol-13-acetate response element by the activated c-raf-1 protein in a manner independent of protein kinase C. *J. biol. Chem.* **264**, 20855–20858.

Karim, F.D., Urness, L.D., Thummel, C.S. *et al.* 1990 The ETS-domain: a new DNA-binding motif that recognizes a purine-rich core DNA sequence. *Genes Dev.* **4**, 1451–1453.

Keleher, C.A., Goutte, C. & Johnson, A.D. 1988 The yeast cell-type-specific repressor alpha 2 acts cooperatively with a non-cell-type-specific protein. *Cell* **53**, 927–936.

LaMarco, K., Thompson, C.C., Byers, B.P., Walton, E.M. & McKnight, S.L. 1991 Identification of Ets- and notch-related subunits in GA binding protein. *Science, Wash.* **253**, 789–792.

Leevers, S.J. & Marshall, C.J. 1992 Activation of extracellular signal-regulated kinase, ERK2, by p21ras oncoprotein. *EMBO J.* **11**, 569–574.

Ma, J. & Ptashne, M. 1988 Converting a eukaryotic transcriptional inhibitor into an activator. *Cell* **55**, 443–446.

Malik, R.K., Roe, M.W. & Blackshear, P.J. 1991 Epidermal growth factor and other mitogens induce binding of a protein complex to the c-fos serum response element in human astrocytoma and other cells. *J. biol. Chem.* **266**, 8576–8582.

Marais, R., Wynne, J. & Treisman, R. 1993 The SRF accessory protein Elk-1 contains a growth-factor-regulated transcriptional activation domain. *Cell* **73,** 395–406.

Marsh, L., Neiman, A.M. & Herskowitz, I. 1991 Signal transduction during pheromone response in yeast. *A. Rev. Cell Biol.* **7**, 699–728.

Mohun, T.J., Garrett, N. & Taylor, M.V. 1989 Temporal and tissue-specific expression of the proto-oncogene c-fos during development in *Xenopus laevis*. *Development* **107**, 835–846.

Mueller, C.G. & Nordheim, A. 1991 A protein domain conserved between yeast MCM1 and human SRF directs ternary complex formation. *EMBO J.* **10**, 4219–4229.

Norman, C., Runswick, M., Pollock, R. & Treisman, R. 1988 Isolation and properties of cDNA clones encoding SRF, a transcription factor that binds to the c-*fos* serum response element. *Cell* **55**, 989–1003.

Pelech, S.L. & Sanghera, J.S. 1992 Mitogen-activated protein kinases: versatile transducers for cell signalling. *TIBS* **17**, 233–238.

Primig, M., Winkler, H. & Ammerer, G. 1991 The DNA binding and oligomerization domain of MCM1 is sufficient for its interaction with other regulatory proteins. *EMBO J.* **10**, 4209–4218.

Qureshi, S.A., Cao, X.M., Sukhatme, V.P. & Foster, D.A. 1991 v-Src activates mitogen-responsive transcription factor Egr-1 via serum response elements. *J. biol. Chem.* **266**, 10802–10806.

Rao, V.N., Huebner, K., Isobe, M., ar-Rushdi, A., Croce, C.M. & Reddy, E.S. 1989 elk, tissue-specific ets-related genes on chromosomes X and 14 near translocation breakpoints. *Science, Wash.* **244**, 66–70.

Rivera, V.M. & Greenberg, M.E. 1990 Growth factor-induced gene expression: the ups and downs of c-fos regulation. *New Biol.* **2**, 751–758.

Sartorelli, V., Webster, K.A. & Kedes, L. 1990 Muscle-specific expression of the cardiac alpha-actin gene requires MyoD1, CArG-box binding factor, and Sp1. *Genes Dev.* **4**, 1811–1822.

Sassone-Corsi, P., Der, C.J. & Verma, I.M. 1989 ras-induced neuronal differentiation of PC12 cells: possible involvement of fos and jun. *Molec. cell. Biol.* **9**, 3174–3183.

Shaw, P. 1992 Ternary complex formation over the c-fos serum response element: p62TCF exhibits dual component specificity with contacts to DNA and an extended structure in the DNA binding domain of p67SRF. *EMBO J.* **11**, 3011–3019.

Shaw, P.E., Schroter, H. & Nordheim, A. 1989 The ability of a ternary complex to form over the serum response element correlates with serum inducibility of the human c-fos promoter. *Cell* **56**, 563–572.

Siegfried, Z. & Ziff, E.B. 1990 Altered transcriptional activity of c-fos promoter plasmids in v-raf-transformed NIH 3T3 cells. *Molec. cell. Biol.* **10**, 6073–6078.

Stacey, D.W., Watson, T., Kung, H.F. & Curran, T. 1987 Microinjection of transforming ras protein induces c-fos expression. *Molec. cell. Biol.* **7**, 523–527.

Taylor, M., Treisman, R., Garrett, N. & Mohun, T. 1989 Muscle-specific (CArG) and serum-responsive (SRE) promoter elements are functionally interchangeable in *Xenopus* embryos and mouse fibroblasts. *Development 106*, 67–78.

Thomas, S.M., DeMarco, M., DArcangelo, G., Halegoua,





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S. & Brugge, J.S. 1992 Ras is essential for nerve growth factor- and phorbol ester-induced tyrosine phosphorylation of MAP kinases. *Cell* **68**, 1031–1040.

Thompson, C.C., Brown, T.A. & McKnight, S.L. 1991 Convergence of Ets- and notch-related structural motifs in a heteromeric DNA binding complex. *Science, Wash.* **253**, 762–768.

Treisman, R. 1987 Identification and purification of a polypeptide that binds to the c-fos serum response element. *EMBO J.* **6**, 2711–2717.

Treisman, R. 1990 The SRE: a growth factor responsive transcriptional regulator. *Semin. Cancer Biol.* **1**, 47–58.

Treisman, R.H., Marais, R.M. & Wynne, J.K. 1992 Spatial flexibility in ternary complex formation by SRF and its accessory proteins. *EMBO J.* **11**, 4631–4640.

Walsh, K. 1989 Cross-binding of factors to functionally different promoter elements in c-fos and skeletal actin genes. *Molec. cell. Biol.* **9**, 2191–2201.

Wood, K.W., Sarnecki, C., Roberts, T.M. & Blenis, J. 1992 ras mediates nerve growth factor receptor modulation of three signal-transducing protein kinases: MAP kinase, Raf-1, and RSK. *Cell* **68**, 1041–1050.

Wynne, J. & Treisman, R. 1992 SRF and MCM1 have related but distinct DNA binding specificities. *Nucl. Acids Res.* **20**, 3297–3303.





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