The EMBO Journal vol.15 no.13 pp.3403–3412, 1996

Activation of the serum response factor by p65/NF-κB

Guido Franzoso, Louise Carlson, Keith Brown, Mary Beth Daucher, Peter Bressler and Ulrich Siebenlist[1]

Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA

[1]Corresponding author

This study demonstrates that the NF-κB subunit p65 can act like an accessory protein for the serum response factor (SRF) in transfection assays. p65 functionally synergizes with SRF to activate the transcription of a reporter construct dependent only on the serum response element (SRE). The synergy of the two factors requires neither a κB motif nor direct contact of p65 with DNA. Consistent with these results, a physical complex containing p65 and SRF is observed *in vitro*. Synergy of the factors is independent of the previously described activation domains present on p65, ruling out indirect effects of p65, but synergy is dependent on the activation domain of SRF. The complexing of p65 and SRF is mediated by a segment of the SRF DNA binding domain, a region of the protein which has also been reported to inhibit its own activation domain. We speculate that p65, upon direct or facilitated interaction with SRF, may relieve the inhibitory activity of this segment, thus enabling the activation domain of SRF to become fully functional. In contrast to p65, the p50 subunit of NF-κB does not interact significantly with SRF, either functionally or physically. The data suggest the intriguing possibility that NF-κB may participate in the regulation of SRE-dependent promoters, expanding the range of activities of this rapidly activatable transcription factor.

Keywords: accessory factors/NF-κB/p65/serum response factor/transcriptional regulation

Introduction

The NF-κB/Rel family of dimeric transcription factors is involved in the regulated expression of a large array of genes, particularly of those participating in immune functions induced in response to pathogen-related stimuli. The NF-κB family of transcription factors also regulates the expression of several viruses, including human immunodeficiency virus (HIV)-1. NF-κB is retained in the cytoplasm of unstimulated cells by the inhibitory IκB-α protein; in response to pathogen- or stress-related stimuli, inhibition is relieved and the transcription factor translocates into the nucleus (reviewed in Siebenlist *et al.*, 1994). Activation involves the site-specific phosphorylation of IκB-α serine residues at positions 32 and 36, followed by proteolytic degradation of the phosphorylated inhibitor (Brockman *et al.*, 1995; Brown *et al.*, 1995; Traenckner *et al.*, 1995; Whiteside *et al.*, 1995). The NF-κB/Rel protein subunits, which include p50 (NF-κB1), p52 (NF-κB2), p65 (RelA), RelB and c-Rel (Rel), can form multiple homo- and heterodimeric complexes. Those dimers containing c-Rel, p65 and RelB can potently transactivate κB site-dependent reporters, whereas homodimers of the p50 and p52 subunit by themselves cannot, because of the absence of known activation domains in the latter proteins (Siebenlist *et al.*, 1994).

In the context of promoters or enhancers, NF-κB dimers can functionally interact with other transcription factors. For example, NF-κB physically associates with both activating transcription factor (ATF)-2 and high mobility group protein (HMG)-I/Y on the interferon-β promoter, and the resulting protein–DNA multicomplex appears to be necessary for efficient transcriptional activation (Thanos and Maniatis, 1992, 1995; Du *et al.*, 1993). It has also been reported that NF-κB may interact both physically and functionally with c-*fos*/c-*jun*, CCAAT/enhancer binding protein (C/EBP), interferon regulatory factor 1 (IRF-1), SP-1 and the glucocorticoid receptor (reviewed in Siebenlist *et al.*, 1994).

We demonstrate here that NF-κB functionally interacts with the serum response factor (SRF). SRF participates in the expression of many immediate-early genes in response to growth factors or other mitogens by binding to serum response elements (SREs) present in these genes (Treisman, 1990, 1992, 1994). Signal-induced transactivation through the SRE motif can occur through the phosphorylation of an accessory factor which is already constitutively associated with SRF in a ternary complex with DNA (Gille *et al.*, 1992; Hill *et al.*, 1993; Janknecht *et al.*, 1993, 1994; Marais *et al.*, 1993; Zinck *et al.*, 1993; Kortenjann *et al.*, 1994; Treisman, 1994).

At the c-*fos* SRE, SRF forms a ternary complex with the ternary complex factor $p62^{TCF}$ (Shaw *et al.*, 1989). Proteins of the Ets family, including Elk-1, SRF accessory protein (SAP)-1a, SAP-1b, SAP-2 and Net, have been shown to exhibit $p62^{TCF}$-like biochemical properties (Hipskind *et al.*, 1991; Dalton and Treisman, 1992; Giovane *et al.*, 1994). Association of the proteins requires the conserved B-box of the Ets proteins and sequences located in the core domain of SRF ($core^{SRF}$), a region that also encodes the DNA binding and dimerization functions of SRF (Normann *et al.*, 1988; Mueller and Nordheim, 1991; Dalton and Treisman, 1992; Janknecht and Nordheim, 1992; Rao and Reddy, 1992; Shaw, 1992; Treisman *et al.*, 1992; Shore and Sharroks, 1994). Furthermore, ternary complex formation requires contact of the Ets proteins with an Ets recognition element directly adjacent to the SRE; in the absence of SRF, interaction of the Ets proteins with this element is insufficient for

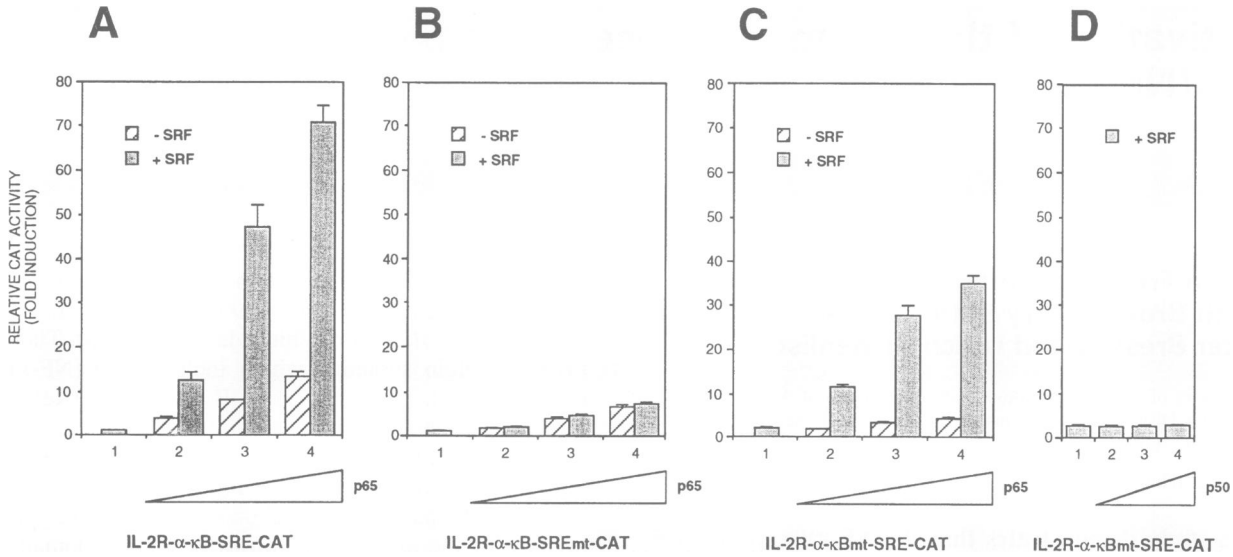

Fig. 1. Synergistic transactivation mediated by p65 and SRF does not require the κB binding element. NTera-2 cells were transfected with increasing amounts of the p65 expression plasmid (A–C; columns 2–4; 0.1, 0.3 and 1.0 μg, respectively) or the p50 expression vector (D; columns 2–4; 0.3, 1.0 and 2.0 μg, respectively) in the absence (hatched columns) or presence of the SRF expression vector (solid columns 1–4; A–C, 0.3 μg; D, 1.0 μg). CAT reporter plasmids are as indicated (6.0 μg; columns 1–4): (**A**) IL-2R–κB–SRE–CAT, (**B**) IL-2R–κB–SREmt–CAT and (**C** and **D**) IL-2R–κBmt–SRE–CAT. The fold induction values represent CAT activity relative to that observed with reporter plasmid alone. Each column represents the mean of three independent experiments after normalization to the protein concentration of the cellular extracts. The total amounts of transfected DNA and expression vector were kept constant throughout by adding compensating amounts of the PMT2T vector without insert.

binding (Hipskind *et al.*, 1991; Dalton and Treisman, 1992). Elk-1-mediated transactivation through the SRE is regulated by MAP kinase, a growth factor-regulated kinase, which phosphorylates the activation domain of the accessory protein to potentiate its activity (Hill *et al.*, 1993; Marais *et al.*, 1993). Phosphorylation may further promote ternary complex formation and DNA binding in conjunction with SRF (Gille *et al.*, 1992, 1995). In addition to Elk-1's activation domain, that of SRF is also required for full signal-induced responses, an indication that its activity may be regulated in some way (Janknecht *et al.*, 1992; Hill *et al.*, 1993, 1994; Johansen and Prywes, 1994). It has been noted that SRF contains sequences, including those within its core domain, that can inhibit its own activation domain (Johansen and Prywes, 1993). This has led to the idea that activation signals may relieve the inhibitory activity. It is possible, for example, that an unknown inhibitory protein is removed; alternatively, the activation domain may be liberated from intramolecular inhibition via conformational changes in SRF (Johansen and Prywes, 1993, 1994; Hill *et al.*, 1994).

In addition to this mechanism, signal-induced transactivation through the SRE motif can occur independently of the known TCF accessory proteins (Treisman, 1990; Graham and Gilman, 1991; Konig, 1991; Sadoshima and Izumo, 1993; Hill *et al.*, 1994; Johansen and Prywes, 1994). This could be explained by the existence of as yet undescribed accessory factors (Treisman, 1990; Mueller and Nordheim, 1991; Hill *et al.*, 1994, 1995; Miranti *et al.*, 1995). Several genes, including the human interleukin (IL)-2R-α gene, contain functional SRE elements in their regulatory regions which lack associated Ets motifs (Mohun *et al.*, 1987; Treisman, 1990, 1992; Latinkic *et al.*, 1991; Kuang *et al.*, 1993; Treisman *et al.*, 1992).

During T-cell activation, expression of the IL-2R-α gene is regulated, at least in part, by NF-κB (Bohnlein *et al.*, 1988; Leung and Nabel, 1988; Ruben *et al.*, 1988; Cross *et al.*, 1989; Tan *et al.*, 1992). Nonetheless, the IL-2R-α-κB site binds NF-κB only poorly and, when used by itself, mediates only a modest NF-κB-dependent transactivation of a linked reporter gene (Franzoso *et al.*, 1993; Kuang *et al.*, 1993). In the context of the IL-2R-α promoter region, however, this element is greatly enhanced in its activity, in particular in T cells, because of the presence of an adjacent SRE motif (Ballard *et al.*, 1989; Cross *et al.*, 1989; Toledano *et al.*, 1990; Kuang *et al.*, 1993; Pierce *et al.*, 1995). Based on the studies presented here, we propose that the NF-κB subunit p65 (and c-Rel) can function as an SRF accessory molecule, leading to stimulation through the SRE–SRF complex. Surprisingly, a functional synergy between the factors does not require the κB binding site in our assays and is mediated only by the activation domain of SRF and not that of p65. It is possible that transactivation functions encoded in SRF are liberated as a consequence of their association with NF-κB/p65 (or c-Rel).

Results

Synergistic transactivation of p65 and SRF through the SRE motif

To investigate whether SRF and NF-κB functionally interact, we transfected NTera-2 cells with expression vectors for these factors (see Materials and methods), together with a chloramphenicol acetyl transferase (CAT) reporter plasmid driven by the κB and SRE elements of the IL-2R-α gene (κB–SRE–CAT; Figure 1A). p65 alone only weakly transactivated the reporter plasmid, even at high concentrations (Figure 1A, hatched columns), consistent with the low affinity of p65/p65 or p50/p65 dimers for this particular κB site (Franzoso *et al.*, 1993; Kuang *et al.*, 1993). SRF alone did not increase CAT

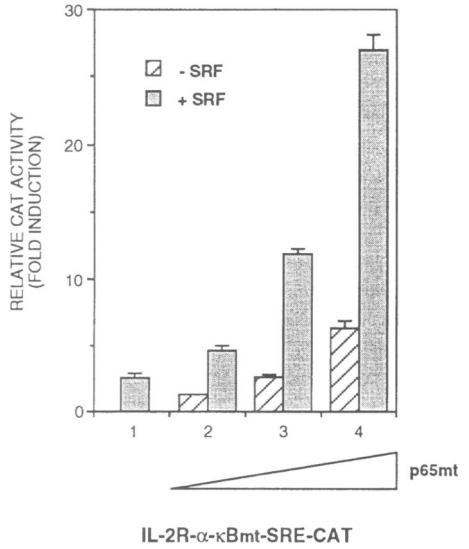

Fig. 2. A p65 mutant unable to bind DNA still synergizes with SRF. NTera-2 cells were transfected with increasing amounts of the p65mt$^{33-34}$ expression vector (columns 2–4; 0.3, 1.0 and 2.0 μg, respectively) in the absence (hatched columns) or presence of the SRF expression vector (solid columns 1–4; 0.3 μg). The reporter (6.0 μg) was the IL-2R–κBmt–SRE–CAT vector (lanes 1–4). See legend to Figure 1 for more details on CAT activity calculations and how DNA amounts were kept constant throughout.

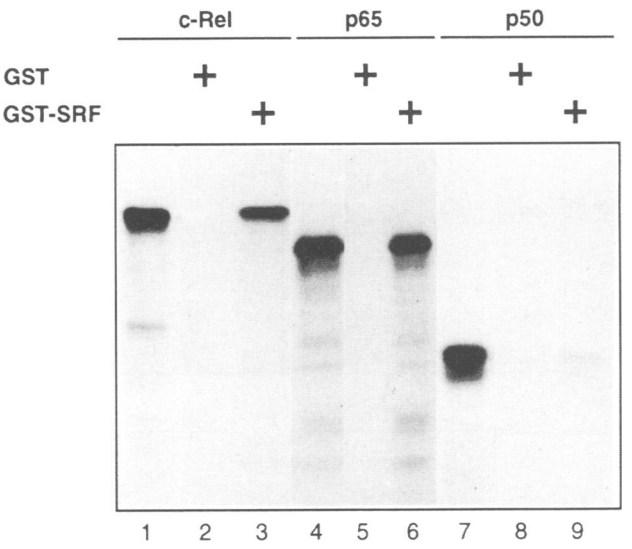

Fig. 3. SRF physically complexes with c-Rel and p65, but not significantly with p50. ^{35}S-labeled c-Rel (lanes 1–3), p65 (lanes 4–6) and p50 (lanes 7–9) are shown as *in vitro* translated products (1.0 μl) (lanes 1, 4 and 7) or after precipitation (of 20 μl) with GST–SRF fusion proteins attached to glutathione–Sepharose beads (lanes 3, 6 and 9) or with GST alone attached to the beads (lanes 2, 5 and 8).

activity above background levels (column 1). However, when SRF was co-transfected together with p65, a marked synergistic and dose-dependent transactivation could be observed (solid columns).

Mutation of the SRE motif in the reporter construct (κB–SREmt–CAT) abrogated the ability of p65 to synergistically transactivate the reporter construct, regardless of the level of exogenous SRF (Figure 1B and data not shown). p65 alone was slightly less efficient in the transactivation of κB–SREmt–CAT than of κB–SRE–CAT (cf. hatched columns in Figure 1A and B), most probably because of detectable endogenous SRF in NTera-2 cells (data not shown). Surprisingly, mutation of the κB motif in the reporter construct (κBmt–SRE–CAT) did not abrogate the ability of p65 to synergistically transactivate in the presence of co-transfected SRF (Figure 1C). The absolute CAT activities observed with the κB mutant vector were slightly reduced (~2-fold) when compared with the κB–SRE–CAT vector, but the magnitude of the synergistic effect was unchanged (compare ratios of hatched and solid columns in Figure 1A with those in Figure 1C).

Qualitatively similar results were obtained by the co-expression of SRF together with p50 and p65 or with c-Rel, but not with p50 alone (data not shown; Figure 1D). Consistent with the observation that synergy is dependent only on the SRE site and does not require the κB site, we found that synergy was unaffected by altering the spacing of the two DNA elements in the vector by inserting two, five, seven or 10 nucleotides (data not shown).

The DNA binding activity of p65 is not required for the transcriptional activation of the SRE element

To explore the possibility that synergy between SRF and p65 does not require the DNA binding ability of p65, we made use of a DNA binding mutant of p65. Previously we showed that mutations of amino acids at positions 56–57 of the NF-κB/p50 subunit abolish DNA binding of that protein (Bressler *et al.*, 1993). These residues are localized in a region which directly contacts DNA (Ghosh *et al.*, 1995; Müller *et al.*, 1995). Because the DNA binding domain is highly conserved among various NF-κB/Rel family members, we generated an analogous mutation in p65 by substitution of the amino acids at positions 33–34 (p65mt). Loss of DNA binding was assessed by an electrophoretic mobility shift assay *in vitro*, and loss of function was confirmed by co-transfection of the p65mt expression vector together with an HIV–κB–CAT reporter construct (data not shown).

When co-transfected with SRF, p65mt consistently caused synergistic transactivation of the κBmt–SRE–CAT reporter (Figure 2), and the magnitude of this effect was similar to that seen with wild-type p65 (see Figure 1C). Qualitatively similar results were obtained with another DNA binding mutant of p65 bearing substitutions of amino acids at positions 36 and 37 (data not shown). Our data suggest a potential activating role for p65 in the context of the SRE–SRF complex, even in the absence of DNA binding by p65.

Physical complexes of SRF and select NF-κB family members

Our data could be explained if p65 was somehow tethered to the SRE element via SRF. To probe for a potential physical interaction between SRF and NF-κB, partially purified glutathione S-transferase (GST)–SRF fusion proteins were mixed with radiolabeled p65, c-Rel or p50 and precipitated with glutathione-coated Sepharose beads (Figure 3). The GST–SRF fusion proteins bound c-Rel and p65, while p50 was not retained to a significant degree (Figure 3, lanes 3, 6 and 9, respectively). This indicates that SRF can specifically complex with p65 (or c-Rel) *in vitro*. It remains to be determined, however, if the

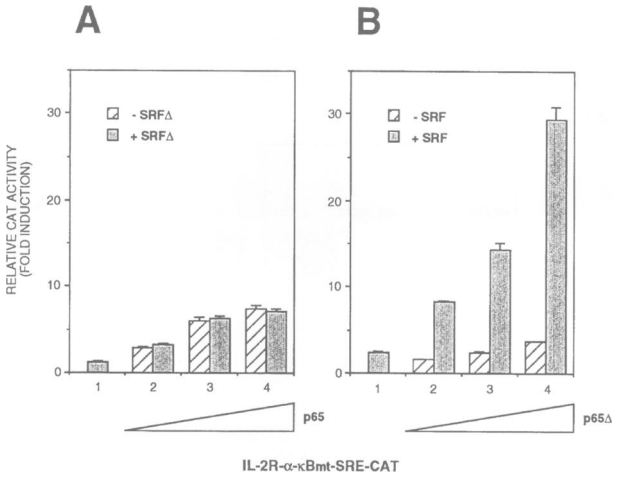

Fig. 4. Synergy requires the SRF activation domain, but not the p65 activation domain. NTera-2 cells were transfected with increasing amounts of p65 (**A**) or p65Δ (**B**) expression vectors alone (hatched columns 2–4; 0.3, 1.0 and 2.0 μg, respectively) or together with SRFΔ (A) or SRF (B) expression vectors (solid columns 1–4; 1.0 μg). The reporter plasmid was IL-2R–κBmt–SRE–CAT. See legends to Figures 1 and 3 for further details.

interaction is direct or requires an additional factor(s) which could have been present in the cell extracts used to synthesize the proteins. As a negative control, GST alone did not retain the radiolabeled proteins (Figure 3, lanes 2, 5 and 8). The lack of significant interaction between p50 and SRF correlates well with the lack of functional synergy between these two factors (Figure 1D).

Synergistic transactivation is mediated by the SRF activation domain

Both SRF and p65 contain well-defined C-terminal activation domains (Schmitz and Baeuerle, 1991; Ballard *et al.*, 1992; Johansen and Prywes, 1993; Blair *et al.*, 1994). Thus, both proteins may have been directly involved in initiating transcription in our experiments. Another possibility is that SRF merely tethered the strong transactivator p65 to DNA. To gain insight into the mechanism of synergistic transactivation, we generated truncations of SRF and p65 which lack activation domains but which still retain the ability to dimerize and bind to DNA [SRFΔ and p65Δ (Kanno *et al.*, 1994), respectively; see Materials and methods and maps in Figures 5 and 6]. SRFΔ abolished synergy and did not increase expression of the reporter above that seen with p65 alone (Figure 4A). The transfection of even higher amounts of SRFΔ actually resulted in a reduction of the CAT activity, presumably because of competition with endogenous SRF for the SRE site (data not shown).

Surprisingly, p65 proteins lacking the C-terminal activation domains (p65Δ) retained the ability to synergistically transactivate together with wild-type SRF (Figure 4B). The ability of both p65Δ (no transactivation domain) and p65mt (no DNA binding) (Figure 2) to synergize with SRF effectively rules out the possibility that p65, upon transfection, may have induced, via κB elements, the expression of another protein, which then acted as an accessory factor for SRF. Our data do show that synergistic transactivation by p65 is mediated through the C-terminal activation domain of SRF. When transfected alone, SRF did not induce significant levels of reporter gene activity (Figure 1, column 1). It is possible that, as a result of its interaction with p65, SRF may undergo a change to fully express its transcriptional activation function, a function which is otherwise inhibited (see Discussion).

Synergy may also have resulted if association with p65 was to significantly increase the DNA binding of SRF. Such a model was proposed for the interaction of Phox1 with SRF (Grueneberg *et al.*, 1992). No increase in binding to SRE was observed in electrophoretic mobility shift assays using extracts of cells transfected with SRF plus p65, compared with SRF alone (data not shown). In addition, these electrophoretic mobility shift assays did not reveal a distinctly migrating complex involving SRE, SRF and p65, nor could such a complex be demonstrated with p65 supershifting antibodies. It is likely that the interaction between the factors was too unstable to be detected with these *in vitro* assays.

Mapping of the regions of SRF and p65 required for physical interaction

To define the domains responsible for complex formation between SRF and p65, a series of N- and C-terminal deletions of SRF and p65 were constructed (maps shown in Figures 5C and 6C, respectively). ^{35}S-labeled full-length and deleted SRF and p65 proteins were produced by *in vitro* transcription/translation (Figures 5A and 6A, respectively). For each series of deletions, roughly equal amounts of the radiolabeled proteins were incubated with GST–p65 (Figure 5B) or with GST–SRF (Figure 6B) bound to glutathione-coated beads; incubation with GST alone served as a negative control. An analysis of SRF truncations revealed that the activation domain and the N-terminal region were both dispensable for the interaction with p65 (summarized in Figure 5C). This indicates that the activation domain of SRF is required only for functional synergy and not for physical association. Most deletions extending into the SRF dimerization and DNA binding domains prevented association with p65. However, fully intact dimerization or DNA binding functions may not be required for interaction with p65 because one C-terminal deletion, which removes a small part of the region previously found necessary for these functions, was still able to associate with p65 (Figure 5B, lane 14; *Pst*I). SRF C- and N-terminal truncations were more efficient in associating with p65 than full-length or near full-length proteins. This may be because of partial shielding of the core^{SRF} domain by intramolecular associations in SRF (see Discussion).

In the case of p65, the C-terminal portion of the Rel homology domain (RHD) was necessary to form a complex with SRF (Figure 6B, and summarized in Figure 6C). Although this region encodes dimerization functions of p65, dimerization *per se* was dispensable for interaction because several deletions known to impair dimerization still allowed for binding to SRF (Figure 6B, lanes 14 and 16; *Pst*I, *Apa*LI). The region of p65 identified here as necessary for association with SRF contains sequences which are most likely exposed on the outside of the protein, away from the DNA and dimerization interfaces (Figure 6D). This prediction is based on the 3-D structure of p50 homodimers, which is presumably very similar to that of p65-containing complexes, given the overall

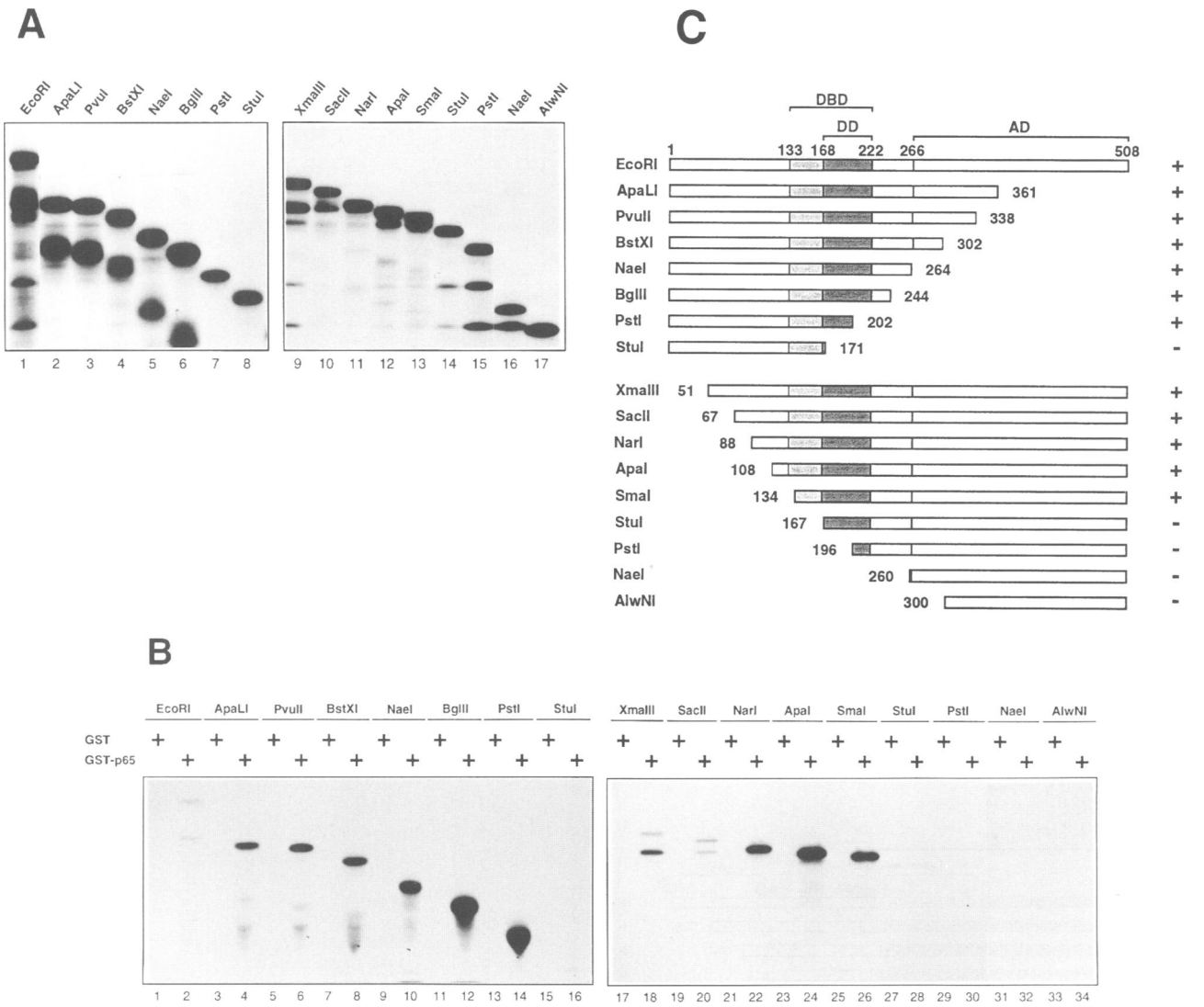

Fig. 5. Mapping of the domain in SRF required to form a complex with p65. (**A**) Roughly equal amounts of ^{35}S-labeled N- (lanes 1–8) and C- (lanes 9–17) terminal truncations of SRF were produced by *in vitro* transcription/translation reactions; truncations are as indicated in (C). (**B**) The SRF-derived proteins shown in (A) were incubated with GST or GST–p65 fusion proteins, as indicated, and precipitated with glutathione-coated Sepharose beads. The bound proteins were analyzed by SDS–PAGE and visualized by autoradiography. (**C**) Schematic representations of the SRF full-length and truncated products, generated with the indicated restriction enzymes as described in Materials and methods, and summary of the data shown in (B). The position number of the first or last amino acid present in the deletion proteins is listed. '+' indicates specific interaction, while '–' indicates the absence of a measurable interaction. DBD, DNA binding domain; DD, dimerization domain; AD, activation domain.

conservation of primary sequence in the relevant domains (Ghosh *et al.*, 1995; Müller *et al.*, 1995; Figure 6D). The exposed sequences appear to be ideally located in space for contacting other proteins.

The region of SRF critical for complexing with p65 may overlap the previously described domain which inhibits transactivation by the SRF activation domain (Johansen and Prywes, 1993; see also Discussion). Part of this region of SRF has also been shown to be involved in the direct interaction with SRF accessory proteins, such as Elk-1 (Mueller and Nordheim, 1991; Shaw, 1992). Co-transfection of Elk-1 along with p65 and SRF in unstimulated cells resulted in a marked decrease in the p65-mediated synergistic transactivation of the reporter (Figure 7). This is consistent with the idea that binding of SRF to Elk-1 and complex formation with p65 are incompatible with each other, possibly due to steric hindrance of the proteins involved, because closely spaced regions of SRF appear to be implicated in the two types of interaction. The synergistic transactivation of p65 and SRF was also efficiently inhibited by co-transfected IκB-α, presumably because this inhibitor retains p65 within the cytoplasmic compartment (Figure 7).

Discussion

We demonstrate here that the NF-κB subunit p65 interacts functionally and, in some way physically, with the SRF transcription factor. SRF and p65 potently synergize to transactivate a reporter gene, dependent on the SRE, the DNA recognition element for SRF, but not dependent on a κB DNA element. Interaction between p65 and DNA appears to be entirely dispensable, at least in our assays, because a mutant of p65 deficient in DNA binding continues to synergize with SRF. Finally, the p65 transactivation domain(s) is not required either, because a C-terminally

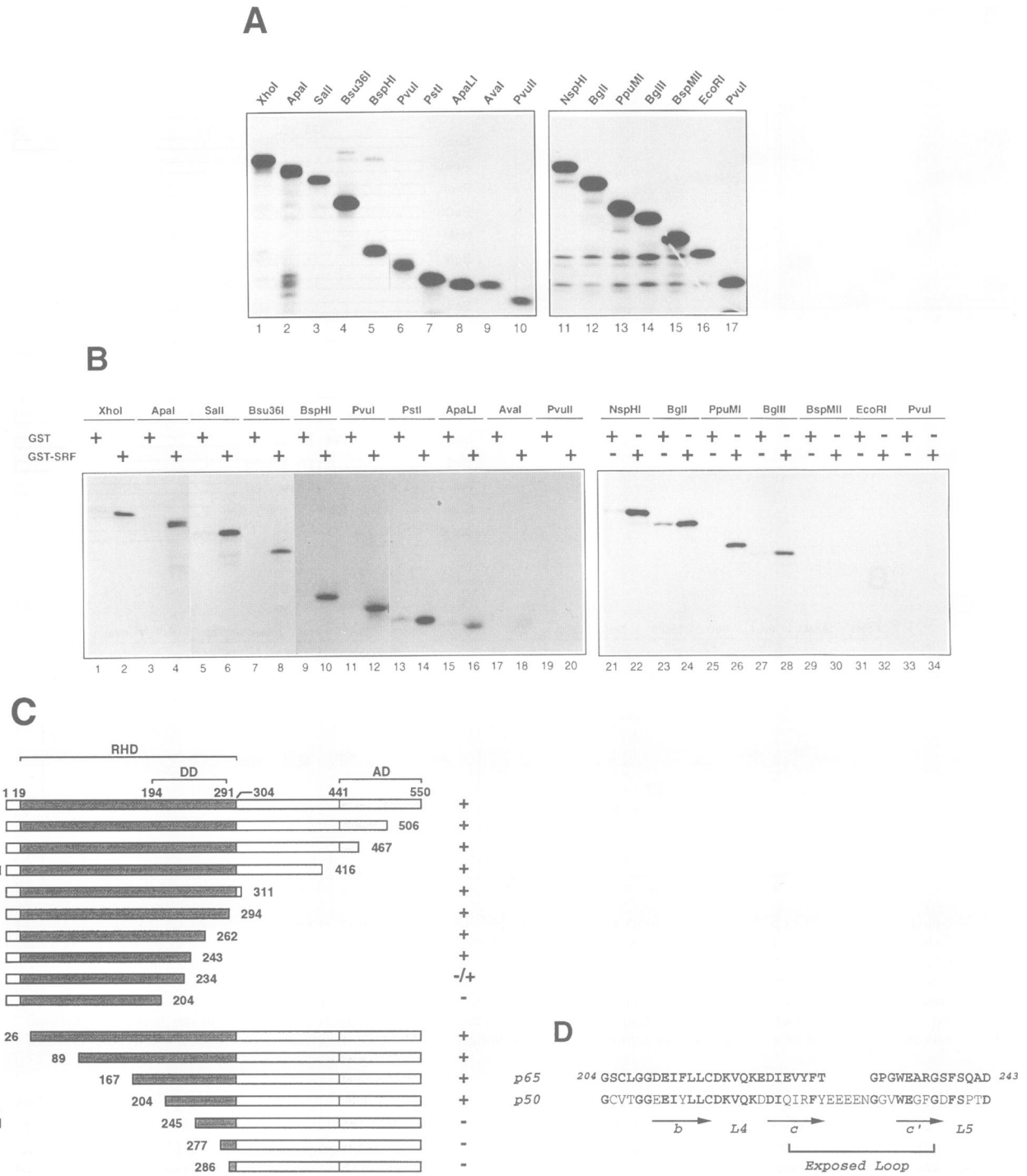

Fig. 6. Mapping of the domain in p65 required to form a complex with SRF. (**A**) Roughly equal amounts of ^{35}S-labeled N- (lanes 1–10) and C- (lanes 11–17) terminal truncations of p65 were produced by *in vitro* transcription/translation reactions; truncations are as indicated in (C). (**B**) The p65-derived proteins shown in (A) were incubated with GST or GST–SRF fusion proteins, as indicated. (**C**) Schematic representations of the p65 full-length and truncated products, position number of the first or last amino acid present, and summary of the data shown in (B). For more details see the legend to Figure 5; the *Ava*I truncation exhibits a weak, but specific, interaction, indicated by '–/+'. RHD, Rel homology domain; AD, activation domain; DD, dimerization domain. (**D**) Sequence of the p65 domain required to form a complex with SRF, as delimited in (B). The corresponding region of p50 is shown for comparison, with identical amino acids printed in bold characters. *b*, *c* and *c'* are β-strands in the protein structure of p50 homodimers, as taken from Ghosh *et al.* (1995). *c* and *c'* interact, pointing in opposite directions, with the segment in between looping out, and *b* is part of the dimerization interface. *L4* and *L5* are loops which contact DNA. The subdomain which should be easily accessible to an interacting protein is indicated as 'Exposed Loop'.

truncated p65 protein (p65Δ), containing only the RHD and missing its activation domain, is fully functional in the SRE site-dependent CAT assays. The data argue strongly against an indirect mechanism whereby p65 could have induced, via a κB site-dependent mechanism, the expression of a protein responsible for the observed synergistic effects; instead, a direct mechanism involving p65 and SRF is indicated. Synergistic transactivation does require the trans-

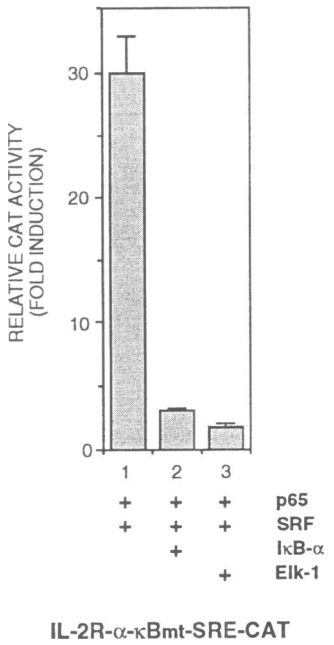

Fig. 7. Inhibition of SRF/p65 transactivation by Elk-1 and IκB-α. NTera-2 cells were transfected with p65 and SRF expression plasmids (columns 1–3; 0.3 μg each) alone or in the presence of Elk-1 (column 3) or IκB-α (column 2) expression vectors (1.0 μg each). The reporter was IL-2R–κBmt–SRE–CAT. Transfections and CAT assays were as described in the legend to Figure 1.

activation domain of SRF. In addition to p65, the NF-κB subunit c-Rel appears to be able to interact with SRF.

The synergistic effect is likely to be the result of a physical complex between SRF and p65 (or c-Rel), which could be demonstrated with GST experiments *in vitro*. Further support for complex formation derives from the observations that neither DNA binding nor transactivation functions of p65 are required for synergy with SRF. This points to a protein contact-mediated interaction of some kind to allow p65 to affect SRF functions. Additional unpublished observations suggest that SRF may need to be bound to its cognate site in cells to permit p65 to interact, possibly because DNA binding induces a necessary conformational change in SRF. It remains to be determined if complex formation of SRF and p65 is facilitated by an additional factor(s) present in cells and extracts. Whatever the nature of the complex, it does not appear to affect the strength of DNA binding of SRF; rather, it appears to affect the transactivation function of SRF.

The physical association with p65 is mediated by a subregion of the SRF DNA binding domain (core^{SRF}; Normann *et al.*, 1988). Sequences in the core region have previously been shown to exert a negative effect on the activity of the SRF transactivation domain (Johansen and Prywes, 1993). We speculate that p65, by complex formation with the SRF core domain, may neutralize these inhibitory functions of the core, thus promoting transactivation by SRF. One may envisage a conformational change in SRF upon association with p65 to expose previously shielded transactivating surfaces, because the SRF activation domain is required for functional synergy but not for physical association. Alternatively, p65 could counteract another factor which otherwise negatively regulates transcription by interaction with SRF (Treisman, 1990; Dalton and Treisman, 1992; Johansen and Prywes, 1993, 1994). In support of the former model, MCM1, a yeast homolog of SRF, appears to undergo conformational changes upon interaction with the accessory protein MATα1 (Tan and Richmond, 1990).

The data point to a possible physiological role for signal-activated NF-κB/p65 (or c-Rel) in transactivation through the SRF–SRE complex. Activated nuclear p65/NF-κB may behave like an accessory protein for SRF in some situations, contributing to the transactivation of SRE-driven target genes, potentially even independent of κB sites. It is conceivable that under normal physiological conditions the presence of a nearby κB site may be needed for p65 to transactivate through the SRE, for example by increasing the local concentration of NF-κB complexes; such a situation may exist in the human IL-2R-α gene, but this is not addressed here. A nearby κB site may not have been required in our assays because the exogenously introduced proteins were expressed at high concentrations. Our data imply an expanded role for NF-κB factors in relaying extracellular stimuli to the nucleus to activate gene transcription.

p65: a putative accessory protein for SRF

After activation through cellular stimuli, NF-κB complexes containing p65 (or c-Rel) may function as p62^{TCF}-independent accessory factors for SRF. Like the established accessory factor Elk-1, p65 appears to associate with SRF through the SRF DNA binding domain (core^{SRF}; Figure 5; Mueller and Nordheim, 1991; Shaw, 1992), although distinct subdomains within the core may be involved (see below). Otherwise, mechanisms for p65 versus Elk-1-mediated activation through SRF differ. Elk-1 is nuclear and can form a ternary complex with SRF prior to cell stimulation (Hipskind *et al.*, 1991; Hill *et al.*, 1993; Marais *et al.*, 1993; Zinck *et al.*, 1993), although stimulation may significantly enhance this (Gille *et al.*, 1992, 1995). p65, on the other hand, could form a complex with SRF only after it is freed from its cytoplasmic inhibitors, primarily IκB-α, a situation which requires either cellular stimulation or the high expression of exogenously introduced, and thus nuclear, p65. Activation by Elk-1 does depend on the signal-induced phosphorylation of its C-terminal activation domain (Hill *et al.*, 1993; Janknecht *et al.*, 1993; Marais *et al.*, 1993; Zinck *et al.*, 1993), while the mere presence of p65 in the nucleus is apparently sufficient for transactivation through the SRE, even when deleted for its known activation domains.

It cannot be determined as yet under what conditions p65-mediated activation via SRE may be physiologically relevant. Significant levels of nuclear Elk-1 may preclude p65-mediated SRF-dependent transactivation because co-transfected Elk-1 effectively interfered in our assays. If one considers the SRE site in the context of a given promoter, however, it is possible that other transcription factors binding to that promoter may interact with, and thus select, only one of several possible accessory factors for SRF.

Further considerations on the mechanism of synergistic transactivation

The functional synergy and physical association of p65 and SRF could conceivably require another protein con-

stitutively present in cells and extracts. Such a protein could, for example, inhibit the transactivation functions of SRF. It is also important to note that p65, upon interaction with SRF, may have contributed directly to transactivation, rather than only indirectly by liberating SRF transactivation functions. On the other hand, the RHD of p65 has never been shown to contain direct transactivation functions of its own (Schmitz and Baeuerle, 1991; Ballard *et al.*, 1992; Blair *et al.*, 1994; Kanno *et al.*, 1994).

While known transactivation functions of p65 appear not to be involved, the SRF activation domain is clearly required for synergy but not for physical association. Full transactivation with the known accessory protein Elk-1 also requires the activation domain of SRF (Janknecht *et al.*, 1992; Hill *et al.*, 1993, 1994; Johansen and Prywes, 1994). Therefore, accessory factors may function, at least in part, by liberating an apparently latent transactivation function of SRF. That SRF contains sequences capable of inhibiting its own transactivation function was established with transfection studies in which a Gal4-dependent reporter was co-transfected together with vectors encoding fusion proteins of various segments of SRF joined to the Gal4 DNA binding domain (Johansen and Prywes, 1994).

Functional and physical interactions of NF-κB with a number of other transcription factors have been documented (see Introduction; reviewed in Siebenlist *et al.*, 1994). In some cases it has been shown that NF-κB can exert its effects by association with a partner protein already prebound to its cognate site, independent of a κB element, a situation similar to that described here (Stein and Baldwin, 1993; Stein *et al.*, 1993a,b), although the mechanisms of action differ.

p65 and SRF structures required for protein–protein interaction

We found that sequences embedded within the SRF DNA binding domain and lying between residues 134 and 202 are necessary for association with p65 (Figure 5). Previously, residues 198–203 of the SRF have been shown to be required for ternary complex formation with Elk-1 (Mueller and Nordheim, 1991; Hill *et al.*, 1994), although additional sequences in the core^{SRF} region may also contribute (Shaw, 1992). Transfection experiments reported here demonstrate that the overexpression of Elk-1 inhibits the p65- and SRF-mediated transactivation (Figure 7), consistent with competition between different complexes involving SRF, although our data do not address this directly. If competition does occur, it may involve steric hindrance rather than the recognition of identical determinants on SRF, because sequences N-terminal to position 167 of SRF are required only for association with p65 and not with Elk-1. Furthermore, p65 does not share B-box-like sequences with Elk-1. The region of SRF implicated in complex formation with p65 is also involved in DNA binding, as revealed by the recently published 3-D structure of the core of SRF bound to DNA (Pellegrini *et al.*, 1995). In space, this region could be accessible for interaction with other proteins.

SRF may act as a DNA adaptor for several transcriptional regulatory proteins, including negative regulators. The function of the SRF–SRE complex in different cells and under different conditions would then be determined by the particular set of protein(s) interacting with it (Tan and Richmond, 1990; Mueller and Nordheim, 1991), possibly analogous to MCM1's interaction with positive- (MATα1 and STE12) and negative-acting (MATα2) factors (Tan and Richmond, 1990; Bruhn and Sprague, 1994).

We mapped the region of p65 required for complex formation with SRF *in vitro* to between residues 204 and 243, a segment located in the C-terminal part of the RHD (Figure 6). A C-terminal deletion at residue 234 within this region greatly reduced but did not completely abolish the interaction, indicating that critical amino acids lie between residues 204 and 234. Recently the structure of the RHD has been resolved for p50 (Ghosh *et al.*, 1995; Müller *et al.*, 1995). The region in p50, corresponding to the domain in p65 which interacts, directly or indirectly, with SRF, contains two associated β-strands, with the sequences in between forming a loop. This segment protrudes from the surface of the protein, away from DNA and dimerization interfaces. Therefore the segment is ideally suited for mediating potential protein–protein interactions with other factors, even when the p65 subunit is bound to DNA in a dimer. In p50, the apex of the loop contains a stretch of five additional amino acids not present in p65 (Figure 6D), and this may account for the absence of synergy between p50 and SRF.

Materials and methods

Plasmids

PMT2T-based expression vectors for p50, p65, IκB-α (Franzoso *et al.*, 1992, 1993; Bours *et al.*, 1993; Brown *et al.*, 1995), p65Δ (Kanno *et al.*, 1994) and SRF (Kuang *et al.*, 1993); Bluescript (BS) vectors for p50, p65 and c-Rel (Ruben *et al.*, 1991; Franzoso *et al.*, 1992; BS–c-Rel was a generous gift from N.Rice); *in vitro* transcription/translation vector pT7ΔATG–SRF (Normann *et al.*, 1988); vectors encoding GST fusions with p50 and p65 (pGEX–p50 and –p65 encoding GST–p50 and GST–p65 respectively) (Franzoso *et al.*, 1992, 1993; Drew *et al.*, 1995) have been described previously.

PMT2T–p65-mt$^{33-34}$ was generated by the site-directed mutagenesis of codons 33 and 34 to express a protein bearing a RF → KA substitution, analogous to a mutation made previously in p50 (see Bressler *et al.*, 1993 for experimental details). The PMT2T–Elk-1 expression vector contains the *Nco*I–*Spe*I fragment derived from the MLV–Elk-1 plasmid (Marais *et al.*, 1993), and the PMT2T–SRFΔ expression vector contains the *Not*I–*Pvu*II fragment derived from PMT2T–SRF; in each case, these fragments were cloned into the *Eco*RI site of PMT2T after fill-in reactions with Klenow enzyme.

The BS–SRF plasmid was obtained by inserting the *Bgl*I–*Hin*dII fragment of SRF into the *Hin*dIII–*Sma*I sites of BS SK$^-$, together with a *Hin*dIII–*Bgl*I linker; the linker contains SRF-derived sequences to allow for the expression of a complete, reconstructed SRF protein in the final vector (linker oligonucleotide: AGCTTGGATTCATGTTACCG-ACCCAAGCTGGGGCCGCGG). The SRF coding sequence was then excised from BS–SRF with *Bam*HI and *Hin*dIII, blunt-ended and cloned into the *Sma*I site of pGEX-1 to generate pGEX–SRF.

The 5′ untranslated region of p65 cDNA in BS–p65 was replaced by a shorter leader and a Kozak consensus sequence to improve *in vitro* transcription/translation (the *Xba*I–*Rsr*II fragment in BS–p65 was replaced by the *Xba*I–*Rsr*II fragment generated by appropriate restriction cutting of the PCR-generated fragment of p65 using the following primers: sense primer (AGCTCTAGAGCCATGGACGAACTGTTCCC); antisense primer (CTGTGGATGCAGCGGTCC).

The 5′ truncated SRF expression plasmids were constructed in the BS vector by replacing the deleted regions with oligonucleotide linkers containing the initiating ATG and, for consistency in translation efficiency, the second amino acid of full-length SRF, followed by three to eight codons lying immediately upstream of the indicated restriction enzyme sites (Figure 5C lists the position number of the first and, in the case of 3′ deletions, of the last amino acid present in the various deletion

proteins). BS–SRF was cut with *Hin*dIII and *Xma*III, *Nar*I, *Sma*I, *Stu*I or *Pst*I to remove the encoded 5′ segments of SRF, which were then replaced by ligation into the vector of the appropriate matching linker (only sense orientation is listed, 5′ end matches *Hin*dIII site): *Xma*III deletion (AGCTTGCCGCCATGTTACTCGGGCCC); *Nar*I deletion (AGCTTGCCGCCATGTTAGAGGAGGAGCTGGG); *Sma*I deletion (AGCTTGCCGCCATGTTAGCCAAGCCGGGTAAGAAGACCC); *Stu*I deletion (AGCTTGCCGCCATGTTAGGCATCATGAAGAAGG); *Pst*I deletion (AGCTTGCCGCCATGTTAACCTTTGCCACCCGAAAAC-TGCA). For the remaining 5′ deletions, fragments encoding the various 3′ segments of SRF (lying between the indicated restriction sites and the *Xba*I site of BS) were cloned into BS opened at *Hin*dIII and *Xba*I, together with the appropriate linker oligonucleotide (sense orientation; 5′ end matches the *Hin*dIII site): *Sac*II deletion (AGCTTGCCGCC-ATGTTAACCCCGGCGCCCACCGC); *Apa*I deletion (AGCTTGCCG-CCATGTTAGGTATGGTGGTCGGTGGGCC); *Nae*I deletion (AGC-TTGCCGCCATGTTAAAGGACACACTGAAGCC); *Alw*NI deletion (AGCTTGCCGCCATGTTAACCAACTACCTGGCACCAGTGT).

The 5′ truncated p65 expression plasmids were constructed by first removing the *Nco*I–*Bam*HI fragment encoding the entire p65 in BS–p65 (generated by PCR, see above), and then replacing it via ligation with various isolated 3′-encoding fragments (lying between one of the indicated restriction sites and the *Bam*HI site of BS–p65), as well as with a linker fragment encoding the initiating ATG and, for consistency in translation efficiency, the second amino acid of full-length p65, followed by three to eight codons lying immediately upstream of the indicated restriction enzyme site (Figure 6C lists the position number of the first, and in the case of the 3′ deletions the last, amino acid present in the various deletion proteins). Linker oligonucleotides (only sense orientation is listed; 5′ end matches the *Nco*I site): *Nsp*HI deletion (CATGGACCAGCCCAAGCAGCGGGGCATG); *Bgl*I deletion (CAT-GGACGAGCTTGTAGGAAAGGACTGCCGGGA); *Bsp*MII deletion (CATGGACCACCGACAAGTGGCCATTGTGTT); *Eco*RI deletion (CATGGACGACCGGGAGCTCAGTGAGCCCATGG); *Pvu*I deletion (CATGGACTTCCAGTACCTGCCAGATACAGACGAT). The *Bgl*II deletion was generated in the same way, except that the *Hin*dIII restriction site in BS–p65 was used instead of the *Bam*HI site, in addition to a *Bgl*II-specific linker (sense orientation; 5′ end matches the *Nco*I site, as above) (CATGGACGGCAGCTGCCTCGGTGGGGATGA). The *Ppu*MI deletion was generated by first removing the p65 coding segment from BS–p65 by restriction with *Nco*I and then replacing it via blunt-end ligation after appropriate Klenow fill-in reactions with the *Ppu*MI fragment of p65, encoding the 3′ part of the protein.

The 3′ deletions of SRF and p65 were all generated by cutting the BS vectors encoding the full-length proteins with the indicated restriction enzymes prior to *in vitro* transcription/translation.

The CAT reporter plasmids are under the regulatory control of wild-type or mutated oligonucleotides derived from the human IL-2R-α promoter, which were inserted upstream of a minimal mouse c-*fos* promoter (Bours *et al.*, 1992, 1993; Franzoso *et al.*, 1992, 1993). The oligonucleotides used were: (i) CA**GGGGAATCTCCCTCTCCTTT-TATGG**GCGT (κB–SRE–CAT; the κB and SRE sites are in bold respectively); (ii) CAGCTCAATCTAGCTCTCCTTTTATGGGCGT (κBmt–SRE–CAT); and (iii) CAGGGGAATCTCCCTCTAACCTAA-GCTGCGT (κB–SREmt–CAT).

Cells, transfections, stimulations and CAT assays

NTera-2 cell assays, calcium phosphate-mediated transient DNA transfection of NTera-2 cells and CAT assays (involving scintillation vial counting) were performed as reported previously (Franzoso *et al.*, 1992, 1993).

GST-based assays and in vitro transcription/translations

GST–p50, GST–p65 and GST–SRF fusion proteins and GST proteins were prepared by glutathione-coated bead purification from appropriate bacterial sonicates (Franzoso *et al.*, 1992, 1993; Bours *et al.*, 1993). BS–p50, BS–cRel, pT7ΔATG–SRF and the BS–SRF constructs were linearized and used to direct protein synthesis in an *in vitro* transcription/translation assay with [^{35}S]methionine (T7 polymerase and rabbit reticulolysates; Promega). The various BS–p65 plasmids were also used to program *in vitro* transcription/translation with the T3 polymerase (Promega). The labeled products were assayed for interaction with the GST fusion proteins attached to glutathione-coated beads, as detailed previously (Franzoso *et al.*, 1992, 1993; Bours *et al.*, 1993).

Acknowledgements

We thank M.Lenardo for the PMT2T–SRF expression vector, N.Rice for the BS–c-Rel plasmid and R.Treisman for the MLV–Elk-1 and pT7ΔATG–SRF constructs. We are grateful to A.S.Fauci for support, encouragement and review of the manuscript. We thank M.Rust for help with the preparation of this manuscript.

References

Ballard,D.W., Bohnlein,E., Hoffman,J.A., Bogerd,H.P., Dixon,E.P., Franza,B.R. and Greene,W.C. (1989) *New Biol.*, **1**, 83–92.
Ballard,D.W., Dixon,E.P., Peffer,N.J., Bogerd,H., Doerre,S., Stein,B. and Greene,W.C. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 1875–1879.
Blair,W.S., Bogerd,H.P., Madore,S.J. and Cullen,B.R. (1994) *Mol. Cell. Biol.*, **14**, 7226–7234.
Bohnlein,E., Lowenthal,J.W., Siekevitz,M., Ballard,D.W., Franza,B.R. and Greene,W.C. (1988) *Cell*, **53**, 827–836.
Bours,V., Burd,P.R., Brown,K., Villalobos,J., Park,S., Ryseck,R.-P. Bravo,R., Kelly,K. and Siebenlist,U. (1992) *Mol. Cell. Biol.*, **12**, 685–695.
Bours,V., Franzoso,G., Azarenko,V., Park,S., Kanno,T., Brown,K. and Siebenlist,U. (1993) *Cell*, **72**, 729–739.
Bressler,P., Brown,K., Timmer,W., Bours,V., Siebenlist,U. and Fauci,A.S. (1993) *J. Virol.*, **67**, 288–293.
Brockman,J.A., Scherer,D.C., McKinsey,T.A., Hall,S.M., Qi,X., Lee,W.Y. and Ballard,D.W. (1995) *Mol. Cell. Biol.*, **15**, 2809–2818.
Brown,K., Gerstberger,S., Carlson,L., Franzoso,G. and Siebenlist,U. (1995) *Science*, **267**, 1485–1488.
Bruhn,L. and Sprague,G.F. (1994) *Mol. Cell. Biol.*, **14**, 2534–2544.
Cross,S.L., Halden,N.F., Lenardo,M.J. and Leonard,W.J. (1989) *Science*, **244**, 466–469.
Dalton,S. and Treisman,R. (1992) *Cell*, **68**, 597–612.
Drew,P.D., Franzoso,G., Becker,K.G., Bours,V., Carlson,L.M., Siebenlist,U. and Ozato,K. (1995) *J. Interferon Cytokine Res.*, **15**, 1037–1045.
Du,W., Thanos,D. and Maniatis,T. (1993) *Cell*, **74**, 887–898.
Franzoso,G., Bours,V., Park,S., Tomita-Yamaguchi,M., Kelly,K. and Siebenlist,U. (1992) *Nature*, **359**, 339–342.
Franzoso,G., Bours,V., Azarenko,V., Park,S., Tomita-Yamaguchi,M., Kanno,T., Brown,K. and Siebenlist,U. (1993) *EMBO J.*, **12**, 3893–3901.
Ghosh,G., Van Duyne,G., Ghosh,S. and Sigler,P.B. (1995) *Nature*, **373**, 303–310.
Gille,H., Sharrocks,A.D. and Shaw,P. (1992) *Nature*, **358**, 414–417.
Gille,H., Kortenjann,M., Thomae,O., Moomaw,C., Slaughter,C., Cobb,M.H. and Shaw,P.E. (1995) *EMBO J.*, **14**, 951–962.
Giovane,A., Pintzas,A., Maira,S.-M., Sobieszczuk,P. and Wasylyk,B. (1994) *Genes Dev.*, **8**, 1502–1513.
Graham,R. and Gilman,M. (1991) *Science*, **251**, 189–192.
Grueneberg,D.A., Natesan,S., Alexandre,C. and Gilman,M.Z. (1992) *Science*, **257**, 1089.
Hill,C.S., Marais,R., John,S., Wynne,J., Dalton,S. and Treisman,R. (1993) *Cell*, **73**, 395–406.
Hill,C.S., Wynne,J. and Treisman,R. (1994) *EMBO J.*, **13**, 5421–5432.
Hill,C.S., Wynne,J. and Treisman,R. (1995) *Cell*, **81**, 1159–1170.
Hipskind,R.A., Rao,V.N., Mueller,C.G.F., Reddy,E.S.P. and Nordheim,A. (1991) *Nature*, **354**, 531.
Janknecht,R. and Nordheim,A. (1992) *Nucleic Acids Res.*, **20**, 3317–3324.
Janknecht,R., Hipskind,R.A., Houthaeve,T., Nordheim,A. and Stunnenberg,H.G. (1992) *EMBO J.*, **11**, 1045–1054.
Janknecht,R., Ernst,W.H., Pingoud,V. and Nordheim,A. (1993) *EMBO J.*, **12**, 5097–5104.
Janknecht,R., Zinck,R., Ernst,W.H. and Nordheim,A. (1994) *Oncogene*, **9**, 1273–1278.
Johansen,F.E. and Prywes,R. (1993) *Mol. Cell. Biol.*, **13**, 4640–4647.
Johansen,F.-E. and Prywes,R. (1994) *Mol. Cell. Biol.*, **14**, 5920–5928.
Kanno,T., Brown,K., Franzoso,G. and Siebenlist,U. (1994) *Mol. Cell. Biol.*, **14**, 6443–6451.
Konig,H. (1991) *Nucleic Acids Res.*, **19**, 3607–3611.
Kortenjann,M., Thomae,O. and Shaw,P.E. (1994) *Mol. Cell. Biol.*, **14**, 4815–4824.
Kuang,A.A., Novak,K.D., Kang,S.M., Bruhn,K. and Lenardo,M.J. (1993) *Mol. Cell. Biol.*, **13**, 2536–2545.
Latinkic,B.V., O'Brien,T.P. and Lau,L.F. (1991) *Nucleic Acids Res.*, **19**, 3261–3267.
Leung,K. and Nabel,G.J. (1988) *Nature*, **333**, 776–778.
Marais,R., Wynne,J. and Treisman,R. (1993) *Cell*, **73**, 381–393.
Miranti,C.K., Ginty,D.D., Huang,G., Chatila,T. and Greenberg,M.E. (1995) *Mol. Cell. Biol.*, **15**, 3672–3684.

Mohun,T., Garrett,N. and Treisman,R. (1987) *EMBO J.*, **6**, 667–673.
Mueller,C.G.F. and Nordheim,A. (1991) *EMBO J.*, **10**, 4219–4229.
Müller,C.W., Rey,F.A., Sodeoka,M., Verdine,G.L. and Harrison,S.C. (1995) *Nature*, **373**, 311–317.
Normann,C., Runswick,M., Pollock,R. and Treisman,R. (1988) *Cell*, **55**, 989–1003.
Pellegrini,L., Tan,S. and Richmond,T.J. (1995) *Nature*, **376**, 490–498.
Pierce,J.W., Jamieson,C.A., Ross,J.L. and Sen,R. (1995) *J. Immunol.*, **155**, 1972–1980.
Rao,V.N. and Reddy,S.P. (1992) *Oncogene*, **7**, 2335–2340.
Ruben,S., Poteat,H., Tan,T.-H., Kawakam,K., Roeder,R., Haseltine,W. and Rosen,C.A. (1988) *Science*, **241**, 84–92.
Ruben,S.M., Dillon,P.J., Schreck,R., Henkel,T., Chen,C.H., Maher,M., Baeuerle,P.A. and Rosen,C.A. (1991) *Science*, **251**, 1490–1493.
Sadoshima,J. and Izumo,S. (1993) *EMBO J.*, **12**, 1681–1692.
Schmitz,M.L. and Baeuerle,P.A. (1991) *EMBO J.*, **10**, 3805–3817.
Shaw,P.E. (1992) *EMBO J.*, **11**, 3011–3019.
Shaw,P.E., Schroter,H. and Nordheim,A. (1989) *Cell*, **56**, 563–572.
Shore,P. and Sharrocks,A.D. (1994) *Mol. Cell. Biol.*, **14**, 3283–3291.
Siebenlist,U., Franzoso,G. and Brown,K. (1994) *Annu. Rev. Cell Biol.*, **10**, 405–455.
Stein,B. and Baldwin,A.S. (1993) *Mol. Cell. Biol.*, **13**, 7191–7198.
Stein,B., Baldwin,A.S., Ballard,D.W., Greene,W.C., Angel,P. and Herrlich,P. (1993a) *EMBO J.*, **12**, 3879–3891.
Stein,B., Cogswell,P.C. and Baldwin,A.S. (1993b) *Mol. Cell. Biol.*, **13**, 3964–3974.
Tan,S. and Richmond,T.J. (1990) *Cell*, **62**, 367–377.
Tan,T.-H., Huang,G.P., Sica,A., Ghosh,P., Young,H.A., Longo,D.L. and Rice,N.R. (1992) *Mol. Cell. Biol.*, **12**, 4067–4075.
Thanos,D. and Maniatis,T. (1992) *Cell*, **71**, 777–789.
Thanos,D. and Maniatis,T. (1995) *Cell*, **80**, 529–532.
Toledano,M.B., Roman,D.G., Halden,N.F., Lin,B.B. and Leonard,W.J. (1990) *Proc. Natl Acad. Sci. USA*, **87**, 1830–1834.
Traenckner,E.B., Pahl,H.L., Henkel,T., Schmidt,K.N., Wilk,S. and Baeuerle,P.A. (1995) *EMBO J.*, **14**, 2876–2883.
Treisman,R. (1990) *Semin. Cancer Biol.*, **1**, 47–58.
Treisman,R. (1992) *Trends Biochem. Sci.*, **17**, 423–426.
Treisman,R. (1994) *Curr. Opin. Genet. Dev.*, **4**, 96–101.
Treisman,R., Marais,R. and Wynne,J. (1992) *EMBO J.*, **11**, 4631–4640.
Whiteside,S.T., Ernst,M.K., LeBail,O., Laurent-Winter,C., Rice,N. and Israel,A. (1995) *Mol. Cell. Biol.*, **15**, 5339–5345.
Zinck,R., Hipskind,R.A., Pingoud,V. and Nordheim,A. (1993) *EMBO J.*, **12**, 2377–2387.

Received on July 28, 1995; revised on March 4, 1996