Gene Ther Mol Biol Vol 3, 355-371. August 1999.

The ETS-domain transcription factors: lessons from the TCF subfamily.

Review Article

Shen-Hsi Yang[1,3], Paula R. Yates[1,3], Yi Mo[2] and Andrew D. Sharrocks[1]

[1]Department of Biochemistry and Genetics, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, NE2 4HH, UK.

[2]The Wistar Institute, Department of Chemistry, University of Pennsylvania. Philadelphia, Pennsylvania 19104, USA.

[3]These authors contributed equally to this work.

Correspondence: A.D. Sharrocks, Ph.D.; Tel: +44-191 222 8800; Fax: +44-191 222 7424; E-mail: a.d.sharrocks@ncl.ac.uk

Key Words: ETS-domain, ternary complex factor, TCF, transcription factors, MAP kinases.

Received: 3 October 1998; accepted: 10 November 1998

Summary

The ternary complex factors (TCFs) represent a subfamily of ETS-domain transcription factors. In recent years, several significant advances have been made in our understanding of their function at the molecular level. Many aspects of TCF function are conserved with other ETS-domain proteins and with transcription factors from other families, therefore these studies contribute more generally to our understanding of transcription factor function. Structural and biochemical studies have furthered our understanding of protein-DNA recognition by the TCFs. Furthermore, significant insights into the regulation of TCF activity by phosphorylation have been achieved. The TCFs have developed as a paradigm for nuclear targets of MAP kinase pathways and further advances have been made into understanding the specificity of kinase action towards the TCFs. The TCFs are known to bind to multiple protein partners which have roles in up- and down-regulating their activity, recruitment to DNA and transmission of transcriptional activation and repression signals. Finally, our understanding of the role of TCFs in specific biological processes is starting to become clearer, with roles defined in immediate-early gene regulation in a neuronal context.

I. Introduction

ETS-domain transcription factors are found in all metazoan organisms investigated to date (Degnan *et al.*, 1993; Laudet *et al*., 1993). However, they appear to be absent in yeast and plant species, indicating that they function in novel regulatory cascades which are specific to the metazoan lineage. The ETS-domain proteins are characterised by the presence of a conserved DNA-binding domain (the ETS-domain; Karim *et al.*, 1990) and are further subclassified on the basis of sequence similarities within this domain and the presence of additional conserved domains (reviewed in Sharrocks *et al*., 1997; Graves and Petersen, 1998).

Members of the ETS-domain transcription factor family have been implicated in a variety of developmental processes in organisms that include, fruit flies, worms, fish, frogs and mice. In mammals, many ETS-domain proteins have also been associated with the adult immune system (reviewed in Bassuk and Leiden, 1996). One common theme appears to be that these transcription factors act as nuclear targets of the MAP kinase signal transduction pathways and thereby convert extracellular cues into nuclear responses (reviewed in Sharrocks *et al*., 1997; Graves and Petersen, 1998). Their importance is further emphasised by their implication in a variety of diseases in mammals, including cancer, in which individual proteins are either inappropriately expressed or expressed as

fusions with other proteins. Such deregulated ETS-domain proteins act to subvert the normal tight cellular controls over the signalling pathways which are required to prevent deregulated cell growth and hence tumourigenesis (reviewed in Dittmer and Nordheim, 1998).

In this review we will summarise recent advances in our understanding of the mechanism of action of the ternary complex factor (TCF) subfamily of ETS-domain transcription factors and relate these to our understanding of the function of other family members and transcription factors in general (for earlier review on the TCFs, see Treisman, 1994). In particular we will focus on how the specificity of promoter targeting is achieved by a combination of protein-DNA and protein-protein interactions. We also discuss the mechanisms by which protein kinase cascades are targeted to the TCFs and how phosphorylation affects their function. Finally, we will review the current understanding of how the TCFs function in a physiological context to convert extracellular signals into specific nuclear responses. Other recent reviews provide a more comprehensive account of the ETS-domain family in general (Bassuk and Leiden, 1996, Sharrocks *et al.*, 1997, Graves and Petersen, 1998, Dittmer and Nordheim, 1998).

II. The modular structure of the TCFs.

The name of the TCF subfamily is derived from the fact that they form ternary DNA-bound complexes with a second transcription factor, serum response factor (SRF), and serum response elements (SREs) such as the one found in the c-*fos* SRE. Three human TCFs (Elk-1, SAP-1 and SAP-2) have been identified (Rao *et al*., 1989; Dalton and Treisman, 1992, Price et al., 1995). Murine Elk-1 and SAP-2 (ERP/Net) homologues also exist (Giovane *et al*., 1994, Lopez *et al.*, 1994) and their conservation in vertebrates is demonstrated by the cloning of a cDNA encoding a TCF homologue (zTCF-1) from zebrafish (Brown and Sharrocks, unpublished data).

In addition to the N-terminal ETS DNA-binding domain, the TCFs share three regions of sequence similarity (**Fig. 1**). This sequence similarity is also reflected in the functional similarity exhibited by these domains. The B-box mediates protein-protein interactions with SRF (Shore and Sharrocks, 1994), the D-domain serves as a docking site for MAP kinases (Yang *et al.*, 1998a, Yang *et al.*, 1998b) and the C-domain is a transcriptional activation domain which also serves as a regulatory domain whose activity is modified upon phosphorylation by MAP kinases (reviewed in Treisman, 1994, Whitmarsh and Davis, 1996; Gille *et al.*, 1995; Kortenjann *et al*., 1994; Janknecht *et al*., 1994; Whitmarsh *et al.*, 1995; Shore *et al*., 1996, Whitmarsh *et al*., 1997, Cavigelli *et al*., 1995). An additional functional domain has been identified in SAP-2 which acts as an autoinhibitory motif (NID) that regulates DNA binding and also mediates transcriptional repression (Maira *et al.*, 1996). SAP-1 and zTCF-1 exhibit some similarity with this motif but this region is not conserved in Elk-1.

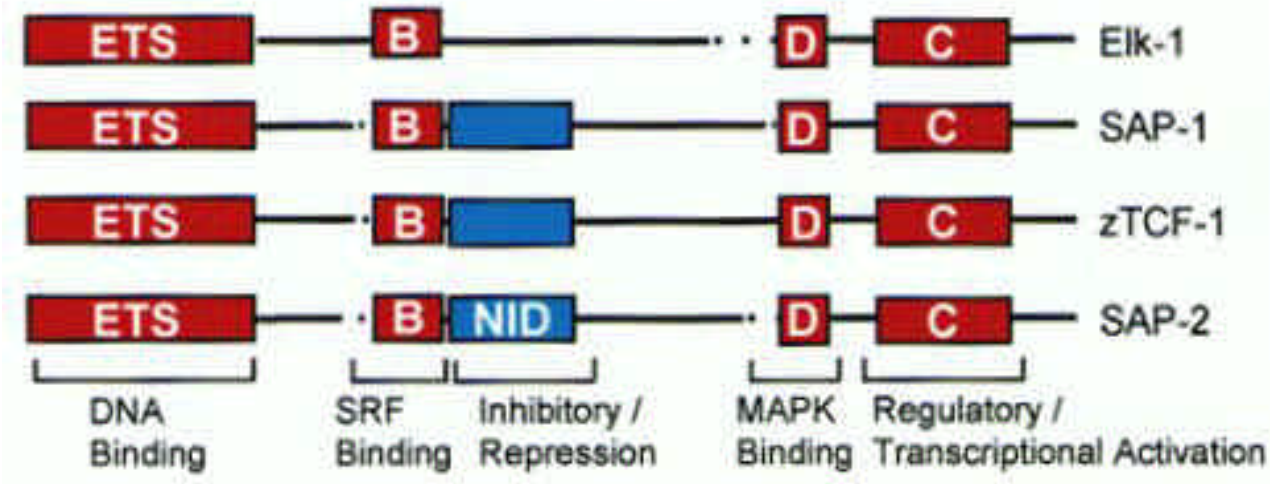

Figure 1. The domain structure of the TCFs. Each TCF contains four conserved domains (ETS-, B-, D- and C-), indicated by red boxes. SAP-2 contains an additional domain (NID), which is partially conserved in SAP-1 (45% identity) and zTCF-1 (40% identity) (indicated by a blue box). The lengths of the inter-domain linker regions vary, therefore additional dotted lines are included between the domains when a shorter linker is present.

Each domain in the TCFs is functionally separable from the rest of the protein and each has been shown to act in an autonomous fashion. The ETS-domain is sufficient for DNA binding (Janknecht and Nordheim, 1992), the B-box is sufficient for binding to SRF (Shore and Sharrocks, 1994), the D-domain is sufficient for binding to ERK2 (Yang *et al.*, 1998a) and the C-domain represents an independent transcriptional activation domain (reviewed in Treisman, 1994). However, it is becoming apparent that within the context of the full-length proteins, significant intramolecular crosstalk occurs between domains. For example, the D-domain is required for efficient phosphorylation of the C-domain (Yang *et al.*, 1998a, Yang *et al*., 1998b) and phosphorylation of the C-domain elicits a conformational change elsewhere in the protein which alters inhibitory interactions with the N-terminal part of the protein (see section III-C). Therefore, although the TCFs are clearly modular in structure, their normal function requires the integrated functioning of their constituent domains.

III. DNA binding properties

A. DNA binding sites

The recognition of DNA target sites is one of the major mechanisms responsible for maintaining the specificity of transcription factor function and is mediated by structural modules referred to as DNA-binding domains. ETS-domain transcription factors share a highly conserved DNA-binding domain of approximately 85 amino acids that mediates binding to DNA target sites known as ets-motifs which harbour a central GGA trinucleotide motif. The DNA binding affinity and specificity for individual family members is further defined by the DNA base-pairs that flank this central core motif (reviewed in Sharrocks *et al.*, 1997 and Graves and Petersen, 1998). However, although the consensus binding sequences for ETS-domain proteins are very similar, it should be emphasised that the binding efficiency of proteins to individual sites may vary considerably. This is evident from the DNA binding properties of the TCFs (**Table 1**, Shore *et al.*, 1996, Brown and Sharrocks, unpublished data). These studies show that sequences corresponding to the site, ACCGGAAGTR, represent high affinity binding sites for the TCFs. However, SAP-1 shows a more relaxed binding site selectivity and can tolerate several nucleotide changes thereby allowing efficient binding to a greater spectrum of sites than Elk-1. SAP-2 also differs and exhibits an intermediate DNA binding specificity (Shore *et al*., 1996; Brown and Sharrocks unpublished data). Moreover, the binding specificity of the recently cloned zebrafish TCF (zTCF-1) closely mirrors that exhibited by Elk-1 (Brown and Sharrocks, unpublished data).

In the context of complex promoters, it is unlikely that autonomous DNA binding will be the sole determinant of binding specificity. Instead, the formation of complexes with partner proteins is also likely to contribute to the affinity and specificity of promoter binding by ETS-domain proteins (see section V). These additional protein-protein interactions likely compensate for the loss of affinity associated with the more degenerate binding sites typically found in promoters. Indeed, in ternary complexes with SRF, Elk-1 recognises a relaxed version of its consensus binding site (RC/aC/aGGAA/tRT/c) which more closely resembles the consensus sequence selected by SAP-1 (Treisman *et al*., 1992). Significantly, the ets-motif within the *c-fos* SRE represents a lower affinity TCF binding site and requires concomitant binding of SRF to permit high affinity binding. This combinatorial binding might act to enhance promoter-specific recognition and/or allow a tighter regulation of TCF DNA binding by MAP kinase phosphorylation (see section III-C). Similarly, binding of Elk-1 and SAP-2 to the ets-motif in the *mb-1* promoter requires binding of Pax-5 to the adjacent site (Fitzsimmons *et al.*, 1996). Autonomous binding of SAP-1 to the *c-fos* SRE is also possible, albeit with much reduced affinity compared to SAP-1 binding in ternary complexes with SRF (Dalton and Treisman, 1992, Shore and Sharrocks, 1995, Masutani *et al.*, 1997). However, although the physiological significance of this observation is questionable, it raises the possibility that binding sites might exist at which the TCFs act as the major determinants of DNA binding specificity such as is seen with the N10 promoter (Latinkic *et al.*, 1996).

B. Structure of the ETS-domain

The ETS-domain transcription factors all contain a common DNA-binding motif which is conserved both in the primary sequence and in the secondary and tertiary structure elements. The three-dimensional structures of the ETS-domains of the family members, Fli-1, Ets-1, PU.1/Spi-1, (reviewed in Graves and Petersen 1998; Sharrocks *et al.*, 1997) and GABPα (Batchelor *et al.*, 1998) have been determined. More recently, the structures of the SAP-1 ETS-domain bound to the high affinity E74 site and the ets motif in the c-*fos* SRE DNA have also been elucidated (**Fig. 2**; Mo *et al.*, 1998). Inspection of the tertiary structure provides insights into the roles of highly-conserved residues within the ETS-domain and the roles of non-conserved residues in mediating the unique activities of each protein. The ETS-domain of SAP-1, like Fli-1, Ets-1, PU.1 and GABPα, forms a winged helix-turn-helix topology and the protein assumes an α/β fold with three α-helices and four anti-parallel β-strands on opposite faces of the protein which pack against one another to form an extensive hydrophobic core.

	DNA binding properties				***Phosphorylation properties***				
	Consensus binding site	***3° complex formation (TCF-SRF-SRE)***	***Auto-inhibitory domain***	***Regulation of DNA binding by phosphorylation***	***Enhanced transcriptional activation***	**[b]** ***Activating kinases***	***Kinases targeted by D-domain binding***	***Phosphoacceptor sites*** *preferred in vivo sites*	***Phosphoacceptor sites*** *regulate transcription activation and DNA binding*
Elk-1	AACC**GGAA**GTG/a **[a]** RC/aC/a**GGA**A/tRT/c	YES	B-box and C-terminal TAD	YES	YES	ERKs/ JNKs/p38α,β_2,γ,δ	**[c]** ERK/JNK	S383/S389(ERK2) T363/T368(JNK2) T417/S422(p38α) **[d]**	S383 /S389
zTCF-1	Elk-1-like	YES	C-terminal TAD	YES	YES	ERK2/p38α	ERK	?	?
SAP-1	NACC**GGAA**/tG/aT/cN	YES	B-box and C-terminal regions	YES	YES	ERK/p38α,β_2/unknown IL-1 & CSF-1 induced kinases	ERK/p38α,β_2	?	S381 /S387
SAP-2	SAP-1-like	Weak	NID and C-terminal regions	NO	YES	ERK2/p38α	?	?	**[e]** S357 /S363

Table 1. Summary of DNA binding and phosphorylation properties of the TCFs. See text for details and references.
[a] Site-selection carried out in ternary complexes with SRF.
[b] These kinases are implicated from *in vivo* studies using overexpressed kinase cascade components or "natural" inducers and pharmacological substrates.
[c] ERK2 and JNK2 require different D-domain residues for optimal binding.
[d] These sites were identified in a single comparative studies of the three TCFs (Price *et al*., 1996). Other studies have analysed the sites phosphorylated by individual kinases.
[e] Phosphorylation at these sites has no apparent effect on DNA binding.

Most of the conserved residues function to stabilise this hydrophobic core and to mediate protein-DNA interactions. The DNA recognition helix (α3) is embedded in the DNA major groove and additional DNA contacts are made by the 'wing' (between the β-strands 3 and 4) and the loop separating helices 2 and 3. These additional binding regions flank the DNA recognition helix and are anchored to opposite strands in the DNA minor groove. An overall bend of 11° is induced in the DNA which facilitates the insertion of the recognition helix into the major groove. Interestingly, the binding site adopts partial A-form character. This may have some biological significance as this requirement would reduce the DNA binding affinity *in vivo*, thereby reducing promiscuous DNA binding by ETS-domain proteins in the absence of appropriate protein partners.

The replacement of two residues (D38 and D69) in Elk-1 by the corresponding residues from SAP-1 (Q37 and V68 respectively) is sufficient to confer the SAP-1 DNA-binding specificity on Elk-1 (Shore *et al*., 1996). V68 is located after the C-terminus of recognition helix whereas Q37 is located between β2 and α2. The locations of these residues suggest that neither is in a position to make direct hydrophobic or H-bond contacts with the DNA (Mo *et al*., 1998). Thus, the effect of these residues must be indirect and be mediated by altering DNA contacts made by other amino acids. More recently, further insights into this recognition process have been achieved from structural studies on the Elk-1-DNA complex (Mo and Marmorstein, unpublished data). Preliminary studies indicated that the structure of the Elk-1 ETS-domain would not differ significantly from other family members (Shore *et al.*, 1995; Bisset, Virden and Sharrocks, unpublished data). Indeed, the overall structure and DNA contacts made by the ETS-domain are very similar to SAP-1. However, there are several key differences which may help to explain the different DNA binding specificities of the TCFs. One of these is that Tyr66 in the recognition helix no longer makes a base contact and instead makes a H-bond contact to a backbone phosphate. The presence of D69 (one of the key specificity determinants) appears to be responsible for this change in interactions by Tyr66. The second residue involved in determining the unique binding specificities of the TCFs (D38) may act to alter the overall orientation of the recognition helix. Hence, a combination of biochemical studies and the structure of the SAP-1/DNA complex demonstrates that these non-conserved residues within a conserved structural framework have a profound effect on DNA binding specificity.

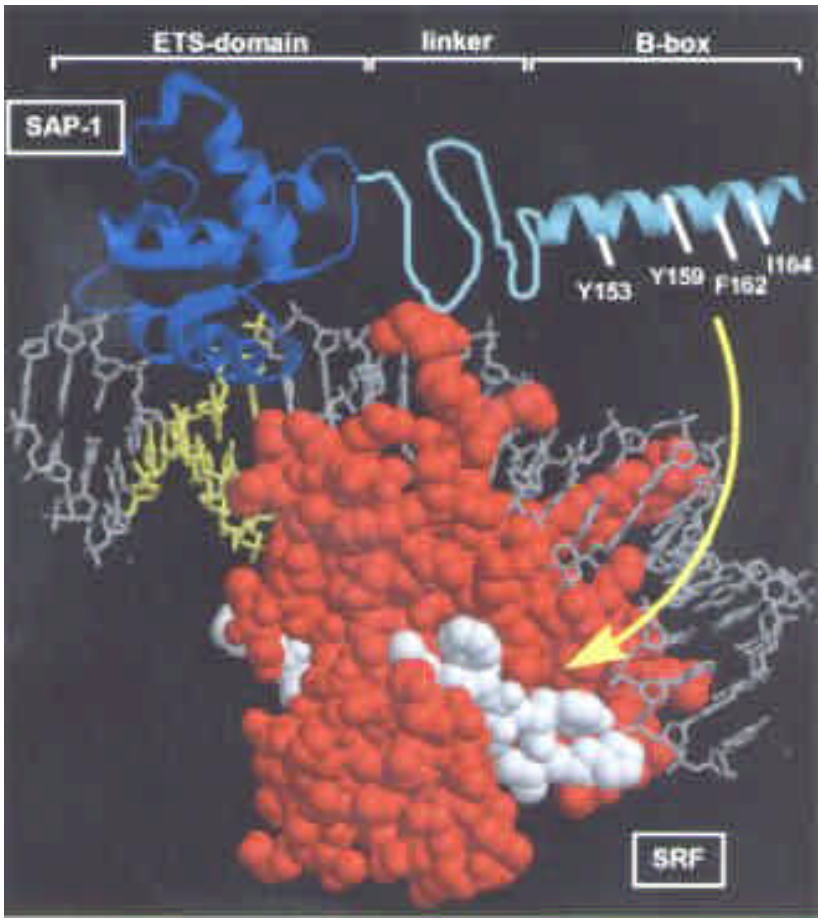

Figure 2. Model of the ternary SAP-1-SRF-SRE complex**.** The structure of the SAP-1 ETS-domain bound to the c-*fos* SRE (Mo *et al*., 1998) and SRF bound to an SRE-like site (Pellegrini *et al*., 1995) have been determined independently and the two structures subsequently combined (Mo *et al*., 1998). The secondary structure elements in the SAP-1 ETS-domain are shown in dark blue whereas a hypothetical structure of the B-box and linker region joining this to the ETS-domain are shown in light blue. Mutagenic studies indicate that the B-box is likely to form an a-helix and that several hydrophobic residues (indicated) play essential roles in the interaction with SRF (Ling *et al*., 1997). The core DNA-binding domain of SRF is shown in red as a space filling model. Residues which have been shown to form the interaction surface with the Elk-1 B-box are indicated in white. The yellow arrow indicates that the B-box interacts with this region of SRF. The DNA is shown in grey and the nucleotides comprising the central GGA of the ets-motif are shown in yellow.

A comparison of the structures of the SAP-1/DNA and Elk-1/DNA complex and the analogous complexes containing the mutant (D38Q/D69V) Elk-1 ETS-domain will provide further insights into this molecular recognition process.

C. Regulation of DNA binding

Many ETS-domain transcription factors are subject to auto-inhibitory mechanisms which regulate their DNA-binding activity (Sharrocks *et al*., 1997 and Graves and Petersen, 1998). This may function to prevent promiscuous DNA-binding because of their relatively non-stringent DNA-binding specificities. However, the auto-inhibitory regions and mechanisms of action vary amongst family members. Recently, the concept of transcription factor modularity has been challenged by the observation that DNA can act as an allosteric activator, thereby implying structural coupling between the DNA-binding and transcriptional activation domains (reviewed in Lefstin and Yamamoto, 1998). In the case of the ETS-domain protein Ets-1, the inhibitory mechanism involves such an allosteric change mediated by cooperating C- and N-terminal modules from outside the ETS-domain (reviewed in Graves and Petersen, 1998). Part of the regulatory mechanism in Ets-1 involves structural changes in the inhibitory elements themselves which may be transduced into changes in the overall protein conformation. Such conformational changes may be activated by phosphorylation and/or coupling with other cofactors and would act to relieve the auto-inhibition, although the regulatory 'switch' within Ets-1 is still unknown. Moreover, these mechanisms may also achieve tighter regulation over transcriptional activation by reciprocal effects on the structure and/or accessibility of the transcriptional activation domain. The TCFs are also regulated by autoinhibitory mechanisms (**Table 1**; reviewed in Sharrocks *et al.*, 1997 and Graves and Petersen, 1998). In Elk-1, DNA binding is inhibited by a combination of the B-box and C-terminal transcriptional activation domain (Janknecht *et al.*, 1994; Yang and Sharrocks, unpublished data). Recently, several advances in our understanding of this inhibitory process have been made (Yang and Sharrocks, unpublished data). Direct

binding of the ETS-domain to the B-box and binding of the N-terminal half of the protein (containing the ETS-domain and B-box) to the C-terminal TAD can be demonstrated, indicating that intramolecular interactions may serve to regulate DNA binding. Partial proteolysis experiments, CD and fluorescence absorption spectra have clearly demonstrated that phosphorylation by ERK2 induces a significant conformational change in Elk-1 which is thought to relieve these inhibitory interactions. Moreover, deletions and disruptive point mutations in the B-box block the induction of DNA binding and concomitant conformational structural changes observed upon phosphorylation. This suggests that this motif acts to couple C-terminal phosphorylation and relief of inhibitory interactions with the N-terminal ETS-domain. Furthermore, experiments with phosphorylated peptide effector molecules (which encompass the key phosphoacceptor motifs, Ser383/Ser389) indicate that phosphorylation triggers an allosteric change in Elk-1 which activates DNA binding. An elegant model emerges in which phosphorylation of the Elk-1 TAD plays a pivotal role in the activation of its DNA binding activity (**Fig. 3**).

Inhibitory interactions are relieved by an intramolecular switch which triggers a conformational changes via the B-box to the ETS DNA-binding domain. In addition to the effects on DNA binding, the conformational changes triggered by phosphorylation are also likely to play a pivotal role in enhancing the transcriptional activation potential of Elk-1 (see section V-C). SAP-1 is also regulated by an autoinhibitory mechanism which involves both B-box and C-terminal regions although these studies are less extensive (Dalton and Treisman, 1992). In contrast, the autoinhibition of DNA binding by SAP-2 appears to be regulated differently to the other TCFs by a unique inhibitory domain (NID) which is located C-terminal to the B-box and is predicted to form a helix-loop-helix (HLH)-like structure (Maira *et al*., 1996). This domain of SAP-2 is also sufficient for mediating transcriptional repression by SAP-2 (see section V-C). The NID exhibits sequence similarity with the HLH domain in the Id proteins.

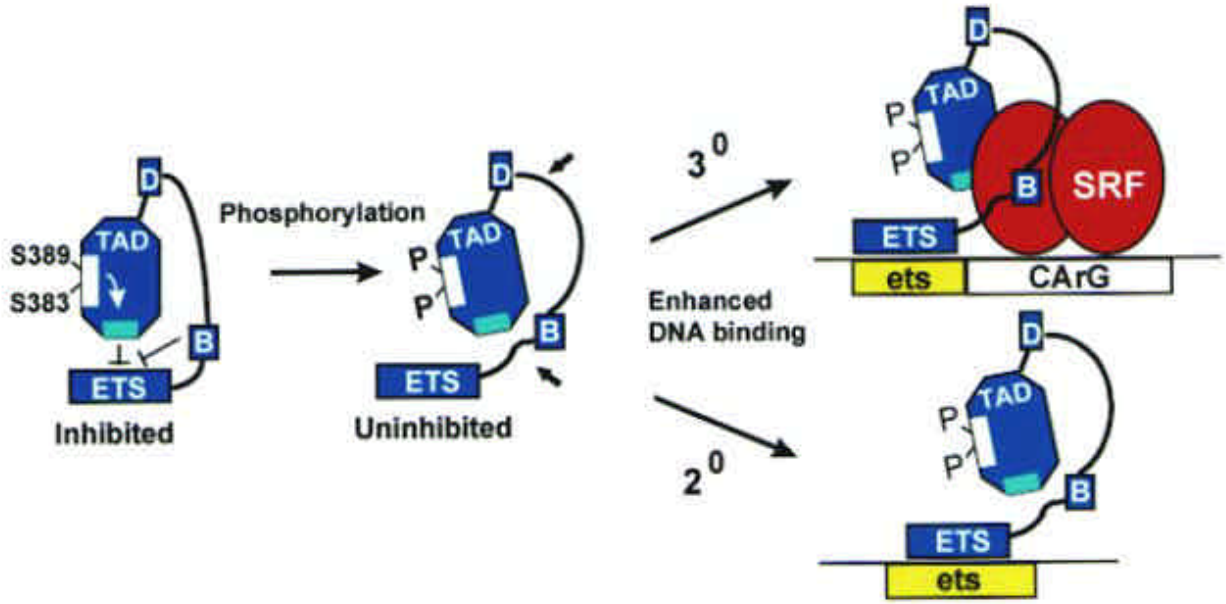

Figure 3. Activation of Elk-1 DNA binding by phosphorylation. In the unphosphorylated state, Elk-1 exists in an inhibited conformation. Upon phosphorylation, Elk-1 undergoes a conformational change (arrows indicate changes in the accessibility of the inter-domain linkers to proteolytic cleavage; Yang and Sharrocks, unpublished data) which is accompanied by a relief of intramolecular inhibition of the ETS-domain. Elk-1 can then efficiently bind to DNA, either on its own in binary complexes (2^0) or in conjunction with SRF in ternary complexes (3^0). The major regulatory sites (Ser383 and Ser389) involved in the activation of DNA binding by MAP kinase-induced phosphorylation are indicated. Phosphorylation of these residues is proposed to trigger a local change in the C-terminal transcriptional activation domain (TAD) which in turn triggers the conformational change and subsequent activation of DNA binding.

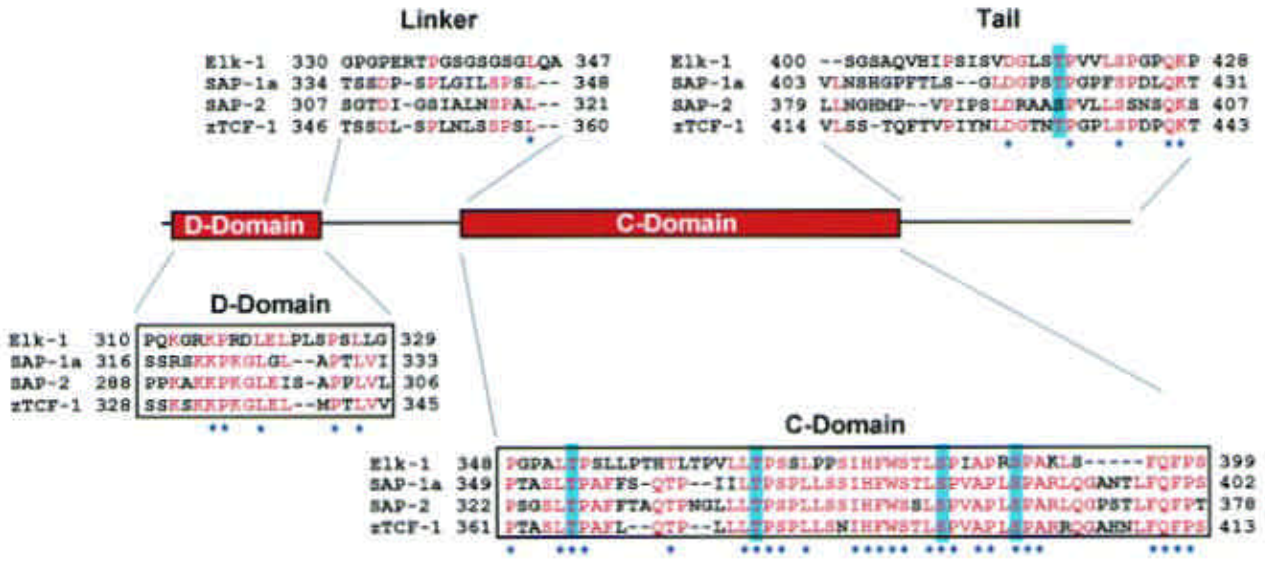

Figure 4. Sequence conservation in the C-terminal domains of the TCFs. The sequences of the D-domain, the C-domain, the linker separating these two domains and the C-terminal tail of Elk-1, SAP-1, SAP-2 and zTCF-1 are shown. The numbers of the amino acids at the ends of each segment with respect to the full-length proteins are shown. Residues which are identical in at least three of the four TCFs are shown in red. Asterisks indicate that the residues are conserved in all four TCFs. Potential MAP kinase sites conserved in all four TCFs are highlighted in blue.

Interestingly, the Ids interact with the TCFs via their ETS-domains (see section V-D) and cause their dissociation from low affinity ets-motifs such as the one contained in the c-*fos* SRE (Yates et al, 1999). Thus helix-loop-helix-like motifs can act both in cis and in trans to inhibit DNA binding by the TCFs.

IV. Regulation by signal transduction pathways

A. Activation of the TCFs by phosphorylation

The MAP kinase pathways play major roles in converting extracellular signals such as mitogens, growth factors, cytokines and stress into nuclear responses (reviewed in Karin, 1994; Treisman, 1996). The activation of MAP kinase signal transduction pathways results in the phosphorylation of transcription factors by the terminal kinases in these cascades. The activity of many ETS-domain transcription factors is regulated by MAP kinase pathways (reviewed in Sharrocks *et al.*, 1997) and may therefore contribute to the generation of certain types of cancer which result from inappropriate triggering of these cascades.

TCFs have been used as a paradigm to study the activation of transcription factors by signal-induced phosphorylation. Phosphorylation of the TCFs, Elk-1 and SAP-1, occurs at multiple conserved carboxy-terminal S/T-P motifs and leads to enhanced DNA binding and TCF-mediated transcriptional activation (**Table 1**; reviewed in Treisman, 1994, Whitmarsh and Davis, 1996; Gille *et al.*, 1995; Kortenjann *et al.*, 1994; Janknecht *et al.*, 1994; Whitmarsh *et al.*, 1995; Shore *et al*., 1996, Whitmarsh *et al.*, 1997, Cavigelli *et al.*, 1995). However, phosphorylation only affects the transactivation potential of SAP-2 and does not appear to stimulate its DNA binding activity (Lopez *et al.*, 1994; Giovane *et al*., 1994; Price *et al.*, 1996). It has also been demonstrated that the phosphorylation enhances the formation of a quaternary complex at the *c-fos* SRE containing two Elk-1 molecules (Gille *et al*., 1996), although the physiological relevance of this observation remains unclear.

Five potential MAP kinase sites are conserved amongst all four family members (**Fig. 4**). Additional sites exist which are either unique to individual proteins or conserved in a subset of the TCFs. However, the role of the individual phoshoacceptor motifs is still unknown. It is also unclear as to whether these motifs are phosphorylated

in a specific or random order and whether phosphorylation occurs cooperatively. However, it is evident that phosphorylation of Ser383 in Elk-1 is critical for transcriptional activation and efficient ternary complex formation with DNA-bound SRF (reviewed in Treisman, 1994). Similarly, phosphorylation of the corresponding serine residues is essential for maximal stimulation of the transcriptional activity of SAP-1 and SAP-2 (Giovane *et al.*, 1994; Strahl *et al.*, 1996; Janknecht and Hunter, 1997). However, it should be emphasised that whilst other sites play less critical roles when analysed individually, in combination, they play important roles in regulating the activity of the TCFs (Treisman, 1994).

Although the TCFs are closely related and exhibit significant sequence conservation in the C-terminal activation domain (**Fig. 4**; Price *et al.*, 1995), they appear to respond differently to MAP kinase signalling pathways. In humans, at least three parallel MAP kinase pathways exist and several distinct MAP kinases have been identified in each class of pathway. The ERK subclass contains ERK1 and ERK2, the JNK subclass contains JNK1, JNK2 and JNK3, and the p38 subclass contains p38α, p38β, p38γ and p38δ (reviewed in Whitmarsh and Davis, 1996, Robinson and Cobb, 1997, Cohen, 1997, Wang *et al.*, 1997). Current evidence suggests that Elk-1 is a target for all three classes of MAP kinase pathways, ERK, JNK and p38α/β_2/γ/δ (reviewed in Treisman, 1994, Whitmarsh and Davis, 1996, Kortenjann *et al.*, 1994, Janknecht *et al.*, 1994; Gille *et al.*, 1995, Whitmarsh *et al.*, 1995; Cavigelli *et al.*, 1995; Enslen *et al.*, 1998; Yang and Sharrocks, unpublished data). However, SAP-1 and SAP-2 appear to only be targeted efficiently by the ERK and p38α/β_2 pathways (Price *et al.*, 1996; Strahl *et al.*, 1996; Whitmarsh *et al.*, 1995; Whitmarsh *et al.*, 1997; Yang, Galanis and Sharrocks, unpublished data). In comparison, SAP-1 and SAP-2 appear to be a poor JNK substrates (Price *et al.*, 1996; Strahl *et al.*, 1996; Whitmarsh *et al.*, 1995, 1997; Yang and Sharrocks, unpublished data) although when overexpressed, JNKs can phosphorylate and activate SAP-1 to some extent (Janknecht and Hunter, 1997a). Similarly, zTCF-1 appears to be differentially targeted by different classes of MAP kinases as it is only efficiently activated by ERK and p38α but is a poor substrate for JNK isoforms both *in vitro* and *in vivo* (Yang and Sharrocks, unpublished data). However, as many of these studies have been carried out in the presence of overexpressed kinase cascade components and/or transcription factor substrates, it is possible that normal specificity determinants are bypassed (e.g. section IV-B). Thus care has to be taken in interpreting these results in a physiological context. Nevertheless, it is clear that multiple MAP kinase pathways can converge on the TCFs. One of the future challenges will be to determine which of these act under normal physiological conditions.

The existence of novel SAP-1 and zTCF-1-specific kinase(s) has been suggested from transient transfection assays. In CHO cells, IL-1 stimulation of Elk-1 is mediated by the JNK pathway. However, a dominant-negative form of MKK4 and overexpression of JIP-1 only have small inhibitory effect on IL-1-induction of reporters regulated by GAL4-SAP-1 and GAL4-zTCF fusions, indicating that IL-1 signalling to SAP-1 and zTCF-1 is mediated by a JNK-independent kinase pathway (Whitmarsh *et al.*, 1997; Brown, Yang and Sharrocks, unpublished data). In the case of SAP-1, this activation is also independent of the p38 pathway (Whitmarsh *et al.*, 1997). Interestingly, the existence of additional signalling pathways to SAP-1 has also been implicated from studies on CSF-1 stimulation of macrophages. In this case, growth factor activation of SAP-1 occurs independently of the Ras/ERK signalling cascade (Hipskind *et al.*, 1994a). Similarly, the existence of novel Elk-1 kinases (other than ERK1/2) which are activated in response to FGF signalling and overexpression of activated Raf-1 proteins has been demonstrated (Chung *et al.*, 1998). Thus further complexities in signalling to the TCFs are likely to be uncovered as the identities of these kinases become known.

The differential utilisation of TCFs and MAP kinase signalling pathways represents a potential mechanism for the determination of cell-type-specific responses to extracellular stimuli. However, cross-cascade signals involving Rac1 and cdc42 (activating the JNK pathway) and Raf-1 co-operate to activate ERK and lead to a synergistic increase in TCF-dependent transcriptional activation (Frost *et al.*, 1997). Similarly, the ERK and p38 cascades cooperate to activate Elk-1 in response to UV stimulation (Price *et al.*, 1996). The TCFs may therefore also allow integration of distinct extracellular signals via a single nuclear effector molecule.

B. MAP kinase targeting motifs

Due to the limited nature of the consensus sequence of the phosphoacceptor motifs, initial binding of the MAP kinases to transcription factors has been proposed as a mechanism to enhance substrate specificity over simple recognition of the local context of the phosphorylation motif (Kallunki *et al.*, 1996). For example, in the case of phosphorylation of c-Jun by the JNK MAPKs, the local context of the phosphoacceptor motifs and the presence of a kinase docking domain on the transcription factor, combine to enhance its substrate specificity (Derijard *et al.*, 1994; Kallunki *et al.*, 1994, 1996; Sluss *et al.*, 1994; Dai *et al.*, 1995; Gupta *et al.*, 1996). Recently, the D-domain homology region has been demonstrated to play an analogous role in the TCFs. This motif is distinct from the conserved phosphoacceptor motifs (**Fig. 4**) and in the case of Elk-1 is responsible for targeting by the ERK and

JNK MAP kinases and is required for its efficient phosphorylation and activation. In contrast, this domain does not appear to play a role in phosphorylation by the p38 subclass (Yang *et al.*, 1998a; Yang *et al.*, 1998b). This suggests that Elk-1 phosphorylation by p38 may differ from that mediated by the ERK and JNK subclasses, and that the p38 MAPKs may phosphorylate Elk-1 in a more constitutive manner which does not require rapid and efficient kinase targeting. This is consistent with the observation that p38 is insufficient for mediating Elk-1 activation in response to UV and requires cooperation with the ERK pathway is required (Price *et al.*, 1996). Phosphorylation of both SAP-1 and zTCF-1 by ERK also appears to be facilitated by the presence of the D-domain (Galanis, Yang and Sharrocks, unpublished data). However, in contrast to Elk-1, SAP-1 appears to be targeted by p38α and p38β_2 via the D-domain (Galanis and Sharrocks, unpublished data) which is consistent with the robust activation of SAP-1 observed with the p38 MAP kinases (Janknecht and Hunter, 1997b). Thus, although the D-domains are highly conserved amongst the TCFs, sequence differences within this motif must be responsible for this differential targeting. In addition to kinase binding, it should be emphasised that the local context of the phosphoacceptor motifs probably plays a role in determining substrate specificity although this has not been investigated. Indeed, each class of MAP kinase exhibits preferences for a different subset of the potential phosphoacceptor sites (**Table 1**; Price *et al.*, 1996).

It is unclear how widespread the docking of kinases to specific motifs on transcription factors is. However, one possible implication is that under physiological conditions, these interactions are an essential component of the specificity determining mechanism and hence explain how different MAP kinases could elicit different effects on highly related transcription factors. Future studies should help resolve the generality and importance of this phenomenon.

C. Downregulation of TCF activity by dephosphorylation

Whilst the activation of TCFs by MAP kinases is relatively well understood, negative regulators such as protein phosphatases have not been studied in detail. In mammalian cells, several subfamilies of serine-threonine phosphatases exist, including PP1A, PP2A, PP2B (calcineurin) and PP2C. Elk-1 phosphatase activity in cell extracts is inhibited by cyclosporin A (a PP2B inhibitor), but not by okadaic acid (PP1 and PP2A inhibitors) *in vitro* indicating that PP2B may be a physiological Elk-1 phosphatase (Sugimoto *et al.*, 1997). Furthermore, cyclosporin A also significantly enhances EGF-induced Ser383 Elk-1 phosphorylation in COS cells following activation of the ERK pathway, providing evidence for a role of this phosphatase *in vivo*. In addition, a novel regulator of MAP kinase signalling, the kinase suppression of Ras (KSR), blocks EGF-induced Elk-1 activity without directly altering the activity of the ERK pathway and may promote the accumulation of dephosphorylated Elk-1 by activating the PP2B phosphatase (Sugimoto *et al.*, 1997). Previously, the okadaic acid-sensitive phosphatases PP1A and PP2A have been implicated in the downregulation of c-*fos* by the ternary TCF-SRF-SRE complex. However, it is unclear whether these phosphatases directly target the TCF component (Hipskind *et al*., 1994b). Further studies are required to determine which phosphatases act upon the different TCFs *in vivo* following activation by each of the MAPK cascades.

D. "Physiological" Elk-1 phosphorylation

To date, most of the studies on Elk-1 phosphorylation have been carried out in fibroblast-derived cell lines with overexpressed proteins. However, there is growing evidence linking Elk-1 phosphorylation with MAP kinase activation in neuronal cells.

In the brain for example, Elk-1 is expressed exclusively in neuronal cell types where it is localised to both the cytoplasm and nucleus (see section VI-B) (Sgambato *et al*., 1998). Phosphorylated (ie activated) Elk-1 can be detected in both these compartments and hyperphosphorylation of ERK and Elk-1 spatiotemporally correlates with *c-fos* mRNA induction in these cells, providing a link between Elk-1 phosphorylation and immediate-early gene induction *in vivo* (Sgambato *et al*., 1998). Furthermore, cAMP can activate Elk-1 (via cAMP → B-Raf → Rap1 → ERK → Elk-1) in a cell type-specific manner and induce neuronal differentiation (Vossler *et al*., 1997). Thus, Elk-1 appears to play an important role in tissue-specific regulation of cell growth and differentiation via the ERK signal pathway in neuronal cells.

V. Protein-protein interactions.

The TCFs have been implicated in interactions with a wide range of proteins including transcription factors, regulatory proteins and coactivators. Such interactions serve to connect the TCFs to upstream regulatory pathways, modify their activities and mediate their effect on the transcriptional apparatus. In addition, these interactions serve to provide an additional level of complexity which enhances their specificity of action. This is particularly important in promoter recognition where protein-protein interactions supplement the DNA-protein interactions mediated by the ETS-domain (see section III).

A. SRF and Pax-5

Multi-component transcription factor complexes play important roles in integrating extracellular signals into promoter-specific transcriptional responses. The TCFs can form complexes on two types of composite binding sites, which contain ets-motifs and either Pax- or SRF-(CArG boxes) binding motifs. The ternary complex at the *c-fos* SRE between TCFs and SRF has developed as a paradigm for the latter type of complex. This ternary complex plays a pivotal role in the activation of immediate-early gene expression upon induction by a series of mitogenic and stress stimuli (reviewed in Cahill *et al*., 1996; see section VI-A).

Direct protein-protein interactions between the TCFs and SRF play an essential role in the formation of the ternary complex. Each TCF possesses a 21 amino acid conserved segment known as the B-box which is required, in addition to the ETS-domain, for complex formation with SRF and the c-*fos* SRE (reviewed in Treisman, 1994, Sharrocks *et al.*, 1997). Recently, *Net-b*, a naturally occurring splice variant of the murine *SAP-2* homologue, has been cloned and found to represent one of the major components of the ternary complexes found *in vivo* (Maira *et al.*, 1996). Net-b represents a dominant-negative form of the TCFs as it contains the ETS-domain and the B-box but lacks the C-terminal transcriptional activation domain. It can therefore still bind to SRF-SRE complexes but is unable to activate transcription. Furthermore, as Net-b also retains the NID, it has the potential to act as an "active" repressor in addition to acting as a competitive inhibitor protein.

The 'grappling hook' model was proposed to describe the mechanism of ternary complex formation in which the B-box is tethered to the ETS-domain by a flexible linker which permits interactions with SRF from variable distances (Treisman *et al.*, 1992). Recent molecular modelling studies of a SAP-1/SRF/DNA complex provide evidence to support this model (**Fig. 2**; Mo *et al*., 1998). In the model, the DNA-binding domains of SRF and SAP-1 bind to opposite sides of the major groove, positioning the C-terminus of the SAP-1 ETS-domain and the B-box interaction surface on SRF more that 45Å apart. However, the linker region between the ETS-domain and the B-box in SAP-1 would be long enough to accommodate this distance in an extended conformation.

The B-box is necessary and sufficient to interact with SRF in the absence of DNA and acts when bound to other proteins as a "portable" protein-protein interaction motif (Hill *et al.*, 1993; Shore and Sharrocks, 1994). In the Elk-1 B-box, five predominantly hydrophobic, residues are thought to constitute a hydrophobic face of an inducible α-helix which forms the interaction surface for SRF (**Fig. 2**, **Fig. 5**; Ling *et al*., 1997). Such short motifs with significant hydrophobic content have been increasingly implicated in interactions between other eukaryotic transcription factors. Recently, the cognate binding surface for the B-box on SRF has been found to be small surface-exposed hydrophobic patch (Ling *et al*., 1998), indicating that the interactions between the two proteins are likely to be predominantly hydrophobic in nature.

The yeast protein MATα2 interacts with the SRF-related protein Mcm1 by using a short motif which shows some sequence similarity to the B-box. Hydrophobic residues in this motif play a key role in these interactions (Mead *et al*., 1996). The crystal structure of the ternary complex formed between Mcm1/MATα2 on DNA was recently solved (Tan and Richmond, 1998) and revealed that the otherwise flexible N-terminal extension of the MATα2 homeodomain, forms a β-hairpin that grips the Mcm1 surface through parallel β-strand hydrogen bonds and closely packed, predominantly hydrophobic, side chains. Several parallels therefore exist with the TCF-SRF interaction with the use of hydrophobic residues and a similar interaction surface on SRF/Mcm1. One key difference is the prediction of an α-helical binding motif on the TCFs but a β-strand structure on MATα2. Interestingly, the crystal structure suggests that the interaction motif on MATα2 can also adopt a mixed α-helical/β-strand structure, raising the possibility that these short motifs might adopt different conformations in different contexts. Future structural and mutagenic studies on the TCF-SRF complex will resolve these issues.

Another interesting implication from the model of the DNA-bound SAP-1/SRF complex is that the SAP-1 ETS-domain might make direct contacts with SRF. The C-terminal region of the α3 recognition helix of the SAP-1 ETS-domain is in position to interact with the N-terminus of the recognition helix of SRF. This interaction has been proposed to affect protein-DNA contacts mediated by the SAP-1 ETS-domain (Mo *et al*., 1998). Thus, this part of the ETS-domain appears to play a key role in mediating the biochemical properties of the TCFs, being involved in interactions with Pax-5 (see below), modifying their DNA binding specificity (see section III-B) and potentially, directly binding to SRF.

Several ETS-domain proteins, including the TCFs Elk-1 and SAP-2 can be recruited by Pax-5 into ternary complexes on the *mb-1* promoter (Fitzsimmons *et al.*, 1996). In contrast to the TCF-SRF complex, ternary complex assembly requires only the ETS DNA-binding domains and the Pax-5 paired box DNA-binding domain.

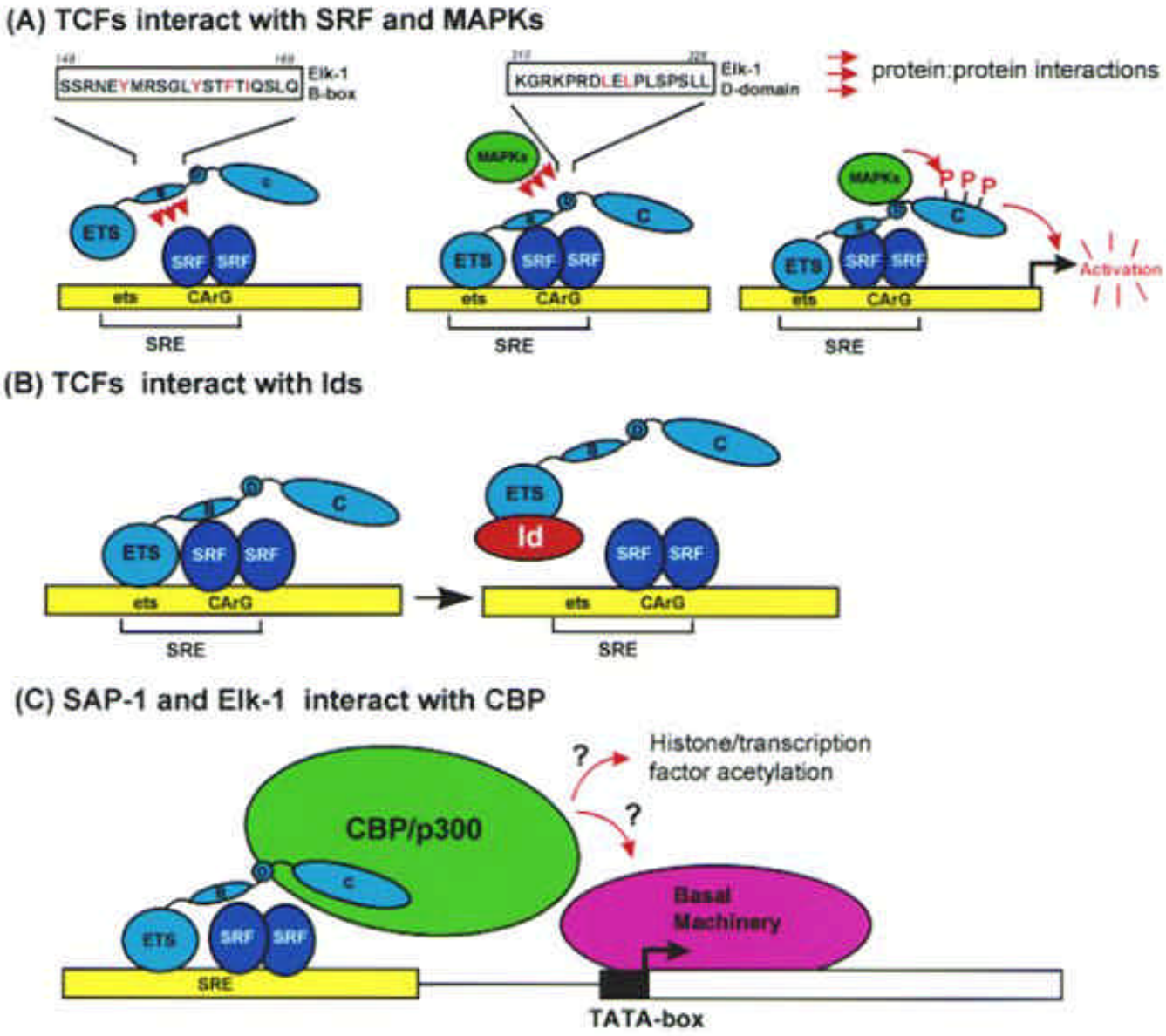

Figure 5. Protein-protein interactions involving TCFs. (A) Interactions with SRF and MAP kinases. The sequences of the SRF-interaction motif (B-box) and MAP kinase interaction motif (D-domain) are shown. Key hydrophobic residues involved in these interactions are shown in red. (B) Interactions with Ids. The Ids bind to the TCFs via the ETS-domain and inhibit binding to the c-*fos* SRE. (C) Elk-1 and SAP-1 bind to CBP. CBP can subsequently affect downstream events by mechanisms such as interacting with the basal transcription machinery or by acetylating histones, coactivator proteins or other transcription factors.

SAP-1 is not recruited by Pax-5, indicating that a specificity-determining mechanism exists. This differential ability to assemble in complexes with Pax-5 on the *mb-1* promoter is defined by a single amino acid in the ETS-domain (V68 in SAP-1 and D69 in Elk-1) (Fitzsimmons *et al.*, 1996). This amino acid also contributes significantly to the differential DNA-binding specificities of the TCFs Elk-1 and SAP-1 (see section III-B), indicating a key role for this amino acid in determining the specificity of complex formation by modifying either protein-DNA or protein-protein interactions.

B. Protein kinases.

The TCF D-domain has been demonstrated to act as a binding site for different classes of MAP kinases (Fig. 5B; see section VI-B). In common with the B-box, this is a very short domain (18-20 amino acids; Fig. 4) acts as a portable protein-protein interaction motif and can direct the binding of MAP kinases to heterologous substrates. Interestingly, this binding site exhibits sequence similarity with several other transcription factors and scaffold proteins which bind to MAP kinases including ATF-2, ATFa, MEF2C, TCFs, Spi-B, c-Jun, NFAT4 and JIP-1 (see Yang *et al.*, 1998b). One common motif found in all these sites is the central "LXL" motif. Indeed, in the case of the TCFs, the two leucine residues appear to be the most critical amino acids for binding to MAP kinases (Yang *et al.*, 1998a, Yang *et al.*, 1998b). This suggests that in common with the B-box motif, hydrophobic interactions may be major determinants of the interface with the MAP kinases. The situation is however clearly more complex as different TCFs are targeted by different kinases, suggesting a role for other residues in specificity determination. For example, the JNK binding epitope within the Elk-1 D-domain overlaps the ERK binding site but extends further downstream (Yang *et al.*, 1998). Further studies are however required to identify these specificity determinants. Hence, the D-domain does not represent a promiscuous MAPK targeting motif but allows discrimination between different classes of MAPKs.

C. Interactions with coactivators and corepressors.

The conserved C-domain of the TCFs represents a phosphorylation-dependent transcriptional activation domain (reviewed in Treisman, 1994; see section IV-A). It is currently unclear how this domain mediates transcriptional activation. Moreover, the role of phosphorylation in activating this process is still unknown although one attractive hypothesis would be recruitment of coactivator proteins in a phosphorylation-dependent manner, either by contributing to or by unmasking an interaction surface. Support for the latter mechanism is provided by the observation that Elk-1 undergoes a phosphorylation-dependent conformational change which might unmask an interaction surface (see section III-C). An increasing number of transcription factors use the coactivator, CBP/p300 to mediate the transactivation of RNA polymerase II (reviewed in Janknecht and Hunter, 1996). Indeed, both SAP-1 and Elk-1 bind directly to the N-terminus of CBP (between amino acids 451-721) in a phosphorylation-independent manner. However, functional cooperation between the two proteins requires the TCFs to become phosphorylated (Janknecht and Nordheim, 1996a, Janknecht and Nordheim, 1996b). Nucleosome positioning occurs on the *c-fos* promoter *in vivo* at a site between -90 and -280 relative to the transcriptional start site (Herrera *et al.*, 1997). It is possible that the recruitment of CBP affects either the general transcription machinery or the nucleosomal structure, either within or downstream from the c-*fos* promoter and thereby facilitates gene activation (**Fig. 5C**).

SAP-2 has been shown to act as a repressor in some contexts (Maira *et al.*, 1996). In this case, the NID is sufficient to mediate this activity but it is currently unknown how this occurs and what relevance the binding of bHLH proteins has to its activity as a repression domain.

D. Helix-loop-helix proteins.

Members of the TCF subfamily have recently been shown to interact with two different classes of proteins containing helix-loop-helix motifs. Firstly, SAP-2 has been shown to bind to bHLH proteins via the NID motif (Maira *et al.*, 1996). However, the functional consequences of this interaction are unclear.

Secondly, the Id HLH proteins can bind to all three TCFs (Yates et al, 1999). Id proteins are characterised by their ability to bind and subsequently inhibit the DNA binding activity of bHLH proteins and thereby inhibit differentiation (reviewed in Norton *et al.*, 1998). In contrast to the bHLH-SAP-2 interaction, the HLH of Id2 interacts with the ETS DNA-binding domain of the TCFs. Binding of the Ids causes the dissociation of TCFs from low affinity ets motifs and from ternary complexes, thereby inactivating ternary complex-mediated transcription of immediate-early genes such as c-*fos* (see section VI-A). Regulation of immediate-early genes in this way may implicate the Ids in resetting or ‘dampening down’ the activity of the SRE in a negative feedback mechanism once cell cycle progression has been initiated. Indeed, consistent with this hypothesis, the Id proteins are themselves expressed from immediate-early responsive promoters and but their expression in response to serum stimulation is slightly delayed in comparison to other genes such as c-*fos* (Yates et al, 1999; reviewed in Norton *et al.*, 1998).

VI. Biological Roles.

A. Immediate-early gene regulation and Cancer.

The precise regulation of the basic cellular processes of proliferation, differentiation and migration is a fundamental aspect of development and malignant disease is associated with loss of control over such events. Hence, an understanding of the role that key proteins play in co-ordinating such behaviour during embryogenesis, as well as the processes by which these proteins are regulated will provide important insights into the molecular basis of cancer. Many of the ETS-domain transcription factors have been shown to have roles in embryonic development and have also been implicated in tumourigenesis (reviewed in Sharrocks *et al.*, 1997 and Dittmer and Nordheim, 1998).

MAP kinase cascades mediate cellular responses by the transduction of mitogenic and stress stimuli into the activation of immediate-early genes such as *c-fos* and *egr-1*. The resultant transcription factors can direct an appropriate cellular response via proliferative or differentiation pathways. As major players in the transduction of signals via the SRE, activated TCFs upregulate *c-fos* expression (reviewed in Treisman, 1994) and increasing evidence suggests a role for TCFs in the upregulation of other immediate-early genes such as *egr-1* (Lim *et al.*, 1998, Watson *et al*., 1997) and *Pip92* (Chung *et al.*, 1998). Permanent activation of the proto-oncoprotein ras, as seen in a high percentage of human tumours and implicated in tumour angiogenesis, is likely to lead to the constitutive activation of TCFs and other ETS proteins by MAPKs (reviewed in Dittmer and Nordheim, 1998). Thus, the TCFs are likely to play an important role in oncogenesis.

The recent observation that SREs containing both SRF- and ets-binding motifs are activated during gastrulation represents the first example of the SRE being directly targeted by MAP kinase signalling pathways during development (Panitz *et al*., 1998). Activation via this composite element, and hence by implication through complexes containing ETS-domain proteins and SRF, was shown to be necessary and sufficient for *Xegr-1* expression in response to MAP kinase pathway activation in *Xenopus* embryos. It is currently unknown whether TCFs or other ETS-domain proteins are directly involved in this process. However, based on work carried out in mammalian systems and the observation that TCF homologues are present in other lower vertebrates (Brown and Sharrocks, unpublished data), it is likely that homologues also exist in *Xenopus* and participate in the activation of *Xegr-1* via the SRE.

B. Neuronal gene regulation

Whilst the expression levels of SRF mRNA and protein are similar in most cell lines tested, levels of the TCFs SAP-1 and Elk-1 are less homogeneous with high levels of one TCF being accompanied by low levels of the other (Price *et al*., 1995, Magnaghi-Jaulin *et al*., 1996). The expression of SAP-2 also differs according to cell-type (Price *et al.*, 1995; Giovane *et al.*, 1997). Interestingly, the relative expression levels of the murine SAP-2 homologue Net and its alternative splice form Net-b vary significantly in different cell lines and tissues. As the products of the two splice forms react differently to Ras-signalling pathways (see section V-A), this alternative splicing likely represents a key regulatory mechanism of TCF activity.

In situ hybridisation studies have revealed that Elk-1 mRNA is expressed in various structures of the adult rat brain but is restricted to neuronal cell types, suggesting a role in the regulation of neuronal function (Sgambato *et al.*, 1998). The Elk-1 protein is found in several intracellular compartments including the soma, dendrites and axon terminals in addition to the nucleus. In contrast, in fibroblasts, Elk-1 appears to be mainly nuclear when overexpressed (Janknecht and Nordheim, 1994) suggesting the possibility that a limiting amount of a cytoplasmic anchoring protein might exist which becomes saturated by Elk-1. Significantly, stimulation of glutamate receptors in the CNS induces ERK expression and a recent *in vivo* model of immediate-early gene induction has linked the stimulation of ERK to the phosphorylation and activation of Elk-1 and *c-fos* mRNA induction (Sgamboto *et al.*, 1998).

Immediate-early genes, and in particular *c-fos*, are also induced in astrocytes during early postnatal stages and at adult age in response to severe injury (axotomy, hypoxia and hypoglycaemia) (reviewed in Arenander and De Vellis, 1995) although Elk-1 is barely detectable in astrocytes *in vivo* (Sgamboto *et al.*, 1998). As Elk-1 is strongly expressed in astrocytoma cell lines (Zinck *et al*., 1995) it is therefore possible that it may be transiently induced in astrocytes and play a role in immediate-early gene activation in this cell-type.

VII. Summary and perspectives.

Studies of the TCFs have provided considerable insights into how other transcription factors belonging to the ETS-domain and other transcription factor families function at the molecular level. For example, the specificity of target promoter binding is determined by a combination of DNA-protein and protein-protein interactions. In the case of ETS-domain proteins, binding in combination with partner proteins appears to be the major mechanism of achieving promoter-specific binding. The elucidation of the structure of the DNA binding domains of several ETS-domain proteins, including SAP-

1, has enhanced our knowledge of their mechanisms of DNA binding. Future studies of higher order complexes (eg TCF-SRF-DNA) will provide further significant insights into their molecular function.

It is becoming increasingly clear that post-translational modifications such as phosphorylation and acetylation play major roles in regulating the activities of transcription factors. Again, studies on the TCFs have provided insights into how phosphorylation can affect DNA binding and transcriptional activation mediated by these proteins. In particular, the observation that phosphorylation triggers conformational changes which are transmitted intramolecularly between different domains, is likely to be a common mechanism shared by many transcription factors.

Another emerging theme is that MAP kinase pathways actually represent compact modules in which the kinases are maintained in complexes to increase the specificity and speed of signal transduction. The observation that transcription factors such as the TCFs possess distinct kinase docking sites which contribute to the specificity of kinase action, further supports the hypothesis that protein-protein interactions play a major role in determining the specificity of signal transduction in the cell. Furthermore, the ability of TCFs to be phosphorylated by different classes of MAP kinases, provides a mechanism by which different signals can be integrated into a single nuclear response by using a common substrate. Subsequent differences in the profiles of gene expression induced by mitogenic and stress stimulation must presumably result from their ability to phosphorylate other nuclear substrates which respond uniquely to different MAP kinase cascades.

Finally, one of the major challenges of the future is to discover which genes and biological processes are regulated by individual transcription factors. This is particularly important in transcription factor families where different family members have presumably evolved to carry out specific tasks. The availability of a large body of data on the molecular action of the TCFs and numerous reagents is likely to ensure that future studies on the TCFs are likely to continue to contribute significantly to our understanding of eukaryotic transcription factor function.

Acknowledgements

We are grateful to members of our laboratories for stimulating discussions and Amanda Greenall for comments on the manuscript. We would also like to thank Ronen Marmorstein for the discussion of data prior to publication. The work in the corresponding Author's laboratory is supported by grants from the Cancer Research Campaign [CRC], the Wellcome Trust, and the BBSRC. ADS is a Research Fellow of the Lister Institute of Preventative Medicine.

References

Arenander, A. and De Vellis, J. (**1995**) Early response gene expression in glial cells. In: Neurologia (Kettenman, H. and Ransom, B.R. eds), pp510-522. New Yok: Oxford UP.

Bassuk, A. and Leiden, J. (**1996**) The role of Ets transcription factors in the development and function of the mammalian immune system. **Adv. Immunol**. 64, 65-104.

Batchelor, A.H., Piper, D.E., de la Brousse, F.C., McKnight, S.L. and Wolberger, C. (**1998**) The structure of GABPa/b: an ETS-domain-ankyrin repeat heterodimer bound to DNA. **Science** 279, 1037-1041.

Cahill, M.A., Janknecht, R and Nordheim, A. (**1996**) Signalling pathways: Jack of all cascades. **Current Biology** 6**,** 16-19.

Cavigelli, M., Dolfi, F., Claret, F.X. and Karin, M. (**1995**) Induction of c-*fos* expression through JNK-mediated TCF/Elk-1 phosphorylation. **EMBO. J.** 14, 5957-5964.

Chung, K-C., Gomes, I., Wang, D., Lau, L.F. and Rosner, M.R. (**1998**) Raf and fibroblast growth factor activate the serum response element of the immediate early gene *pip92* by mitogen-activated protein kinase-independent as well as -dependent signalling pathways. **Mol. Cell. Biol**. 18, 2272-2281.

Cohen, P. (**1997**) The search for physiological substrates of MAP and SAP kinases in mammalian cells. **Trends In Cell. Biol**. 7, 353-361.

Dai, T., Rubie, E., Franklin, C.C., Kraft, A., Gillespie, D.A.F., Avruch, J., Kyriakis, J.M. and Woodgett, J.R. (**1995**) Stress-activated protein kinases bind directly to the δ domain of c-*Jun* in resting cells: implications for repression of c-*jun* function. **Oncogene** 10**,** 849-855.

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

Degnan, B.M., Degnan, S.M., Naganuma, T. and Morese, D.E. (**1993**) The *ets* multigene family is conserved throughout the Metazoa. **Nucleic Acids Res**. 21, 3479-3484.

Dérijard, B., Hibi, M., Wu, I-H., Barrett, T., Su, B., Deng, T., Karin, M. and Davis, R.J. (**1994**) JNK1: A protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-*jun* activation domain. **Cell** 76**,** 1025-1037.

Dittmer, J. and Nordheim, A. (**1998**) Ets transcription factors and human disease. Biochim. **Biophys. Acta** 1377, F1-F11.

Enslen, H., Raingeaud, J., and Davis, R.J. (**1998**) Selective activation of p38 MAP kinase isoforms by the MAP kinase kinases MKK3 and MKK6. **J. Biol. Chem**. 273, 1741-1748.

Fitzsimmons, D., Hodsdon, W., Wheat, W., Maira, S-M., Wasylyk, B. and Hagman, J. (**1996**) Pax-5 (BSAP) recruits Ets proto-oncogene family proteins to form

functional ternary complexes on a B-cell-specific promoter. **Genes Dev.** 10, 2198-2211.

Frost, J.A., Steen, H., Shapiro, P., Lewis, T., Ahn, N., Shaw, P.E. and Cobb, M.H. (**1997**) Cross-cascade activation of ERKs and ternary complex factors by Rho family proteins. **EMBO J**. 16, 6426-6438.

Gille, H., Kortenjann, M., Strahl, T. and Shaw, P.E. (**1996**) Phosphorylation-dependent formation of a quaternary complex at the c-*fos* SRE. **Mol. Cell. Biol.** 16, 1094-1102.

Gille, H., Kortenjann, M., Thomae, O., Moomaw, C., Slaughter, C., Cobb, M.H. and Shaw P.E. (**1995a**) Phosphorylation-dependent formation of a quaternary complex at the *c-fos* SRE. **EMBO J.** 14, 951-962.

Gille, H., Strahl, T. and Shaw, P.E. (**1995b**) Activation of ternary complex factor Elk-1 by stress-activated protein kinases. **Current Biology 5**, 1191-1200.

Giovane, A., Pintzas, A., Maira, S-M., Sobieszcuk, P. and Wasylyk, B. (**1994**) Net, a new *ets* transcription factor that is activated by Ras. **Genes Dev.** 8, 1502-1513.

Giovane, A., Sobieszczuk, P., Ayadi, A., Maira, S-M. and Wasylyk, B. (**1997**) Net-b, a Ras-insensitive factor that forms ternary complexes with the serum response element of the *fos* promoter. **Mol. Cell. Biol**. 17, 5667-5678.

Graves, B.J. and Petersen, J.M. (**1998**) Specificity within the *ets* family of transcription factors. In *Adv. Cancer Res.*, G. van de Woude and G. Klein, Eds. (Academic Press, San Diego, 1998).

Gupta, S., Barrett, T., Whitmarsh, A.J., Cavanagh, J., Sluss, H.K., Dérijard, B. and Davis, R.J. (**1996**) Selective interaction of JNK protein kinase isoforms with transcription factors. **EMBO J**. 15**,** 2760-2770.

Herrera, R.E., Nordheim, A. and Stewart, A.F. (**1997**) Chromatin structure analysis of the human c-*fos* promoter reveals a centrally positioned nucleosome. **Chromosoma** 106, 284-292.

Hill, C.S., Marais, R., John, S., Wynne, J., Dalton, S. and Treisman, R. (**1993**) Functional analysis of a growth factor-responsive transcription factor complex. **Cell** 73, 395-406.

Hipskind, R.A., Baccarini, M. and Nordheim, A. (**1994b**) Transient activation of RAF-1, MEK and ERK2 coincides kinetically with TCF phosphorylation and IEG promoter activity *in vivo*. **Mol. Cell. Biol**. 14, 6219-6231.

Hipskind, R.A., Buscher, D., Nordheim, A. and Baccarini, M. (**1994a**) Ras/MAP kinase-dependent and -independent signalling pathways target distinct ternary complex factors. **Genes Dev**. 8, 1803-1816.

Janknecht, R. and Hunter, T. (**1996**) A growing coactivator network. **Nature** 383, 22-23.

Janknecht, R. and Hunter, T. (**1997a**) Convergence of MAP kinase pathways on the ternary complex factor SAP-1a. **EMBO J**. 16**,** 1620-1627.

Janknecht, R. and Hunter, T. (**1997b**) Activation of the SAP-1a transcription factor by the c-Jun N-terminal kinase (JNK) mitogen-activated protein kinase. **J. Biol. Chem**. 272**,** 4219-4224.

Janknecht, R. and Nordheim, A. (**1992**) Elk-1 protein domains required for direct and SRF-assisted DNA-binding. **Nucleic Acids Res**. 20, 3317-3324.

Janknecht, R. and Nordheim, A. (**1996a**) Regulation of the c-*fos* promoter by the ternary complex factor SAP-1a and its coactivator CBP. **Oncogene** 12, 1961-1969.

Janknecht, R. and Nordheim, A. (**1996b**) MAP kinase-dependent transcriptional coactivation by Elk-1 and its cofactor CBP. **Biochem. Biopys. Res. Comm.** 228, 831-837.

Janknecht, R., Zinck, R., Ernst, W.H. and Nordheim, A. (**1994**) Functional dissection of the transcription factor Elk-1. **Oncogene** 9, 1273-1278.

Kallunki, T., Deng, T., Hibi, M. and Karin, M. (**1996**) c-Jun can recruit JNK to phosphorylate dimerization partners via specific docking interactions. **Cell** 87**,** 929-939.

Kallunki, T., Su, B. Tsigelny,I., Sluss, H.K., Dérijard, B., Moore, G., Davis, R.J., and Karin, M. (**1994**) JNK2 contains a specificity-determining region responsible for efficient c-jun binding and phosphorylation. **Genes Dev**. 8**,** 2996-3007.

Karim, F.D., Urness, L.D., Thummel, C.S., Klemsz, M.J., McKercher, S.R., Celada, A., Van Beueren, C., Maki, R.A., Gunther, C.V., Nye, J.A. and Graves, B.J. (**1990**) The ETS-domain: a new DNA-binding motif that recognises a purine-rich core DNA sequence. **Genes Dev**. 4, 1451-1453.

Karin, M. (**1994**) Signal transduction from the cell surface to the nucleus through the phosphorylation of transcription factors. **Curr. Opin. Cell Biol**. 6**,** 415-424.

Kortenjann, M., Thomae, O. and Shaw, P.E. (**1994**) Inhibition of v-*raf*-dependent c-*fos* expression and transformation by a kinase-defective mutant of the mitogen activated protein kinase Erk2. **Mol. Cell. Biol**. 14, 4815-4824.

Latinkic, B.V., Zeremski, M. and Lau, L.F. (**1996**) Elk-1 can recruit SRF to form a ternary complex upon the serum response element. **Nucleic Acids. Res**. 24, 1345-1351.

Laudet, V., Niel, C., Duterque-Coquillaud, M., Leprince, D. and Stehelin, D. (**1993**) Evolution of the *ets* gene family. **Biochem. Biophys. Res. Comm.** 190, 8-14.

Lefstin, J.F. and Yamamoto. K.R. (**1998**) Allosteric effect of DNA on transcriptional regulator. **Nature** 392, 885-888.

Lim, C.P., Jain, N. and Cao, X. (**1998**) Stress-induced immediate-early gene, egr-1, involves activation of p38/JNK1. **Oncogene** 16, 2915-2926.

Ling, Y., Lakey, J.H., Roberts, C.E. and Sharrocks, A.D. (**1997**) Molecular characteristics of the B-box protein-protein interaction motif of the ETS-domain transcription factor Elk-1. **EMBO. J**. 16, 2431-2440.

Ling, Y., West, A.G., Roberts, E.C., Lakey, J.H. and Sharrocks, A.D. (**1998**) Interaction of transcription factors with SRF: identification of the Elk-1 binding surface. **J. Biol. Chem**. 273**,** 10506-10514.

Lopez, M., Oettgen, P., Akbarali, Y., Fendorter, U. and Liberman, T.A. (**1994**) ERP, a new member of the *ets* transcription factor/oncoprotein family: cloning, characterization, and differential expression during B-lymphocyte development. **Mol. Cell. Biol**. 14, 3292-3309.

Magnaghi-Jaulin, L., Masutani, H., Lipinski, M. and Harel-Bellan, A. (**1996**) Analysis of SRF, SAP-1 and Elk-1 transcripts and proteins in human cell lines. **FEBS Lett**. 391, 247-251.

Maira, S-M., Wurtz, J-M. and Wasylyk, B. (**1996**) Net (ERP/SAP2), one of the Ras-inducible TCFs, has a novel inhibitory domain with resemblance to the helix-loop-helix motif. **EMBO J**. 15, 5849-5865.

Masutani, H., Magnaghi-Jaulin, L., Ali, S.A.S., Groisman, R., Robin, P. and Harel-Bellan, A. (**1997**) Activation of the *c-fos* SRE through SAP-1a. **Oncogene** 15, 1661-1669.

Mead, J., Zhong, H., Acton, T.B. and Vershon, A.K. (**1996**) The yeast α2 and Mcm1 proteins interact through a region similar to a motif found in homeodomain proteins of higher eukaryotes. **Mol. Cell. Biol**. 16**,** 2135-2143.

Mo, Y., Vaessen, B., Johnston, K. and Marmorstein, R. (**1998**) Structures of SAP-1 bound to DNA sequences from the E74 and c-fos promoters provide insights into how ETS proteins discriminate between related DNA targets. **Molecular Cell** 8, 210-212.

Norton, J.D., Deed, R.W., Craggs, G. and Sablitzky, F. (**1998**) Id helix-loop-helix proteins in cell growth and differentiation. **Trends Cell. Biol**. 8, 58-65.

Panitz, F., Krain, B., Hollemann, T., Nordheim, A. and Pieler, T. (**1998**) The Spemann organiser-expressed zinc finger gene *Xerg-1* responds to MAP kinase/Ets-SRF signal transduction pathway. **EMBO J**. 17, 4414-4425.

Price, M.A., Cruzalegui, F.H. and Treisman, R. (**1996**) The p38 and ERK MAP kinase pathways co-operate to activate ternay complex factors and c-*fos* transcription in response to UV light. **EMBO. J**. 15, 6552-6563.

Price, M.A., Rogers, A.E. and Treisman, R. (**1995**) Comparative analysis of the ternary complex factors Elk-1, SAP-1a and SAP-2 (ERP/NET). **EMBO J.** 14, 2589-2601.

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

Robinson, M.J. and Cobb, M.H. (**1997**) Mitogen-activated protein kinase pathways. **Current Opinion in Cell Biology** 9**,** 180-186.

Sgamboto, V., Vanhoutte, P., Pages, C., Rogard, M., Hipskind, R., Besson, M-J. and Caboche, J. (**1998**) *In vivo* expression and regulation of Elk-1, a target of the extracellular-regulated kinase signalling pathway, in the adult rat brain. **J. Neuroscience** 18, 214-226.

Sharrocks, A.D., Brown, A.L., Ling, Y. and Yates, P.R. (**1997**) The ETS-domain transcription factor family. **Int. J. Biochem. Cell Biol.,** 29, 1371-1387.

Shore, P. and Sharrocks, A.D. (**1995**) The ETS-domain transcription factors Elk-1 and SAP-1 exhibit differential DNA binding specificities. **Nucleic Acids Res**. 23, 4698-4706.

Shore, P. and Sharrocks, A.D. (**1994**) The transcription factors Elk-1 and serum response factor interact by direct protein-protein contacts mediated by a short region of Elk-1. **Mol. Cell. Biol**. 14, 3283-3291.

Shore, P., Bisset, L., Lakey, J., Waltho, J.P., Virden, R. and Sharrocks, A.D (**1995**) Characterization of the Elk-1 ETS DNA-binding domain. **J. Biol. Chem**. 270, 5805-5811.

Shore, P., Whitmarsh, A.J., Bhaskaran, R., Davis, R.J., Waltho, J.P. and Sharrocks, A.D. (**1996**) Determinants of DNA-binding specificity of ETS-domain transcription factors. **Mol. Cell. Biol**. 16, 3338-3349.

Sluss, H.K., Barrett, T., Derijard, B. and Davis, R.J. (**1994**) Signal transduction by tumor necrosis factor mediated by JNK protein kinases. **Mol. Cell. Biol**. 14**,** 8376-8384.

Strahl, T., Gille, H. and Shaw, P.E. (**1996**) Selective response of ternary complex factor Sap-1a to different mitogen-activated protein kinase sub-groups. **Proc. Natl. Acad. Sci**. 93, 11563-11568.

Sugimoto, T., Stewart, S. and Guan, K-L. (**1997**) The calcium/calmodulin-dependent protein phosphatase calcineurin is the major Elk-1 phosphatase. **J. Biol. Chem**. 272, 29415-29418.

Tan, S. and Richmond, T.J. (**1998**) Crystal structure of the yeast MATa2/MCM1/DNA ternary complex. **Nature** 391**,** 660-666

Treisman, R. (**1994**) Ternary complex factors: growth regulated transcriptional activators. **Curr. Opin. Gen. Dev**. 4, 96-101.

Treisman, R. (**1996**) Regulation of transcription by MAP kinase cascades. **Curr. Opin. Cell Biol**. 8, 205-215.

Treisman, R., Marais, R. and Wynne, J. (**1992**) Spatial flexibility in complexes between SRF and its accessory proteins. **EMBO J.** 11, 4631-4640.

Vossler, M.R., Yao, H., York, R.D., Pan, M-G., Rim, C.S. and Stork, P.J.S. (**1997**) cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. **Cell** 89, 73-82.

Wang, X.S., Diener, K., Manthey, C.L., Wang, S-W., Rosenzweig, B., Bray, J., Delaney, J., Cole, C.N., Chan-Hui, P-Y., Mantlo, N., Lichenstein, H.S., Zukowski, M. and Yao, Z. (**1997**) Molecular cloning and characterization of a novel p38 mitogen-activated protein kinase. **J. Biol. Chem**. 272, 23668-23674.

Watson, D.K., Robinson, L., Hodge, D.R., Kola, I., Papas, T.K. and Seth, A. (**1997**) FLI1 and EWSFLI1 function as ternary complex factors and ELK1 and SAP1a function as ternary and quaternary complex factors on the Egr1 promoter serum response elements. **Oncogene**. 14, 213-221.

Whitmarsh, A.J. and Davis, R.J. (**1996**) Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. **J. Mol. Med**. 74**,** 589-607.

Whitmarsh, A.J., Shore, P., Sharrocks, A.D. and Davis, R.J. (**1995**) Integration of MAP kinase signal transduction pathways at the serum response element. **Science** 269, 403-407.

Whitmarsh, A.J., Yang, S-H., Su, M. S-S., Sharrocks, A.D. and Davis, R.J. (**1997**) Role of p38 and JNK MAP kinases in the activation of ternary complex factors. **Mol**. **Cell. Biol**. 17, 2360-2371.

Yang, S-H., Whitmarsh, A.J., Davis, R.J. and Sharrocks, A.D. (**1998a**) The Elk-1ETS-domain transcription factor contains a MAP kinase targeting motif. **Mol. Cell. Biol**. 18, 710-720.

Yang, S-H., Whitmarsh, A.J., Davis, R.J. and Sharrocks, A.D. (**1998b**) Differential targeting of MAP kinases to the ETS-domain transcription factor Elk-1. **EMBO J**. 17, 1740-1749.

Yates PR, Atherton GT, Deed RW, Norton JI and Sharrocks AD (**1999**) Id helix-loop-helix proteins inhibit nucleoprotein complex formation by the TCF ETS-domain transcription factors. **EMBO J**. In press.

Zinck, R., Cahill, M.A., Kracht, M., Sachsenmaier, C., Hipskind, R.A. and Nordheim, A. (**1995**) Protein synthesis inhibitors reveal differential regulation of mitogen-activated protein kinase and stress-activated protein kinase pathways that converge on Elk-1. **Mol. Cell. Biol.** 15, 4930-4938.