ERK2/p42 MAP kinase stimulates both autonomous and SRF-dependent DNA binding by Elk-1

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Abstract A ternary complex comprised of SRF, ternary complex factor (TCF) and the c-*fos* SRE is the target of several extracellular signal regulated pathways. Phosphorylation of the TCF Elk-1 is a key event in the activation of this complex. We demonstrate that ERK2/p42 phosphorylation of Elk-1 stimulates its recruitment into ternary complexes with SRF. Moreover, phosphorylation of Elk-1 also stimulates its autonomous SRF-independent binding to high affinity binding sites. Thus part of the effect of ERK2/p42 phosphorylation is to stimulate DNA-binding by the ETS DNA-binding domain of Elk-1.

Key words: MAP kinase; TCFs; Serum response element; Transcription factor; ETS-domain

1. Introduction

Extracellular growth factors cause the rapid and transient transcriptional induction of the c-*fos* gene. These signals are transduced in part through the serum response element SRE within the c-*fos* promoter [1]. A ternary complex assembles at this site both in vivo and in vitro that contains two protein components, the serum response factor (SRF) and ternary complex factor (TCF) [1,2].

The TCFs represent a sub-group of the ETS-domain transcription factor family [3,4] that is comprised of at least 3 members, Elk-1 [5], SAP1 [6], and SAP2/ERP/Net [6–8]. The TCFs utilise both protein/protein and protein/DNA contacts to bind to the binary SRF/SRE complex. Three domains in the TCFs have been identified on the basis of primary sequence similarity; the N-terminal ETS-domain, which is involved in DNA-binding [6,9,10]; the B-domain which mediates direct contacts with SRF [11] and is required for ternary complex formation [6,9]; and the C-terminal domain which is the target for MAP kinases [12–14]. Indeed, extracellular signals are transduced via the MAP kinase pathway through the TCF component of this ternary complex [2,15]. Phosphorylation by MAP kinase of TCFs purified from nuclear extracts causes an induction of their binding to SRF/SRE complexes [16]. This stimulation of binding activity is also observed using purified Elk-1 and p44/ERK1 [17]. In contrast, others have observed a change in mobility of the ternary complex following MAP kinase phosphorylation of Elk-1 [12,13]. Furthermore phosphorylation of the C-terminal domain of Elk-1 by MAP kinase causes an increase in its transcriptional activation properties [12,14]. In this study, we investigated the effect of p42/ERK2 phosphorylation on purified Elk-1. We show that recruitment of Elk-1 into a ternary complex with SRF is stimulated by its phosphorylation by p42/ERK2 MAP kinase. Moreover, we also demonstrate that the phosphorylated form of Elk-1 shows enhanced autonomous binding to a site containing a high affinity ets- motif. These results indicate that at least part of the function of p42/ERK2 phosphorylation of Elk-1 is to stimulate the binding of the ETS-domain to DNA.

2. Materials and methods

2.1. Gel retardation analysis

DNA binding reactions were carried out essentially as described previously [18] for 20 min at room temperature in the presence of 125 mM KCl. Proteins were bound to either the c-*fos* SRE or the *Drosophila* E74 site [11]. The protein/DNA complexes and free DNAs were resolved by electrophoresis through 5% polyacrylamide gels cast in 1 × Tris-borate-EDTA (TBE) buffer.

2.2. Protein production

The DNA-binding domain of SRF (core^{SRF}) was purified from *E. coli* as described previously [11]. Full-length non-phosphorylated Elk-1 (amino acids 1–428) was purified as a C-terminally histidine-tagged protein from *E. coli* transformed with the plasmid pQE6/16Elk as described elsewhere [17]. MAP kinase (p42/ERK2) was expressed as a fusion with GST (GST:p42^{MAPK}) and purified from *E. coli* by a modification of the procedure described by Smith and Johnson [19]. Cells were lysed and GST:p42^{MAPK} was bound to reduced glutathione-agarose beads in 50 mM Tris-Cl, pH 7.4, 150 mM NaCl, 1 mM PMSF, 1 mM DTT. Beads were washed in the same buffer and the purified GST:p42^{MAPK} was eluted in 100 mM HEPES, pH 8, 15 mM reduced glutathione. Finally, the purified GST:p42^{MAPK} was dialysed against 20 mM HEPES, pH 8, 20% glycerol, 0.2 mM EDTA, 0.1 M KCl, 0.5 mM PMSF, 0.5 mM DTT. Active MEK (MAP kinase kinase) was partially purified from rat L6 cell extracts on a Mono-Q column using FPLC (Pharmacia).

Phosphorylation of Elk-1 by MAP kinase was carried out in 25 μl reaction mixtures containing ~4 ng GST:p42^{MAPK}, 1 μl MEK extract, 0.1 mM ATP, 0.1 mM sodium vanadate, 40 mM Tris-Cl pH 7.4, 1 mM DTT, 10 mM $MgCl_2$, 10 μCi [γ-^{32}P]ATP, ~10 ng bacterially expressed Elk-1. Reactions were allowed to proceed for 1 h at 30°C. The presence of ATP, MEK and MAP kinase was required for maximal stimulation of Elk-1 phosphorylation. 25 × MAP kinase inhibitor buffer (MIB) was 50 mM EDTA [12], 20 × Phosphatase inhibitor buffer (PIB) was 20 mM sodium vanadate, 200 mM pNPP, 200 mM β-glycerophosphate [16]. 0.04 units of potato acid phosphatase (Boehringer-Mannheim) were added where indicated.

3. Results

Purified MAP kinase (ERK2/p42) preparations phosphorylate TCFs purified from nuclear extracts and induce their recruitment into ternary complexes with SRF and the c-*fos* SRE [16]. We investigated whether the same effect could be brought about in vitro using purified components. The DNA binding domain of SRF (core^{SRF}) was expressed in *E. coli* as a GST fusion protein and subsequently purified in the absence of the GST moiety [11]. Full-length Elk-1 was purified from

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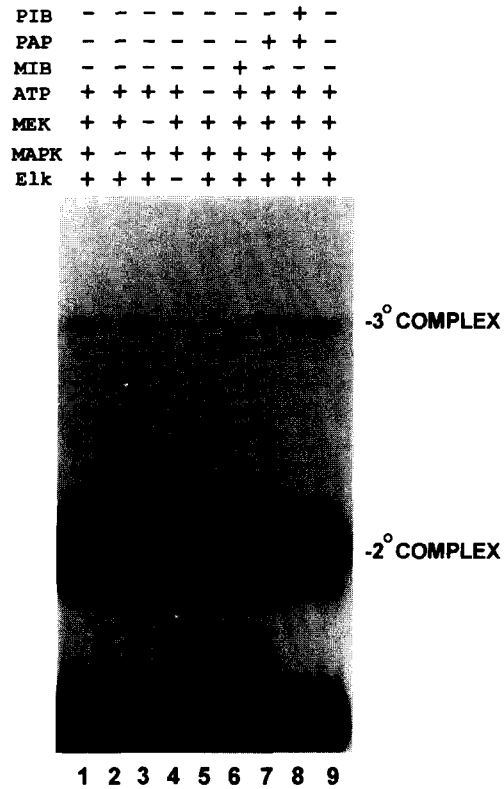

Fig. 1. ERK2/p42 phosphorylation of Elk-1 stimulates its recruitment into ternary complexes with SRF and the c-*fos* SRE. Elk-1 was pre-treated in the presence of the components indicated above each lane. The following components were omitted; GST MAP kinase (lane 2), MEK extract (lane 3), Elk-1 (lane 4), ATP (lane 5). MAP kinase inhibitor buffer (MIB) was included in lane 6, Phosphatase inhibitor buffer (PIB) was included in lane 8. Potato acid phosphatase was included in lanes 7 and 8. Elk-1 was subsequently bound to SRF and ^{32}P-labelled SRE. The mobilities of the binary (2°) SRF:SRE and ternary (3°) Elk:SRF:SRE complexes are indicated.

E. coli using a hexa-histidine C-terminal tag [17]. ERK2/p42 MAP kinase was purified in an inactive form as a GST fusion protein from *E. coli*. Activation of GST-ERK2 was carried out in vitro using extracts containing active MEK. Purified Elk-1 was phosphorylated in vitro using the coupled MEK/GST-ERK2 system. Subsequently, the ability of the TCF Elk-1 to form ternary complexes was monitored using the gel retardation assay in the presence of core^{SRF} and a ^{32}P-labelled SRE probe. Under these conditions Elk-1 forms a discrete ternary complex (Fig. 1, lanes 1 and 9). The function of this ternary complex is dependent upon the inclusion of Elk-1 in the binding reaction (Fig. 1, lane 4). However, if either the MAP kinase (Fig. 1, lane 2), MEK (Fig. 1, lane 3) or ATP (Fig. 1, lane 5) are omitted from the pre-incubation reaction, the recruitment of Elk-1 into the ternary complex is clearly reduced. Moreover, the inclusion of MAP kinase inhibitor buffer (MIB) (Fig. 1, lane 6) also reduces ternary complex formation. Potato acid phosphatase (PAP) reverses the effect of MAP kinase phosphorylation (Fig. 1, lane 7) whereas the inclusion of phosphatase inhibitor buffer (PIB) blocks this reversal (Fig. 1, lane 8). These data clearly demonstrate that phosphorylation of Elk-1 by ERK2/p42 MAP kinase stimulates its recruitment into ternary complexes with SRF and the SRE.

Elk-1 utilises both protein–protein interactions [11] and protein–DNA interactions [9,20] to bind specifically to the c-*fos* SRE. One or both of these interactions might be stimulated by MAP kinase phosphorylation of Elk-1. Although Elk-1 cannot bind autonomously to the c-*fos* SRE, it can bind to other higher affinity sites that contain ets-motifs such as the *Drosophila* E74 site [9,10,20]. We therefore took advantage of this observation to investigate the effect phosphorylation of Elk-1 has on its DNA-binding activity. Maximal DNA binding of both phosphorylated and non-phosphorylated Elk-1 to the E74 site occurs within 30 s of probe addition (data not shown). This rapid attainment of equilibrium is indicative of a rapid on-rate. However, differences in the DNA binding affinity were apparent. Elk-1 that had been pre-phosphorylated by ERK2/p42 MAP kinase, bound to the E74 site (Fig. 2, lane 1; closed arrow). However, the omission of either the MAP kinase (Fig. 2, lane 2), the MEK (Fig. 2, lane 3) or ATP (Fig. 2, lane 5) caused a marked reduction in the binding of Elk-1. The inclu-

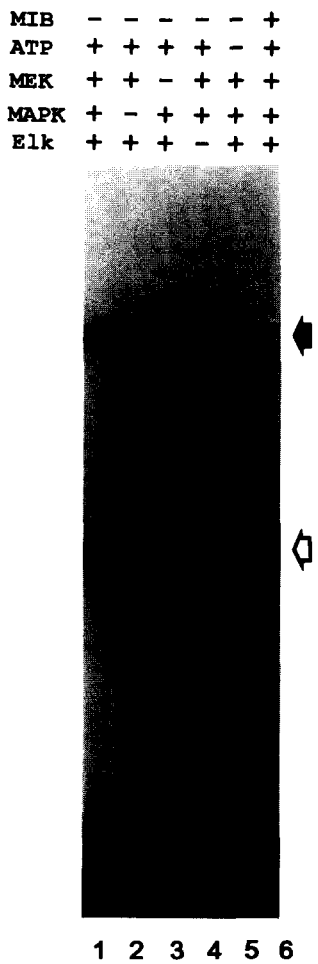

Fig. 2. ERK2/p42 phosphorylation of Elk-1 stimulates its autonomous DNA binding to high affinity ETS-sites. Elk-1 was pre-treated in the presence of the components indicated above each lane. The following components were omitted; GST MAP kinase (lane 2), MEK extract (lane 3), Elk-1 (lane 4), ATP (lane 5). MAP kinase inhibitor buffer (MIB) was included in lane 6. Elk-1 was subsequently bound to a ^{32}P-labelled E74 site. The mobilities of full-length Elk-1/E74 complexes are indicated by a closed arrow. The DNA–protein complex indicated by an open arrow probably represents a degradation product of Elk-1. The level of binding of this complex does not fluctuate in a phosphorylation-dependent manner.

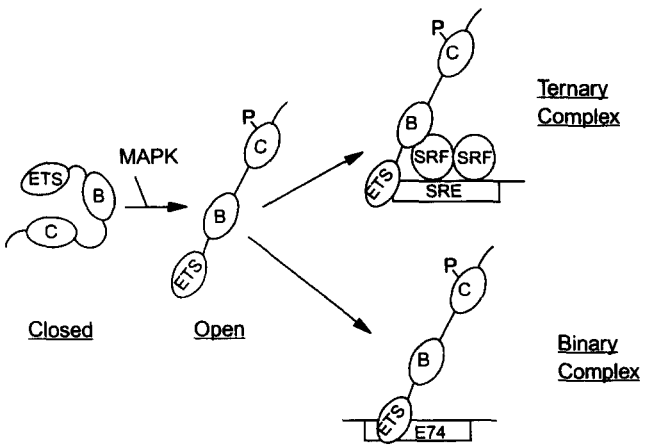

Fig. 3. Model depicting possible mechanisms whereby ERK2/p42 phosphorylation of Elk-1/TCF stimulates its DNA-binding activity. In the non-phosphorylated state, Elk-1/TCF exists in a closed conformation. Upon phosphorylation of the C-domain by ERK2/p42 MAP kinase, Elk-1 reverts to an open conformation in which the ETS-domain and B-domain become accessible to the DNA and SRF, respectively. Elk-1 can then subsequently bind either autonomously to ETS-motifs to form a binary complex or in conjunction with SRF to the SRE to form a ternary complex.

sion of MAP kinase inhibitor buffer also blocked the stimulation of Elk-1 binding (Fig. 2, lane 6). These results essentially mirror those observed with the recruitment of Elk-1 into the ternary complex and indicate that ERK2/p42 phosphorylation of Elk-1 stimulates DNA-protein interactions by the Elk-1 ETS DNA-binding domain. A second faster mobility complex is also observed in the tracks containing Elk-1. The mobility of this complex is consistent with that of a truncated version of Elk-1 (Elk1-168) which lacks the regulatory C-terminal domain and binds constitutively to the E74 site [11]. This complex is therefore likely to represent a proteolytic degradation product of Elk-1. The intensity of this complex does not vary in manner that is consistent with a phosphorylation-dependent induction of DNA binding and is variable in different experiments.

4. Discussion

The phosphorylation of TCFs correlates with the rapid induction of c-*fos* transcription [15,16,21]. This is therefore a pivotal step in the transduction of extracellular signals into a nuclear response. Several groups have proposed that a change in ternary complex mobility observed following phosphorylation of Elk-1 reflects the induction of a large conformational change in this complex [12,19]. The majority of this effect is apparently mediated through the protein components rather than a change in DNA conformation (our unpublished data). However, others have observed a clear phosphorylation-dependent stimulation of TCF/Elk-1 recruitment into a ternary complex with SRF and the SRE [16,22].

We demonstrate, using purified components, that ERK2/p42 MAP kinase phosphorylation of Elk-1 stimulates the formation of ternary complexes with SRF and the SRE. This is consistent with recent work that shows that ERK1/p44 MAP kinase also induces similar changes in the properties of Elk-1. Thus at least two MAP kinase isoforms cause similar alterations in the biochemical properties of Elk-1. It is possible that other more divergent members of the MAP kinase family may target and elicit similar effects on Elk-1.

TCFs utilise both protein–protein and protein–DNA contacts to bind to the binary SRF/SRE complex [9,11,23]. Experiments in which the ETS-domain of Elk-1 is replaced with the heterologous DNA-binding domain of GAL4 suggest that it is the protein–protein interaction component that is stimulated by MAP kinase phosphorylation [14,17]. However, such experiments might underscore the importance of the ETS DNA-binding domain in regulating such interactions. Indeed we clearly demonstrate that SRF-independent autonomous binding of Elk-1 to DNA is enhanced by ERK2/p42 phosphorylation. This implies that the stimulation of binding is via an enhancement of the ETS-domain:DNA interactions. Moreover, clear differences in DNA-binding specificity of the ETS-domains of different TCFs have been identified (our unpublished data), suggesting a possible wider role of the ETS-domain in determining target specificity. It is therefore likely that both protein–DNA and protein–protein interactions mediated by Elk-1 are stimulated by MAP kinase phosphorylation.

Our data are consistent with the model presented in Fig. 3. This is a refinement of the grappling hook model [24]. In the absence of phosphorylation, TCF/Elk-1 exists in a closed conformation in which the accessibility of the B-domain and ETS-domain to SRF and DNA, respectively, are blocked (closed conformation). Phosphorylation of the C-domain of Elk-1 by MAP kinases subsequently induces a conformational change in which the protein–DNA and protein–protein interfaces are exposed (open conformation) to allow recruitment of Elk-1/TCF to high affinity ETS-motifs or SRF:SRE complexes. Such putative conformational changes have been inferred by others [12,13]. Moreover, the changes in mobility of binary and ternary complexes containing Elk-1 after phosphorylation (visible upon overexposure of Figs. 1 and 2) are also suggestive of phosphorylation induced conformational changes in TCF/Elk-1. Further experiments are required to formally prove the existence of such putative phosphorylation-dependent conformational changes in Elk-1.

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