PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Regulation of AP-1 Activity by Mitogen-Activated Protein Kinases [and Discussion]

Michael Karin and P. T. Hawkins

Phil. Trans. R. Soc. Lond. B 1996 **351**, 127-134
doi: 10.1098/rstb.1996.0008

The regulation of AP-1 activity by mitogen-activated protein kinases

MICHAEL KARIN

Department of Pharmacology, Program in Biomedical Sciences, University of California, San Diego School of Medicine, 9500 Gilman Drive, La Jolla, California 92093, U.S.A.

SUMMARY

AP-1 is a collection of dimeric sequence specific, DNA binding, transcriptional activators composed of Jun and Fos subunits. The composition, the level and the activity of AP-1 complexes are regulated in response to extracellular stimuli. An important role in this regulation is played by mitogen-activated protein kinases (MAPKs). The specific roles of three MAPKs, namely ERK, JNK and FRK, in modulation of both the level and activity of AP-1, are discussed.

1. INTRODUCTION

AP-1 is a collection of sequence-specific transcriptional activators composed of members of the Jun and Fos families (for review see Angel & Karin 1991). These proteins, which belong to the bZIP superfamily of DNA binding proteins (for review see Johnson & McKnight 1989), form a variety of homo- and heterodimers that bind to a common DNA recognition site (Angel & Karin 1991). First identified by its role in human metallothionein II_A gene regulation (Lee *et al.* 1987), AP-1 was also found as a transcription factor that mediates gene induction by the phorbol ester tumour promoter 12–0-tetradecanoyl phorbol-13-acetate (TPA) and therefore its recognition site is also known as the TRE (TPA response element) (Angel *et al.* 1987). After its discovery, AP-1 activity was found to be induced by many other stimuli, including growth factors, cytokines, T cell activators, neurotransmitters and UV irradiation (Angel & Karin 1991). Several mechanisms mediate the induction of AP-1 activity, some of which increase the abundance of AP-1 components whereas others modulate their activity. A complete discussion of all the mechanisms that regulate AP-1 activity, either positively or negatively, is beyond the scope of this short review (for a more comprehensive review, see Angel & Karin 1991). This review focuses on the role of mitogen-activated protein kinases (MAPKs) in regulation of AP-1 activity. The regulation and functions of these important signal-transducing enzymes were recently reviewed by Marshall (1995) and Cobb & Goldsmith (1995).

One should be aware, however, that although AP-1 DNA binding activity is conveniently measured by electrophoretic mobility shift or footprinting assays, changes in AP-1 DNA binding activity do not mirror the regulation of transcriptional activity of this complex factor. Therefore, when dealing with AP-1, it is critical to measure its ability to activate transcription of an AP-1 dependent reporter gene. A useful promoter for such experiments is that of the human collagenase gene, one of the two AP-1 target genes initially identified (Angel *et al.* 1987). The reasons for this discrepancy are several. First and foremost, several proteins form complexes that bind to AP-1 sites. These proteins, however, differ considerably in their ability to activate transcription of target genes. For instance, both c-Fos and Fra-1 form stable heterodimers with any of the Jun proteins and these heterodimers have similar DNA binding activities and specificities, yet c-Fos has a potent transactivation domain that is absent from the smaller Fra-1 protein (Suzuki *et al.* 1992; Yoshioka *et al.* 1995). Second, phosphorylation at specific sites enhances the transactivating potential of several AP-1 proteins, including c-Jun and c-Fos, without changing their DNA binding activities or dimerization abilities (Smeal *et al.* 1992; Deng & Karin 1994).

2. TRANSCRIPTIONAL REGULATION OF AP-1 ACTIVITY

Most of the genes which encode AP-1 components are 'immediate-early' genes i.e. genes whose transcription is rapidly induced in response to extracellular stimuli, independently of *de novo* protein synthesis. Amongst these, the regulation of *c-fos* and *c-jun* transcription is best understood. Several *cis* elements mediate *c-fos* induction in response to a diverse spectrum of extracellular stimuli (reviewed in Treisman 1992). A cAMP response element (CRE), recognized by members of the CREB (CRE binding proteins) family, mediates *c-fos* induction in response to neurotransmitters and polypeptide hormones, stimuli that use either cAMP or Ca^{2+} as second messengers to activate either protein kinase A (PKA) or calmodulin dependent protein kinases (CaMKs), respectively (Sheng *et al.* 1994). A serum response element (SRE)

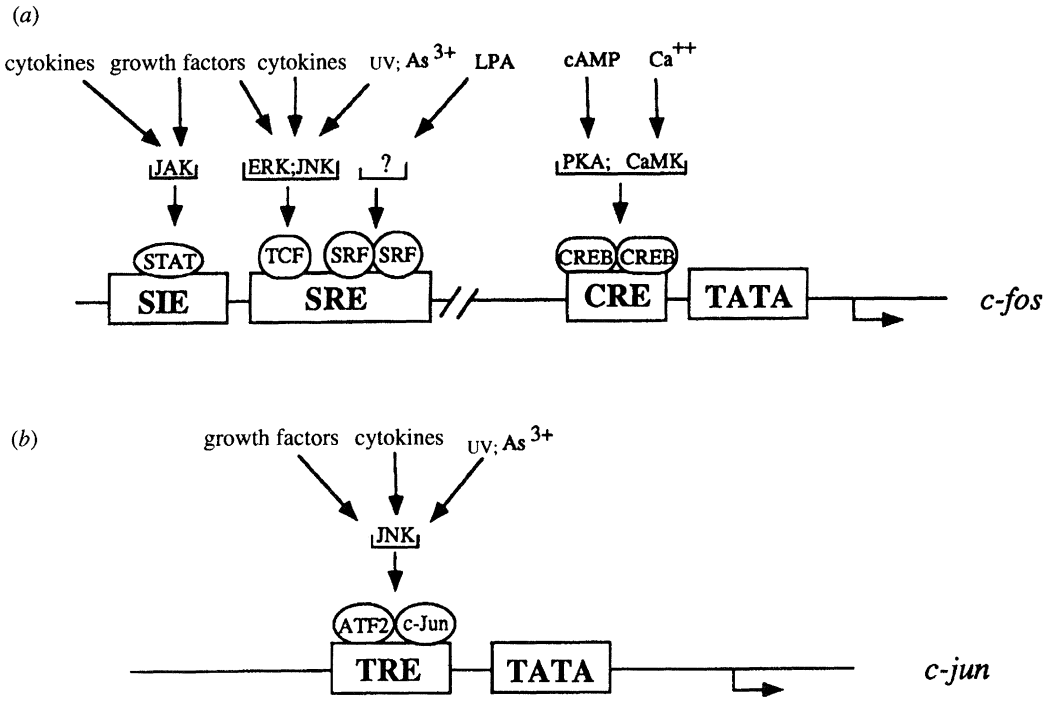

Figure 1. Regulation of *c-fos* and *c-jun* transcription in response to extracellular stimuli. The cis acting elements in the *c-fos* and *c-jun* promoters and the transcription factors that mediate their induction in response to extracellular stimuli are illustrated. The protein kinases that phosphorylate the transcription factors that interact with these elements are indicated. LPA-lysophosphatidylic acid. ? -unknown kinase.

mediates *c-fos* induction by serum, growth factors, cytokines and other stimuli that activate MAPKs (Treisman 1992) and a Sis inducible enhancer (SIE) mediates induction by growth factors and cytokines that activate the JAK group of protein kinases (Darnell *et al.* 1994). Recently the SRE was shown to be a target for another signalling pathway activated by mitogens like lysophosphatidylic acid (LPA), which is not activated by any of the currently known MAPKs (Hill *et al.* 1995). The transcription factor that mediates this response remains to be identified. It is likely to be another SRF interacting protein, different from the known TCFs (Hill *et al.* 1995). Given this complexity, it is not surprising that *c-fos* transcription is rapidly induced in response to almost any imaginable extracellular stimulus (see figure 1*a*).

The SRE is recognized by the dimeric serum response factor (SRF) whose binding results in recruitment of ternary complex factors (TCF), which cannot bind to the SRE by themselves (Treisman 1992). After mitogenic stimulation Elk-1, one of several candidate TCFs (Treisman 1994), is rapidly phosphorylated, probably by members of the ERK group of MAPKs (Gille *et al.* 1992; Marais *et al.* 1993). Phosphorylation of Elk-1 was reported to facilitate formation of the ternary complex composed of itself, the SRF and the SRE (Gille *et al.* 1992) and to stimulate its ability to activate transcription, without affecting its DNA binding properties (Marais *et al.* 1993). Because *in vivo* the SRE appears to be constitutively occupied (Treisman 1992), increased Elk-1 or other TCF transcriptional activity is the most likely mechanism by which ERK activation causes *c-fos* induction. The sites at which Elk-1 is phosphorylated are clustered within its C-terminal activation domain and are conserved in other candidate TCFs, such as SAP-1 (Treisman 1994). Recently the activity of other TCFs was shown to be regulated by the same mechanism involving the phosphorylation of their activation domain by MAPKs (Price *et al.* 1995). Because the SRE also mediates *c-fos* induction in response to stimuli such as UV irradiation (Büscher *et al.* 1988), which has only a marginal effect on ERK activity (Minden *et al.* 1994*a*), it is possible that Elk-1 or other TCFs are also phosphorylated by other MAPKs (see below). Nevertheless, ERK activation leads to elevated AP-1 activity via *c-fos* induction. This results in increased c-Fos synthesis which translocates to the nucleus where it combines with pre-existing Jun proteins to form AP-1 dimers that are more stable than those formed by Jun proteins alone (Smeal *et al.* 1989). Increased dimer stability results in higher levels of AP-1 DNA binding activity because more of the Jun and Fos proteins are found in the dimeric state that is essential for DNA binding.

By comparison to *c-fos*, the *c-jun* promoter is simpler and most of its inducers operate through one major *cis* element, the *c-jun* TRE (see figure 1*b*). This TRE differs from the consensus TRE sequence by 1 b.p. insertion (Angel *et al.* 1988) and due to this subtle change it is recognized by c-Jun:ATF2 heterodimers rather than conventional AP-1 complexes (van Dam *et al.* 1993). ATF2 is a constitutively expressed protein, and despite its inducible expression, most cell types contain some c-Jun protein before their stimulation. Like the *c-fos* SRE, the *c-jun* TRE is constitutively occupied *in vivo* (Rozek & Pfeifer 1993). After exposure to stimuli that activate members of the JNK group of MAPKs (Dérijard *et al.* 1992; Minden *et al.* 1994*a*; Su *et al.* 1994), both c-Jun (Devary *et al.* 1992; Minden *et*

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

al. 1994*a*; Su *et al.* 1994) and ATF2 (van Dam *et al.* 1995; Gupta *et al.* 1995; Livingstone *et al.* 1995) are rapidly phosphorylated. The constitutive occupancy of the *c-jun* TRE indicates that this phosphorylation occurs while the proteins are bound to the *c-jun* promoter. Similarly, in the case of the *c-fos*, Elk-1 must be phosphorylated while bound to DNA. Phosphorylation of c-Jun and ATF2 stimulates their transactivation ability, thereby leading to *c-jun* induction. Thus part of the increase in AP-1 activity in response to JNK activating stimuli (such as TNFα, UV irradiation), is due to increased c-Jun synthesis and possibly c-Fos synthesis (as the JNKs may also phosphorylate and activate Elk-1; see below). Another part of the increase in AP-1 activity is due to c-Jun phosphorylation.

3. POST-TRANSLATIONAL REGULATION OF AP-1 ACTIVITY

The activities of both pre-existing and newly synthesized AP-1 components are modulated through their phosphorylation. So far, this form of post-translational control was demonstrated for c-Jun, c-Fos and ATF2, but it is likely that other Jun and Fos proteins are similarly regulated. In the case of c-Jun, phosphorylation at a cluster of sites located next to its basic region inhibits DNA binding by c-Jun homodimers, but not by c-Jun:c-Fos heterodimers (Boyle *et al.* 1991; Lin *et al.* 1992). Most likely the phosphates on c-Jun lead to electrostatic repulsion from phosphates on DNA, thereby inhibiting DNA binding. On the other hand, phosphorylation of c-Jun at Ser73 and Ser63, located within its transactivation domain, potentiates its ability to activate transcription as either a homodimer (Pulverer *et al.* 1991; Smeal *et al.* 1991, 1992) or a heterodimer with c-Fos (Deng & Karin 1994). These residues, which do not affect DNA binding activities, are phosphorylated by the newly discovered members of the MAPK family, the Jun kinases or JNKs (Hibi *et al.* 1993; Dérijard *et al.* 1994). So far, the JNKs are the only protein kinases found to efficiently phosphorylate the N-terminal sites of c-Jun. Interestingly, neither ERK1 nor ERK2 phosphorylate the N-terminal stimulatory sites of c-Jun and instead phosphorylate one of the inhibitory sites located next to the C-terminal DNA binding domain (Chou *et al.* 1992; Minden *et al.* 1994*a*). Using an altered specificity mutant of c-Jun that is phosphorylated by PKA instead of JNK, phosphorylation of Ser73 (and Ser63 to a lesser extent) was shown to be directly responsible for potentiating the transactivation function (Smeal *et al.* 1994). Phosphorylation at Ser73 may potentiate c-Jun transcriptional activity through recruitment of CBP (CREB binding protein), a protein which was originally identified by virtue of its binding to phospho-CREB (Arias *et al.* 1994; Kwok *et al.* 1994). After phosphorylation of its N-terminal sites, but not the C-terminal sites, c-Jun can bind CBP and CBP can potentiate its ability to activate transcription (Arias *et al.* 1994). CBP is thought to act as an adaptor protein connecting the phosphorylated activation domains of CREB or c-Jun to the basal transcriptional machinery.

The amino acids that surround the N-terminal phosphoacceptors of c-Jun are conserved in the C-terminal activation domain of c-Fos (Sutherland *et al.* 1992), suggesting that phosphorylation at Thr232, the homologue of Ser73 of c-Jun, potentiates c-Fos transcriptional activity. This prediction was confirmed, but despite the considerable similarity between the two phosphoacceptor sites, Thr232 of c-Fos is not phosphorylated by either JNK1 or JNK2, but by a novel 88kD MAPK termed FRK (Deng & Karin 1994). Like the ERKs and the JNKs, FRK is a proline-directed kinase whose activity is rapidly stimulated in response to Ha-Ras activation by growth factors. Although the mechanism by which phosphorylation at Thr232 stimulates c-Fos transcriptional activity is not clear, it appears that in the context of a c-Jun:c-Fos heterodimer phosphorylation of each protein makes a similar contribution to stimulation of transcriptional activity.

A similar situation may apply for c-Jun:ATF2 heterodimers, as ATF2 phosphorylation at Thr63 and Thr71 within its N-terminal activation domain was recently shown to stimulate its transcriptional activity (van Dam *et al.* 1995; Gupta *et al.* 1995; Livingstone *et al.* 1995).

Like c-Jun, ATF2 is phosphorylated by the JNKs (Gupta *et al.* 1995), but at least *in vitro* it is also phosphorylated by another MAPK that does not phosphorylate c-Jun, p38/MPK2 (Dérijard *et al.* 1995). It remains to be determined whether p38/MPK2 phosphorylates ATF2 *in vivo* and can thus contribute to *c-jun* induction. Transactivation by ATF2 is also potentiated upon binding of Rb or E1A, probably through recruitment of additional activation domains to the DNA bound ATF2 dimer (Kim *et al.* 1992). Both E1A and Rb act in concert with phosphorylation of ATF2 (Gupta *et al.* 1995). Although E1A can induce *c-jun* transcription (van Dam *et al.* 1993) it represses AP-1 activity (Offringa *et al.* 1990). This repression could be mediated through competition for CBP, which is very similar to the p300 E1A binding protein (Arany *et al.* 1994). Indeed, it was recently demonstrated that p300 and CBP are functionally interchangeable and that E1A can inhibit the co-activator function of both factors (Arany *et al.* 1995; Lundblad *et al.* 1995).

4. REGULATION AND SPECIFICITY OF MAPK ACTIVITY

As described above, at least three different types of MAPKs, the ERKs, the JNKs and FRKs, contribute to induction of AP-1 activity after exposure to a diverse array of extracellular stimuli. It is of considerable interest that each of these types of MAPKs is affecting AP-1 activity through phosphorylation of a different substrate (see figure 2). Although the ERKs phosphorylate TCF/Elk-1 and thereby induce c-Fos synthesis, they do not phosphorylate c-Jun or c-Fos on the sites that potentiate their transcriptional activities (Chou *et al.* 1992; Deng & Karin 1994; Minden *et al.* 1994*a*). In addition, the ERKs do not appear to be involved in ATF2 phosphorylation (van Dam *et al.*

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

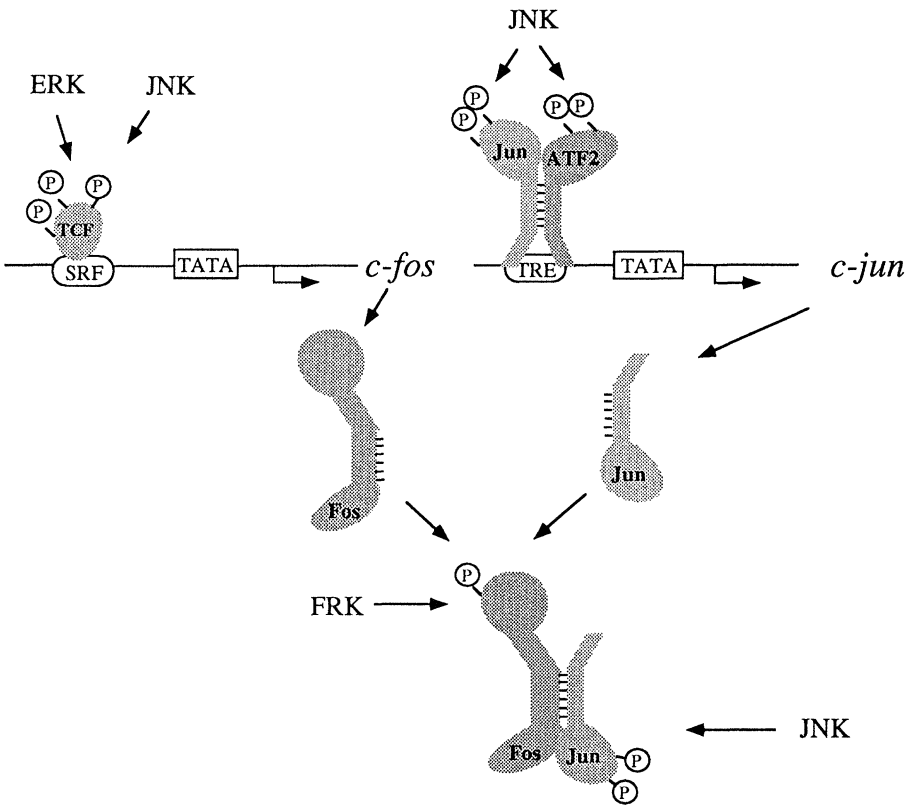

Figure 2. Three distinct MAPKs contribute to induction of AP-1 activity. Phosphorylation of TCF/Elk-1 bound to the *c-fos* promoter by the ERKs stimulates its transcriptional activity, thus leading to *c-fos* induction. JNK-mediated phosphorylation of ATF2 and c-Jun bound to the *c-jun* promoter stimulates their transcriptional activities leading to *c-jun* induction. JNK can also phosphorylate Elk-1 and thus cause *c-fos* induction. The newly synthesized c-Fos and c-Jun proteins combine to form stable AP-1 heterodimers. A further increase in AP-1 activity is brought about by the JNKs and FRK, which phosphorylate c-Jun and c-Fos, respectively, on sites that augment their transcriptional activities.

1995; Gupta *et al.* 1995; Livingstone *et al.* 1995). The JNKs, on the other hand, phosphorylate the stimulatory sites of c-Jun and ATF2 (Hibi *et al.* 1993; Su *et al.* 1994; van Dam *et al.* 1995; Gupta *et al.* 1995; Livingstone *et al.* 1995), but do not phosphorylate c-Fos (Deng & Karin 1994). The JNKs are also capable of phosphorylation and activation of TCF/Elk-1, suggesting they may be involved in *c-fos* induction under certain circumstances, such as exposure to UV irradiation which results in marginal ERK activation (Cavigelli *et al.* 1995). So far, FRK is only known to affect c-Fos activity (Deng & Karin 1994).

These results indicate that MAPKs are highly specific in their choice of substrates and do not phosphorylate just any Ser or Thr that is followed by a Pro residue, as previously assumed. The molecular mechanisms underlying this high degree of substrate specificity are being explored with the Jun:JNK interaction as a paradigm. Efficient phosphorylation by the JNKs requires a docking site located between residues 30 to 60 of c-Jun (Adler *et al.* 1992; Hibi *et al.* 1993). *In vitro*, this site mediates binding of c-Jun to the JNKs, and although c-Jun:JNK complexes were not yet isolated from living cells, the integrity of the docking site is essential for phosphorylation and stimulation of c-Jun activity (Hibi *et al.* 1993). The docking site is not the only feature of c-Jun required for efficient phosphorylation by the JNKs. *In vitro* JunB also binds to the JNKs through the same region used by c-Jun, but is not phosphorylated by them (T. Deng, T. Kallunki & M. Karin, unpublished results). JunB is not phosphorylated by the JNKs because its equivalents of Ser63 and Ser73 of c-Jun are not followed by prolines. Once prolines are inserted after these serines in JunB, the resulting variant becomes JNK responsive (T. Deng & M. Karin, unpublished results). In addition to the docking site and proline at the P+1 position, efficient phosphorylation of Jun proteins by the JNKs requires specific residues that surround the phosphoacceptor site. These residues, however, are not a part of the docking site and do not affect JNK binding (T. Deng, T. Kallunki & M. Karin, unpublished results). When the binding of the two human JNKs to c-Jun was compared, JNK2 was found to bind much better than JNK1 does (Kallunki *et al.* 1994). Consequently, the Km of JNK2 towards c-Jun is lower than the Km of JNK1 towards c-Jun and its Vmax is higher (Kallunki *et al.* 1994). The catalytic properties of JNK2 measured with other substrates are not considerably different from those of JNK1 and JNK1 may be the more effective kinase for other substrates. The molecular basis for the higher affinity of JNK2 towards c-Jun was traced to a small region of approximately 23 residues located near its catalytic pocket (Kallunki *et al.* 1994). This region, which is variable amongst all MAPKs, is not a part of the

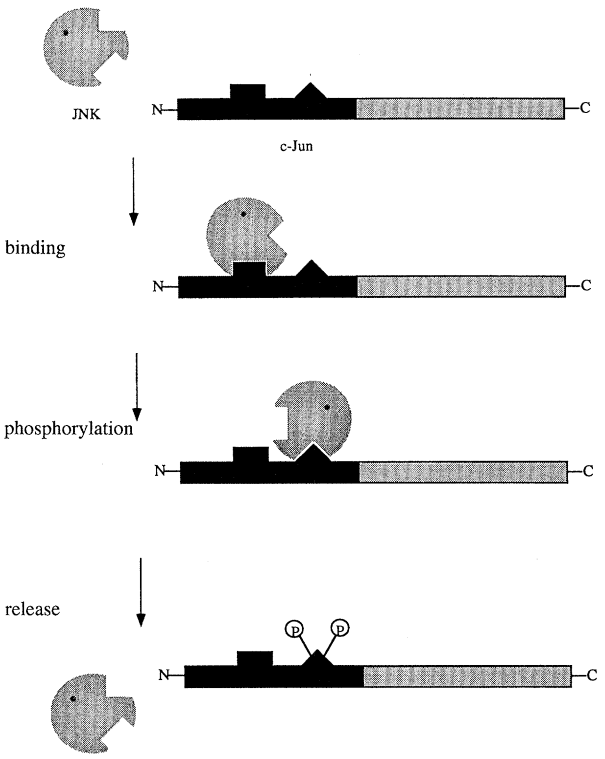

Figure 3. Mechanism of c-Jun phosphorylation by JNK2. The first step in this reaction involves the binding of JNK2 through its substrate recognition site (rectangular indentation) to the docking site (rectangular protrusion) in the N-terminal activation domain of c-Jun (black box). Next JNK2 dissociates from the docking site on c-Jun but, due to the high local concentration of its substrate (c-Jun) it binds through its catalytic pocket (triangular indentation) to a peptide loop that contains the phosphoacceptor sites of c Jun (triangular protrusion). This results in c-Jun phosphorylation and dissociation of JNK2, allowing the phosphorylation of another substrate molecule.

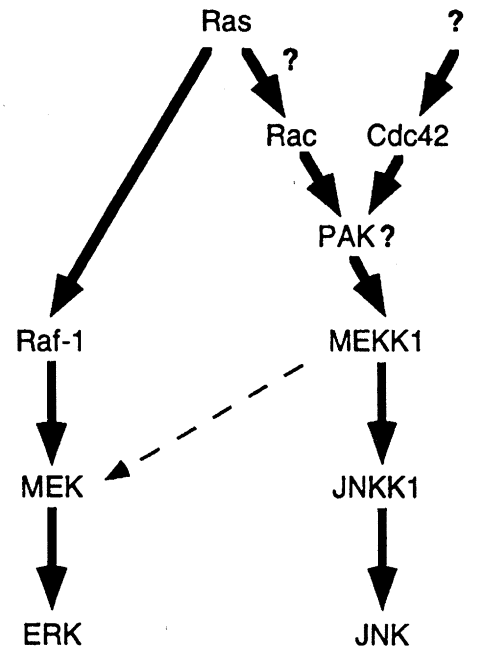

Figure 4. Two distinct Ras-dependent protein kinase cascades lead to ERK and JNK activation. The relations between the different signalling proteins involved in the Ras-dependent activation of ERK and JNK in response to growth factors are illustrated. While Ras directly interacts with Raf-1 to cause its activation, the activation of MEKK1 is mediated through Rac, another small GTP binding protein. It is not clear how Ras leads to Rac activation. Once activated Rac binds to and activates PAK65, but it is not known yet whether PAK65 or a related kinase are direct activators of MEKK1. MEKK1 can lead to MEK activation, as indicated by the broken arrow. *In vivo*, however, this does not result in ERK activation. The major function of MEKK1 is therefore JNKK1 activation and consequently JNK activation.

catalytic pocket itself. Most likely, it is the element of JNK2 which interacts with the docking sites on c-Jun, as illustrated in figure 3. Interestingly this region in JNK2 is encoded by two alternatively spliced exons, suggesting that alternative splicing of the primary JNK2 transcript generates two isozymes that differ in their choice of substrates (K. Yoshioka, T. Kallunki & M. Karin, unpublished results).

In addition to the differences in their substrate specificities, the three types of MAPKs that affect AP-1 activity differ in their responses to extracellular stimuli and regulation. The ERKs are most efficiently stimulated by growth factors and phorbol esters (Kyriakis *et al.* 1994; Minden *et al.* 1994*a*, *b*; Cobb & Goldsmith 1995), whereas FRK is activated after exposure to growth factors but not to phorbol esters (Deng & Karin 1994). Neither FRK nor ERK activities are considerably stimulated by exposure to UV irradiation or tumour necrosis factor, stimuli that cause efficient JNK activation (Deng & Karin 1994; Kyriakis *et al.* 1994; Minden *et al.* 1994*a*, *b*). Compared to the FRKs and the ERKs, JNK activity is modestly stimulated by growth factors (Kyriakis *et al.* 1994; Minden *et al.* 1994*a*, *b*). The largest increases in JNK activity are observed after UV irradiation (Hibi *et al.* 1993; Dérijard *et al.* 1994) or costimulatory activation of T cells (Kallunki *et al.* 1994; Su *et al.* 1994). Although the activities of all three MAPK types are stimulated in response to Ras activation (Robbins *et al.* 1992; Thomas *et al.* 1992; Minden *et al.* 1994*b*, 1995), the JNKs also respond to Ras independent signals (Minden *et al.* 1994*b*, 1995). Even Ras activation, however, affects ERK and JNK through different kinase cascades (see figure 4). The major pathway leading from Ras to ERK is based on the Ras mediated recruitment of Raf-1 to the plasma membrane (Leevers *et al.* 1994). This results in activation of Raf-1, a Ser/Thr kinase which phosphorylates and activates the dual specificity kinases MEK1 and MEK2 (Dent *et al.* 1992). The latter are responsible for phosphorylation and activation of the ERKs (Crews *et al.* 1992). Raf-1 activation, however, does not lead to direct JNK activation. Instead, it is activated by another MAPK kinase kinase called MEKK1 (Minden *et al.* 1994*b*). Whereas MEKK1 related in its primary structure to Raf, it is even closer in sequence to yeast MAPK kinase kinases, such as STE11 (Lange-Carter *et al.* 1993). Although MEKK1 is an efficient MEK activator (Lange-Carter *et al.* 1993; Lange-Carter & Johnson 1994), its activation results in JNK instead of ERK activation (Minden *et al.* 1994*b*; M. Cobb, personal communication). It is not yet clear why MEK

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

activation by Raf-1 results in efficient ERK activation, although a similar increase in its activity through MEKK1 does not translate into ERK activation (M. Cobb, personal communication). One explanation for this paradox is the existence of specific adaptor proteins that are required for effective organisation of MAPK cascades (Herskowitz 1995).

MEKK1 is believed to act downstream of Ras, yet unlike Raf-1 it is not activated through direct interaction with Ras (Lange-Carter & Johnson 1994). Recently it was found that Ras-dependent activation of the JNK cascade by growth factors requires activation of another member of the Ras superfamily of small G proteins, Rac (Coso *et al.* 1995; Minden *et al.* 1995). Although constitutively activated Rac mutants activate the JNK cascade, they have no effect on ERK activity (Coso *et al.* 1995; Minden *et al.* 1995). In addition to acting downstream of Ras on the pathway leading to JNK activation, Rac may also act in parallel to Ras, as activated Ras and Rac mutants have a synergistic effect on JNK activity (Minden *et al.* 1995). JNK activation also occurs after expression of activated Cdc42Hs, a relative of Rac, but not in response to another member of that group of small G proteins, Rho (Coso *et al.* 1995; Minden *et al.* 1995). Interestingly, both Rac and Cdc42Hs bind and activate in a GTP-dependent manner, a protein kinase called PAK65 (Manser *et al.* 1994). PAK65 is closely related to a yeast protein kinase called STE20, which is required for activation of STE11 (Herskowitz 1995). As MEKK1 is a homolog of STE11, it is likely that its activity is directly stimulated by PAK65 mediated phosphorylation. Thus, unlike Ras-dependent ERK activation which requires only two upstream protein kinases, Raf and MEK, the Ras-dependent activation of JNK, as illustrated in figure 4, appears to require one additional small GTP binding protein, Rac, and three upstream protein kinases, PAK65 (or a close relative), MEKK1 and a JNK activating kinase called JNKK1, XMEK2, SEK1 or MKK4 (Sanchez *et al.* 1994; Dérijard *et al.* 1995; Lin *et al.* 1995). Although JNKK1 phosphorylates and activates the JNKs, it does not phosphorylate the ERKs. Likewise the MEKs do not phosphorylate the JNKs. Thus, the JNK and ERK cascades are clearly separated at the level of their MAPK kinases. However, in addition to the JNKs, JNKK1 phosphorylates and activates p38/MPK2 (Dérijard *et al.* 1995; Lin *et al.* 1995). Curiously, however, MEKK1 can strongly potentiate the activation of JNK by JNKK1 *in vivo*, while hardly having an effect on the activation of p38 by JNKK1 (Lin *et al.* 1995). Yet, *in vitro* JNKK1, that was activated by coexpression with MEKK1, phosphorylates and activates both JNK and p38 with similar efficiency (Lin *et al.* 1995). These useful findings are similar to those described above regarding the inability of MEKK-activated MEK to activate ERK. The simplest explanation for these findings is to invoke the existence of STE5-like scaffolding proteins that organise mammalian MAPK cascades in the same manner used by STE5 to organise the pheromone-responsive MAPK cascade in yeast. It has been known for a while that the three kinases within the pheromone responsive MAPK cascade, STE11, STE7 and FUS3 or KSS1, are necessary but not sufficient for an effective pheromone response, which requires an additional component called STE5 (reviewed by Herskowitz 1995). It was recently shown that STE5 is a large protein that contains separate binding sites for STE11, STE7 and FUS3/KSS1 (Choi *et al.* 1994). By binding to all three protein kinases STE5 forms a large multi-component complex whose integrity appears to be necessary for effective signal transduction through the cascade (Choi *et al.* 1994). Based on this example, it is postulated that effective signalling through mammalian MAPK cascades also requires STE5-like scaffolding proteins. One such protein may bind Raf-1, MEK and ERK but does not allow MEKK1 binding. On the other hand, the scaffolding protein that binds MEKK1, JNKK1 and JNK does not bind p38. Another scaffolding protein and another MEKK-like kinase will be required for effective stimulation of p38 activity through JNKK1. It is therefore a major challenge to find such proteins and determine their mechanism of activation. As both ERK and JNK can translocate to the nucleus after their activation, whereas their upstream activation remains in the cytoplasmic or in membrane attached complexes, it is likely that the multisubunit complexes allow the release of the MAPK component after its activation.

5. PERSPECTIVE

Although a great deal remains to be learned about the mechanisms that contribute to the regulation of AP-1 activity and most of the important target genes whose expression is modulated by the different forms of AP-1 are yet to be identified, quite a lot has been revealed so far by focusing on this transcription factor and its response to extracellular stimuli. The investigation of AP-1 regulation had revealed some of the general mechanisms by which protein phosphorylation modulates transcription factor activity (reviewed in Hunter & Karin 1992) and the strategies used by cell surface receptors to communicate with the nucleus (reviewed in Hill & Treisman 1995). In addition to the identification of important AP-1 target genes that will explain the physiological functions of the different forms of this transcription factor, a major challenge for the future is understanding the mechanisms that confer biological specificity to the actions of protein kinases and transcription factors. It is clear that even generic and ubiquitous signalling proteins like AP-1 and the MAPK cascades can be involved in highly specific biological responses.

I thank Ms P. Alford for preparation of the manuscript and Dr T. Kallunki, Dr B. Su and Dr A. Lin for preparation of the figures. Work in author's laboratory was supported by the National Institutes of Health, Department of Energy and Council for Tobacco Research.

REFERENCES

Adler, V., Franklin, C. C. & Kraft, A. S. 1992 Phorbol esters stimulate the phosphorylation of c-Jun but not v-Jun: regulation by the N-terminal delta domain. *Proc. natn Acad. Sci. U.S.A.* **89**, 5341–5345.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Angel, P., Hattori, K., Smeal, T. & Karin, M. 1988 The jun proto-oncogene is positively autoregulated by its product, Jun/AP1. *Cell* **55**, 875–885.

Angel, P., Imagawa, M., Chiu, R. *et al.* 1987 Phorbol ester-inducible genes contain a common cis element recognized by a TPA-modulated trans-acting factor. *Cell* **49**, 729–739.

Angel, P. & Karin, M. 1991 The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochem. biophys. Acta.* **1072**, 129–157.

Arany, F., Sellers, W. R., Livingstone, D. M. & Eickner, R. 1994 E1A-associated p300 and CREB-associated CBP belong to a conserved family of coactivators. *Cell* **97**, 799–800.

Arany, Z., Newsome, D., Oldread, E., Livingston, D. M. & Eckner, R. 1995 A family of transcriptional adaptor proteins targeted by the E1A oncoprotein. *Nature*, *Lond.* **374**, 81–84.

Arias, J., Alberts, A. S., Brindle, P. *et al.* 1994 Activation of cAMP and mitogen responsive genes relies on a common nuclear factor. *Nature*, *Lond.* **370**, 226–229.

Boyle, W. J., Smeal, T., Defize, L. H. K. *et al.* 1991 Activation of protein kinase C decreases phosphorylation of c-Jun at sites that negatively regulate its DNA-binding activity. *Cell* **64**, 573–584.

Büscher, M., Rahmsdorf, H. J., Liftin, M., Karin, M. & Herrlich, P. 1988 Activation of the *c-fos* gene by UV and phorbol ester: different signal transduction pathways converge to the same enhancer element. *Oncogene* **3**, 301–311.

Cavigelli, M., Dolbi, F., Claret, F. X. & Karin, M. 1995 *EMBO J.* **14**.

Choi, K. Y., Satterberg, B., Lyons, D. M. & Elion, E. A. 1994 STE5 tethers multiple protein kinases in the MAP kinase cascade required for mating in S-Cerevisiae. *Cell* **78**, 499–512.

Chou, S. Y., Baichwal, V. & Ferrell, J. E., Jr 1992 Inhibition of cJun DNA binding by mitogen activated protein kinase. *Molec. Biol. Cell.* **3**, 1117–1130.

Cobb, M. H. & Goldsmith, E. J. 1995 How MAP kinases are regulated. *J. biol. Chem.* **270**, 14843–14846.

Coso, O. A., Chiariello, M., Yu, J. C., Teramoto, H., Crespo, P., Xu, N., Miki, T. & Gutkind, J. S. 1995 The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. *Cell* **81**, 1137–1146.

Crews, C. M., Alessandrini, A. & Erickson, R. L. 1992 The primary structure of MEK, a protein kinase that phosphorylates the ERK gene product. *Science*, *Wash.* **258**, 478–480.

Darnell, J. E., Jr, Kerr, I. M. & Stark, G. R. 1994 Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science*, *Wash.* **264**, 1415–1421.

Deng, T. & Karin, M. 1994 c-Fos transcriptional activity stimulated by h-Ras-activated protein kinase distinct from JNK and ERK. *Nature*, *Lond.* **371**, 171–175.

Dent, P., Hasar, W., Haystead, T., Vincent, L., Roberts, T. & Sturgill, T. 1992 Activation of mitogenic activated protein kinase kinase by v-raf in NIH3T3 cells and *in vitro*. *Science*, *Wash.* **254**, 1404–1407.

Dérijard, B., Hibi, M., Wu, I. -H. *et al.* 1994 JNK1: A protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* **76**, 1–20.

Dérijard, B., Raingeaud, J., Barrett, T. *et al.* 1995 Independent human MAP-kinase signal transduction pathways defined by MEK and MKK isoforms. *Science*, *Wash.* **267**, 682–685.

Devary, Y., Gottlieb, R., Smeal, T., Bauskin, A. R., Ben-Neriah, Y. & Karin, M. 1992 The mammalian UV-response is triggered by the activation of protein tyrosine kinases. *Cell* **71**, 1081–1091.

Gille, H., Sharrocks, A. & Shaw, P. 1992 Phosphorylation of p62TCF by MAP kinase stimulates ternary complex formation at c-Fos promoter. *Nature*, *Lond.* **358**, 414–417.

Gupta, S., Campbell, D., Derijard, B. & Davis, R. J. 1995 Transcription factor ATF2 regulation by the JNK signal transduction pathway. *Science*, *Wash.* **267**, 389–393.

Herskowitz, I. 1993 MAP kinase pathways in yeast; for mating and more. *Cell* **80**, 187–197.

Hibi, M., Lin, A., Smeal, T., Minden, A. & Karin, M. 1993 Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Dev.* **7**, 2135–2148.

Hill, C. S. & Treisman, R. 1995 Transcriptional regulation by extracellular signals: mechanisms and specificity. *Cell* **80**, 199–211.

Hill, C. S., Wynne, J. & Treisman, R. 1995 The Rho family GTPases RhoA, Rac1 and Cdc42Hs regulate transcriptional activation by SRF. *Cell* **81**, 1159–1170.

Hunter, T. & Karin, M. 1992 The regulation of transcription by phorphorylation. *Cell* **70**, 375–387.

Johnson, P. F. & McKnight. S. L. 1989 Eukaryotic transcriptional regulatory proteins. *A. Rev. Biochem.* **58**, 799–839.

Kallunki, T., Su, B, Tsigelny, I. *et al.* 1994 JNK2 contains a specificity-determining region responsible for efficient c-Jun binding and phosphorylation. *Genes Dev.* **8**, 2996–3007.

Kim, S. J., Wagner, S., Liu, F., O'Reilly, M. A., Robbins, P. D. & Green, M. R. 1992 Retinoblastoma gene product activates expression of the human TGF-beta 2 gene through transcription factor ATF-2. *Nature*, *Lond.* **358**, 331–334.

Kwok, R. P., Lundblad, J. R., Chrivia, J. C. *et al.* 1994 Nuclear protein CBP is a coactivator for the transcription factor CREB. *Nature*, *Lond.* **370**, 223–226.

Kyriakis, J. M., Banerjee, P., Nikolakaki, E. *et al.* 1994 The stress-activated protein kinase subfamily of c-Jun kinases. *Nature*, *Lond.* **369**, 156–160.

Lange-Carter, C. A., Pleiman, C., Gardner, A., Blumer, K. & Johnson, G. 1993 A divergence in the MAP kinase regulatory network defined by MEK kinase and Raf. *Science*, *Wash.* **260**, 315–319.

Lange-Carter, C. A. & Johnson, G. L. 1994 Ras-dependent growth factor regulation of MEK kinase in PC12 cells. *Science*, *Wash.* **265**, 1458–1461.

Lee, W., Haslinger, A., Karin, M. & Tjian, R. 1987 Activation of transcription by two factors that bind promoter and enhancer sequences of the human metallothionein gene and SV40. *Nature*, *Lond.* **352**, 368–372.

Leevers, S. J., Paterson, H. F. & Marshall, C. J. 1994 Requirement for Ras in Raf activation is overcome by targeting Raf to the plasma membrane. *Nature*, *Lond.* **369**, 411–414.

Lin, A., Frost, J., Deng, T. *et al.* 1992 Casein kinase II is a negative regulator of c-Jun DNA binding and AP-1 activity. *Cell* **70**, 777–789.

Lin, A., Minden, A., Martinetto, H. *et al.* 1995 Identification of a dual specificity kinase that activates the Jun kinases and p38-Mpk2. *Science*, *Wash.* **268**, 286–290.

Livingstone, C., Patel, G. & Jones, N. 1995 ATF-2 contains a phosphorylation-dependent transcriptional activation domain. *EMBO J.* **14**, 1785–1797.

Lundblad, J. R., Kwok, R. P. S., Laurance, M. E., Harter, M. L. & Goodman, R. H. 1995 Adenoviral E1A-associated protein p300 as a functional homologue of the transcriptional co-activator CBP. *Nature*, *Lond.* **374**, 85–88.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Manser, E., Leung, T., Salihuddin, H., Zhao, Z. & Lim, L. 1994 A brain serine/theronine protein kinase activated by Cdc42 and Rac1. *Nature, Lond.* **367**, 40–46.

Marais, R., Wynne, J. & Treisman, R. 1993 The SRF accessory protein ELS-1 contains a growth factor regulated transcription domain. *Cell* **73**, 381–393.

Marshall, C. J. 1995 Specificity of receptor tyrosine signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell* **80**, 179–185.

Minden, A., Lin, A., Smeal, T. *et al.* 1994*a* c-Jun N-terminal phosphorylation correlates with activation of the JNK subgroup but not the ERK subgroup of mitogen-activated protein kinases. *Molec. Cell Biol.* **14**, 6683–6688.

Minden, A., Lin, A., McMahon, M. *et al.* 1994*b* Differential activation of ERK and JNK mitogen-activated protein kinases by Raf-1 and MEKK. *Science, Wash.* **266**, 1719–1723.

Minden, A. J., Lin, A., Claret, F. X., Abo, A. & Karin, M. 1995 Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. *Cell* **81**, 1147–1157.

Offringa, R. 1990 A novel function of the transforming domain of E1a: repression of AP-1 activity. *Cell* **62**, 527–538.

Price, M. A., Rogers, A. & Treisman, R. 1995 Comparative analysis of the ternary complex factors Elk-1, Sap-1A and Sap-2 (ERP/Net). *EMBO J.* **14**, 2589–2601.

Pulverer, B. J., Kyriakis, J. M., Avruch, J., Nikolakaki, E. & Woodgett, J. R. 1991 Phosphorylation of c-jun mediated by MAP kinases. *Nature, Lond.* **353**, 670–674.

Robbins, D. J. Cheng, M., Zhen, E., Vanderbilt, C. A., Feig, L. A. & Cobb, M. H. 1992 Evidence for a Ras-dependent extracellular signal-regulated protein kinase (ERK) cascade. *Proc. natn Acad. Sci. U.S.A.* **89**, 6924–6928.

Rozek, D. & Pfeifer, G. P. 1993 *in vivo* protein-DNA interactions at the *c-jun* promoter: Performed complexes mediate the UV response. *Molec. Cell. Biol.* **13**, 5490–5491.

Sanchez, I., Hughes, R. T., Mayer, B. J. *et al.* 1994 Role of SAPK/ERK kinase-1 in the stress-activated pathway regulating transcription factor c-Jun. *Nature, Lond.* **372**, 794–798.

Sheng, M. E., Thompson, M. A. & Greenberg, M. E. 1991 CREB: a Ca^{2+}-regulated transcription factor phosphorylated by calmodulin-dependent kinases. *Science, Wash.* **252**, 1427–1430.

Smeal, T., Binetruy, B., Mercola, D. *et al.* 1992 Oncoprotein-mediated signalling cascade stimulates c-Jun activity by phosphorylation of serines 63 and 73. *Molec. Cell. Biol.* **12**, 3507–3513.

Smeal, T., Angel, P., Meek, J. & Karin, M. 1989 Different requirements for formation of Jun:Jun jun:Fos complexes. *Genes Dev.* **3**, 2091–2100.

Smeal, T., Binetruy, B., Mercola, D. A., Birrer, M. & Karin, M. 1991 Oncogenic and Transcriptional Cooperation with Ha-Ras Requires Phosphorylation of c-Jun on Serines 63 and 73. *Nature, Lond.* **354**, 494–496.

Smeal, T., Hibi, M. & Karin, M. 1994 Altering the specificity of signal transduction cascades: positive regulation of c-Jun transcriptional activity by protein kinase A. *Embo J.* **13**, 6006–6010.

Su, B., Jacinto, E., Hibi, M., Kallunki, T., Karin, M. & Ben-Neriah, Y. 1994 JNK is involved in signal integration during costimulation of T lymphocytes. *Cell* **77**, 727–736.

Sutherland, J. A., Cook, A., Bannister, A. J. & Kouzarides, T. 1992 Conserved motifs in Fos and Jun define a new class of activation domain. *Genes Dev.* **6**, 1810–1819.

Suzuki, T., Okuno, H., Yoshida, T., Endo, T., Nishina, H. & Iba, H. 1992 Difference in transcriptional regulatory function between c-Fos and FRA-2. *Nucl. Acids Res.* **19**, 5537–5542.

Thomas, S. M., De Marco, M., D'Arcangelo, G., Halegoua, S. & Brugge, J. S. 1992 Ras is essential for nerve growth factor and phorbol ester induced tyrosine phosphorylation of MAP kinases. *Cell* **68**, 1031–1040.

Treisman, R. 1992 The serum response element. *Trends Biochem. Sci.* **17**, 423–426.

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

van Dam, H., Duyndam, M., Rottier, R. *et al.* 1993 Heterodimer formation of cJun and ATF-2 is responsible for induction of *c-jun* by the 243 amino acid adenovirus E1A protein. *EMBO J.* **12**, 479–487.

van Dam, H., Wilhelm, D., Herr, I., Steffen, A., Herrlich, P. & Angel, P. 1995 ATF-2 is preferentially activated by stress-activated protein kinases to mediate *c-jun* induction in response to genotoxic agents. *EMBO J.* **14**, 1798–1811.

Yoshioka, K, Deng, T., Cavigelli, M. & Karin, M. 1995 Anti-tumor promotion by phenolic antioxidants: Inhibition of AP-1 activity through induction of Fra expression. *Proc. natn Acad. Sci. U.S.A.* **92**, 4972–4976.

Discussion

P. T. Hawkins (*The Babraham Institute, Cambridge, U.K.*). Does Professor Karin know whether wortmannin blocks the activation of JNK by EGF or NGF?

M. Karin. We have not seen any inhibition by wortmannin but we have not run a positive control to show that phosphoinositide 3-kinase is blocked in the same cells. However, we have looked at a mutant FGF receptor from Jonathan Cooper that does not couple to phosphoinositide 3-kinase, and it activated JNK as well as the wild-type receptor. However, both sets of experiments have some problems, and the question of whether phosphoinositide 3-kinase feeds into the JNK pathway remains an open question.

Question. In terms of control of the AP1 complex, how important are the relative quantities of the different components and the effects of post-translational modification?

M. Karin. The contributions will vary in different cell-types in response to different stimuli. We really shall not have answers until knockouts for the various Jun and Fos proteins and the various kinase pathways have been fully analysed. So far, the reverse genetic experiments suggest that nearly everything is important, but they do not tell us which are the most important players in particular circumstances.