Transcriptional control by protein phosphorylation: signal transmission from the cell surface to the nucleus

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Two general mechanisms have evolved for the rapid and accurate transmission of signals from cell-surface receptors to the nucleus, both involving protein phosphorylation. One mechanism depends on the regulated translocation of activated protein kinases from the cytoplasm to the nucleus, where they phosphorylate target transcription factors. In the second mechanism, transcription factors are kept in a latent state in the cytoplasm and are translocated into the nucleus upon activation.

Introduction

The gene expression repertoire of a cell is constantly modulated by a variety of extracellular stimuli. Excluding those signals delivered by small lipophilic molecules that cross the plasma membrane and allosterically affect ligand-binding transcription factors, a common way that transcription-factor activity is regulated in both prokaryotes and eukaryotes is by protein phosphorylation. Protein phosphorylation appears to be the post-translational modification of choice for situations where rapid modulation of transcription-factor activity is required in response to signals that are perceived by receptors situated at the cell's surface.

Whereas in prokaryotes the modification of transcription factors in response to cell surface receptor activation is facilitated by the membrane attachment of bacterial transcription complexes, in eukaryotes the nuclear membrane and relatively large cell size constitute considerable obstacles to communication between the cell surface and the nucleus. During the past several years, the basic principles that govern the transmission of such information have emerged, and these are the subject of the present review.

Instead of providing an exhaustive review of all the relevant transcytoplasmic signalling pathways and the affected transcription factors, we shall restrict our discussion to a small number of well developed experimental systems that illustrate the mechanisms of communication between the surface of a eukaryotic cell and its nucleus. For a discussion of the basic mechanisms by which protein phosphorylation modulates the activities of transcription factors, please refer to an earlier review [1].

Protein kinase A and CRE-binding proteins

One of the simpler modes of signal transmission from surface receptors to the nucleus is illustrated by proteins that bind to DNA sequences known as cAMP-response elements (CREs); these are known, naturally enough, as CRE-binding proteins (CREBs). The activation of certain G-protein-coupled receptors causes a build up of intracellular cAMP. The binding of cAMP to the regulatory subunit of the tetrameric protein kinase A (PKA) liberates its catalytic subunit, which then translocates to the nucleus [2]. Once in the nucleus, the PKA catalytic subunit phosphorylates CREB — which is thought to bind constitutively to functional CREs upstream of certain, cAMP-regulated genes — on residue Ser 133, thereby stimulating its ability to activate transcription [3]. PKA also stimulates the transactivating potential of the related protein CREM (CRE modulator) by phosphorylation at a similar, functionally equivalent, site [4].

It is not yet clear which genes are affected by CREB and which are affected by CREM, as both factors recognize the same CREs. Phosphorylation of Ser 133 greatly enhances the affinity of CREB for a large nuclear protein, termed CREB-binding protein (CBP), and thus may facilitate the recruitment of CBP, which does not bind DNA by itself, to the promoter region [5]. As microinjection of anti-CBP antibodies blocks CRE-mediated gene activation, the binding of CBP to CREB (and possibly CREM) is essential for transcriptional activation [6]. Overexpression of CBP modestly potentiates gene activation by phospho-CREB [6,7].

Once recruited to the promoter region, CBP is proposed to activate transcription by interacting with the general

transcription factor TFIIB [7]. Thus, CBP is thought to act as an adaptor that bridges between phospho-CREB (and other phosphorylated transcription factors, such as c-Jun [6]) and the transcriptional machinery. It was recently noticed that CPB is highly similar to the p300 E1A-binding protein [8], which is also known to be capable of activating a gene upon recruitment to the gene's promoter [9]. In fact, CBP and p300 were shown to be interchangeable in their ability to interact with CREB, and the co-activation function of both is repressed by E1A [9,10]. These findings explain the known ability of E1A to interfere with cAMP-mediated gene activation [11].

Information transfer by mitogen-activated protein kinase cascades

A more complex mechanism involving the activation of protein kinase cascades is used to transmit signals from growth-factor receptors to the immediate–early genes — genes induced by a stimulus in the absence of *de novo* protein synthesis — that are regulated by transcription factors such as ternary complex factor (TCF)/Elk-1 and AP-1. The major function of these cascades is to activate members of the mitogen-activated protein kinase (MAPK) family of serine/threonine protein kinases, and downstream protein kinases such as ribosomal S6 kinase (RSK or MAPKAP kinase-1) and MAPK-activated protein kinase-2 (MAPKAP kinase-2) [12,13]. These cascades are activated by a large number of extracellular stimuli, including mating pheromones and osmotic stress in yeast [14], cell–cell interactions in *Drosophila* [15], and mitogens [16,17], cytokines [13,18–21], and UV irradiation and other stresses [12,18,22–26] in vertebrates.

Currently, three distinct MAPK cascades are known in vertebrates, but it is possible that additional examples will be found, as there are known to be at least four MAPK pathways in the budding yeast, *Saccharomyces cerevisiae*. In vertebrates, the target MAPKs are the extracellular signal-regulated kinases (ERKs), the Jun N-terminal kinases (JNKs)/stress-activated protein kinases (SAPKs), and the p38/Mpk2/cytokine-suppressive anti-inflammatory drug binding protein (CSBP; related to the *S. cerevisiae* kinase Hog1p). In each case, the MAPKs are activated in response to activation of a dual-specificity MAPK kinase (MAPKK). The activating MAPKKs phosphorylate their target MAPKs on conserved threonine and tyrosine residues, which are separated by an intervening amino acid that is characteristic for each MAPK family [12,14,16,18,20–22,25,26].

The upstream MAPKKs and MAPKK kinases (MAPKKKs) are distinct for each pathway and, although some crosstalk may be possible, specificity could be ensured by association of the protein kinases in each pathway with a scaffolding protein [27]. A cascade of three or four protein kinases, in theory, should provide a considerable degree of amplification of low-level surface signals, greatly increasing the sensitivity of the response. However, the possible existence of scaffolding proteins suggests that the protein kinases within any given cascade interact stoichiometrically, at least during the activation process, thereby limiting signal amplification while gaining specificity.

Upon activation, members of the ERK group of MAPKs and the RSK group of MAPKAP kinases translocate from the cytoplasm to the nucleus [28–30]. Regulated nuclear translocation upon activation of JNK1 has also been observed recently (M. Cavigelli, F. Dolfi and M.K., unpublished data). The MAPKKK and MAPKK — Raf and MEK, respectively — that operate upstream of the ERKs remain in the cytoplasm when activated [29–31]. Once in the nucleus, the ERKs phosphorylate several substrates including TCF, the transcription factor that mediates *c-fos* induction [32,33]. Although the available data are most consistent with the nucleus being the site of TCF phosphorylation, at present cytoplasmic phosphorylation followed by rapid nuclear transfer, although rather unlikely, cannot be excluded. Genomic footprinting suggests that TCF (or another factor binding to the same site) binds constitutively to the serum response element (SRE) of the *c-fos* promoter, forming a ternary complex which also includes SRF (SRE-binding factor) [34]. The most parsimonious mechanism of TCF activation, therefore, is one in which phosphorylation occurs within the nucleus while TCF is already bound to DNA.

Following mitogenic stimulation, TCF is phosphorylated on a cluster of serines near its carboxyl terminus [33,35]. The kinetics of TCF phosphorylation are rapid, and phosphorylated protein with altered electrophoretic mobility can be detected within 5 minutes [33]. Consistent with its constitutive binding to the SRE, phosphorylation of TCF potentiates its transactivating function, and the region containing the phosphorylation sites functions as a mitogen-responsive activation domain [33,35]. In addition, although phosphorylation of TCF does not affect its DNA-binding activity under conventional binding conditions, under stringent conditions TCF phosphorylation facilitates formation of TCF–SRF–SRE complexes *in vitro*, possibly because phosphorylation increases TCF's propensity to interact with SRF [32,35]. Although phosphorylation may stabilize the SRE-bound ternary complex, it is not expected to affect the apparent occupancy of the SRE. In this respect, it should be noted that SRF is rapidly phosphorylated by RSK upon mitogenic stimulation on a site that enhances its affinity to the SRE [36]. As the SRE is constitutively occupied, however, the contribution of this event to *c-fos* induction is not clear.

In vitro, the sites that stimulate TCF activity are efficiently phosphorylated by ERK1 and ERK2 [35], and in-gel kinase assays identify two growth-factor-activatable TCF kinases, the sizes of which match those of ERK1 and ERK2 [33]. Furthermore, overexpression of catalytically-inactive mutant forms of ERK1 and ERK2 attenuates *c-fos* promoter activation by mitogenic signals [37]. However, it remains to be shown that these mutants

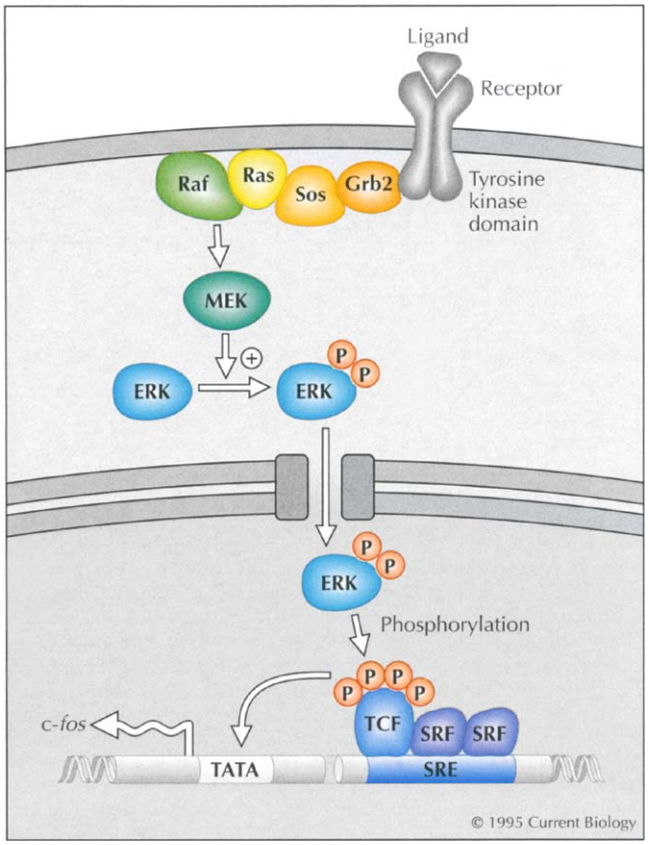

Fig. 1. The pathway by which signals are transmitted from growth factor receptor protein-tyrosine kinases to the SRE of the *c-fos* promoter. Ligand-induced receptor dimerization results in receptor autophosphorylation, which is followed by recruitment of signalling mediators, such as Grb2, to the receptor's tyrosine phosphorylated cytoplasmic domain. This leads to translocation of the Grb2-associated Ras guanine nucleotide exchange factor Sos to the plasma membrane, where Ras is located, followed by Ras activation. Subsequently, the serine/threonine protein kinase Raf-1 binds to Ras and becomes activated, leading to phosphorylation and activation of the dual-specificity ERK-kinase, MEK. MEK activates the MAPKs ERK1 and ERK2, which translocate to nucleus once activated. In the nucleus, the ERKs phosphorylate transcription factor TCF, which is bound together with SRF to the SRE of the *c-fos* promoter. Phosphorylation of TCF at a cluster of sites located next to its carboxyl terminus stimulates its transactivation function, probably by enhancing its ability to interact with the basal transcription machinery. This pathway leads to rapid activation of *c-fos* transcription.

prevent TCF phosphorylation. It is likely that other MAPKs also phosphorylate and stimulate TCF in response to stimuli that do not activate the ERK cascade, thus providing a partial explanation for the almost universal induction of *c-fos* upon cell stimulation. As the signalling pathway responsible for ERK activation is relatively well understood, its identification as the major mediator of TCF phosphorylation provides a clearly delineated pathway connecting growth factor receptors to the transcriptional machinery (Fig. 1). Constitutive activation of any component of this cascade can lead to *c-fos* induction followed by stimulation of AP-1 activity (see below).

Recently, three members of a second subfamily of MAPKs, known as JNKs [20,22,26] or SAPKs [18], were molecularly cloned. Like the ERKs, to which they are 40 % identical, the JNK/SAPKs can be activated by growth factors, through a pathway dependent on Ras but not Raf [38]. JNK/SAPKs are, however, even more responsive to stressful stimuli, such as ultraviolet (UV) irradiation, protein synthesis inhibitors, TNFα and agonists of certain G-protein-coupled receptors, which have only a small effect on ERK activity [18,20,22–24,39,40]; this activation pathway appears to be Ras-independent. The Ras-dependent activation of JNK/SAPKs involves MEKK and a novel MEK-related kinase known as SEK1 [41,42], MKK4 [43] or JNKK [44]. However, the mechanism through which MEKK activity is regulated is not clear.

Another potential complication is the presence of multiple MEKK isoforms, most of which remain to be characterized. Interestingly, SEK1/MKK4/JNKK1 can also activate p38/Mpk2/CSBP [43,44]. It is worth noting that, despite its ability to activate the MEKs, MEKK does not activate the ERK pathway unless overexpressed [38,42]. This specificity in MEKK action could be due to the presence of scaffolding proteins, as discussed above. The JNK/SAPKs are present in both the cytoplasm and nucleus [45], and recently it was found that JNK1 (and presumably also other JNK/SAPKs) is concentrated in the nucleus following its activation in response to UV irradiation (M. Cavigelli, F. Dolfi and M.K., unpublished data).

The JNK/SAPKs appear to be responsible for stimulation of c-Jun transcriptional activity [45,46], by phosphorylation of residues Ser 63 and Ser 73 in c-Jun's activation domain [47,48]. Although both of these serines are followed by prolines, and therefore are potential MAPK targets, neither the ERK nor p38/Mpk2/CSBP MAPKs phosphorylate these sites effectively [12,39,49]. Indeed, the ERK MAPKs instead phosphorylate a site located in the carboxy-terminal domain of c-Jun, associated with inhibition of DNA binding [39,49]. So far, only the JNK/SAPKs, which can bind to the c-Jun activation domain, have been found to phosphorylate the c-Jun activating sites efficiently [18,20,22,26,45,46,50]. Although this interaction has been demonstrated only *in vitro*, deletion of c-Jun's JNK/SAPK docking site, located between amino acids 30 and 60, greatly diminishes its ability to be phosphorylated *in vivo* in response to a variety of JNK/SAPK activators [45,51,52]. Such mutants are no longer activated by JNK/SAPK-activating signals [29]. The stimulation of c-Jun amino-terminal phosphorylation is rapid and correlates well with JNK/SAPK activation [39,51,52].

c-Jun is one of the components of transcription factor AP-1, its best known partner being c-Fos [53]. The activity known as AP-1 can actually consist of heterodimers between any of the Jun proteins (which also include JunB and JunD) and any of the Fos proteins (which also include FosB, Fra1 and Fra2). Although their stability is not as high as those of c-Jun–c-Fos heterodimers, c-Jun homodimers can also bind to AP-1 sites

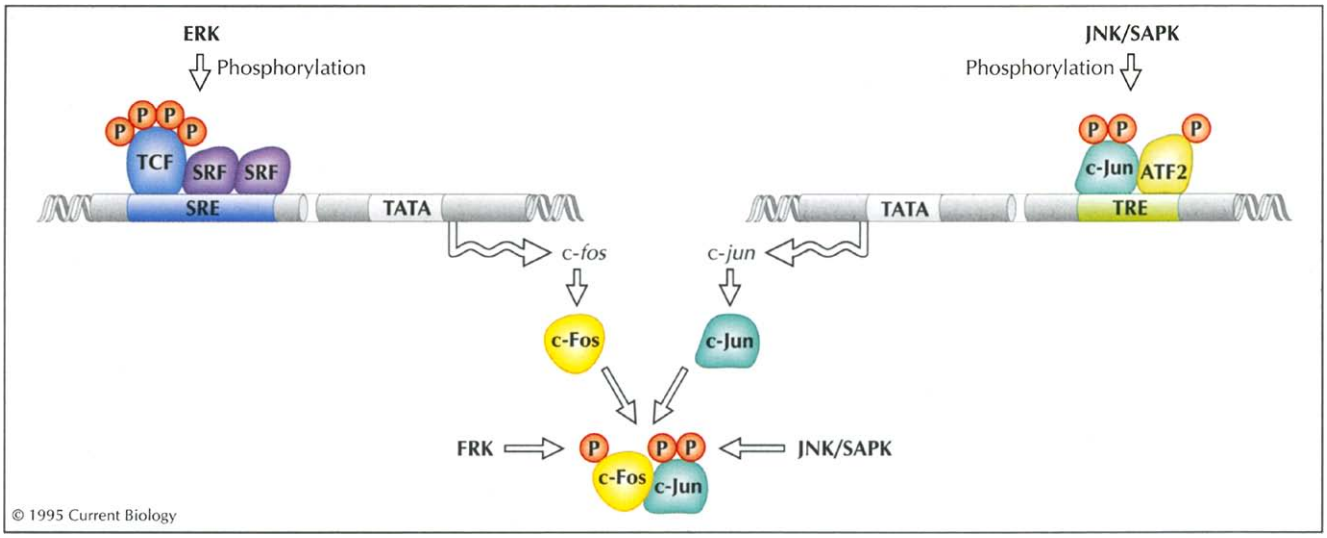

Fig. 2. Stimulation of AP-1 activity by MAP kinases. Three different types of MAP kinase are involved in induction of AP-1 activity, through three different mechanisms. ERK1 and ERK2 are involved in *c-fos* induction through phosphorylation of TCF bound to the *c-fos* promoter. Increased *c-fos* expression drives formation of Jun–Fos heterodimers, which are more stable than the pre-existing Jun–Jun homodimers. Increased dimer stability results in higher levels of AP-1 DNA-binding activity. JNK/SAPKs are involved in *c-jun* induction through phosphorylation of c-Jun and ATF2; these two transcription factors form a heterodimer, which is pre-bound to the TRE of the *c-jun* promoter in non-stimulated cells. Phosphorylation of c-Jun and ATF2 increases their transcriptional activity, leading to enhanced *c-jun* transcription followed by increased c-Jun synthesis. The newly synthesized c-Jun may combine with newly synthesized c-Fos or other proteins such as ATF2, or form homodimers, all of which can contribute to increased AP-1 activity. In addition to stimulation of c-Jun synthesis, the JNK/SAPKs contribute to elevated AP-1 activity by phosphorylating the activation domain of c-Jun and thereby enhancing its transcriptional activity. A further increase in the activity of c-Fos containing AP-1 complexes is achieved upon phosphorylation of the c-Fos activation domain by the growth-factor-activated protein kinase FRK (by all biochemical criteria, FRK appears to be a MAP kinase).

[53]. Several different pathways and mechanisms, including *c-fos* and *c-jun* induction as well as the post-translational modifications of their products, contribute to induction of AP-1 activity (Fig. 2). While it is plausible to assume that each pathway makes some contribution to general AP-1 activity, it is also possible that some genes may be more responsive to *c-fos* induction and ERK activation, whereas others may be more responsive to c-Jun phosphorylation and JNK/SAPK activation.

One possible target for the JNK/SAPK pathway is the positively autoregulated *c-jun* promoter [53], the induction of which correlates better with JNK/SAPK activation than with ERK activation [39,45,52]. Like the *c-fos* SRE, the *c-jun* TRE (TPA response element), which is most probably recognized by a c-Jun–ATF2 heterodimer [54], is constitutively occupied [55]. Phosphorylation of transcription factors already bound to their target promoters therefore appears to be a common mechanism providing for rapid gene induction in response to protein kinase activation. As shown for CREB, upon phosphorylation at Ser 73, c-Jun gains the ability to bind CBP and overexpression of CBP enhances its transcriptional activity [6].

The transcriptional activity of a c-Jun mutant, the amino-terminal sites of which are phosphorylated by PKA instead of JNK/SAPK, is stimulated following PKA activation and is no longer responsive to JNK/SAPK activating signals [56]. This shows that it is the phosphorylation of c-Jun itself, and not of another protein that interacts with its activation domain, which is directly responsible for enhancing its transactivating function. Interestingly, ATF2 is also an efficient substrate for JNK/SAPK, which phosphorylates it on sites that stimulate its transcriptional activity [57,58]. The JNK/SAPK also appears to be able to phosphorylate and activate TCF/Elk-1 (L. Mahadevan, personal communication; M. Cavigelli and M.K., unpublished data), which would provide another way to induce *c-fos* and increase AP-1 activity. This connection may be important in the induction of *c-fos* in response to UV irradiation and stress.

In addition to being stimulated by *c-fos* induction, AP-1 activity is also stimulated by phosphorylation of c-Fos at Thr 232, which enhances its transcriptional activity, in response to Ras activation by growth factors [59]. Despite the striking similarity between the sequences flanking the activating phosphorylation sites of c-Jun and c-Fos [60], Thr 232 of c-Fos is phosphorylated by neither JNK/SAPK nor ERK MAPKs, but by a novel 88 kD Ras-activated protein kinase termed FRK (Fos-regulating kinase) [59]. Although FRK has yet to be cloned, its rapid activation by growth factors and its recognition of threonines or serines followed by a proline strongly suggest that it is also a member of the MAPK family.

In summary, three distinct MAPK types contribute to induction of AP-1 activity (Fig. 2). Some of the MAPKs are involved in increasing the amount of AP-1 complexes, whereas other MAPKs increase their specific activity. The ERKs stimulate AP-1 activity through

induction of c-Fos synthesis, which leads to formation of stable Jun–Fos heterodimers. The JNK/SAPKs stimulate c-Jun and ATF2 transcriptional activities and can thereby also enhance *c-jun* transcription. FRK activation results in a further increase in AP-1 activity by enhancing the transactivation function of c-Fos. As each of these MAPKs has a unique activation pattern, this multilayered regulation allows a large variety of extracellular stimuli to induce AP-1 activity, which serves as a general nuclear transducer. The differential activation of these protein kinases and their distinct effects on *c-jun* and *c-fos* transcription and on AP-1 composition may also affect the selection of AP-1 target genes.

Like vertebrates, yeast also contains multiple MAPKs situated at the ends of different signal transduction cascades [14]. Of the four known cascades, the pheromone signalling pathway of *S. cerevisiae* is best understood and its connection to transcription-factor activation most apparent. Binding of pheromone to cell-surface receptors results in activation of two closely related MAPKs, FUS3 and KSS1. Despite extensive genetic analysis, the subcellular localization of these MAPKs and its modulation by pheromones have not been investigated. Nonetheless, it is clear that these protein kinases have at least two nuclear targets. One target, common to both FUS3 and KSS1, is STE12, a transcription factor that activates mating-specific genes [61]. Although it has not been formally proven, activation of STE12 correlates with its phosphorylation, presumably by either FUS3 or KSS1. In support of this possibility, STE12 can be coprecipitated with FUS3 and phosphorylated by it *in vitro* [62]. While FUS3 and KSS1 are fully interchangeable in the regulation of STE12, they differ in their ability to induce cell-cycle arrest [63]. This specific effect of FUS3 is mediated by FAR1, a negative regulator of the G1 form of Cdc2 (Cln2–Cdc28) [64,65]. FUS3 has been shown to associate physically with FAR1 and phosphorylate it [62,64]. Once phosphorylated, FAR1 inhibits Cln2/Cdc28 activity more efficiently [65]. This mechanism, however, remains to be tested *in vivo*.

NF-κB activation: regulated subcellular compartmentalization

An alternative strategy for transmitting signals from the cell surface to the nucleus is regulated nuclear translocation of transcription factors that are stored as inactive cytoplasmic complexes, first demonstrated for NF-κB (Fig. 3) [66]. In non-stimulated cells, the different NF-κB complexes are held in the cytoplasm by interaction with the IκB inhibitors. The latter may function by masking the nuclear translocation sequence within the Rel-homology domain of NF-κB proteins [67]. Following cell stimulation by inflammatory mediators, the NF-κB–IκB complex dissociates and NF-κB dimers are rapidly translocated to the nucleus, by an as yet unknown mechanism.

It was suggested that the key step in NF-κB activation is the inducible proteolysis of IκB [68], but the main evidence for this conclusion was obtained by using protease inhibitors which are alkylating agents that block IκBα phosphorylation, an event that precedes its degradation [69]. Hence, the original suggestion that the first step in NF-κB activation is IκB phosphorylation [70,71], although not proven, remained valid. Recently, Ser 32 and Ser 36 were suggested to be possible inducible phosphorylation sites in IκBα [72]. Substitution of these serines with alanines blocks both phosphorylation and degradation of IκBα, providing the best evidence so far that phosphorylation is required for degradation of IκBα. Phosphorylation of IκBα does not, however, induce its dissociation from NF-κB; rather, it appears to mark it for degradation while attached to NF-κB [69,73,74]. Recent evidence suggests that IκBα degradation may involve the ubiquitin/proteasome system [69,74,75].

A role for protein phosphorylation in activation of NF-κB-like transcription factors is well established in *Drosophila*. Dorsal, a member of the Rel family, is held in the cytoplasm of the early embryo (which is a syncytium) through interaction with the IκB-like protein Cactus [76]. In a similar fashion to the IκB–NF-κB interaction, Cactus binds next to and probably masks the nuclear translocation sequence within the Rel-homology domain of Dorsal [76]. Although in oocytes or early embryos Cactus is bound to Dorsal, it remains to be shown that later in development, or in mutants where Dorsal is constitutively nuclear, the two are no longer associated. Like IκB, Cactus is a phosphoprotein whose phosphorylation state is modulated during embryonic development [76], and Cactus degradation is required for Dorsal activation [77]. Moreover, a role for phosphorylation in Dorsal activation emerged with the isolation of the *pelle* gene, which encodes a serine/threonine protein kinase [78]. In loss-of-function *pelle* mutants, Dorsal remains cytoplasmic and the embryo is dorsalized. Injection of wild-type Pelle RNA into *pelle* mutants rescues their dorsalized phenotype, whereas microinjection of catalytically-inactive Pelle RNA does not [78]. Although it has not been formally established that Pelle is the Cactus-kinase, epistasis analysis suggests that it is likely to function in this manner. Alternatively, Pelle may be located upstream of a putative Cactus-kinase in a phosphorylation cascade leading to Dorsal activation.

Genetically, *pelle* acts downstream of *toll*, dominant gain-of-function mutations of which cause ventralization, a phenotype associated with nuclear translocation of Dorsal [79]. Toll is a transmembrane protein with certain homology to the interleukin-1 (IL-1) receptor [79]. This is interesting, as IL-1 is an inflammatory mediator that activates NF-κB in mammals. The parallels between the Dorsal and NF-κB activation pathways are illustrated in Figure 3.

An even more striking resemblance to NF-κB is displayed by a second Rel family member from *Drosophila*, Dif, an activator of insect immunity genes [80]. Unlike

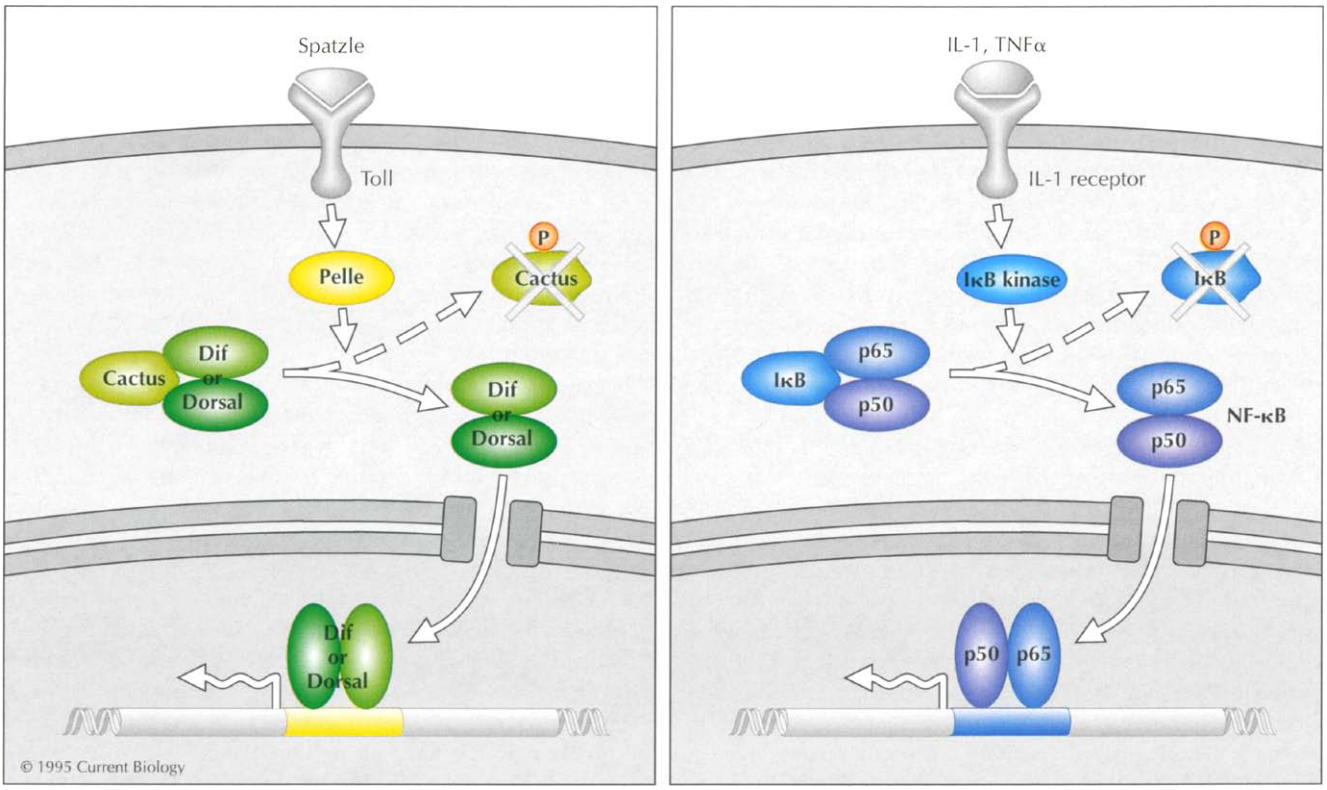

Fig. 3. Similarities between the signalling pathways involved in activation of Dorsal and Dif in *Drosophila*, and of NF-κB in vertebrates. Toll is a cell-surface receptor that exhibits modest, but significant, similarity to the IL-1 receptor. Toll is activated by a maternal signal deposited into the egg, most likely the Spatzle protein. In addition, Toll may be the receptor for the inflammatory signal leading to Dif activation. The IL-1 receptor is activated by IL-1, a cytokine that is secreted upon inflammation. Activation of Toll results in activation of the Pelle protein kinase, whereas activation of IL-1 receptor activates the yet-to-identified IκB-kinase. Activation of this latter kinase results in phosphorylation and degradation of IκB, the inhibitor which keeps NF-κB in the cytoplasm. It appears likely that Cactus, the inhibitor that keeps Dorsal and Dif in the cytoplasm, is similarly regulated. These signalling pathways lead to nuclear translocation of NF-κB, Dorsal and Dif, and thereby to the induction of their corresponding target genes.

Dorsal, which is primarily expressed in embryos, Dif is expressed in later stages. Dif is also stored in the cytoplasm, and in response to injury or infection it is rapidly translocated to the nucleus [80]. Interestingly, Dif is also activated by the Toll pathway, because in gain-of-function *toll* mutants it is nuclear. However, it remains to be established whether Toll is a receptor for inflammatory mediators. It is also not clear whether Cactus is the authentic Dif inhibitor, or whether Dif is kept in the cytoplasm by another IκB-like protein. If Toll and Cactus are directly involved in Dif activation by inflammatory signals, it will be important to know whether and how *Drosophila* distinguishes between these and developmental signals. One simple solution is the temporal separation between *dorsal* and *dif* expression.

Membrane–nucleus communication through JAKs and STATs

A second family of transcription factors whose activity is regulated by their compartmentalization are the STATs (signal transducers and activators of transcription) [81]. Originally identified as components of the interferon (IFN)-activated transcription factor known as interferon-stimulated gene factor 3 (ISGF3), the STATs are activated in response to a large variety of cytokines and growth factors [81]. Currently, six STAT genes are known, but it appears likely that more will be discovered. The mechanism of STAT activation has been most extensively studied in the case of IFN signalling (Fig. 4). Treatment of cells with IFN-α or IFN-β rapidly induces nuclear ISGF3 DNA-binding activity [82]. Although the exact composition of ISGF3 remains to be determined, it is most likely a trimeric complex composed of heterodimers of STAT1α (p91), or its alternatively spliced form STAT1β (p84), and STAT2 (p113), associated with a p48 DNA-binding subunit [81,83].

Although the exact subcellular location of p48 in unstimulated cells is controversial, the STATs are cytoplasmic prior to IFN-α exposure, which causes their rapid nuclear translocation [84]. IFN-γ on the other hand, induces a different DNA-binding activity, GAF (gamma-interferon-activating factor), composed mostly of STAT1α dimers [85]. Exposure to IFN-α results in rapid phosphorylation of STAT1α, STAT1β, and STAT2 on a tyrosine located at the carboxy-terminal edge of a Src-homology-2 (SH2) domain, which is conserved among these proteins [86] (SH2 domains are protein modules that bind with sequence specificity to phosphotyrosine-containing peptides within, for example, activated receptor protein

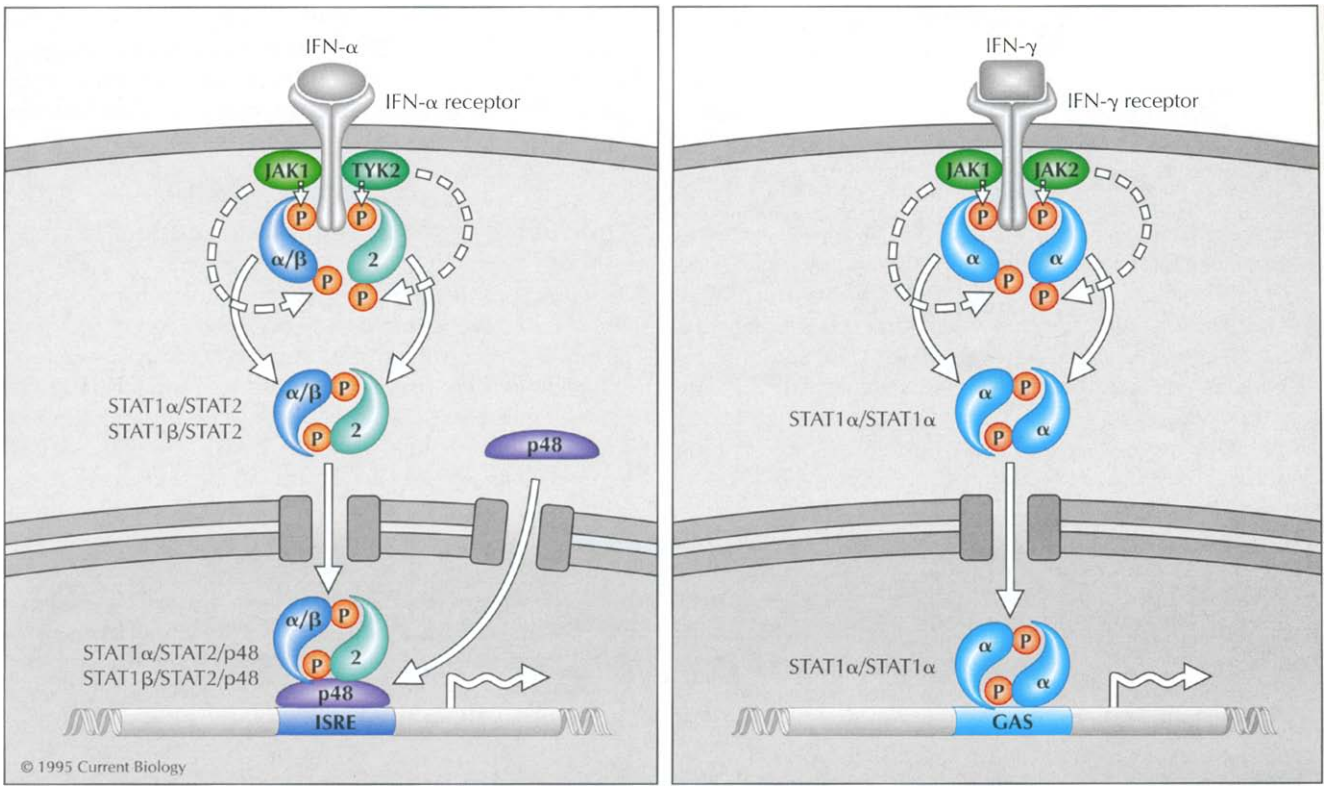

Fig. 4. Membrane to nucleus signalling in response to interferons (IFNs). By analogy to other receptors, it is proposed that binding of IFN-α or IFN-γ to their respective receptors results in recruitment and activation of JAK1 and TYK2 or JAK1 and JAK2, respectively. Activation of JAK1 and TYK2 results in phosphorylation of the IFN-α receptor followed by association of STAT1α/STAT1β and STAT2 with specific phosphotyrosines on the receptor via their SH2 domains. The receptor-bound STATs are then phosphorylated, presumably by the receptor-associated JAKs, and this results in dimerization and nuclear translocation of the STAT1α/β–STAT2 dimer. In the nucleus, these activated STAT dimers associate with p48 to form trimeric ISGF3 complexes which bind to ISREs (interferon-stimulated gene response elements) and activate transcription of IFN-α inducible genes. In this complex, p48 and STAT1 make specific contacts with the ISRE, whereas STAT2 makes general contacts [102]. Activation of JAK1 and JAK2 by IFN-γ, on the other hand, results in phosphorylation, dimerization and nuclear translocation only of STAT1α. STAT1α homodimers bind to GAS elements and activate transcription of IFN-γ-inducible genes.

tyrosine kinases [87]). IFN-γ, on the other hand, induces only STAT1α phosphorylation and does not affect STAT2 [85,86]. In both cases, tyrosine phosphorylation precedes nuclear translocation. Whereas non-phosphorylated STAT1α is monomeric, its tyrosine phosphorylated form is dimeric [88]. At least part of the dimerization energy is provided by interactions between phosphotyrosine residues on each STAT and the SH2 domain on its partner [88]. STAT1α can also heterodimerize with STAT3 in response to epidermal growth factor (EGF), which causes the simultaneous tyrosine phosphorylation of both proteins [89].

At this point, how dimerization of the STATs triggers their nuclear translocation is unknown, but as dimerization and nuclear translocation are essential for DNA binding, the tyrosine phosphorylation of STAT proteins is the critical event underlying the subsequent activation of their target genes. Recently, the binding of STAT3 homodimers to DNA was shown to be dependent on serine phosphorylation [90]. The location of this serine residue has not been determined, and it is not yet clear whether it is constitutively phosphorylated or whether some intracellular stimuli may promote its phosphorylation and thereby modulate STAT3 activity. In addition, phosphorylation of residue Ser 727 in STAT1α and STAT3 by an unknown protein kinase has been shown to be required for maximal transcriptional activation by STAT1α and STAT3 (J. Darnell, personal communication). Phosphorylation of Ser 727 in STAT1α can be induced by IFN-γ, consistent with it playing a role in IFN-induced transcription.

The regulation of STAT compartmentalization by tyrosine phosphorylation provides for rapid information transfer from the cell surface to the nucleus, because one of the first events following occupancy of many receptors is activation of protein-tyrosine kinases [87,91]. In response to interferons and many other cytokines, the STATs are probably phosphorylated by members of the JAK (Janus kinase) family of receptor-associated protein-tyrosine kinases [92–94] (STATs may be also be directly phosphorylated by activated receptor protein-tyrosine kinases upon binding via their SH2 domains). The JAK family has four known members [81], two of which, JAK1 and TYK2, are rapidly activated by IFN-α or IFN-β. This activation presumably requires the association of the JAKs with one or more subunits of the multi-subunit

receptor for these IFNs [92,95], which in the case of other cytokine receptors is known to occur through a proline-rich region in the cytoplasmic domain. Activation is brought about through ligand-induced receptor oligomerization, in a fashion analogous to the activation of receptor protein-tyrosine kinases.

Although both JAK1 and TYK2 are required for activation of ISGF3 and phosphorylation of STAT1α, STAT1β and STAT2 in response to IFN-α or IFN-β [94], it remains to be demonstrated that these STATs are directly phosphorylated by these JAKs. The response to IFN-γ, on the other hand, does not involve TYK2, but requires JAK1 and JAK2 activation [93], through inducible association with the IFN-γ receptor [96]. IFN-γ induces rapid phosphorylation of its receptor on Tyr 440 in the cytoplasmic domain of the α subunit, which provides a specific binding site for STAT1α through its SH2 domain [96]. Moreover, it has recently been shown that the activation of STAT3 by the gp130 cytokine receptor signalling subunit can be elicited individually by four out of the five tyrosines in the cytoplasmic domain, all of which lie in a YXXQ consensus sequence [97]. In addition, when one of these motifs is appended to the erythropoietin receptor cytoplasmic domain, the chimeric receptor can now activate STAT3, whereas the wild-type receptor cannot [97]. Finally, SH2 domain-swap experiments between STAT1 and STAT2 indicate that it is the STAT SH2 domain that provides receptor specificity [98].

Thus, the emerging view of specificity in the JAK/STAT system is that this is primarily a result of STAT SH2 domain-mediated interactions with specific tyrosines in the cytoplasmic domains of the receptor subunits that are presumably phosphorylated by receptor-associated JAKs (Fig. 4). Some degree of specificity is also achieved at the level of STAT–STAT dimer assembly, as only low levels of STAT1α dimers are found in IFN-α-treated cells, and genes containing a GAS element but lacking an ISRE are only weakly induced by IFN-α. This could be because the STAT1α SH2 domain has a higher affinity for the STAT2 phosphotyrosine than for its own phosphotyrosine, but this could also be mediated at the receptor level. For instance, STAT1 is only weakly phosphorylated in response to IFN-α in cells lacking STAT2, suggesting that there may be sequential or at least interdependent phosphorylation of different STATs [99].

So far there is no evidence for specificity at the level of JAK–STAT interactions, and the bound STATs are apparently phosphorylated by whichever JAKs are present within the activated receptor complex. Overall, this system provides a basis for the activation of individual STAT proteins in response to different ligands, thus generating different STAT oligomers with distinct DNA-binding specificities. Although the number of components that connect receptor activation to transcriptional activation is considerably smaller than those involved in MAPK signaling, the kinetics of STAT activation are not considerably faster than those of TCF or c-Jun activation. This may be because the JAK/STAT pathway has a lower degree of amplification than the MAPK pathways, or because the nuclear import of activated components is a rate-limiting step in both cases.

Concluding remarks: generation of specificity

A common feature of all the regulatory systems described above is the specific phosphorylation of a transcription factor (or an inhibitor thereof) in response to extracellular cues that activate distinct protein kinases (or a small number of similarly regulated protein kinases). The ability of such protein kinases to discriminate between relevant and irrelevant substrates, which is not fully apparent from *in vitro* studies using non-physiological substrates, is extremely important for ensuring biological specificity. While specificity can be generated at many different levels, starting with the receptor–ligand interaction, the final outcome must be determined by the constellation of protein kinases that are activated by a given signal and the spectrum of transcription factors that they can phosphorylate.

Several mechanisms may ensure specificity of transcription factor phosphorylation. In the case of c-Jun, STE12, FAR1, and possibly the STAT proteins, phosphorylation and activation appear to require physical interactions with their respective protein kinases or an intermediary docking protein. Efficient phosphorylation of c-Jun and ATF2 by JNK/SAPKs requires JNK/SAPK-docking sites, which are separate from the actual phosphoacceptor sites [26,45,46]. Phosphorylation of STAT1α requires an intact SH2 domain, which in addition to its role in dimerization [88] may anchor the transcription factor to the activated receptor complex in close proximity to the relevant JAK [96–98]. Although the protein domains that ensure proper STE12 and FAR1 phosphorylation have not been defined, both proteins coprecipitate with FUS3, suggesting the existence of a complex between the kinase and its substrates [62].

For most protein kinases, the specificity offered by the sequence surrounding the phosphoacceptor site is limited [100]. It is not, therefore, surprising that certain proteins whose phosphorylation has to occur very rapidly and with a high degree of fidelity, such as transcription factors, have evolved to contain protein-kinase-docking sites that ensure such specificity. The contribution of the JNK/SAPK-docking site to the regulation of c-Jun activity is quite apparent. *In vitro*, wild-type c-Jun is phosphorylated by JNK/SAPK 30-times more efficiently than a mutant lacking the docking site. However, both wild-type c-Jun and the mutant are phosphorylated with the same very low efficiency by the ERKs [22,45]. In addition, overexpression of a fusion protein containing the JNK docking site decreases expression of an AP-1 target gene, functioning most likely as a competitive inhibitor of JNK/SAPK [52].

Although so far there are only a few examples of such interactions, we predict that specific interactions between transcription factors and their cognate protein kinases via regions distinct from those containing the target hydroxy-amino acid will turn out to be widespread. Otherwise, it is difficult to explain how closely related protein kinases, such as JNK/SAPK and ERK, or JAK2 and TYK2, have distinct biological functions. The requirement for such specificity is best illustrated by KSS1 and FUS3. These two MAPKs are very similar to each other and are activated by a common protein kinase cascade [14]. Despite this high degree of similarity, the two enzymes have only partially overlapping biological functions [63]. As discussed, this specificity is most likely due to physical interactions between FUS3 and FAR1, which may not occur with KSS1 [62]. It will be of great interest to determine the structural basis for this interaction.

As even closely related protein kinase catalytic domains exhibit hypervariable loop regions [101], it is likely that these regions mediate specific substrate recognition. Indeed, one such surface-exposed loop was found to account for the more efficient phosphorylation of c-Jun by JNK2 (SAPKα), relative to the closely related JNK1 (SAPKγ), by allowing tighter binding to c-Jun [26]. Such interactions would provide even closely related protein kinases with distinct biological functions, mediated through association with different substrates, which in the case of transcription factors could activate distinct target genes.

In summary, two general mechanisms have evolved to ensure rapid and accurate transfer of information from cell-surface receptors to the nucleus. One mechanism depends on the regulated translocation of activated protein kinases from the cytoplasm to the nucleus, whereas the other is based on the regulated subcellular localization of transcription factors. While the components of these signalling cascades are being elucidated at a very fast pace, much remains to be learned about the basic mechanisms involved in regulated nuclear translocation of both protein kinases and transcription factors. One amazing feature of these processes, which has to be accounted for when thinking about possible mechanisms, is their rapidity and efficiency. In most of the cases described here increased transcription of relevant target genes may be detected within 10 minutes of signal reception.

References

1. Hunter T, Karin M: **The regulation of transcription by phosphorylation.** *Cell* 1992, **70:**375–387.
2. Nigg EA, Hilz H, Eppenberger HM, Dutly F: **Rapid and reversible translocation of the catalytic subunit of cAMP-dependent protein kinase type II from the Golgi complex to the nucleus.** *EMBO J* 1985, **4:**2801–2806.
3. Gonzalez GA, Montminy MR: **Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133.** *Cell* 1989, **59:**675–680.
4. de Groot RP, den Hertog J, Vandenheede JR, Goris J, Sassone-Corsi P: **Multiple and cooperative phosphorylation events regulate the CREM activator function.** *EMBO J* 1993, **12:**3903–3911.
5. Chrivia JC, Kwok RP, Lamb N, Hagiwara M, Montminy MR, Goodman RH: **Phosphorylated CREB binds specifically to the nuclear protein CBP.** *Nature* 1993, **365:**855–859.
6. Arias J, Alberts AS, Brindle P, Claret FX, Smeal T, Karin M, Feramisco J, Montminy M: **Activation of cAMP and mitogen responsive genes relies on a common nuclear factor.** *Nature* 1994, **370:**226–229.
7. Kwok RP, Lundblad JR, Chrivia JC, Richards JP, Bachinger HP, Brennan RG, Roberts SG, Green MR, Goodman RH: **Nuclear protein CBP is a coactivator for the transcription factor CREB.** *Nature* 1994, **370:**223–226.
8. Arany Z, Sellers WR, Livingston DM, Eckner R: **E1A-associated p300 and CREB-associated CBP belong to a conserved family of coactivators.** *Cell* 1994, **77:**799–800.
9. Arany Z, Newsome D, Oldread E, Livingston DM, Eckner R: **A family of transcriptional adaptor proteins targeted by the E1A oncoprotein.** *Nature* 1995, **374:**81–84.
10. Lundblad JR, Kwok RP, Laurance ME, Harter ML, Goodman RH: **Adenoviral E1A-associated protein p300 as a functional homologue of the transcriptional co-activator CBP.** *Nature* 1995, **374:**85–88.
11. Janaswami PM, Kalvakolanu DV, Zhang Y, Sen GC: **Transcriptional repression of interleukin-6 gene by adenoviral E1A proteins.** *J Biol Chem* 1992, **267:**24886–24891.
12. Rouse J, Cohen P, Trigon S, Morange M, Alonso-Llamazares A, Zamanillo D, Hunt T, Nebreda AR: **A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins.** *Cell* 1994, **78:**1027–1037.
13. Freshney NW, Rawlinson L, Guesdon F, Jones E, Cowley S, Hsuan J, Saklatvala J: **Interleukin-1 activates a novel protein kinase cascade that results in the phosphorylation of Hsp27.** *Cell* 1994, **78:** 1039–1049.
14. Ammerer G: **Sex, stress and integrity: the importance of MAP kinases in yeast.** *Curr Opin Genet Dev* 1994, **4:**90–95.
15. Brunner D, Oellers N, Szabad J, Biggs WH, III, Zipursky SL, Hafen E: **A gain-of-function mutation in Drosophila MAP kinase activates multiple receptor tyrosine kinase signaling pathways.** *Cell* 1994, **76:**875–888.
16. Ahn NG, Seger R, Krebs EG: **The mitogen-activated protein kinase activator.** *Curr Opin Cell Biol* 1992, **4:**992–999.
17. Cooper JA: **MAP kinase pathways — straight and narrow or tortuous and intersecting.** *Curr Biol* 1994, **4:**1118–1121.
18. Kyriakis JM, Banerjee P, Nikolakaki E, Dai T, Rubie EA, Ahmad MF, Avruch J, Woodgett JR: **The stress-activated protein kinase subfamily of c-Jun kinases.** *Nature* 1994, **369:**156–160.
19. Westwick JK, Weitzel C, Minden A, Karin M, Brenner DA: **Tumor necrosis factor α stimulates AP-1 activity through prolonged activation of the c-Jun kinase.** *J Biol Chem* 1994, **269:**26396–26401.
20. Sluss HK, Barrett T, Dérijard B, Davis RJ: **Signal transduction by tumor necrosis factor mediated by JNK protein kinases.** *Mol Cell Biol* 1994, **14:**8376–8384.
21. Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Kumar S, Green D, McNulty D, Blumenthal MJ, Heys JR, Landvatter SW: **A protein kinase involved in the regulation of inflammatory cytokine biosynthesis.** *Nature* 1994, **372:**739–746.
22. Dérijard B, Hibi M, Wu IH, Barrett T, Su B, Deng T, Karin M, Davis RJ: **JNK1: A protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain.** *Cell* 1994, **76:**1025–1037.
23. Pombo CM, Bonventre JV, Avruch J, Woodgett JR, Kyriakis JM, Force T: **The stress-activated protein kinases are major c-Jun amino-terminal kinases activated by ischemia and reperfusion.** *J Biol Chem* 1994, **269:**26546–26551.
24. Cano E, Hazzalin CA, Mahadevan LC: **Anisomycin-activated protein kinases p45 and p55 but not mitogen-activated protein kinases ERK-1 and -2 are implicated in the induction of c-fos and c-jun.** *Mol Cell Biol* 1994, **14:**7352–7362.
25. Han J, Lee JD, Bibbs L, Ulevitch RJ: **A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells.** *Science* 1994, **265:**808–811.
26. Kallunki T, Su B, Tsigelny I, Sluss HK, Dérijard B, Moore G, Davis R, Karin M: **JNK2 contains a specificity-determining region responsible for efficient c-Jun binding and phosphorylation.** *Genes Dev* 1994, **8:**2996–3007.
27. Choi KY, Satterberg B, Lyons DM, Elion EA: **Ste5 tethers multiple protein kinases in the MAP kinase cascade required for mating in *S. cerevisiae*.** *Cell* 1994, **78:**499–512.
28. Chen RH, Sarnecki C, Blenis J: **Nuclear localization and regulation of *erk*- and *rsk*-encoded protein kinases.** *Mol Cell Biol* 1992, **12:**915–927.
29. Lenormand P, Sardet C, Pages G, L'Allemain G, Brunet A, Pouyssegur J: **Growth factors induce nuclear translocation of MAP kinases**

($p42^{mapk}$ and $p44^{mapk}$) but not of their activator MAP kinase kinase ($p45^{mapkk}$) in fibroblasts. *J Cell Biol* 1993, **122:**1079–1088.

30. Gonzalez FA, Seth A, Raden DL, Bowman DS, Fay FS, Davis RJ: **Serum-induced translocation of mitogen-activated protein kinase to the cell surface ruffling membrane and the nucleus.** *J Cell Biol* 1993, **122:**1089–1101.
31. Zheng ZF, Guan KL: **Cytoplasmic localization of the mitogen-activated protein kinase activator MEK.** *J Biol Chem* 1994, **269:**19947–19952.
32. Gille H, Sharrocks AD, Shaw PE: **Phosphorylation of transcription factor $p62^{TCF}$ by MAP kinase stimulates ternary complex formation at *c-fos* promoter.** *Nature* 1992, **358:**414–417.
33. Marais R, Wynne J, Treisman R: **The SRF accessory protein Elk-1 contains a growth factor-regulated transcriptional activation domain.** *Cell* 1993, **73:**381–393.
34. Herrera RE, Shaw PE, Nordheim A: **Occupation of the *c-fos* serum response element *in vivo* by a multi-protein complex is unaltered by growth factor induction.** *Nature* 1989, **340:**68–70.
35. Gille H, Kortenjann M, Thomae O, Moomaw C, Slaughter C, Cobb MH, Shaw PE: **ERK phosphorylation potentiates Elk-1-mediated ternary complex formation and transactivation.** *EMBO J* 1995, **14:**951–962.
36. Rivera VM, Miranti CK, Misra RP, Ginty DD, Chen RH, Blenis J, Greenberg ME: **A growth factor-induced kinase phosphorylates the serum response factor at a site that regulates its DNA-binding activity.** *Mol Cell Biol* 1993, **13:**6260–6273.
37. Kortenjann M, Thomae O, Shaw PE: **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* 1994, **14:**4815–4824.
38. Minden A, Lin A, McMahon M, Lange-Carter C, Dérijard B, Davis RJ, Johnson GL, Karin M: **Differential activation of ERK and JNK mitogen-activated protein kinases by Raf-1 and MEKK.** *Science* 1994, **266:**1719–1723.
39. Minden A, Lin A, Smeal T, Dérijard B, Cobb M, Davis R, Karin M: **c-Jun N-terminal phosphorylation correlates with activation of the JNK subgroup but not the ERK subgroup of mitogen-activated protein kinases.** *Mol Cell Biol* 1994, **14:**6683–6688.
40. Coso OA, Chiariello M, Kalinec G, Kyriakis JM, Woodgett J, Gutkind JS: **Trimeric G protein-coupled receptors potently activate JNK (SAPK). Evidence for a divergence from the tyrosine kinase signaling pathway.** *J Biol Chem* 1995, **270:**5620–5624.
41. Sanchez I, Hughes RT, Mayer BJ, Yee K, Woodgett JR, Avruch J, Kyriakis JM, Zon LI: **Role of SAPK-ERK kinase-1 in the stress-activated pathway regulating transcription factor c-Jun.** *Nature* 1994, **372:**794–798.
42. Yan M, Dai T, Deak JC, Kyriakis JM, Zon LI, Woodgett JR, Templeton DJ: **Activation of stress-activated protein kinase by MEKK1 phosphorylation of its activator SEK1.** *Nature* 1994, **372:**798–800.
43. Dérijard B, Raingeaud J, Barrett T, Wu IH, Han J, Ulevitch RJ, Davis RJ: **Independent human MAP-kinase signal transduction pathways defined by MEK and MKK isoforms.** *Science* 1995, **267:**682–685.
44. Lin A, Minden A, Martinetto H, Claret FX, Lange-Carter C, Mercurio F, Johnson GL, Karin M: **Identification of a dual specificity kinase that activates the Jun kinases and p38-Mpk2.** *Science* 1995, **268:**286–290.
45. Hibi M, Lin A, Smeal T, Minden A, Karin M: **Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain.** *Genes Dev* 1993, **7:**2135–2148.
46. Adler V, Polotskaya A, Wagner F, Kraft AS: **Affinity-purified c-Jun amino-terminal protein kinase requires serine/threonine phosphorylation for activity.** *J Biol Chem* 1992, **267:**17001–17005.
47. Pulverer BJ, Kyriakis JM, Avruch J, Nikolakaki E, Woodgett JR: **Phosphorylation of c-Jun mediated by MAP kinases.** *Nature* 1991, **353:**670–674.
48. Smeal T, Binetruy B, Mercola DA, Birrer M, Karin M: **Oncogenic and transcriptional cooperation with Ha-Ras requires phosphorylation of c-Jun on serines 63 and 73.** *Nature* 1991, **354:**494–496.
49. Alvarez E, Northwood IC, Gonzalez FA, Latour DA, Seth A, Abate C, Curran T, Davis RJ: **Pro-Leu-Ser/Thr-Pro is a consensus primary sequence for substrate protein phosphorylation. Characterization of the phosphorylation of *c-myc* and *c-jun* protein by an epidermal growth factor receptor threonine 669 protein kinase.** *J Biol Chem* 1991, **266:**15277–15285.
50. Adler V, Unlap T, Kraft AS: **A peptide encoding the c-Jun delta domain inhibits the activity of a c-Jun amino-terminal protein kinase.** *J Biol Chem* 1994, **269:**11186–11191.
51. Adler V, Franklin CC, Kraft AS: **Phorbol esters stimulate the phosphorylation of c-Jun but not v-Jun: regulation by the N-terminal delta domain.** *Proc Natl Acad Sci USA* 1992, **89:**5341–5345.
52. Su B, Jacinto E, Hibi M, Kallunki T, Karin M, Ben-Neriah Y: **JNK is involved in signal integration during costimulation of T lymphocytes.** *Cell* 1994, **77:**727–736.
53. Angel P, Karin M: **The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation.** *Biochem Biophys Acta* 1991, **1072:**129–157.
54. van Dam H, Duyndam M, Rottier R, Bosch A, de Vries-Smits L, Herrlich P, Zantema A, Angel P, van der Eb AJ: **Heterodimer formation of c-Jun and ATF-2 is responsible for induction of *c-jun* by the 243 amino acid adenovirus E1A protein.** *EMBO J* 1993, **12:**479–487.
55. Rozek D, Pfeifer GP: **In vivo protein-DNA interactions at the *c-jun* promoter: preformed complexes mediate the UV response.** *Mol Cell Biol* 1993, **13:**5490–5491.
56. Smeal T, Hibi M, Karin M: **Altering the specificity of signal transduction cascades: positive regulation of c-Jun transcriptional activity by protein kinase A.** *EMBO J* 1994, **13:**6006–6010.
57. Gupta S, Campbell D, Dérijard B, Davis RJ: **Transcription factor ATF2 regulation by the JNK signal transduction pathway.** *Science* 1995, **267:**389–393.
58. van Dam H, Wilhelm D, Herr I, Steffen A, Herrlich P, Angel P: **ATF-2 is preferentially activated by stress-activated protein kinases to mediate c-*jun* induction in response to genotoxic agents.** *EMBO J* 1995, **14:**1798–1811.
59. Deng T, Karin M: **c-Fos transcriptional activity stimulated by H-Ras-activated protein kinase distinct from JNK and ERK.** *Nature* 1994, **371:**171–175.
60. Sutherland JA, Cook A, Bannister AJ, Kouzarides T: **Conserved motifs in Fos and Jun define a new class of activation domain.** *Genes Dev* 1992, **6:**1810–1819.
61. Song O, Dolan JW, Yuan YL, Fields S: **Pheromone-dependent phosphorylation of the yeast STE12 protein correlates with transcriptional activation.** *Genes Dev* 1991, **5:**741–750.
62. Elion EA, Satterberg B, Kranz JE: **FUS3 phosphorylates multiple components of the mating signal transduction cascade: evidence for STE12 and FAR1.** *Mol Biol Cell* 1993, **4:**495–510.
63. Elion EA, Brill JA, Fink GR: **FUS3 represses CLN1 and CLN2 and in concert with KSS1 promotes signal transduction.** *Proc Natl Acad Sci USA* 1991, **88:**9392–9396.
64. Tyers M, Futcher B: **Far1 and Fus3 link the mating pheromone signal transduction pathway to three G1-phase Cdc28 kinase complexes.** *Mol Cell Biol* 1993, **13:**5659–5669.
65. Peter M, Gartner A, Horecka J, Ammerer G, Herskowitz I: **FAR1 links the signal transduction pathway to the cell cycle machinery in yeast.** *Cell* 1993, **73:**747–760.
66. Baeuerle PA, Baltimore D: **IκB: A specific inhibitor of the NF-κB transcription factor.** *Science* 1988, **242:**540–546.
67. Beg AA, Baldwin AS Jr: **The IκB proteins: multifunctional regulators of Rel/NF-κB transcription factors.** *Genes Dev* 1993, **7:**2064–2070.
68. Henkel T, Machleidt T, Alkalay I, Krönke M, Ben-Neriah Y, Baeuerle PA: **Rapid proteolysis of IκB-α is necessary for activation of transcription factor NF-κB.** *Nature* 1993, **365:**182–185.
69. DiDonato JA, Mercurio F, Karin M: **Phosphorylation of IκBα precedes but is not sufficient for its dissociation from NF-κB.** *Mol Cell Biol* 1995, **15:**1302–1311.
70. Shirakawa F, Mizel SB: **In vitro activation and nuclear translocation of NF-κB catalyzed by cyclic AMP-dependent protein kinase and protein kinase C.** *Mol Cell Biol* 1989, **9:**2424–2430.
71. Ghosh S, Baltimore D: **Activation *in vitro* of NF-κB by phosphorylation of its inhibitor IκB.** *Nature* 1990, **344:**678–682.
72. Brown K, Gerstberger S, Carlson L, Franzoso G, Siebenlist U: **Control of IκBα proteolysis by site-specific, signal-induced phosphorylation.** *Science* 1995, **267:**1485–1488.
73. Miyamoto S, Maki M, Schmitt MJ, Hatanaka M, Verma IM: **Tumor necrosis factor α-induced phosphorylation of IκBα is a signal for its degradation but not dissociation from NF-κB.** *Proc Natl Acad Sci USA* 1994, **91:**12740–12744.
74. Traenckner EB, Wilk S, Baeuerle PA: **A proteasome inhibitor prevents activation of NF-κB and stabilizes a newly phosphorylated form of IκBα that is still bound to NF-κB.** *EMBO J* 1994, **13:**5433–5441.
75. Palombella VJ, Rando OJ, Goldberg AL, Maniatis T: **The ubiquitin-proteasome pathway is required for processing the NF-κB1 precursor protein and the activation of NF-κB.** *Cell* 1994, **78:**773–785.
76. Kidd S: **Characterization of the *Drosophila cactus* locus and analysis of interactions between cactus and dorsal proteins.** *Cell* 1992, **71:**623–635.
77. Belvin P, Jin Y, Anderson KV: **Cactus protein degradation mediates *Drosophila* dorsal-ventral signaling.** *Genes Dev* 1995, **9:**783–793.
78. Shelton CA, Wasserman SA: ***pelle* encodes a protein kinase required to establish dorsoventral polarity in the *Drosophila* embryo.** *Cell* 1993, **72:**515–525.

79. Schneider DS, Hudson KL, Lin TY, Anderson KV: **Dominant and recessive mutations define functional domains of Toll, a transmembrane protein required for dorsal-ventral polarity in the *Drosophila* embryo.** *Genes Dev* 1991, **5**:797–807.
80. Ip YT, Reach M, Engstrom Y, Kadalayil L, Cai H, Gonzalez-Crespo S, Tatei K, Levine M: **Dif, a dorsal-related gene that mediates an immune response in Drosophila.** *Cell* 1993, **75**:753–763.
81. Darnell JE, Kerr IM, Stark GR: **Jak–STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins.** *Science* 1994, **264**:1415–1421.
82. Levy DE, Kessler DS, Pine R, Reich N, Darnell JE: **Interferon-induced nuclear factors that bind a shared promoter element correlate with positive and negative transcriptional control.** *Genes Dev* 1988, **2**:383–393.
83. Fu XY, Schindler C, Improta T, Aebersold R, Darnell JE: **The proteins of ISGF-3, the interferon α-induced transcriptional activator, define a gene family involved in signal transduction.** *Proc Natl Acad Sci USA* 1992, **89**:7840–7843.
84. Schindler C, Shuai K, Prezioso VR, Darnell JE: **Inteferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor.** *Science* 1992, **257**:809–813.
85. Shuai K, Schindler C, Prezioso VR, Darnell JE: **Activation of transcription by IFN-γ: tyrosine phosphorylation of a 91 kD DNA binding protein.** *Science* 1992, **258**:1808–1812,
86. Shuai K, Stark GR, Kerr IM, Darnell JE: **A single phosphotyrosine residue of Stat91 required for gene activation by interferon-gamma.** *Science* 1993, **261**:1744–1746.
87. Pawson T, Schlessinger J: **SH2 and SH3 domains.** *Curr Biol* 1993, **3**:434–444.
88. Shuai K, Horvath CM, Huang LH, Qureshi SA, Cowburn D, Darnell JE: **Inteferon activation of the transcription factor Stat1 involves dimerization through SH2-phosphotyrosyl peptide interactions.** *Cell* 1994, **76**:821–828.
89. Zhong Z, Wen Z, Darnell JE: **Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6.** *Science* 1994, **264**:95–98.
90. Zhang X, Blenis J, Li HC, Schindler C, Cheng-Kiang S: **Requirement of serine phosphorylation for formation of STAT-promoter complexes.** *Science* 1995, **267**:1990–1994.
91. van der Geer P, Hunter T, Lindberg RA: **Receptor protein-tyrosine kinases and their signal transduction pathways.** *Annu Rev Cell Biol* 1994, **10**:251–337.
92. Velazquez L, Fellous M, Stark GR, Pellegrini S: **A protein tyrosine kinase in the interferon α/β signaling pathway.** *Cell* 1992, **70**: 313–322.
93. Watling D, Guschin D, Muller M, Silvennoinen I, Witthuhn BA, Quelle FW, Rogers NC, Schindler C, Stark GR, Ihle JN: **Complementation by the protein tyrosine kinase JAK2 of a mutant cell line defective in the interferon-γ signal transduction pathway.** *Nature* 1993, **366**:166–170.
94. Müller M, Briscoe J, Laxton C, Guschin D, Ziemiecki A, Silvennoinen O, Harpur AG, Barbieri G, Witthuhn BA, Schindler C: **The protein tyrosine kinase JAK1 complements defects in interferon-α, β and γ signal transduction.** *Nature* 1993, **366**:129–135.
95. Igarashi KI, Garotta G, Ozmen L, Ziemiecki, A, Wilks AE, Harpur AG, Larner AC, Finbloom DS: **Interferon-γ induces tyrosine phosphorylation of interferon-γ receptor and regulated association of protein tyrosine kinases, Jak1 and Jak2, with its receptor.** *J Biol Chem* 1994, **269**:14333–14336.
96. Greenlund AC, Farrar MA, Viviano BL, Schreiber RD: **Ligand-induced IFN-γ receptor tyrosine phosphorylation couples the receptor to its signal transduction system (p91).** *EMBO J* 1994, **13**:1591–1600.
97. Stahl N, Farruggella TJ, Boulton TG, Zhong Z, Darnell JE, Yancopoulos GD: **Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors.** *Science* 1995, **267**:1349–1353.
98. Heim MH, Kerr IM, Stark GR, Darnell JE: **Contribution of STAT SH2 groups to specific interferon signaling by the Jak-STAT pathway.** *Science* 1995, **267**:1347–1349.
99. Leung S, Qureshi SA, Kerr IM, Darnell JE, Stark GR: **Role of STAT2 in the alpha interferon signaling pathway.** *Mol Cell Biol* 1995, **15**:1312–1317.
100. Kemp BE, Pearson RB: **Protein kinase recognition sequence motifs.** *Trends Biochem Sci* 1990, **15**:342–346.
101. Hanks SK, Hunter T: **The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification.** *FASEB J* 1995, **9**:576–596.
102. Qureshi SA, Salditt-Georgieff M, Darnell JE: **Tyrosine-phosphorylated Stat1 and Stat2 plus a 48-kDa protein all contact DNA in forming interferon-stimulated gene factor 3.** *Proc Natl Acad Sci USA* 1995, **92**:3829–3833.

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