MAP kinases as structural adaptors and enzymatic activators in transcription complexes

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Summary

Mitogen-activated protein kinase (MAPK) pathways regulate eukaryotic gene expression in response to extracellular stimuli. MAPKs and their downstream kinases phosphorylate transcription factors, co-regulators and chromatin proteins to initiate transcriptional changes. However, the spatial context in which the MAPKs operate in transcription complexes is poorly understood. Recent findings in budding yeast show that MAPKs can form integral components of transcription complexes and have novel structural functions in addition to phosphorylating local substrates. Hog1p MAPK is stably recruited to target promoters by specific transcription factors in response to osmotic stress, and acts as both a structural adaptor and enzymatic activator driving the assembly and activation of the transcription complex. We review the evidence that suggests a similar bifunctional role for MAPKs in mammalian transcription complexes.

Key words: MAP kinase, Transcription complexes, Hog1/p38

Introduction

Mitogen-activated protein kinases (MAPKs) are a family of evolutionarily conserved enzymes that respond to a plethora of extracellular stimuli by regulating gene expression (transcription and post-transcriptional processes), movement, metabolism, cell death, proliferation and differentiation (Chang and Karin, 2001; Kyriakis and Avruch, 2001; Posas et al., 1998; Widmann et al., 1999). They are activated by MAPK kinases (MAPKKs), which in turn are activated by MAPKK kinases (MAPKKKs), themselves controlled by often poorly understood mechanisms linked to cell-surface receptor activation. MAPKs mediate their effects through phosphorylation, and hence activation/inactivation of a range of protein substrates including diverse enzymes, transcription factors, cytoskeletal proteins and downstream signaling proteins such as MAPK-activated protein kinases (MAPKAPKs) and other kinases (Fig. 1).

The budding yeast Saccharomyces cerevisiae uses at least five different MAPK cascades (shown schematically in Fig. 1), each transducing signals about different aspects of its environment (Herskowitz, 1995; Posas et al., 1998; Widmann et al., 1999). These are the mating-pheromone response pathway, filamentous growth pathway, cell wall integrity/protein kinase C pathway, sporulation/nutritional deprivation pathway and high osmolarity glycerol (HOG) response pathway (Posas et al., 1998; Widmann et al., 1999). In mammals, at least four parallel MAPK cascades exist that respond differentially to distinct extracellular stimuli (Chang and Karin, 2001; Johnson and Lapadat, 2002; Kyriakis and Avruch, 2001). Furthermore, each MAPK subtype exists in multiple forms, reflecting the increased complexity and proliferation of these signalling pathways during evolution (Fig. 1). These include the extracellular-signal-regulated kinase (ERK1 and ERK2), p38 kinase (p38α, p38β, p38δ and p38γ) and Jun N-terminal kinase (JNK1, JNK2 and JNK3) subfamilies, and the more recently identified big mitogen-activated protein kinase 1 (BMK1)/ERK5. These are activated by specific upstream MAPKKs: MEK1/MEK2, MKK3/MKK6, MKK4/MKK7 and MEK5, respectively (Fig. 1). Although there is some cross-talk and cell-type specificity, the JNK and p38 MAPK cascades are more strongly activated by stress stimuli and inflammatory cytokines, whereas the ERK pathway is strongly activated by polypeptide growth factors through receptor tyrosine kinases (Kyriakis and Avruch, 2001). The BMK1/ERK5 pathway is activated both by stress and by mitogenic stimuli (Kyriakis and Avruch, 2001). There is clear evolutionary conservation of MAPK function and regulation between yeast and humans (Widmann et al., 1999). This is evident from complementation experiments in which certain human MAPKs can functionally replace their homologues in yeast (Atienza et al., 2000; Galcheva-Gargova et al., 1994). Therefore, MAPKs might have originated as intracellular phosphorylation cascades playing a crucial role in the adaptation of a microorganism to its frequently variable environment. In mammals, MAPKs have acquired a wider and more complex role in controlling cell-cell communication, cell division and differentiation during development, as well as metabolic and genetic reprogramming in response to extracellular factors, stress and chemical stimuli. The complexity of these multiple parallel MAPK pathways (summarized in Fig. 1), as well as their regulation and their evolutionary conservation, has been extensively reviewed elsewhere and we do not dwell upon these areas here.

MAPK cascades control transcription at several levels, most notably by phosphorylating transcription factors and co-regulators, but also by initiating chromatin modifications along inducible genes. The best-understood mechanism is the direct phosphorylation of promoter-specific transcription factor...
targets (Karin and Hunter, 1995; Kyriakis and Avruch, 2001; Treisman, 1996). This can regulate their activity by several mechanisms, including control of protein levels, regulation of binding to DNA, nucleocytoplasmic shuttling and altering their ability to transactivate (Yang et al., 2003). However, it is increasingly obvious that MAPK cascades do not modulate transcription by targeting transcription factors alone. Recent evidence implicates co-regulatory proteins as targets of MAPK cascades (Yang et al., 2003). Furthermore, MAPK cascades also target nucleosomal proteins at inducible genes. The best-studied example of this is the phosphorylation of histone H3 and HMG14 concomitant with induction of the immediate-early (IE) genes mediated through the ERK or p38 MAPK cascades (Thomson et al., 1999). The finding that the downstream kinases MSK1 and MSK2 phosphorylate these nucleosomal proteins (Clayton and Mahadevan, 2003; Thomson et al., 1999) provided the first direct link between a MAPK cascade and chromatin modification during gene

(a) Yeast MAPK pathways

Pathway      | Stimuli            | MAPKKs      | MAPKs       | Substrates   | Pathway      | Stimuli            | MAPKKs      | MAPKs       | Substrates   |
-------------|--------------------|-------------|-------------|--------------|-------------|--------------------|-------------|-------------|--------------
Mating       | Mating pheromones  | Ste7p       | Fus3p/Kss1p | Ste12p       | Cell wall integrity | Mkk1/2p      | Mpk1p       | Rlm1p        |
N limitation  |                    | Ste7p       | Kss1p/Fus3p | Ste12p       | High osmolarity glycerol (HOG) | Hog1p       | Hot1p       | Smp1p        |
Growth factors stress | MKK1/2p        | Mpk1p       | Rlm1p       |        | Starvation            | Snk1p       |            | Sko1p        |

(b) Mammalian MAPK pathways

Pathway      | Stimuli            | MAPKKs      | MAPKs       | Substrates   | Pathway      | Stimuli            | MAPKKs      | MAPKs       | Substrates   |
-------------|--------------------|-------------|-------------|--------------|-------------|--------------------|-------------|-------------|--------------
ERK          | Growth factors     | MEK1/2      | ERK1/2      | Elk1         | p38          | Inflammatory cytokines stress | Mkk3/6      | p38a/38c    | Jun-         |
JNK          |                    |            |            | Rsk          | JNK1/2/3     |                      | Mkk4/7      |            | Mnk1/2       |
BMK          |                    |            |            | MsK1         |              |                      | MEK5        |            | Mnk1/2       |

Fig. 1. Mammalian and yeast MAPK pathways. Schematic overview of yeast (a) and mammalian (b) mitogen-activated protein kinase (MAPK) pathways and some of their downstream substrates. MAPKK/MKK, MAPK kinase; ERK, extracellular-signal-regulated kinase; JNK, Jun N-terminal kinase; BMK, big MAPK; MEK, MAPK/ERK kinase. Colour code as follows: red, MAPKKs; orange, MAPKs; yellow, downstream kinases; green, transcription factors; light blue, nucleosomal proteins; dark blue, coregulators.
induction. However, indirect routes by which MAPK cascades can cause localized nucleosomal modification at inducible genes – for example, by promoting association of histone acetyltransferases (HATs) with transcription factors in a phosphorylation-dependent manner (Mayer and Montminy, 2001; Yang et al., 2003) – have also emerged, linking localized histone acetylation with MAPK activation. A recently reported variation of this involves the interplay between MAPK-regulated SUMOylation of the transcription factor Elk1 and its interaction with histone deacetylases (Yang and Sharrock, 2004). Finally, recent evidence from yeast indicates a novel role for MAPKs as components of promoter-bound transcription complexes. In such complexes, they appear to act as enzymatically functioning structural adaptors, involved both in phosphorylating local substrates and in recruitment of chromatin-remodelling complexes, other transcription factors and the general transcription machinery. Here, we review evidence suggesting a similar bifunctional role for MAPKs in mammals and discuss the implications of these findings for the way we view the role of MAPKs in transcriptional regulation.

**Hog1p kinase as a central component of yeast transcription complexes**

The HOG MAPK pathway in *S. cerevisiae* is involved in the adaptation of cells to hyperosmotic environmental conditions (O’Rourke et al., 2002). Increases in osmolarity lead to the activation of the Pbs2p MAPKK via two upstream branches originating at different transmembrane osmosensors. Pbs2p then activates (through phosphorylation of threonine and tyrosine) the Hog1p MAPK, which phosphorylates effector molecules involved in the regulation of transcription, translation and cell-cycle progression to bring about the osmoadaptive response (de Nadal et al., 2002; O’Rourke et al., 2002).

Upon osmotic-stress-induced phosphorylation by Pbs2p, Hog1p translocates from the cytoplasm to the nucleus (Ferrigno et al., 1998; Reiser et al., 1999b). Genome-wide transcript profiling shows that Hog1p controls expression of ~600 genes in response to osmostress (O’Rourke and Herskowitz, 2003; Posas et al., 2000; Rep et al., 2000). It regulates several transcription factors, each responsible for controlling expression of a subset of osoresponsive genes. These are the Hot1p, Smplp, Msnlp, Msnp2 and Msnp4p activators and the Sko1p repressor (de Nadal et al., 2003; Martinez-Pastor et al., 1996; Proft and Serrano, 1999; Rep et al., 2000; Rep et al., 2001; Rep et al., 1999). We have known for some time that the nuclear retention of certain MAPKs upon stress requires the presence of their transcription factor substrates (Reiser et al., 1999a). The fraction of Hog1p resident in the nucleus following osmotic stress is reduced in the absence of the transcriptional activators Msnlp, Msnp2, Msnp4p and Hot1p (Reiser et al., 1999b; Rep et al., 1999). In addition, yeast two-hybrid experiments indicate that Hog1p and Hot1p physically interact (Rep et al., 1999). Such data suggest that transcription factor targets can act as nuclear anchors for their corresponding MAPKs by engaging in stable interactions with them.

The breakthrough came with the recent application of chromatin immunoprecipitation (ChIP) assays to address this problem. This showed that Hog1p could be stably recruited to promoters in a transcription-factor- and osmostress-dependent manner (Alepuz et al., 2001; Proft and Struhl, 2002). The transcription factor Hot1p was shown to bind constitutively to the *GPD1* promoter and recruit Hog1p in response to osmotic stress (Alepuz et al., 2001). Similarly, promoters of the *Msn2p/Msn4p*-dependent genes *CTT1* and *HSP12* (Rep et al., 1999) associate with catalytically active Hog1p in an Msn2p- and Msn4p-dependent manner upon osmostress (Alepuz et al., 2001). Interestingly, active Hog1p recruitment to these particular promoters is a precondition for the additional recruitment of Hot1p (Alepuz et al., 2001), which is thought to play a minor regulatory role at these genes (Rep et al., 1999). Also, catalytically active Hog1p and Hot1p exhibit interdependent recruitment to the *STLI* promoter (Alepuz et al., 2001). Thus, in addition to being recruited by transcription factors to specific promoters, Hog1p seems to be able to play a part in transcription factor recruitment and complex assembly.

The phosphorylation of transcription factors by MAPKs is well documented and is reviewed elsewhere (Yang et al., 2003). Phosphorylation of the MEF2-like activator Smplp by Hog1p in response to osmostress is required for transactivation (de Nadal et al., 2003). By contrast, osmotic-stress-dependent phosphorylation of Hot1p by Hog1p appears to be unnecessary (Alepuz et al., 2003; Alepuz et al., 2001). The physical presence of Hog1p at Hot1p-controlled promoters is instead required for the direct recruitment of the general transcription machinery (Alepuz et al., 2003). RNA polymerase II (pol II) and other components of the general transcription machinery are recruited to the *STLI* promoter in an osmotic-stress-, Hot1p- and active-Hog1p-dependent manner (Alepuz et al., 2003). This relies on Hog1p, but not Hot1p, interacting with subunits of mediator, pol II and general transcription factors (Alepuz et al., 2003). Finally, artificial tethering of Hog1p to a reporter gene leads to its transcription in response to osmostress (Alepuz et al., 2003). These findings suggest that Hot1p simply acts as an anchor, targeting Hog1p to specific genes to recruit and activate the general transcription machinery in response to osmotic stress. Such a mechanism might not be limited to Hot1p target genes. Hog1p, Msn2p and Msn4p (but not Hot1p) are required for osmostress-induced recruitment of pol II to the promoter of *ALD3*, which is an Msn2p/Msn4p-dependent gene (Alepuz et al., 2003).

Parallel studies examining regulation of the transcriptional repressor Sko1p have revealed more about Hog1p function in transcription complexes. An ATF/CREB-family member, Sko1p, represses expression of a subset of osmostress-induced genes by recruiting the co-repressor complex Cyc8p-Tup1p (Garcia-Gimeno and Struhl, 2000; Proft and Serrano, 1999; Rep et al., 2001). Osmotic stress leads to binding of active Hog1p to Sko1p at these promoters and the subsequent phosphorylation of Sko1p by Hog1p (Proft et al., 2001; Proft and Struhl, 2002). This is required for conversion of Sko1p-Cyc8p-Tup1p from a repressor into an activator that can recruit SAGA and SWI/SNF chromatin-modifying/remodelling complexes (Proft and Struhl, 2002). Recruitment of SAGA and SWI/SNF coincides with an increase in TATA-binding protein (TBP) association and histone H3 acetylation at the promoter (Proft and Struhl, 2002). Although Hog1p-mediated phosphorylation of Sko1p is required for derepression at these promoters, it is not required for transcriptional activation in the
absence of Cyc8p-Tup1p (Proft and Struhl, 2002). These observations led to the proposal that, after derepression triggered by phosphorylation of Sko1p, tethered Hog1p functions in the recruitment and activation of the general transcription machinery similarly to its proposed role at Hot1p-regulated promoters (Alepuz et al., 2003).

More recent evidence has shown that Hog1p directly recruits the Rpd3p-Sin3p histone deacetylase complex to osmoreponsive gene promoters in response to osmotic stress (De Nadal et al., 2004). Microarray analysis revealed that Rpd3p is important for expression of >90% of osmoreponsive genes in response to osmostress (De Nadal et al., 2004). This is at least partly owing to the role of Rpd3p-Sin3p in decreasing acetylation of histone H4 at these promoters (De Nadal et al., 2004). Interestingly, recruitment of pol II to Rpd3p-dependent genes is substantially reduced in Rpd3p mutant strains (De Nadal et al., 2004). This suggests a model where recruitment of chromatin-remodelling/modifying activities such as Rpd3p-Sin3p (De Nadal et al., 2004), SAGA and SWI/SNF (Proft and Struhl, 2002) by Hog1p leads to alteration of local chromatin structure and/or recruitment of other coactivators, facilitating the additional role of Hog1p in recruitment and activation of the pol II holoenzyme (Fig. 2).

Are MAPKs bifunctional in mammalian transcription complexes?

Alepuz et al. have investigated whether the interaction between p38, the mammalian equivalent of Hog1p, and pol II is conserved, using HeLa cells (Alepuz et al., 2003). In contrast to the osmotic-stress-induced interaction seen in yeast, they found that p38 coprecipitates with pol II constitutively and irrespective of osmotic conditions (Alepuz et al., 2003). Although regulated distinctively, this interaction does therefore appear to be conserved. In vitro evidence suggests that p38 and JNK can interact with the DNA-bound transcription factors ATF-2 and Jun, respectively (Read et al., 1997). This study reports that the interactions are constitutive in mammalian
cells, unlike those described above in yeast (Alepuz et al., 2001). Some ChIP evidence also shows constitutive tethering of JNK to the AP1 (Jun/Fos family) response elements in the c-Jun promoter in vivo (Bruna et al., 2003). Such data suggest that p38 and JNK can be activated in the nucleus while pre-assembled in transcription complexes. However, it must be stressed that, in mammalian cells, there is as yet little evidence that transcription-factor-dependent tethering of MAPKs to target promoters is a common mechanism of transcriptional regulation. Despite this, given the evidence in yeast and the conservation of function in higher eukaryotes, mammalian p38 might well function as a structural adaptor that recruits the transcription machinery or chromatin-modifying enzymes to specific promoters.

Phosphorylation of Hot1p by Hog1p is not required for Hot1p-mediated gene expression in yeast, although the catalytic activity of Hog1p is required (Alepuz et al., 2003; Alepuz et al., 2001), which suggests that there are other phosphorylatable substrates for Hog1p, such as the pol II holoenzyme. Observations in mammalian cells hint at a similar function for p38. For example, in macrophages, p38-mediated phosphorylation and activation of TBP is implicated in NF-κB- and API-dependent gene expression (Carter et al., 1999; Carter et al., 2001). Phosphorylation of TBP by p38 increases binding of TBP to the TATA box and its interaction with NF-κB (the p65 subunit) and Jun (Carter et al., 1999; Carter et al., 2001). Perhaps active p38 is recruited to promoters by NF-κB and API to activate TBP locally and hence to initiate protein-protein interactions leading to assembly of the transcription initiation complex. More recently, Ramsauer et al. implicated stress-activated p38 in enhancing interferon (IFN)-γ-induced stimulation of gene expression by STAT1 independently of its ability to phosphorylate gene-specific transcription factors (Ramsauer et al., 2002). They suggested therefore that p38 might phosphorylate components of the general transcription machinery to enhance transcription (Ramsauer et al., 2002). Alternatively, the possibility is now raised that p38 might be tethered to STAT1-regulated gene promoters and recruit transcription complexes or promote local histone modification through downstream enzymes such as MSKs (Fig. 1).

Studies on other mammalian MAPKs also reveal a possible function in transcription complexes. The C-terminal region of BMK1 contains a strong transcriptional activation domain and an interaction domain for the transcription factor MEF2D (a member of the myocyte enhancer factor subfamily) (Kasler et al., 2000). When tethered to the promoter of a reporter construct, this region of BMK1 can activate transcription (Kasler et al., 2000). Active BMK1 can interact with MEF2D and stimulate transcription by a mechanism that requires its kinase activity (Kasler et al., 2000). In this study, the MEF2D region (amino acids 1-92) crucial for mediating coactivation by BMK1 did not include the serine 179 residue (Kasler et al., 2000) that had previously been reported to be phosphorylated by BMK1 (Kato et al., 2000). This suggests the kinase activity of BMK1 is required elsewhere. It is possible that active BMK1 is anchored to specific genes by MEF2D to recruit and/or phosphorylate the general transcription machinery.

The mammalian ERK MAPK subfamily might also function within the context of transcription activation complexes. Using ChIP assays, Benkoussa et al. found that activation of ERKs by 12-O-tetradecanoylphorbol-13-acetate (TPA) leads to recruitment of ERK1 and ERK2, CREB-binding protein (CBP) and pol II to an API1-responsive reporter gene in vivo (Benkoussa et al., 2002). This is accompanied by an increase in Jun-D-FosB binding and a decrease in Jun-D-Fra2 binding at the API1 site (Benkoussa et al., 2002). Constitutively tethering RAR (all-trans retinoic acid receptor) to the promoter, together with treatment with atRA (all-trans retinoic acid) and TPA, led to decreased recruitment of ERKs, CBP and pol II (Benkoussa et al., 2002). It was therefore suggested that RAR might regulate ERK access to the API1 complex and hence modulate phosphorylation of its substrate and CBP association (Benkoussa et al., 2002). It is conceivable that the loss of TPA-induced pol II recruitment upon atRA treatment is partly due to lack of interactions with ERK1/ERK2 that is excluded from the API1 complex by ligand-bound RAR. If ERKs do form stable components of transcription complexes, perhaps they too phosphorylate and activate the general transcription machinery. Indeed, ERKs have been implicated in the phosphorylation of TBP and the C-terminal domain (CTD) of pol II (Biggs et al., 1998; Oelegeslager, 2002).

Interestingly, ERKs stimulate transcription by RNA polymerase I (pol I) and RNA polymerase III (pol III) by phosphorylating general transcription factors. For example, ERK-mediated phosphorylation of TIF-IA and the BRF1 subunit of TFIIIB induces transcription by pol I and pol III, respectively (Felton-Edkins et al., 2003; Zhao et al., 2003). Both ERK2 and the downstream kinase RSK interact with TIF-IA in vivo, ERK2 association being dependent on mitogenic stimulation (Zhao et al., 2003). Active ERK2 also interacts with a docking domain of BRF1, and recruitment of BRF1 and pol III to rRNA genes is greatly reduced upon ERK inactivation (Felton-Edkins et al., 2003). These studies raise the possibility that MAPK function in transcription complexes might not be limited to pol II transcription complexes.

Although little conclusive evidence supports the stable association of mammalian MAPKs and downstream kinases with specific promoters, this is highly likely because they stably interact with various specific DNA-binding transcription factors (Table 1), most of which are also substrates for the bound kinase. Indeed, stable interaction between MAPKs and transcription factors forms the basis of the "pull-down" kinase assay, in which fusions of glutathione-S-transferase (GST) with transcription factor proteins are used as affinity matrices to isolate MAPKs and this is followed by in vitro kinase assays to detect their activity (Derijard et al., 1994). Questions that remain to be conclusively answered relate to the function of mammalian MAPKs once recruited to the promoter and to what extent this has been conserved during evolution to accommodate multicellular complexity.

Protein domains potentially involved in stable and specific interactions of MAPKs within transcription complexes

If MAPKs are recruited to transcription complexes, what protein motifs are involved in the molecular interactions? MAPKs can recognize and bind certain transcription factor targets by means of a docking (D) domain consisting of a region of basic residues followed by an LXL motif and a hydrophobic region (Sharrocks et al., 2000; Tanoue and Nishida, 2003). Such docking domains, alone or in
combination with a conserved FXFP motif downstream of the MAPK phosphorylation site in certain transcription factors, are required for the specificity, efficiency and accuracy of MAPK-mediated phosphorylation (Sharrocks et al., 2000; Tanoue and Nishida, 2003). Another MAPK-docking motif, the LXLXXXF motif, exists within the Pointed (PNT) domain of a subset of ETS transcription factors (Seidel and Graves, 2002). Interaction of these docking sites with corresponding motifs in MAPKs could provide a molecular basis for stable and specific MAPK recruitment. A conserved MAPK domain, the common

Table 1. Mammalian MAP kinase-transcription factor interactions

| MAPK/ downstream kinase | Transcription factor | Evidence | References |
|------------------------|---------------------|----------|------------|
| ERK                    | Elk1                | GST pulldown, yeast two-hybrid, CIP | Cano et al., 1995; Yang et al., 1998c; Yang et al., 1998b; Rao and Reddy, 1994 |
|                        | Ets-1               | Affinity chromatography | Seidel and Graves, 2002 |
|                        | SAP-1               | Peptide competition | Galanis et al., 2001 |
|                        | SAP-2               | GST pulldown | Ducret et al., 2000 |
|                        | BMAL1               | Yeast two-hybrid, GST pulldown | Sanada et al., 2002 |
|                        | THF-I               | CIP and GST pulldown | Kim and Cochran, 2000 |
|                        | AML-1               | GST pulldown | Tanaka et al., 1996 |
|                        | HSF-1               | CIP | Dai et al., 2000 |
|                        | p53                 | CIP | She et al., 2000 |
|                        | TIF-IA              | Pulldown, CIP | Zhao et al., 2003 |
|                        | Myc                 | GST pulldown | Gupta and Davis, 1994 |
|                        | SPI                 | CIP | Liu et al., 2001 |
|                        | TFIIB (BRF1 subunit) | GST pulldown, CIP | Felton-Edkins et al., 2003 |
| JNK                    | Elk1                | Peptide competition, GST pulldown | Yang et al., 1998b; Gupta et al., 1996 |
|                        | Jun                 | GST pulldown, CIP | Kallunki et al., 1996; Adler et al., 1992; Dai et al., 1995; Cano et al., 1995; Read et al., 1997; Gupta et al., 1996; Kallunki et al., 1994; Sluss et al., 1994; Hibi et al., 1993; Derijard et al., 1994 |
|                        | SAP-2               | GST pulldown | Ducret et al., 2000 |
|                        | ATF-2               | GST pulldown | Livingstone et al., 1995; Gupta et al., 1995; Gupta et al., 1996; Raingeaud et al., 1995 |
|                        | JunB                | GST pulldown | Gupta et al., 1996; Kallunki et al., 1996 |
|                        | Myc                 | GST pulldown, CIP | Noguchi et al., 1999 |
|                        | RARα, RARγ          | GST pulldown | Adam-Sittah et al., 1999 |
|                        | TCFβ1               | GST pulldown | Kasibhatla et al., 1999 |
|                        | NFAT4               | Yeast two-hybrid, CIP, GST pulldown | Chow et al., 1997 |
|                        | NFATc1              | GST pulldown | Chow et al., 2000 |
|                        | DP1                 | GST pulldown, CIP | Wang et al., 1999 |
|                        | HSF-1               | CIP | Dai et al., 2000 |
|                        | p53                 | CIP | Fuchs et al., 1998 |
|                        | GR                  | CIP, GST pulldown | Bruna et al., 2003 |
|                        | DNA polymerase II   | CIP | Alepuz et al., 2003 |
| p38                    | SAP-1               | Peptide competition | Galanis et al., 2001 |
|                        | SAP-2               | GST pulldown | Ducret et al., 2000 |
|                        | MEF2A               | Peptide competition, immune complex kinase assay | Yang et al., 1999; Baryshe-Lovejoy et al., 2002 |
|                        | MEF2C               | Yeast two-hybrid, GST pulldown | Han et al., 1997 |
|                        | SP1                 | CIP | D’Addario et al., 2002 |
|                        | ATF2                | GST pulldown, CIP | Raingeaud et al., 1995; Read et al., 1997 |
|                        | CDX2                | GST pulldown, immune complex kinase assay, CIP | Houde et al., 2001 |
|                        | NFAT                | CIP | Gomez del Arco et al., 2000 |
|                        | p53                 | CIP | She et al., 2000 |
|                        | RPA                  | CIP | Alepuz et al., 2003 |
| ERK5                   | MEF2D               | CIP, GST pulldown, mammalian two-hybrid | Kasler et al., 2000; Yang et al., 1998a |
|                        | MEF2C               | GST pulldown, CIP | Yang et al., 1998a |
|                        | MEF2A               | CIP | Yang et al., 1998a |
| MSK1                   | p65 (NF-xB)         | GST pulldown, CIP | Vermeulen et al., 2003 |
| RSK                    | ER81                | CIP, GST pulldown | Wu and Janknecht, 2002 |
|                        | Mi                  | CIP | Wu et al., 2000 |
|                        | TIF-1A              | Pulldown, CIP | Zhao et al., 2003 |
|                        | ERα                 | CIP | Joel et al., 1998 |
| MAPKAPK2               | E47                 | CIP | Neufeld et al., 2000 |
| Mnk2                   | ERβ                 | Mammalian two-hybrid | Slentz-Kesler et al., 2000 |

A non-comprehensive list of stable interactions between mammalian MAP kinases and transcription factors and some of the supporting evidence. CIP, co-immunoprecipitation.
docking (CD) domain, is often used in the interaction with docking sites in MAPKs, MKPs (MAPK-phosphatases) and MAPKAPks (Tanoue and Nishida, 2003). However, whether or not the D domain of particular transcription factors can interact with the CD domain is not known.

The main function of MAPK docking sites in transcription factors appears be in promoting the MAPK-mediated phosphorylation of a nearby phosphoacceptor motif (S/TP) within the same transcription factor (Sharrock et al., 2000; Tanoue and Nishida, 2003). However, docking sites have also been implicated in recruiting MAPKs to phosphorylate other proteins in trans. For example, JunD lacks a JNK-docking site, but can be phosphorylated by JNK through heterodimerization with docking-competent partners such as Jun (Kallunki et al., 1996). This appears to operate at Hot1p-target genes in S. cerevisiae, where the catalytic activity of the Hog1p MAPK is required in trans for phosphorylation of other proteins after recruitment by Hot1p (Alepuz et al., 2003; Alepuz et al., 2001).

Conclusions
Emerging evidence from yeast shows that MAPKs can be functional components of transcription complexes and recruit additional factors, as demonstrated by the recruitment of pol II by Hog1p to Hot1p-regulated promoters (Alepuz et al., 2003). Although this mode of gene regulation is less well established in mammalian systems, circumstantial evidence supports a similar role in higher organisms. MAPKs might interact with docking sites present in components of the general transcription machinery through their CD domains or through novel as-yet-uncharacterized domains. If docking motifs present on particular transcription factors, such as the D domain and LXXLXXXF motif, were to interact with a MAPK domain other than the CD domain, this would leave the CD domain available for recruitment of additional factors. In addition to general transcription factors, these factors could include histone-modifying enzymes such as MSK1, which is an ERK- and p38-activated kinase that has a conserved MAPK-docking motif (Tanoue and Nishida, 2003). Further investigation of these interactions might provide conclusive evidence that the unique mode of Hog1p function observed in yeast extends more generally to the multiplicity of other MAP kinases that have arisen in mammalian cells.

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