PERSPECTIVE

SAPKs and transcription factors do the nucleocytoplasmic tango

Marc G. Wilkinson and Jonathan B.A. Millar¹

Division of Yeast Genetics, National Institute for Medical Research, The Ridgeway, London NW7 1AA, UK

A common mechanism by which eukaryotic cells sense and respond to extracellular stimuli is via activation of a mitogen-activated protein (MAP) kinase (MAPK) cascade, which consists of three sequentially acting kinases. The MAPK is activated by phosphorylation on conserved tyrosine and threonine residues by a dual-specificity MAPK kinase (MAPKK or MEK). In turn, the MAPKK is activated by phosphorylation on conserved serine and threonine residues by the MAP kinase kinase kinase (MAPKKK or MEKK). The most widely studied of the MAPKs in mammalian cells is the extracellular signal-regulated protein kinase (ERK) family of kinases, which are activated by a wide range of peptide growth factors and hormones (for review, see Robinson and Cobb 1997). Signal transmission from plasma membrane-associated receptors requires not only catalytic activation of these kinases but a transient spatial redistribution within the cell. Indeed, activation of both ERK1 and ERK2 is accompanied by their rapid relocation from the cytoplasm to the nucleus, and phosphorylation of target transcription factors (Chen et al. 1992; Gonzalez et al. 1993; Lenormand et al. 1993). Evidence that persistent localization of active ERK to the nucleus increases the frequency of cellular transformation in fibroblasts, or promotes differentiation rather than proliferation in PC12 cells, suggests that the duration of nuclear localization of active ERK is critical for the ultimate cellular response (Traverse et al. 1992, 1994; Nguyen et al. 1993; Dikic et al. 1994; Fukuda et al. 1996, 1997a). The mechanisms that promote nuclear accumulation of MAPKs, control the duration of nuclear localization, or finally determine export of MAPKs back to the cytoplasm, however, are largely unknown. Furthermore, it is known that MAPKs are activated by a wide range of environmental factors, but this activation leads to very different cellular responses. This raises the question of how activated MAPK phosphorylates the correct substrate(s). In particular, how is transcriptional specificity attained by the activated MAPK such that the correct pattern of gene expression occurs in response to a specific stimulus? Two papers in this issue (Gaits et al. 1998; Toone et al. 1998) suggest that regulated and interdependent nucleo-

cytoplasmic transport of both the MAPK and transcription factor play an important role in the answer to these questions.

Although most studies of MAPK activation and translocation have focused on ERKs, a new family of MAPKs recently has been identified that is activated by multiple environmental stresses, inflammatory cytokines, and certain vasoactive neuropeptides (Dérjard et al. 1994; Freshney et al. 1994; Galcheva-Gargova et al. 1994; Han et al. 1994; Kyriakis et al. 1994; Lee et al. 1994; Rouse et al. 1994). At least two subfamilies of stress-activated MAP kinases (SAPKs) exist; the c-Jun-N (amino)-terminal kinases (JNKs) and the p38/CSBP enzymes. Pharmacological, biochemical, and genetic evidence indicates that SAPKs control a wide variety of physiological and pathological conditions including development, control of cell proliferation, cell death, inflammation and response to ischemic injury. Consequently, the SAPK pathways have been attracting a great deal of attention as potential targets for novel therapeutics. Although far fewer studies of SAPK localization have been performed, JNK does undergo nuclear translocation following UV irradiation of human fibroblasts, suggesting that similar mechanisms of nuclear translocation may be operative (Cavigelli et al. 1995). The functional dissection of SAPKs has been aided considerably by the identification of similar pathways in genetically tractable organisms, namely fruit flies and yeast. In particular, work from several groups has revealed a SAPK pathway in the unicellular fission yeast, *Schizosaccharomyces pombe*, the central elements of which are the Sty1/Spc1 MAPK and the Wis1 MAPKK (Millar et al. 1995; Shiozaki and Russell 1995; Kato et al. 1996). Importantly, the fission yeast Sty1/Spc1 MAPK is activated by a similar range of environmental insults as are the mammalian SAPKs, including osmotic stress, oxidative stress, UV light, certain DNA-damaging agents, heat shock, and the protein synthesis inhibitor anisomycin (Millar et al. 1995; Shiozaki and Russell 1995; Degols et al. 1996; Degols and Russell 1997; Shieh et al. 1997).

How does the MAPK go nuclear?

In contrast to the ERKs, MEK is permanently located in the cytoplasm (Lenormand et al. 1993; Zheng and Guan

¹Corresponding author.
E-MAIL j-millar@nimr.mrc.ac.uk; FAX (0181) 913 8536.

1994). This localization depends on a short leucine-rich sequence, located in the amino terminus, called the nuclear export signal (NES), that when deleted leads to nuclear accumulation of MEK (Fukuda et al. 1996, 1997a). The NES has recently been shown to interact with the nuclear export receptor Crm1/Exportin1 in the presence of the small Ran GTPase (Fukuda et al. 1997b; Ossareh-Nazari et al. 1997; Fornerod et al. 1997; Stade et al. 1997). Because Ran GAP is localized in the cytoplasm and the Ran guanine nucleotide exchange factor (GEF) RCC1 is located in the nucleus, a model has been proposed whereby RanGTP in the nucleus stimulates the formation of Crm1/NES complexes leading to nuclear export. Upon translocation into the cytoplasm, Crm1/NES complexes are dissociated in the presence of RanGDP (for review, see Ullman et al. 1997). This has led to the suggestion that ERK is retained in the cytoplasm by association with its activator, MEK, and that dissociation from MEK leads to its nuclear accumulation. This model raises a number of questions that have yet to be answered satisfactorily. First, does phosphorylation of ERK cause its dissociation from MEK? Second, is ERK phosphorylation or activity necessary for nuclear import? Third, as ERK does not have a nuclear localization sequence, how does it actually enter the nucleus?

In this issue Russell and colleagues demonstrate that the fission yeast Sty1/Spc1 MAPK translocates to the nucleus in response to osmotic stress, whereas its activator, Wis1, remains cytoplasmic (Gaits et al. 1998). As is the case with other MAPKs, Sty1/Spc1 is activated by dual phosphorylation on conserved threonine and tyrosine residues in subdomain VIII of the catalytic kinase domain (Millar et al. 1995; Shiozaki and Russell 1995). Russell and colleagues find that mutation of either residue inactivates the kinase and prevents its translocation to the nucleus. Surprisingly, when phosphorylation site mutant Sty1/Spc1 proteins are expressed in heterozygous diploids, which contain one fully functional copy of Sty1/Spc1, the mutant proteins remain cytoplasmic. These results indicate that SAPK activity alone is not sufficient to drive nonphosphorylatable forms of Sty1/Spc1 into the nucleus. However, these experiments do not distinguish whether SAPK phosphorylation rather than SAPK activity per se is the critical determinant of nuclear relocalization. To address this question, it would be necessary to know whether a phosphorylatable but catalytically inactive Sty1/Spc1 is induced to undergo nuclear translocation. Regardless, these results suggest that the phosphorylated kinase must either be released from a cytoplasmic retainer and hence enter the nucleus by default, or “piggy-back” on a component of the nucleocytoplasmic transport apparatus. As Sty1/Spc1 does not accumulate in the nuclei of cells deleted for *wis1*, Russell and colleagues suggest that in contrast to the MEK/ERK model, Wis1 is unlikely to act as a cytoplasmic anchor. However, as Sty1/Spc1 is not phosphorylated in these cells, it would not be recognized by the nucleocytoplasmic transport machinery and therefore would not accumulate in the nucleus. If release of Sty1/Spc1 from Wis1 occurs independently of Sty1/Spc1

phosphorylation, Wis1 may contribute to the retention of Sty1/Spc1 in the cytoplasm. Time will tell which of these hypotheses is correct. This still leaves the problem of how phosphorylated Sty1/Spc1 actually gets into the nucleus. One is left to postulate the existence of protein(s) that promote nuclear import that can bind only the doubly phosphorylated form of the MAPK (Fig. 1). The results by Russell and colleagues contrast directly with evidence from mammalian cells that nonphosphorylated forms of ERK are still competent for nuclear entry (Gonzalez et al. 1993; Lenormand et al. 1993). It is important to point out that the former results were obtained with mutant kinases under the control of the natural Sty1/Spc1 promoter and are therefore more likely to reflect physiological conditions than are overexpression studies using transient transfection. Alternatively, there may be an intrinsic difference between the mechanism of SAPK and ERK translocation—the former requiring dual MAPK phosphorylation that is irrelevant to the latter, or a difference between MAPK translocation in yeast and mammalian cells.

MAPK nuclear retention

Once in the nucleus, the activated MAPK must phosphorylate target proteins and then be exported back to the cytoplasm. A number of nuclear targets for the SAPKs have been described (for review, see Treisman 1996). These include the b-ZIP-containing transcription factors c-Jun and ATF2, which are components of AP-1, a dimeric transcriptional activator implicated in the control of proliferation and apoptosis (for review, see Karin 1997). Phosphorylation of both c-Jun and ATF2 by JNK kinases occurs in amino-terminally located transactivation domains and results in increased transcriptional activity (Pulverer et al. 1991; Smeal et al. 1991; Kallunki et al. 1994; Gupta et al. 1995, 1996; Livingstone et al. 1995). This occurs by direct binding of JNK kinases to specific docking domains of c-Jun and ATF2 distinct from the phosphoacceptor sites (Hibi et al. 1993; Dérijard et al. 1994; Gupta et al. 1995; Livingstone et al. 1995; Kallunki et al. 1996). A remarkable similarity between the fission yeast and mammalian SAPK pathways is highlighted by the fact that one of the direct phosphorylation targets of fission yeast Sty1/Spc1 is Atf1, a structural homolog of human ATF2 (Shiozaki and Russell 1996; Wilkinson et al. 1996). As is the case in mammalian cells, the Sty1/Spc1 SAPK binds directly to the amino terminus of Atf1 to phosphorylate it and, by inference, activate its transcriptional activity.

Surprisingly, Russell and colleagues now demonstrate that Sty1/Spc1 does not accumulate in the nucleus in cells lacking Atf1. Because Atf1 is constitutively localized in the nucleus, these investigators suggest that Atf1 must act as a nuclear anchor for Sty1/Spc1 (Gaits et al. 1998). An alternative explanation is that Atf1 is required for the expression of proteins needed for MAPK import or nuclear retention. However the observation that inhibiting protein synthesis with cycloheximide prior to challenge with an osmotic stress does not block nuclear

Figure 1. A speculative model for stimulus-specific gene expression by the Sty1/Spc1 MAP kinase in fission yeast. The stress-activated Sty1/Spc1 MAP kinase targets two transcription factors: Atf1, a homolog of mammalian ATF2; and Pap1, a homolog of c-Jun. (Left) In unstressed cells Sty1/Spc1 and Pap1 are cytoplasmic, whereas Atf1 is nuclear. Pap1 is maintained in the cytoplasm via active nuclear export by Crm1/Exportin 1. (Middle) After osmotic stress Sty1/Spc1 becomes doubly phosphorylated on threonine and tyrosine and enters the nucleus, possibly as a result of binding a nuclear import protein (green). Once in the nucleus Sty1/Spc1 phosphorylates and activates the Atf1 transcription factor to induce transcription of a subset of Atf1-dependent genes. (Right) In response to oxidative stress, Pap1 physically associates with doubly phosphorylated Sty1/Spc1 in the cytoplasm to induce its nuclear entry but is not exported to the cytoplasm because its interaction with Crm1/Exportin is disrupted. In this case, Sty1/Spc1 would be redirected to Pap1-dependent promoters and cause the induction of a distinct set of genes including thioredoxin (*trx2*), thioredoxin reductase (*trr1*), and two ABC transporters (*hba2* and *pmd1*).

accumulation of Sty1/Spc1 (P. Russell, pers. comm.), suggests that this interpretation may not be correct. To formally distinguish between these scenarios, it should be possible, based on studies of JNK and ATF2, to express an Atf1 mutant protein that is able to bind Sty1/Spc1 correctly but that is transcriptionally inactive. These studies raise the question of whether the duration of nuclear localization depends on mechanisms controlling the interaction of MAPK and transcription factor, or on the phosphorylation state of the MAPK. Indeed, it is not known whether inactivation of the MAPK by MAPK phosphatases is a prerequisite for, or occurs as a consequence of, nuclear exit.

MAPK target selection

The studies by Russell and colleagues deal exclusively with the control of Sty1/Spc1 function in response to osmotic stress (Gaits et al. 1998). However, induction of several genes, including *ctt1* (catalase), *trx2* (thioredoxin), and *trr1* (thioredoxin reductase), by oxidative

stress in fission yeast requires Sty1/Spc1 but not Atf1, suggesting that Sty1/Spc1 targets an additional transcription factor. A clue to the identity of this factor was provided by the observation that induction of the same enzymes by oxidative stress in budding yeast, namely *CTT1*, *TRX2*, and *TRR1*, requires the AP-1 like transcription factor Yap1. This focused attention on a c-Jun homolog in fission yeast, the Pap1 transcription factor (Toda et al. 1991). Jones and colleagues now show that Pap1 is specifically required for the induction of *ctt1*, *trx2*, and *trr1* in response to oxidative stress, and that Pap1 is also controlled by the Sty1/Spc1 MAPK (Toone et al. 1998). These studies further emphasize the structural and functional similarity between the fission yeast and mammalian SAPK pathways.

One is now led to ask how activated Sty1/Spc1 can be directed to either Atf1-dependent or Pap1-dependent promoters depending on the stimulus. A potential answer to this conundrum was provided by the observation that Yap1 enters the nucleus in response to oxidative stress in budding yeast (Kuge and Jones 1994; Kuge et al. 1997). In

this issue, Jones and colleagues demonstrate that Pap1 enters the nucleus when fission yeast cells are challenged with an oxidative stress. Remarkably, entry of Pap1 into the nucleus is abolished in cells lacking the Sty1/Spc1 MAPK (Toone et al. 1998). At first sight, these observations suggest that Sty1/Spc1 either phosphorylates Pap1 or a component of the nucleocytoplasmic transport machinery to induce Pap1 translocation to the nucleus. However, neither osmotic stress nor overexpression of the Wis1 MAPKK (which fully activates Sty1/Spc1) is sufficient to drive Pap1 into the nucleus, indicating that activation of Sty1/Spc1 alone is insufficient for Pap1 nuclear localization. This suggests that oxidative stress triggers Pap1 to undergo nuclear translocation by a second Sty1-independent signal. In principle, relocation of a protein from the cytoplasm to the nucleus could result either from increased nuclear import or by inhibition of nuclear export. Importantly, the cysteine-rich domain (CRD) in the carboxyl terminus of budding yeast Yap1 contains a functional NES that binds a homolog of the nuclear export factor Crm1 (Kuge et al. 1997; S. Kuge, T. Toda, N. Jones, N. Iizuka, and A. Nomoto, in prep.). Mutations of the conserved cysteine residues within the NES or removal of the entire sequence prevents interaction of Yap1 with Crm1 and results in constitutive nuclear localization (S. Kuge, T. Toda, N. Jones, N. Iizuka, and A. Nomoto, in prep.). In one model, oxidative stress may cause a conformational change in the NES by oxidation of Cys–Cys disulfide bridges such that interaction with Crm1 is disrupted and nuclear export of Yap1 is inhibited. Pap1 contains a homologous cysteine-rich NES in its carboxyl terminus and inactivation of the fission yeast Crm1 homolog results in nuclear accumulation of Pap1, suggesting that Pap1 is regulated in a similar manner by the nuclear export machinery (Toda et al. 1992). Because Sty1/Spc1 physically interacts with Pap1 (W. Toone, M. Samuels, and N. Jones, pers. comm.), it is possible that Sty1/Spc1 carries Pap1 into the nucleus. What then would be the role of Sty1/Spc1? As Pap1 nuclear entry is enhanced in oxidatively stressed cells overexpressing the Wis1 MAPKK, it seems likely that either dual phosphorylation or activity of Sty1/Spc1 contributes to the transport process. To distinguish between these possibilities it is necessary to determine whether a catalytically inactive Sty1/Spc1 that has its phosphoacceptor sites intact can support Pap1 entry into the nucleus.

A model of stimulus-specific gene transcription

In their simplest interpretation, the results from both the Jones group and the Russell group provide an attractive means by which interdependent nucleocytoplasmic transport of MAPKs and transcription factors determines specificity at the level of gene transcription. An attractive model is that in response to osmotic stress, doubly phosphorylated Sty1/Spc1 enters the nucleus and is preferentially directed to Atf1-dependent promoters (Fig. 1). On the other hand, oxidative stress induces a conformational change of Pap1 such that it can no longer bind

Crm1 but can bind to doubly phosphorylated Sty1/Spc1 in the cytoplasm. After entering the nucleus the Pap1–Sty1/Spc1 complex would be redirected to Pap1-dependent promoters (Fig. 1). One can make certain predictions based on this model. If Atf1 plays no role in oxidative stress-induced gene transcription it presumably is not required either for the translocation of Pap1 or for the retention of Sty1/Spc1 in the nucleus in response to oxidative stress. Conversely, as Pap1 is not required for induction of genes in response to osmotic stress, Pap1 is unlikely to be required for Sty1/Spc1 translocation or nuclear retention in response to this stress. Unfortunately this model is likely to be too simplistic because induction of some genes by oxidative stress, for instance, the Pyp2 MAPK phosphatase, also requires Atf1 (Wilkinson et al. 1996). Clearly many of the mechanistic details underlying these processes have yet to be unraveled, but answers may not be too far away.

It should be noted that although Pap1 shares significant similarity to c-Jun, the mechanism by which Sty1/Spc1 stimulates oxidative stress-induced gene transcription differs from the mechanism by which JNK activates c-Jun, in that Pap1 does not appear to be a direct Sty1/Spc1 substrate and AP-1 complexes generally appear to be constitutively bound to the promoters of target genes (Rozek and Pfeifer 1993; Herr et al. 1994; W. Toone, M. Samuels, and N. Jones, pers. comm.). One is led to ask whether Sty1/Spc1 plays any direct role in stimulating Pap1-dependent gene transcription in addition to its role in regulating Pap1 nuclear entry. This is not an unreasonable notion as c-Jun can recruit JNK to phosphorylate dimerization partners via specific docking interactions (Kallunki et al. 1996). At least three ATF family members have been identified in fission yeast in addition to Atf1, namely Pcr1, Atf21, and Cbp1 (Takeda et al. 1995; Kanoh et al. 1996; Shiozaki and Russell 1996; Watanabe and Yamamoto 1996; J.B.A. Millar and N. Jones, unpubl.), although it is not yet known whether any of these can serve as Sty1/Spc1 substrates *in vivo*. Nevertheless, these proteins can potentially form a wide range of homodimers and heterodimers between themselves and Pap1, each of which may have a distinct function.

The Sty1/Spc1 MAP kinase pathway, in addition to its role in controlling the cellular response to environmental stress, is required for sexual conjugation and differentiation in fission yeast. The sexual differentiation process is triggered by poor nutrient conditions that cause activation of Sty1/Spc1 (Shiozaki and Russell 1996). Under these conditions, activation of Sty1/Spc1 is required for induction of the HMG box-containing transcription factor Ste11, which is critical for both sexual conjugation and meiotic progression (Shiozaki and Russell 1996; Wilkinson et al. 1996). Remarkably, induction of Ste11 also requires Atf1 and cells that lack Atf1 are sterile (Takeda et al. 1995; Kanoh et al. 1996). These results indicate that Sty1/Spc1 controls the cellular differentiation process by controlling Atf1. However, Ste11 and the sexual differentiation process are not induced by environmental stress even though Sty1/Spc1 is activated under both conditions and each is dependent on Atf1. Like-

wise, targets of Atf1 that are induced by environmental stress, such as the Pyp2 MAPK phosphatase, are not induced by nutrient limitation (M. Wilkinson and J.B.A. Millar, unpubl.). A possible explanation for these results may also lie in the stress-specific nucleocytoplasmic transport of MAPK and transcription factor. In response to nutrient limitation, the key player may be the ATF family member Pcr1, whose functions appear limited to the sexual conjugation and differentiation process (Watanabe and Yamamoto 1996). By analogy with Pap1, one could envisage a scenario in which Pcr1 receives a signal initiated by nutrient limitation (possibly by lowered cAMP levels) that causes Pcr1 to bind to Sty1/Spc1 and redirect it to Pcr1/Atf1-dependent promoters. However, the observation that Pcr1 is required for nuclear localization of Atf1 suggests that alternative explanations are also possible (Gaits et al. 1998).

Sty1/Spc1 as a model of mammalian SAPKs

These studies emphasize that the fission yeast Sty1/Spc1 kinase pathway is more closely related to the mammalian SAPK pathway rather than the budding yeast HOG1 pathway. Indeed, as both the mammalian and fission yeast SAPKs are activated by a similar range of environmental stimuli, one may speculate that the pathways are controlled by an evolutionarily conserved stress sensor. Curiously, despite the fact that budding yeast HOG1 is 83% identical in amino acid sequence to Sty1/Spc1, it is activated solely by osmotic stress, the reason for which is unclear (Schüller et al. 1994). Moreover, HOG1 targets one of two zinc finger-containing transcription factors, Msn2 and Msn4, that are unrelated to either ATF-2 or c-Jun (Schüller et al. 1994; Martinez Pastor et al. 1996). Indeed, there appear to be no ATF family members in the budding yeast genome.

Indirect evidence suggests that the similarity between fission yeast and mammalian SAPK pathways extends beyond a structural conservation of transcription factors. For example, Jones and colleagues show that two genes under the control of the Sty/Spc1-Pap1 pathway encode the Hba2/Bfr1 and Pmd1 ATP-binding cassette (ABC) transporters. Hba2/Bfr1 and Pmd1 are homologous to the human *MDR1* gene (multidrug resistance gene), a P glycoprotein involved in energy-dependent export of drugs used in cancer chemotherapy (Higgins et al. 1992; Nagao et al. 1995). Significantly, *MDR1* is induced in mammalian cells by many of the same stresses that activate JNK and p38. It is conceivable then that inappropriate activation of SAPK pathways contributes to the multidrug resistant phenotype of many cancer cells. Inhibiting SAPK-mediated induction of ABC transporters may be an effective means of sensitizing tumorigenic cells to chemotherapeutic agents by impairing the ability of cells to exclude such drugs. A word of caution though—some cancer therapeutic agents, such as cis-platinum, that cause DNA damage can promote programmed cell death via activation of the SAPK pathways (Zanke et al. 1996). By inhibiting SAPK function one may also abrogate the ability of tumorigenic cells to un-

dergo apoptosis. These observations illustrate the importance of understanding how the stimulus, mechanism, and duration of SAPK activation determines the ultimate cellular response. Intriguingly, fission yeast cells lacking Sty1/Spc1 are also sensitive to DNA-damaging agents such as UV light or cis-platinum, which raises the exciting possibility that the Sty1/Spc1 and SAPK pathways control a common aspect of the DNA repair process (Degols and Russell 1997; Toone et al. 1998). Further analysis of the fission yeast Sty1/Spc1 MAPK pathway will undoubtedly help us understand not only the mechanisms that determine stress-regulated nucleocytoplasmic transport but also the regulation and function of SAPKs in various pathological and physiological conditions in mammals.

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References

- Cavigelli, M., F. Dolfi, F.X. Claret, and M. Karin. 1995. Induction of *c-fos* expression through JNK-mediated TCF/Elk-1 phosphorylation. *EMBO J.* **14**: 5957–5964.
- Chen, R.H., C. Sarnecki, and J. Blenis. 1992. Nuclear localization and regulation of erk- and rsk-encoded protein kinases. *Mol. Cell. Biol.* **12**: 915–927.
- Degols, G. and P. Russell. 1997. Discrete roles of the Spc1 kinase and the Atf1 transcription factor in the UV response of *Schizosaccharomyces pombe*. *Mol. Cell. Biol.* **17**: 3356–3363.
- Degols, G., K. Shiozaki, and P. Russell. 1996. Activation and regulation of the Spc1 stress-activated protein kinase in *Schizosaccharomyces pombe*. *Mol. Cell. Biol.* **16**: 2870–2877.
- Dérjard, B., M. Hibi, I.-H. Wu, T. Barrett, B. Su, T. Deng, M. Karin, and R.J. Davis. 1994. JNK1: A protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-jun activation domain. *Cell* **76**: 1025–1037.
- Dikic, I., J. Schlessinger, and I. Lax. 1994. PC12 cells overexpressing the insulin receptor undergo insulin-dependent neuronal differentiation. *Curr. Biol.* **4**: 702–708.
- Fornerod, M., M. Ohno, M. Yoshida, and I.W. Mattaj. 1997. CRM1 is an export receptor for leucine-rich nuclear export signals. *Cell* **90**: 1051–1060.
- Freshney, N.W., L. Rawlinson, F. Guesdon, E. Jones, S. Cowley, J. Hsuan, and J. Saklatvala. 1994. Interleukin-1 activates a novel protein kinase cascade that results in the phosphorylation of hsp27. *Cell* **78**: 1039–1049.
- Fukuda, M., I. Gotoh, Y. Gotoh, and E. Nishida. 1996. Cytoplasmic localization of mitogen-activated protein kinase kinase directed by its NH₂-terminal, leucine-rich short amino acid sequence, which acts as a nuclear export signal. *J. Biol. Chem.* **271**: 20024–20028.
- Fukuda, M., I. Gotoh, M. Adachi, Y. Gotoh, and E. Nishida. 1997a. A novel regulatory mechanism in the mitogen-activated protein (MAP) kinase cascade. *J. Biol. Chem.* **272**: 32642–32648.
- Fukuda, M., S. Asano, T. Nakamura, M. Adachi, M. Yoshida, M.

- Yanagida, and E. Nishida. 1997b. CRM1 is responsible for intracellular transport mediated by the nuclear export signal. *Nature* **390**: 308–311.
- Gaits, F., G. Degols, K. Shiozaki, and P. Russell. 1998. Phosphorylation and association with the transcription factor Atf1 regulate localization of Spc1 stress-activated kinase in fission yeast. *Genes & Dev.* (this issue).
- Galcheva-Gargova, Z., B. Dérjard, I.-H. Wu, and R.J. Davis. 1994. An osmosensing signal transduction pathway in mammalian cells. *Science* **265**: 806–808.
- Gonzalez, F.A., A. Seth, D.L. Raden, D.S. Bowman, F.S. Fay, and R.J. Davis. 1993. Serum-induced translocation of mitogen-activated protein kinase to the cell surface ruffling membrane and the nucleus. *J. Cell Biol.* **122**: 1089–1101.
- Gupta, S., D. Campbell, B. Dérjard, and R.J. Davis. 1995. Transcription factor ATF2 regulation by the JNK signal transduction pathway. *Science* **267**: 389–393.
- Gupta, S., T. Barrett, A.J. Whitmarsh, J. Cavanagh, H.K. Sluss, B. Dérjard, and R.J. Davis. 1996. Selective interaction of JNK protein kinase isoforms with transcription factors. *EMBO J.* **15**: 2760–2770.
- Han, J., J.-D. Lee, L. Bibbs, and R.J. Ulevitch. 1994. A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* **265**: 808–811.
- Herr, I., H. van Dam, and P. Angel. 1994. Binding of promoter-associated AP-1 is not altered during induction and subsequent repression of the c-jun promoter by TPA and UV irradiation. *Carcinogenesis* **15**: 1105–1113.
- Hibi, M., L. Anning, T. Smeal, A. Minden, and M. Karin. 1993. Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes & Dev.* **7**: 2135–2148.
- Higgins, C.F. 1992. ABC transporters: From microorganisms to man. *Annu. Rev. Cell Biol.* **8**: 67–113.
- Kallunki, T., T. Deng, M. Hibi, and M. Karin. 1996. c-Jun can recruit JNK to phosphorylate dimerization partners via specific docking interactions. *Cell* **87**: 929–939.
- Kallunki, T., B. Su, I. Tsigelny, H.K. Sluss, B. Dérjard, G. Moore, R. Davis, and M. Karin. 1994. JNK2 contains a specificity-determining region responsible for efficient c-Jun binding and phosphorylation. *Genes & Dev.* **8**: 2996–3007.
- Kanoh, J., Y. Watanabe, M. Ohsugi, Y. Iino, and M. Yamamoto. 1996. *Schizosaccharomyces pombe gad7⁺* encodes a phosphoprotein with a bZIP domain, which is required for proper G₁ arrest and gene expression under nitrogen starvation. *Genes Cells* **1**: 391–408.
- Karin, M., Z.-G. Liu, and E. Zandi. 1997. AP-1 function and regulation. *Curr. Opin. Cell Biol.* **9**: 240–246.
- Kato, T., K. Okazaki, H. Murakami, S. Stettler, P. Fantes, and H. Okayama. 1996. Stress signal, mediated by a HOG1-like MAP kinase, controls sexual development in fission yeast. *FEBS Lett.* **378**: 207–212.
- Kuge, S. and N. Jones. 1994. YAP1 dependent activation of TRX2 is essential for the response of *Saccharomyces cerevisiae* to oxidative stress by superoxides. *EMBO J.* **13**: 655–664.
- Kuge, S., N. Jones, and A. Nomoto. 1997. Regulation of yAP-1 nuclear localization in response to oxidative stress. *EMBO J.* **16**: 1710–1720.
- Kyriakis, J.M., P. Banerjee, E. Nikolakaki, T. Dai, E.A. Rubie, M.F. Ahmad, J. Avruch, and J.R. Woodgett. 1994. The stress-activated protein kinase family of c-jun kinases. *Nature* **369**: 156–160.
- Lee, J.C., J.T. Laydon, P.C. McDonnell, T.F. Gallagher, S. Kumar, D. Green, D. McNulty, M.J. Blumenthal, J.R. Heys, S.W. Landvatter, J.E. Strickler, M.M. McLaughlin, I.V. Siemens, S.M. Fisher, G.P. Livi, J.R. White, J.L. Adams, and P.R. Young. 1994. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* **372**: 739–746.
- Lenormand, P., C. Sardet, G. Pages, G. L'Allemain, A. Brunet, and J. Pouyssegur. 1993. Growth factors induce nuclear translocation of MAP kinases (p42mapk and p44mapk) but not of their activator MAP kinase kinase (p45mapkk) in fibroblasts. *J. Cell Biol.* **122**: 1079–1088.
- Livingstone, C., G. Patel, and N. Jones. 1995. ATF-2 contains a phosphorylation-dependent transcriptional activation domain. *EMBO J.* **14**: 1785–1797.
- Martinez Pastor, M.T., G. Marchler, C. Schüller, A. Marchler Bauer, H. Ruis, and F. Estruch. 1996. The *Saccharomyces cerevisiae* zinc finger proteins Msn2p and Msn4p are required for transcriptional induction through the stress response element (STRE). *EMBO J.* **15**: 2227–2235.
- Millar, J.B.A., V. Buck, and M.G. Wilkinson. 1995. Pyp1 and Pyp2 PTPases dephosphorylate an osmosensing MAP kinase controlling cell size at division in fission yeast. *Genes & Dev.* **9**: 2117–2130.
- Nagao, K., Y. Taguchi, M. Arioka, H. Kadokura, A. Takatsuki, K. Yoda, and M. Yamasaki. 1995. bfr1+, a novel gene of *Schizosaccharomyces pombe* which confers brefeldin A resistance, is structurally related to the ATP-binding cassette superfamily. *J. Bacteriol.* **177**: 1536–1543.
- Nguyen, T.T., J.C. Scimeca, C. Filloux, P. Peraldi, J.L. Carpentier, and E. Van Obberghen. 1993. Co-regulation of the mitogen-activated protein kinase, extracellular signal-regulated kinase 1, and the 90-kDa ribosomal S6 kinase in PC12 cells. Distinct effects of the neurotrophic factor, nerve growth factor, and the mitogenic factor, epidermal growth factor. *J. Biol. Chem.* **268**: 9803–9810.
- Ossareh-Nazari, B., F. Bachelier, and C. Dargemont. 1997. Evidence for a role of CRM1 in signal-mediated nuclear protein export. *Science* **278**: 141–144.
- Pulverer, B.J., J.M. Kyriakis, J. Avruch, E. Nikolakaki, and J.R. Woodgett. 1991. Phosphorylation of c-jun mediated by MAP kinases. *Nature* **353**: 670–674.
- Robinson, M.J. and M.H. Cobb. 1997. Mitogen-activated protein kinase pathways. *Curr. Opin. Cell Biol.* **9**: 180–186.
- Rouse, J., P. Cohen, S. Trigon, M. Morange, A. Alonso-Llamazares, D. Zamanillo, T. Hunt, and A. Nebreda. 1994. A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. *Cell* **78**: 1027–1037.
- Rozek, D. and G.P. Pfeifer. 1993. In vivo protein-DNA interactions at the c-jun promoter: preformed complexes mediate the UV response. *Mol. Cell. Biol.* **13**: 5490–5499.
- Schüller, C., J.L. Brewster, M.R. Alexander, M.C. Gustin, and H. Ruis. 1994. The HOG pathway controls osmotic regulation of transcription via the stress response element (STRE) of the *Saccharomyces cerevisiae* CTT1 gene. *EMBO J.* **13**: 4382–4389.
- Shieh, J.-C., M.G. Wilkinson, V. Buck, B. Morgan, K. Makino, and J.B.A. Millar. 1997. The Mcs4 response regulator coordinately controls the stress activated Wak1-Wis1-Sty1 MAP kinase pathway and fission yeast cell cycle. *Genes & Dev.* **11**: 1008–1022.
- Shiozaki, K. and P. Russell. 1995. Cell cycle control linked to extracellular environment by MAP kinase pathway in fission yeast. *Nature* **378**: 739–743.
- . 1996. Conjugation, meiosis, and the osmotic stress response are regulated by Spc1 kinase through Atf1 transcription factor in fission yeast. *Genes & Dev.* **10**: 2276–2288.
- Smeal, T., B. Binetruy, D.A. Mercola, M. Birrer, and M. Karin. 1991. Oncogenic and transcriptional cooperation with Ha-

- Ras requires phosphorylation of c-Jun on serines 63 and 73. *Nature* **354**: 494–496.
- Stade, K., C.S. Ford, C. Guthrie, and K. Weis. 1997. Exportin 1 (Crm1p) is an essential nuclear export factor. *Cell* **90**: 1041–1050.
- Takeda, T., T. Toda, K. Kominami, A. Kohnosu, M. Yanagida, and N. Jones. 1995. *Schizosaccharomyces pombe atf1⁺* encodes a transcription factor required for sexual development and entry into stationary phase. *EMBO J.* **14**: 6193–6208.
- Toda, T., M. Shimanuki, and M. Yanagida. 1991. Fission yeast genes that confer resistance to staurosporine encode an AP-1-like transcription factor and a protein kinase related to the mammalian ERK1/MAP2 and budding yeast FUS3 and KSS1 kinases. *Genes & Dev.* **5**: 60–73.
- Toda, T., M. Shimanuki, Y. Saka, H. Yamano, Y. Adachi, M. Shirakawa, Y. Kyogoku, and M. Yanagida. 1992. Fission yeast pap1-dependent transcription is negatively regulated by an essential nuclear protein, crm1. *Mol. Cell. Biol.* **12**: 5474–5484.
- Toone, W.M., S. Kuge, M. Samuels, B.A. Morgan, T. Toda, and N. Jones. 1998. Regulation of the fission yeast transcription factor Pap1 by oxidative stress: Requirement for the nuclear export factor Crm1 (Exportin) and the stress activated MAP kinase Sty1. *Genes & Dev.* (this issue).
- Traverse, S., N. Gomez, H. Paterson, C. Marshall, and P. Cohen. 1992. Sustained activation of the mitogen-activated protein (MAP) kinase cascade may be required for differentiation of PC12 cells. Comparison of the effects of nerve growth factor and epidermal growth factor. *Biochem. J.* **288**: 351–355.
- Traverse, S., K. Seedorf, H. Paterson, C.J. Marshall, P. Cohen, and A. Ullrich. 1994. EGF triggers neuronal differentiation of PC12 cells that overexpress the EGF receptor. *Curr. Biol.* **4**: 694–701.
- Treisman, R. 1996. Regulation of transcription by MAP kinase cascades. *Curr. Opin. Cell Biol.* **8**: 205–215.
- Ullman, K.S., M.A. Powers, and D.J. Forbes. 1997. Nuclear export receptors: from importin to exportin. *Cell* **90**: 967–970.
- Watanabe, Y. and M. Yamamoto. 1996. *Schizosaccharomyces pombe pcr1⁺* encodes a CREB/ATF protein involved in regulation of gene expression for sexual differentiation. *Mol. Cell. Biol.* **16**: 704–714.
- Wilkinson, M.G., M. Samuels, T. Takeda, T. Toda, M.W. Toone, J.-C. Shieh, J.B.A. Millar, and N.C. Jones. 1996. The Atf1 transcription factor is a target for the Sty1 stress activated MAP kinase pathway in fission yeast. *Genes & Dev.* **10**: 2289–2301.
- Zanke, B.W., K. Boudreau, E. Rubie, E. Winnett, L.A. Tibbles, L. Zon, J. Kyriakis, F.-F. Liu, and J.R. Woodgett. 1996. The stress activated protein kinase pathway mediates cell death following injury induced by cis-platinum, UV irradiation of heat. *Curr. Biol.* **6**: 606–613.
- Zheng, C.F. and K.-L. Guan. 1994. Cytoplasmic localization of the mitogen-activated protein kinase activator MEK. *J. Biol. Chem.* **269**: 19947–19952.

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Marc G. Wilkinson and Jonathan B.A. Millar

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