The EMBO Journal Vol.17 No.13 pp.3556–3564, 1998

Yeast PKA represses Msn2p/Msn4p-dependent gene expression to regulate growth, stress response and glycogen accumulation

Angela Smith, Mary P.Ward[1] and Stephen Garrett[2,3]

Department of Biochemistry, Duke University Medical Center, Durham, NC 27710, [1]Department of Biochemistry and Biophysics, School of Medicine at UNC, Chapel Hill, NC 27599-7260 and [2]Department of Microbiology and Molecular Genetics, UMDNJ, New Jersey Medical School, Newark, NJ 07103-2714, USA

[3]Corresponding author
e-mail: garretst@umdnj.edu

Yeast cAMP-dependent protein kinase (PKA) activity is essential for growth and antagonizes induction of the general stress response as well as accumulation of glycogen stores. Previous studies have suggested that the PKA effects on the two latter processes result in part from transcription repression. Here we show that transcription derepression that accompanies PKA depletion is dependent upon the presence of two redundant Zn^{2+}-finger transcription factors, Msn2p and Msn4p. The Msn2p and Msn4p proteins were shown previously to act as positive transcriptional factors in the stress response pathway, and our results suggest that Msn2p and Msn4p also mediate PKA-dependent effects on stress response as well as glycogen accumulation genes. Interestingly, PKA activity is dispensable in a strain lacking Msn2p and Msn4p activity. Thus, Msn2p and Msn4p may antagonize PKA-dependent growth by stimulating expression of genes that inhibit growth. In agreement with this model, Msn2p/Msn4p function is required for expression of a gene, *YAK1*, previously shown to antagonize PKA-dependent growth. These results suggest that Msn2p/Msn4p-dependent gene expression may account for all, or at least most, of the pleiotropic effects of yeast PKA, including growth regulation, response to stress and carbohydrate store accumulation.

Keywords: growth/Msn2p/PKA/*Saccharomyces cerevisiae*/*YAK1*

Introduction

All living cells employ mechanisms that allow survival under conditions of environmental stress. The best studied of these has been termed the heat shock response and involves the induction of heat shock gene transcription in response to temperature shift. In eukaryotes, temperature shift results in activation of the ubiquitous heat shock transcription factor (HSF) which recognizes specific elements (HSEs) in HSF-dependent promoters (Wu *et al*., 1994; Mager and De Kruijff, 1995). The yeast *Saccharomyces cerevisiae* also employs a general, or global, stress response pathway that functions independently of HSF (and HSE) and is stimulated by a wide variety of stresses. Such stresses include temperature shift, osmotic shock, nutrient starvation, as well as DNA and oxidative damage (Mager and De Kruijff, 1995; Ruis and Schuller, 1995). The general stress response also differs from the heat shock response in that the spectrum of genes induced by these environmental stimuli is broad. Genes considered part of the general stress response include the cytosolic catalase T gene *CTT1*, the DNA damage-responsive gene *DDR2*, the heat shock gene *HSP12* and genes (*TSP2* and *GLC3*) involved in carbon store accumulation (Mager and De Kruijff, 1995; Ruis and Schuller, 1995).

Although the precise mechanism of the general stress response pathway has not been elucidated, recent studies have implicated the related Zn^{2+}-finger transcription factors Msn2p and Msn4p (Martinez-Pastor *et al*., 1996; Schmitt and McEntee, 1996) in this process. Strains lacking *MSN2* and *MSN4* are sensitive to various forms of stress and fail to accumulate stress-regulated messages following heat and osmotic stress, as well as nutrient starvation and DNA damage (Martinez-Pastor *et al*., 1996; Schmitt and McEntee, 1996). Moreover, Msn2p binds specifically to DNA sequences, referred to as the stress response element or STRE (Martinez-Pastor *et al*., 1996; Schmitt and McEntee, 1996), previously shown to be necessary and sufficient to impart stress-induced expression on a heterologous reporter gene (Marchler *et al*., 1993). One attractive hypothesis is that Msn2p and the STREs represent the 'integration point' of multiple signaling pathways that respond to diverse stresses and induce the same set of genes (Martinez-Pastor *et al*., 1996). In support of this hypothesis, osmotic induction of the stress response genes is impaired by mutations that inactivate the high osmolarity glycerol response (HOG) pathway or Msn2p/Msn4p function. By contrast, heat shock- and nitrogen starvation-dependent expression of the same genes is independent of the HOG pathway (Schuller *et al*., 1994; Martinez-Pastor *et al*., 1996). However, the HOG pathway has not been linked directly to Msn2p-dependent gene expression, and substantial Msn2p-dependent osmotic induction remains in a *hog1*Δ background (Martinez-Pastor *et al*., 1996). Moreover, the stress response signaling mechanism may be more complicated than first appreciated because STRE-dependent transcription, as well as Msn2p nuclear localization, is negatively regulated by yeast cAMP-dependent protein kinase (PKA) activity (Marchler *et al*., 1993; Gorner *et al*., 1998).

Yeast PKA activity has been implicated in numerous cellular processes, including growth, carbon storage, response to stress and differentiation (Cameron *et al*., 1988; Broach and Deschenes, 1990; Gimeno *et al*., 1992). PKA-deficient cells arrest in G_1, accumulate storage carbohydrates (glycogen and trehalose) and become resistant to heat and oxidative stress. These phenotypes are similar to the phenotypes displayed by wild-type cells deprived of

© Oxford University Press

nutrients (Johnston *et al*., 1977). In contrast, cells with elevated PKA activity fail to store carbohydrate reserves and are exquisitely sensitive to various forms of stress (Cannon and Tatchell, 1987; Cameron *et al*., 1988). These phenotypes have been taken as evidence that the yeast PKA pathway plays a central role in coordinating cell growth and metabolism in response to environmental stimuli (Broach and Deschenes, 1990; Markwardt *et al*., 1995).

At least some of the physiological effects of PKA can be accounted for by changes in transcription. For example, PKA activity inhibits both STRE-dependent expression of stress response genes (Belazzi *et al*., 1991; Engleberg *et al*., 1994; Varela *et al*., 1995) and expression of genes (*GAC1*, *GSY2*) essential to glycogen synthesis (Francois *et al*., 1992; Hardy *et al*., 1994). By contrast, the mechanism by which PKA affects yeast growth is unknown. Although ribosomal protein synthesis is positively regulated by PKA (Klein and Struhl, 1994; Neuman-Silberberg *et al*., 1995), PKA effects on ribosomal protein synthesis cannot account for the PKA growth requirement (Neuman-Silberberg *et al*., 1995).

One hint that PKA might regulate growth, stress response and glycogen accumulation by a common mechanism came from studies of the high-copy suppressor *SOK2* (Ward *et al*., 1995). Like PKA, Sok2p affected growth, sensitivity to stress and glycogen stores. Moreover, the effects of Sok2p on the latter two processes were associated with transcriptional repression. Thus, we suggested that Sok2p was a PKA-dependent repressor that regulated expression of one or more genes antagonistic to cell growth and division (Ward *et al*., 1995). Indeed, one candidate for such a growth-inhibitory function had been identified previously as a recessive suppressor of a PKA-deficient strain (Garrett and Broach, 1989). Strains lacking the three, redundant PKA catalytic subunit genes (*TPK1*, *TPK2* and *TPK3*) grew slowly if the *YAK1* gene was also deleted (Garrett and Broach, 1989). However, Yak1p has not been assigned definitively to the PKA pathway (Hartley *et al*., 1994), and PKA effects on growth, stress and glycogen accumulation are much greater than can be accounted for by disruption or overexpression of *SOK2* (Ward *et al*., 1995).

Here we describe the characterization of three, allelic suppressors of the *tpk*(Ts) growth defect. Although these suppressors are semi-dominant in diploid analysis, they result from the loss of function of the stress-related transcriptional activator, Msn2p (Martinez-Pastor *et al*., 1996; Schmitt and McEntee, 1996). Interestingly, the growth defect of a *tpk*Δ deletion strain is alleviated by the inactivation of Msn2p and its functional homolog, Msn4p. This is the first indication that Msn2p and Msn4p proteins play a role in PKA-dependent growth. Interestingly, *YAK1* may be one of the growth-related genes under Msn2p and Msn4p control because *YAK1* expression is induced by PKA depletion only in strains containing Msn2p (and Msn4p) activity.

Results

Dominant suppressors of the tpk(Ts) growth defect

Sixteen temperature-resistant revertants of haploid *tpk2-63*(Ts) strain SGY398 contained dominant (or semi-dominant) suppressors as judged by diploid analysis (unpublished results). Three of the 16 suppressors (*SOK3-218*, *SOK3-234* and *SOK3-244*; suppressor of kinase) were weak, semi-dominant, tightly linked (no recombinants in 10 tetrads each) and mapped within 20 cM of the *tpk2-63*(Ts) high-copy suppressor gene *SOK2* (data not shown). Three point crosses placed the *SOK3* alleles within 2.2 cM of the chromosome XIII gene *MIH1*, in the order *MIH1–SOK3–MCM1* (Materials and methods; Figure 1). This corresponds to a physical distance of ~6.6 kb (Olson, 1991).

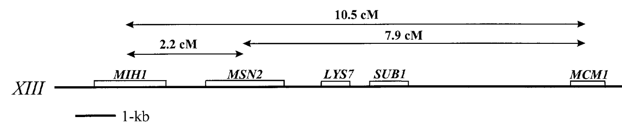

Fig. 1. Genetic mapping of *SOK3* (*msn2*). A bold line denotes the physical region surrounding the *SOK3-234* suppressor. Open boxes above the bold line depict labeled genes. Genetic distances measured in three point crosses between *mih1*::*LEU2*, *SOK3-234* (*msn2-234*) and *MCM1-URA3* are indicated by lines with arrows at either end.

The dominant SOK3 suppressor results from the loss of MSN2 function

Despite varied approaches (random genomic libraries, PCR and gap repair of chromosomal DNA from the region predicted by genetic mapping to contain the *SOK3* alleles), we were unable to clone any of the *SOK3* alleles on the basis of their dominant suppression of the *tpk2-63*(Ts) growth defect. One explanation for this result was that *tpk2*(Ts) suppression resulted from the loss of *SOK3* function. In that scenario, the semi-dominance of the spontaneous *SOK3* suppressors could reflect the limiting nature of the Sok3 protein in diploids (haploinsufficiency). Genetic mapping data suggested that *SOK3* might be identical to *MSN2*, a gene previously shown to encode a non-essential protein involved in the general stress response (Estruch and Carlson, 1991; Martinez-Pastor *et al*., 1996; Schmitt and McEntee, 1996). Interestingly, *msn2*Δ::*HIS3* transformants of *tpk2-63*(Ts) strain SGY446 grew at 33 and 35°C (Figure 2; see below). Growth was the result of the *msn2*Δ deletion because suppression was complemented by the low-copy *MSN2* plasmid pAS15, but not by either its gapped version (pAS16) or a derivative containing chromosomal DNA from the *SOK3-234* strain (pAS24) (data not shown). Thus, the *tpk2-63*(Ts) growth defect is alleviated by loss of Msn2p function. Moreover, a *tpk2-63*(Ts)/*tpk2-63*(Ts) *msn2*Δ::*HIS3*/*MSN2* diploid strain exhibited poor, but demonstrable, growth at the non-permissive temperature of 35°C (data not shown). Thus, inactivation of a single allele of *MSN2* is sufficient to partially alleviate the conditional growth of a *tpk*(Ts)/*tpk*(Ts) diploid. Finally, temperature-resistant growth of the three dominant *tpk2-63*(Ts) revertants (*SOK3-218*, *SOK3-234* and *SOK3-244*) was reversed by the *MSN2* plasmid pAS15, consistent with the notion that, at least by plasmid complementation, the three suppressors are recessive. By these criteria, *SOK3* is allelic with *MSN2* and we have renamed the three alleles *msn2-218*, *msn2-234* and *msn2-244* to reflect their recessive nature in the 'plasmid' complementation assay.

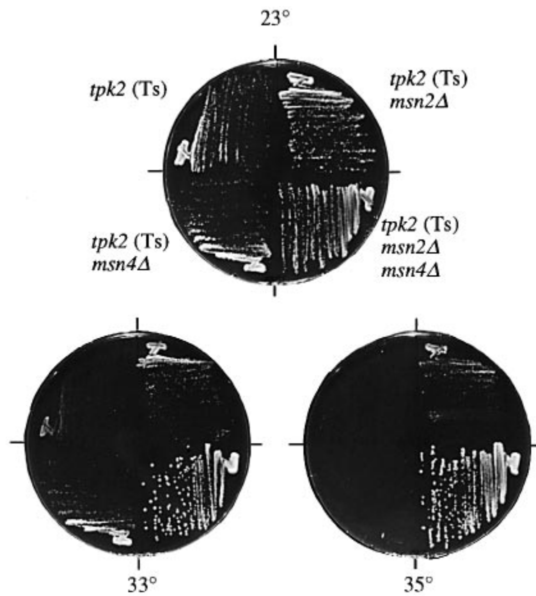

Fig. 2. Suppression of the *tpk*(Ts) growth defect by deletion of *MSN2* and *MSN4*. Isogenic derivatives of the haploid *tpk2-63*(Ts) strain were streaked onto rich medium agar and incubated at 23, 33 and 35°C. The strains were *tpk2*(Ts) [*tpk2-63*(Ts), SGY446], *tpk2*(Ts) *msn2*Δ [*tpk2-63*(Ts) *msn2*Δ::*HIS3*, ASY21], *tpk2*(Ts) *msn2*Δ *msn4*Δ [*tpk2-63*(Ts) *msn2*Δ::*HIS3 msn4*Δ::*LEU2*, ASY29] and *tpk2*(Ts) *msn4*Δ [*tpk2-63*(Ts) *msn4*Δ::*LEU2*, ASY30].

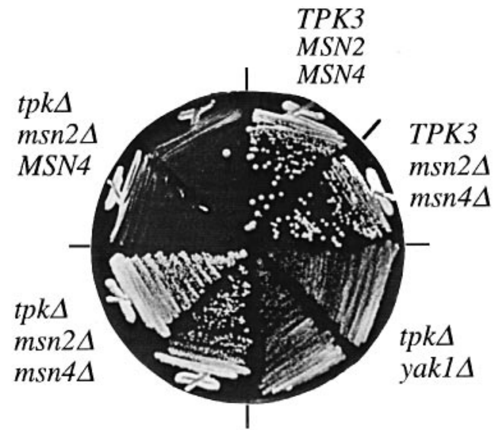

Fig. 3. PKA is dispensable in a strain lacking Msn2p/Msn4p function. Strains were incubated on rich medium agar for 3 days at 30°C. All strains (with the exception of SGP406) were haploid segregants of the heterozygous diploid ASY48 [*tpk1*::*URA3*/*tpk1*::*ADE8 tpk2-63*(Ts)/*tpk2*::*HIS3 tpk3*::*TRP1*/*TPK3 msn2*Δ::*HIS3*/*MSN2 msn4*Δ::*LEU2*/*MSN4*]. The strains were *TPK3 MSN2 MSN4* [*TPK3 MSN2 MSN4*, ASY59], *TPK3 msn2*Δ *msn4*Δ [*TPK3 msn2*Δ::*HIS3 msn4*Δ::*LEU2*, ASY58], *tpk*Δ *yak1*Δ [*tpk*Δ *yak1*Δ::*LEU2*, SGP406], *tpk*Δ *msn2*Δ *msn4*Δ [*tpk*Δ *msn2*Δ::*HIS3 msn4*Δ::*LEU2*, ASY62] and *tpk*Δ *msn2*Δ *MSN4* [*tpk*Δ *msn2*Δ::*HIS3 MSN4*, ASY61].

PKA activity is dispensable in a msn2Δ msn4Δ mutant

Although *MSN2* accounts for the majority of STRE-dependent transcription, the structural homolog, *MSN4*, also plays a significant role (Martinez-Pastor *et al*., 1996; Schmitt and McEntee, 1996). To determine the contribution of both genes to PKA-dependent growth, the *tpk2-63*(Ts) strain SGY446 was transformed with *msn2*Δ::*HIS3* and *msn4*Δ::*LEU2* deletions (Materials and methods). As shown in Figure 2, the conditional growth defect was partially alleviated by either mutation alone (the *msn4*Δ mutation suppressed weakly even at 33°C); however, the *tpk2-63*(Ts) strain lacking all Msn2p and Msn4p activity exhibited robust growth at the elevated temperature (Figure 2). Thus, both Msn2p and Msn4p antagonize PKA-dependent growth.

To test whether deletion of *MSN2*, *MSN4* or both would suppress the growth defect of a strain lacking all PKA activity, haploid strains ASY29 [*MAT*α *tpk1*::*ADE8 tpk2-63*(Ts) *tpk3*::*TRP1 msn2*Δ::*HIS3 msn4*Δ::*LEU2*] and MWY155 (*MAT**a** tpk1*::*URA3 tpk2*::*HIS3 TPK3 MSN2 MSN4*) were mated and sporulated at 23°C. Because the *msn2*Δ and *tpk2*Δ deletions were both marked with *HIS3*, we focused on tetrads that segregated two His^+ colonies and two His^- colonies (the His^+ colonies in these tetrads could be assumed to contain both *tpk2*Δ::*HIS3* and *msn2*Δ::*HIS3*). Eight of 37 tetrads formed two His^+ colonies and two His^- colonies, and nine of the His^+ colonies within this group were Trp^+ (*tpk3*::*TRP1*). Interestingly, five of these His^+ Trp^+ colonies were Leu^+ (*tpk1 tpk2*Δ::*HIS3 tpk3*::*TRP1 msn2*Δ::*HIS3 msn4*Δ::*LEU2*) (Figure 3) and formed colonies that were similar in size to the *TPK3* strains (*TPK3 MSN2 MSN4* and *TPK3 msn2*Δ *msn4*Δ) from the same cross (Figure 3). The other His^+ colonies were Leu^- (*tpk1 tpk2*Δ::*HIS3 tpk3*::*TRP1 msn2*Δ::*HIS3 MSN4*) and formed slow-growing colonies that were smaller than colonies of the *tpk*Δ *yak1*Δ strain, SGP406 (Figure 3). Thus, the *tpk*Δ growth defect is weakly suppressed by Msn2p inactivation and completely alleviated by loss of Msn2p and Msn4p function (the doubling times of strains ASY62 and ASY63 were $<5\%$ longer than the doubling times of ASY58, data not shown). Finally, a strain lacking PKA activity as a result of the deletion of the adenylate cyclase activator genes *RAS1* and *RAS2* (Broach and Deschenes, 1990) exhibited robust growth if *MSN2* and *MSN4* were deleted (data not shown).

MSN2 overproduction exacerbates growth of a tpk(Ts) strain

Because the *tpk*(Ts) growth defect was suppressed by Msn2p inactivation, we determined if the growth defect was exacerbated by *MSN2* overexpression. The *tpk2-63*(Ts) strain SGY448 was transformed with the high-copy *MSN2* plasmid (pAS27), as well as the pRS305-2μ vector control, and purified on selective agar at 23, 30 and 33°C. Even at the 'permissive temperature' of 23°C, the high-copy *MSN2* transformants formed much smaller colonies than the vector transformants (Figure 4). In contrast, the high-copy *MSN2* plasmid had little effect on the growth of the isogenic wild-type *TPK* strain (Figure 7), consistent with previous observations (Estruch and Carlson, 1991). Thus, Msn2p overproduction exacerbates the growth of a PKA-compromised strain. Similar, although less dramatic, results were observed when a high-copy *MSN4* plasmid was transformed into the *tpk2-63*(Ts) strain (data not shown), consistent with the notion that *MSN2* and *MSN4* carry out similar functions.

The *YAK1* gene previously was identified as a loss-of-function suppressor of defects in the PKA pathway (Garrett and Broach, 1989; Hartley *et al*., 1994). The epistasis relationship between Msn2p and Yak1p was tested by asking if the effect of *MSN2* overexpression on *tpk2-63*(Ts) growth required Yak1p function. As shown in

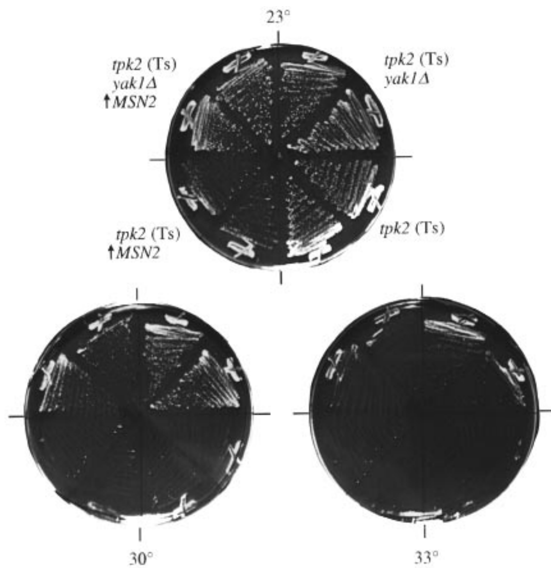

Fig. 4. *MSN2* overproduction exacerbates growth of a *tpk2*(Ts) strain. Strains containing the high-copy *MSN2* plasmid pAS27 (↑*MSN2*) or the control vector pRS305-2μ were streaked onto selective minimal medium agar and incubated at 23, 30 or 33°C. The strains were *tpk2*(Ts) *yak1*Δ [*tpk2-63*(Ts) *yak1*Δ::*ADE8*, SGY470] and *tpk2*(Ts) [*tpk2-63*(Ts), SGY448].

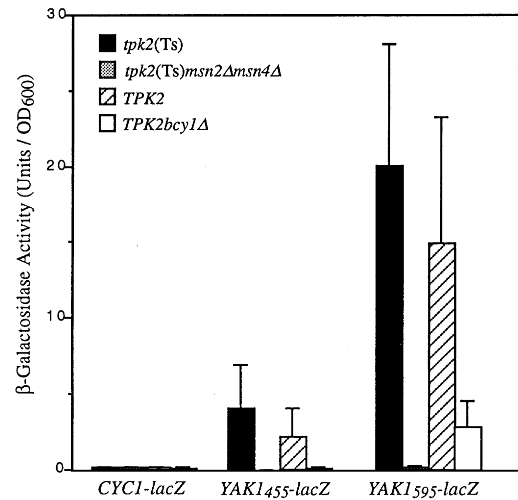

Fig. 5. *YAK1* regulation by PKA and Msn2p/Msn4p. β-Galactosidase activities of strains containing the indicated promoter fusions on high-copy plasmids. Each number represents the average of at least four independent measurements. Plasmids are described in Materials and methods and are based on the promoter-less *CYC1–lacZ* vector pJLB. The strains were *tpk2*(Ts) [*tpk2-63*(Ts), SGY446], *tpk2*(Ts) *msn2*Δ *msn4*Δ [*tpk2-63*(Ts) *msn2*Δ::*HIS3 msn4*Δ::*LEU2*, ASY29], *TPK2* [*TPK2*, ASY18] and *TPK2 bcy1*Δ [*TPK2 bcy1*Δ::*LEU2*, ASY54].

Figure 4, deletion of *YAK1* relieved the debilitating effect of the high-copy *MSN2* plasmid at 23°C, but was not completely epistatic to *MSN2* overexpression at 30 or 33°C. A model in which Yak1p is on a pathway parallel with Msn2p most easily explains this result. However, it can be reconciled with a model in which Yak1p functions downstream of Msn2p as long as Yak1p is not the only Msn2p target affecting growth. This second model is at least consistent with the different strengths of *tpk*Δ suppression by mutations in *MSN2* and *MSN4* versus mutations in *YAK1* (Figure 3).

Msn2p/Msn4p activity is necessary for expression of YAK1

The pleiotropic effects of mutations in *MSN2* and *MSN4* (Figure 3; Martinez-Pastor *et al.*, 1996; Schmitt and McEntee, 1996) can be contrasted with the more specific effects of *YAK1* inactivation (Garrett and Broach, 1989; Garrett *et al.*, 1991; Hartley *et al.*, 1994). This difference is consistent with a model in which Msn2p and Msn4p are required for expression of genes involved in growth inhibition (*YAK1*) as well as sensitivity to stress (*HSP12*, *CTT1* and *DDR2*). In such a model, modulating Msn2p and Msn4p activity would alter *YAK1* expression. To determine if Msn2p and Msn4p regulate *YAK1* expression, we measured β-galactosidase activities of strains containing two different *YAK1–lacZ* promoter fusion plasmids. The *YAK1–lacZ* promoter fusions ($UAS_{YAK1-455}$ and $UAS_{YAK1-595}$) contain the translation start and putative TATA box of *YAK1*, as well as 134 and 274 bp upstream of the putative TATA box, respectively. Whereas the promoter-less control plasmid (*CYC1–lacZ*) was expressed inefficiently in all strains tested, both *YAK1–lacZ* fusions were expressed efficiently in the *tpk2*(Ts) strain as judged by high β-galactosidase activities (Figure 5). Interestingly, neither *YAK1* fusion was expressed in the *tpk2*(Ts) strain lacking Msn2p and Msn4p function (Figure 5). This difference was not due simply to better growth of the *tpk2*(Ts) *msn2*Δ *msn4*Δ strain because both fusions were expressed in an isogenic *TPK2* strain (Figure 5). Moreover, introduction of the *bcy1*Δ::*LEU2* deletion into the *TPK2* strain reduced *YAK1–lacZ* expression, even though strains with elevated PKA activity grow more slowly than wild-type strains. Finally, an unrelated promoter fusion *RCE–lacZ* (Cuevo *et al.*, 1997) was not affected by PKA or Msn2p/Msn4p activity (data not shown).

To confirm that the effects of Msn2p and Msn4p on *YAK1* expression were a reflection of *in vivo* function rather than an artifact of the *YAK1–lacZ* promoter fusions, we monitored mRNA levels of *YAK1*, the glycogen metabolism gene *GLC3* and the heat shock gene *HSP12*. As judged by Northern analyses, the mRNA levels of all three genes were greater in the *tpk2*(Ts) strain than in the *TPK2* strain, and this increase was completely dependent upon Msn2p and Msn4p function (Figure 6A). mRNA levels of the control gene, *ACT1*, are unaffected by either PKA or Msn2p/Msn4p function (Martinez-Pastor *et al.*, 1996) and serve as loading controls. Quantification showed that *YAK1*, *GLC3* and *HSP12* expression was >3-, 6- and 11-fold greater, respectively, in the *tpk2-63*(Ts) strain, SGY446, than in the isogenic *TPK2* strain, ASY18 (Figure 6B). Moreover, deletion of *MSN2* and *MSN4* reduced expression of all three genes to levels that were below (*YAK1*) or similar to (*GLC3* and *HSP12*) levels of the wild-type *TPK2* strain, ASY18 (Figure 6B). Thus, *YAK1* expression is repressed by PKA and is dependent upon Msn2p/Msn4p activity, similarly to expression of the stress-responsive genes *HSP12* and *CTT1* (Figure 6B; Martinez-Pastor *et al.*, 1996).

Msn2p and Msn4p regulate glycogen accumulation

PKA-depleted strains accumulate elevated levels of glycogen, due in part to an increase in expression of genes involved in glycogen synthesis (Figure 6; Francois *et al.*,

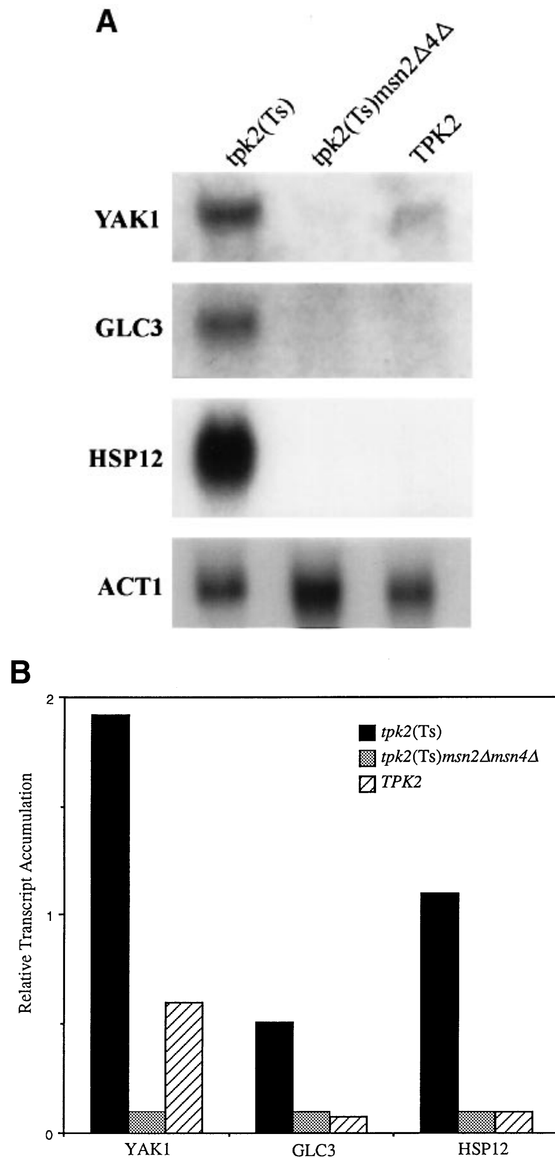

Fig. 6. Transcription regulation by PKA and Msn2p/Msn4p. (**A**) Total RNAs from strains *tpk2*(Ts) [*tpk2-63*(Ts), SGY446], *tpk2*(Ts) *msn2*Δ *msn4*Δ [*tpk2-63*(Ts) *msn2*Δ::*HIS3 msn4*Δ::*LEU2*, ASY29] and *TPK2* [*TPK2*, ASY18] were probed sequentially with the indicated DNAs. (**B**) Quantitation of *YAK1*, *GLC3* and *HSP12* mRNA levels from (A).

1992; Ward *et al.*, 1995). Because induction of at least one (*GLC3*) of those genes was dependent upon Msn2p/Msn4p function (Figure 6), we measured glycogen accumulation by inverting colonies over iodine vapors. Colonies of the *tpk2*(Ts) strain SGY446 stained much darker than colonies of the *tpk2*(Ts) *msn2*Δ::*HIS3 msn4*Δ::*LEU2* strain ASY29, indicating that glycogen accumulation was dependent upon Msn2p/Msn4p function (Figure 7). Indeed, by this measure, the *tpk2*(Ts) *msn2*Δ::*HIS3 msn4*Δ::*LEU2* strain accumulated as little glycogen as the strain (*TPK3 bcy1*Δ::*LEU2*) with elevated PKA activity (Figure 7), suggesting that Msn2p and Msn4p account for all of the effects of PKA on glycogen accumulation.

Diploid strains containing elevated PKA activity fail to sporulate (Broach and Deschenes, 1990). To examine if this effect can also be accounted for by PKA regulation of Msn2p/Msn4p-dependent gene expression, we determined

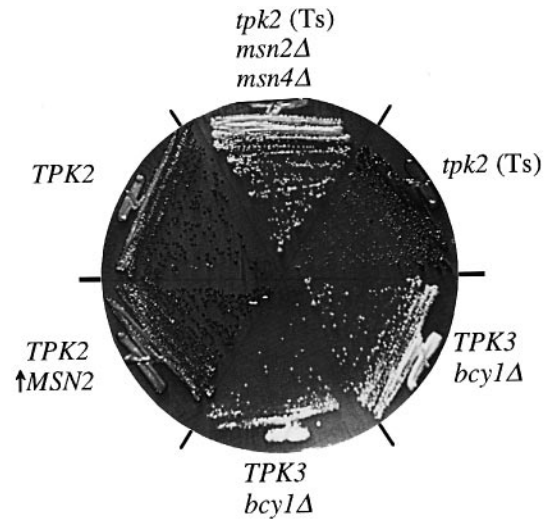

Fig. 7. Msn2p control of glycogen accumulation. Strains were incubated on minimal medium agar for 4 days at 23°C and then exposed to iodine vapors as described (Garrett and Broach, 1989). The strains were *tpk2*(Ts) [*tpk2-63*(Ts), SGY446], *TPK3 bcy1*Δ [*TPK3 bcy1*Δ::*LEU2*, MWY161 and MWY162], *TPK2* ↑*MSN2* [*TPK2* pRS305-2μ-*MSN2*, ASY67], *TPK2* [*TPK2*, ASY18] AND *tpk2*(Ts) *msn2*Δ *msn4*Δ [*tpk2-63*(Ts) *msn2*Δ::*HIS3 msn4*Δ::*LEU2*, ASY29].

the sporulation frequencies of diploid strains containing different levels of Msn2p activity. The homozygous *msn2*Δ/*msn2*Δ *msn4*Δ/*msn4*Δ diploid strain SGY584 was transformed with the low-copy *MSN2* plasmid pAS15 or the vector control (pRS316) and placed in sporulation medium at 30°C. After 3 days, four-spored asci formed 16 and 15% of the population of cells from the pAS15- and pRS316-containing strains, respectively. Thus, neither Msn2p nor Msn4p are required for sporulation, suggesting that PKA does not seem to inhibit sporulation by inactivating Msn2p/Msn4p-dependent transcription.

Msn2p and Sok2p both contribute to PKA-dependent growth regulation

The *SOK2* gene was isolated previously as a pleiotropic high-copy suppressor of the *tpk2-63*(Ts) growth defect (Ward *et al.*, 1995). To determine the relative contribution and order of function of Sok2p and Msn2p in PKA-dependent growth, we asked if *SOK2* overexpression or deletion affected the growth of *tpk2*(Ts) strains containing various levels of Msn2p/Msn4p activity. *SOK2* overexpression enhanced growth of the *tpk2*(Ts) *msn2*Δ *MSN4* strain (Table I); however, this could be explained by an effect on Msn4p function, rather than an effect on a separate pathway. Because the *tpk2*(Ts) *msn2*Δ *msn4*Δ strain did not exhibit a growth defect (Figures 2 and 3), it was not surprising that *SOK2* overexpression had no effect on growth of a strain lacking all Msn2p/Msn4p activity (Table I). Nevertheless, deletion of *SOK2* compromised growth of the *tpk2*(Ts) *msn2*Δ *msn4*Δ strain (Table I), consistent with the notion that Sok2p and Msn2p/Msn4p affect PKA-dependent growth by separate, although perhaps related, mechanisms.

Discussion

Previous reports suggested that the PKA effects on glycogen accumulation and stress response resulted from trans-

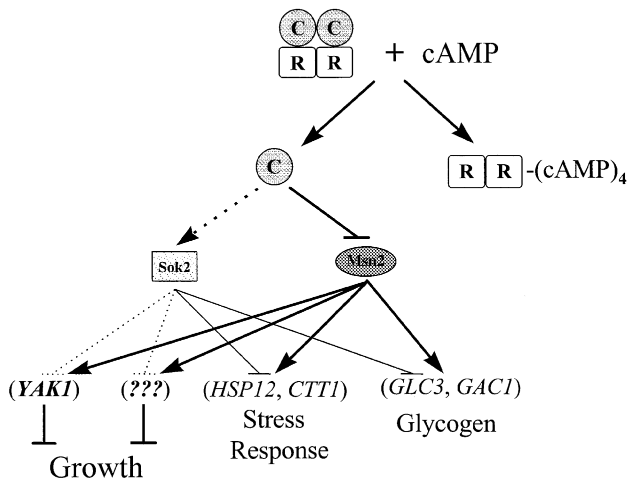

Fig. 8. Model of PKA regulation of genes involved in growth, stress response and glycogen accumulation.

Table I. Tests of epistasis with *SOK2* and *MSN2*

Genotype[a]	Growth at			
	23°C	30°C	33°C	34.5°C
tpk2(Ts) *MSN2 MSN4 SOK2*	+++	++	–	–
tpk2(Ts) *MSN2 MSN4* ↑*SOK2*[b]	+++	+++	++	+
tpk2(Ts) *msn2*Δ *MSN4 SOK2*	+++	+++	++	+
tpk2(Ts) *msn2*Δ *MSN4* ↑*SOK2*	+++	+++	+++	++
tpk2(Ts) *msn2*Δ *msn4*Δ *SOK2*	+++	+++	+++	+++
tpk2(Ts) *msn2*Δ *msn4*Δ ↑*SOK2*	+++	+++	+++	+++
tpk2(Ts) *msn2*Δ *msn4*Δ *sok2*Δ	+++	++	+	–
tpk2(Ts) *MSN2 MSN4 sok2*Δ	++	–	–	–

[a]All strains were derived by transformation of *tpk1 tpk2*(Ts) *tpk3 MSN2 MSN4 SOK2* strain SGY446.
[b]↑*SOK2* is pMW61 (YEp-*SOK2*).

cription repression (Broach and Deschenes, 1990; Ruis and Schuller, 1995), although the precise mechanism was not known. This report shows that transcription repression can also explain the growth requirement for PKA. PKA activity is largely dispensable in a strain lacking the transcription activators Msn2p and Msn4p (Figure 4), suggesting that the single essential function of PKA is to inhibit expression or function of growth-inhibitory genes under Msn2p/Msn4p control (Figure 8). Msn2p and Msn4p were identified recently as the major transcription factors of the yeast multistress response (Martinez-Pastor *et al.*, 1996; Schmitt and McEntee, 1996), and our results show that they play a significant role in the expression of genes involved in glycogen metabolism (Figure 7). Because both PKA and Msn2p/Msn4p regulate these processes at the level of transcription, it seems likely that their opposing effects on growth also reflect antagonistic roles in gene expression. If that model is correct, it suggests that PKA is an integral part of the stress response, which normally involves transient arrest, increased resistance to stress and accumulation of carbon stores (Werner-Washburne *et al.*, 1993; Ruis and Schuller, 1995). Such a model would also fit well with the exquisite stress sensitivity displayed by strains with elevated PKA activity (Broach and Deschenes, 1990).

One prediction of the model shown in Figure 8 is that genes that inhibit PKA-dependent growth should be repressed by PKA and induced by Msn2p/Msn4p. The *YAK1* gene was identified originally as a recessive suppressor of the PKA-deficient growth defect (Garrett and Broach, 1989) and thus behaves as one would expect for an inhibitor of PKA-dependent growth (Hartley *et al.*, 1994). Consistent with the model, *YAK1* expression is inversely correlated with PKA activity (Figures 5 and 6). This result provides a molecular explanation for the observation that Yak1p protein (and activity) is elevated in PKA-depleted strains (Garrett *et al.*, 1991). In addition, *YAK1* expression is absolutely dependent upon Msn2p/Msn4p function, consistent with the presence of three, closely spaced STRE sites within the 150 bp region upstream of the putative *YAK1* TATA box (Garrett and Broach, 1989). Finally, the model is consistent with the observation that an increase in *YAK1* expression restricts growth of a PKA-compromised strain (Garrett *et al.*, 1991; Hartley *et al.*, 1994). Thus, it seems likely that Yak1p activation, by PKA depletion and Msn2p/Msn4p activation, inhibits a cellular process that is essential for growth. Although the nature of that process is unknown, these results should provide additional impetus for further investigation into the physiological and biochemical role of the Yak1p protein kinase.

Msn2p/Msn4p-dependent *YAK1* expression cannot explain all of the growth defects of a PKA-deficient strain. In contrast to the robust growth of a *tpk*Δ *msn2*Δ *msn4*Δ strain (Figure 3), the *tpk*Δ *yak1*Δ strain grows slowly (Figure 3; Garrett and Broach, 1989; Thompson-Jaeger *et al.*, 1992). Moreover, an increase in Yak1p activity is not sufficient to inhibit growth of a wild-type strain (Garrett *et al.*, 1991; Hartley *et al.*, 1994), suggesting that one or more additional genes must be derepressed to restrict growth. Finally, *YAK1* function contributes to, but is not essential for, the toxic effect of *MSN2* overexpression on *tpk*(Ts) growth (Figure 4). Together, these results suggest that *YAK1* is not the only PKA-regulated gene that can inhibit growth. One attractive candidate gene encodes the stress-induced transcriptional repressor, Xbp1p (Mai and Breeden, 1997). The *XBP1* gene contains several STRE consensus sites and is expressed in a manner expected of Msn2p/Msn4p-regulated genes (Mai and Breeden, 1997). Moreover, *XBP1* overexpression results in a slow-growth phenotype, lengthening of G_1 and repression of G_1 cyclin expression (Mai and Breeden, 1997), phenotypes consistent with a factor involved in PKA-dependent growth (Broach and Deschenes, 1990). It remains to be seen if *XBP1* and *YAK1* can together account for the growth defect of a *tpk*Δ strain.

The model presented in Figure 8 suggests that PKA blocks Msn2p-dependent expression through a direct effect on Msn2p function. In support of this interpretation, Gorner and collegues recently have shown that the nuclear localization of Msn2p is correlated inversely with PKA activity (Gorner *et al.*, 1998). Although there is as yet no evidence that PKA phosphorylates Msn2p directly, these results suggest that PKA exerts its effect on Msn2p-dependent gene expression by directly inhibiting Msn2p function. Interestingly, nuclear localization of Msn2p is also induced rapidly by conditions of stress, suggesting that PKA activity is modulated by exposure to stress or that other signaling pathways also impinge upon Msn2p

Table II. List of yeast strains

Strain	Genotype
SGY398[a]	*MATα tpk1Δ::ADE8 tpk2-63*(Ts) *tpk3::TRP1 bcy1::LEU2 ura3-52 his3 leu2-3,112 trp1 ade8*
JPY218	SGY398 *msn2-218*
JPY234	SGY398 *msn2-234*
JPY244	SGY398 *msn2-244*
SGY446	*MATα tpk1Δ::ADE8 tpk2-63*(Ts) *tpk3::TRP1 BCY1 ura3-52 his3 leu2-3,112 trp1 ade8*
SGY448	*MAT***a** *tpk1::URA3 tpk2-63*(Ts) *tpk3::TRP1 BCY1 ura3-52 his3 leu2-3,112 trp1 ade8*
ASY18	SGY446 *TPK2*
ASY54	ASY18 *bcy1::LEU2*
ASY21	SGY446 *msn2Δ::HIS3*
ASY30	SGY446 *msn4Δ::LEU2*
ASY29	SGY446 *msn2Δ::HIS3 msn4Δ::LEU2*
MWY155	*MAT***a** *tpk1::URA3 tpk2::HIS3 TPK3 MSN2 MSN4 ura3-52 his3 leu2-3,112 trp1 ade8 lys2*
ASY48	ASY29×MWY155
ASY58	*MAT***a** *tpk1Δ::ADE8 tpk2::HIS3 TPK3 msn2Δ::HIS3 msn4Δ::LEU2 ura3-52 his3 leu2-3,112 trp1 ade8*
ASY59	*MATα tpk1::URA3 tpk2::HIS3 TPK3 MSN2 MSN4 ura3-52 his3 leu2-3,112 trp1 ade8*
ASY61	*MATα tpk1Δ::ADE8 tpk2::HIS3 tpk3::TRP1 msn2Δ::HIS3 MSN4 ura3-52 his3 leu2-3,112 trp1 ade8*
ASY62	*MAT***a** *tpk1Δ::ADE8 tpk2::HIS3 tpk3::TRP1 msn2Δ::HIS3 msn4Δ::LEU2 ura3-52 his3 leu2-3,112 trp1 ade8*
ASY63	*MAT***a** *tpk1Δ::ADE8 tpk2::HIS3 tpk3::TRP1 msn2Δ::HIS3 msn4Δ::LEU2 ura3-5 his3 leu2-3,112 trp1 ade8*
SGY584	*MAT***a***/MATα tpk1Δ::ADE8/tpk1Δ::ADE8 tpk2-63/TPK2 tpk3::TRP1/tpk3::TRP1 msn2Δ::HIS3/msn2Δ::HIS3 msn4Δ::LEU2/msn4Δ::LEU2 ura3-52/ura3-52 his3/his3 leu2-3,112/leu2-3,112 trp1/trp1 ade8/ade8 lys2/LYS2*
SGY470	SGY448 *yak1::ADE8*
SGP406[b]	*MAT***a** *tpk1::URA3 tpk2::HIS3 tpk3::TRP1 yak1::LEU2 ura3-52 his3 leu2-3,112 trp1 ade8*
MWY161	*MAT***a** *tpk1::URA3 tpk2::HIS3 TPK3 bcy1::LEU2 ura3-52 his3 leu2-3,112 trp1 ade8*
MWY162	*MAT***a** *tpk1::URA3 tpk2::HIS3 TPK3 bcy1::LEU2 ura3-52 his3 leu2-3,112 trp1 ade8*

All strains were from this study, with the exception of [a]Ward and Garrett (1995); [b]Garrett and Broach (1989).

function. It is interesting to note that other protein kinase activities have been implicated in the stress response, including Hog1p (Schuller *et al.*, 1995; Varella *et al.*, 1995; although see Martinez-Pastor *et al.*, 1996), Pho85p (Timblin and Bergman, 1997) and Snf1p (Thompson-Jaeger *et al.*, 1991; Hubbard *et al.*, 1992). In certain respects, Snf1p is a prime candidate for a regulator of Msn2p/Msn4p function because it affects glycogen accumulation (Thompson-Jaeger *et al.*, 1992) and *MSN2* was first identified as a high-copy suppressor of the carbohydrate utilization defect of an *snf1*(Ts) strain (Estruch and Carlson, 1992). However, Snf1p is not required for stress induction of STRE-dependent genes (Martinez-Pastor *et al.*, 1996). Thus, it will be interesting to determine the contribution of each of these pathways to Msn2p translocation and function. Finally, it is important to note that the Sok2p repressor protein also regulates at least a subset of the genes under PKA and Msn2p/Msn4p control (Ward *et al.*, 1995). Thus, PKA might regulate gene expression through its actions on the repressor Sok2p as well as by its modulation of Msn2p/Msn4p function (Figure 8).

Msn2p and Msn4p originally were identified as transcriptional activators of stress response genes; however, Ruis and Schuller (1995) pointed out the presence of STREs within regions upstream of a number of genes involved in the synthesis of glycogen (and trehalose). Several of those genes were known, or suspected, to be repressed by PKA activity (Francois *et al.*, 1992; Hardy *et al.*, 1994; Ward *et al.*, 1995), so it was assumed that those genes, and as a result glycogen synthesis, would also be regulated by Msn2p and Msn4p. Consistent with that hypothesis, inactivation of *MSN2* and *MSN4* restricts the expression (Figure 6) and function (Figure 7) of at least one gene (*GLC3*) involved in the synthesis of glycogen. Schmitt and McEntee (1996) have observed similar effects of Msn2p inactivation on stress-induced expression of the trehalose synthesis gene *TPS2*.

Materials and methods

Media and growth conditions

Media used, including yeast rich and minimal media, as well as bacterial media, were prepared as described previously (Ward *et al.*, 1995). Glycogen accumulation and sporulation frequency were monitored by methods described previously (Garrett and Broach, 1989).

Strains and plasmids

Yeast strains are listed in Table II. Bacterial strains MC1066 [Δ(*lac*)*X74 galU galK strA*^{r} *hsdR trpC9830 leuB6 pyrF*::Tn*5*] and DH5α {F′/*endA hsdR17*(r_K m_K) *supE44* thi-*1 recA1 gyrA relA1* Δ(*lacZYA-argF*)*U169* [φ80d*lac*Δ(*lacZ*)*M15*]} have been described (Casadaban *et al.*, 1983; Woodcock *et al.*, 1989). The bacterial vector pBSKII$^+$ has been described (Stratagene Product catalog). Plasmid pRS316 is a low-copy *URA3* yeast vector and has been described (Sikorski and Hieter, 1989). The high-copy *LEU2* yeast vector pRS305-2μ has been described (Ward *et al.*, 1995). The high-copy, *URA3*-based plasmid (pJLB; Finley *et al.*, 1990) containing a UAS-less cytochrome c (*CYC1*) promoter upstream of the *lacZ* gene was a kind gift from Jon Horowitz (North Carolina State University). The pNUT1a derivative of pJLB1 contains an octameric p180-binding site upstream of the *CYC1* promoter and has been described (Cuevo *et al.*, 1997). The *yak1*::*ADE8* disruption has been described (Ward and Garrett, 1994). The Yip5 (*URA3*)-based *MCM1* plasmid pGC155-2 (Christ and Tye, 1991) was digested with *Sph*I to mark *MCM1*. The *MIH1* gene was disrupted with *mih1*::*LEU2* by digesting plasmid pDLB337 with *Nhe*I as described (Russell *et al.*, 1989). Plasmid pGS127 contains the *YAK1* coding region on the high-copy *URA3* plasmid YEp24 (Garrett and Broach, 1989), and the high-copy *SOK2* plasmid pMW61 (YEp-*SOK2*) has been described (Ward *et al.*, 1995).

DNA manipulations

All DNA manipulations were done according to published procedures (Kaiser *et al.*, 1994) or according to the recommendations of the manufacturers. The low-copy *MSN2* plasmid (pAS15) was constructed by inserting the 4.4 kb *Hin*dIII fragment from pGS222 (YCp50-*MIH1 MSN2 LYS7 SUB1*) into the *Hin*dIII site of pRS316. The related *msn2*Δ and *msn2*Δ::*HIS3* deletion plasmids, pAS16 and pAS19, were constructed by digesting pAS15 with *Bgl*II and religating or inserting the 1.7 kb *Bam*HI *HIS3* fragment, respectively. Chromosomal integration of the *msn2*Δ::*HIS3* deletion was achieved by transforming the indicated strains with pAS19 that had been digested with *Eco*RV and *Cla*I. A gap-repaired derivative of pAS16 was constructed by transforming *sok3-234* strain SGY455 with *Bgl*II-digested pAS16. The gap-repaired plasmid, pAS24, contained the *MSN2* region as judged by size and restriction pattern. The high-copy *MSN2* plasmid, pAS27, consists of the 2.9 kb *Hin*dIII–*Eag*I fragment *MSN2* inserted into the corresponding sites of pRS305-2μ. The *MSN4* plasmid pAS25 was generated by cloning the 3.2 kb *Bam*HI–*Eco*RI *MSN4* fragment (from PCR-amplified chromosomal DNA) into the corresponding sites of pBSKII$^+$. The high-copy *MSN4* plasmid (pAS28) was constructed by placing the 3.3 kb *Bam*HI–*Xho*I fragment of pAS25 into the same sites of pRS305-2μ. An *msn4*Δ::*LEU2* disruption was created by inserting the 2.8 kb *LEU2* fragment of YEp13

into the *Pst*I–*Xmn*I sites of pAS25 to create plasmid pAS26. Plasmid pAS26 was digested with *Bam*HI and *Hin*dIII to liberate the *msn4*Δ::*LEU2* deletion for transplacement.

The *YAK1–lacZ* fusion plasmids (pDS455 and pDS595) were constructed in two steps. Plasmid pDS1 is a derivative of pJLB1 in which the 300 bp *Bam*HI fragment (containing the translation start site and TATA sequence of *CYC1*) was removed and replaced with an 8 bp *Xho*I linker after blunting with Klenow fragment. Plasmids pDS455 and pDS595 were constructed by ligating *Xho*I linkers to the blunt ends of 455 bp *Sna*BI–*Stu*I and 595 bp *Sna*BI–*SnaBI* fragments of *YAK1* and inserting those fragments in the proper orientation into the *Xho*I site of pDS1.

Genetic mapping

The *SOK3-234* mutation was genetically mapped by crossing a *mih1*::*LEU2* derivative of SGY455 [*tpk2-63*(Ts) *SOK3-234*] with a *MCM1-URA3*-marked derivative of strain SGY446 [*tpk2-63*(Ts)]. The results of those crosses (*SOK3-234*×*MIH1-mih1*::*LEU2* = 109 PD, 0 NPD, 5 T), (*SOK3-234*×*MCM1-URA3* = 96 PD, 0 NPD, 18 T), and (*MIH1-mih1*::*LEU2*×*MCM1-URA3* = 90 PD, 0 NPD, 24 T) were used to determine the map distance based on the formula of Perkins (1949).

β-Galactosidase assays

β-Galactosidase activity was measured according to a published procedure (Kaiser *et al*., 1994).

Northern (RNA) analyses

Total RNA was isolated by the LETS/phenol/chloroform extraction method (Kaiser *et al*., 1994) from cells grown at 30°C to an OD_{600} of between 0.5 and 1.0, separated by electrophoresis and transferred to nylon membranes. DNA fragments to be used as probes (*YAK1*, 548 bp *Xba*I–*Eco*RV fragment from pGS127; *HSP12*, a 358 bp PCR fragment; *GLC3*, a 612 bp PCR fragment; and *ACT1*, a 320 bp *Bgl*II fragment from pSP72-*ACT1*) were gel purified and then radiolabeled by random priming. Radioactivity was quantified on a Molecular Dynamics PhosphorImager.

Acknowledgements

The authors thank J.Posey, D.Seo and D.Cortez for preliminary observations, as well as M.Brandriss for critical comments on an early version of the manuscript. D.Mitchell, G.Sprague, J.Fassler and D.Lew generously provided plasmids. This work was supported by grant GM44666 from the National Institutes of Health.

References

Belazzi,Y., Wagner,A., Wieser,R., Schanz,M., Adam,G., Hartig,A. and Ruis,H. (1991) Negative regulation of transcription of the *Saccharomyces cerevisiae* catalase T (*CTT1*) gene by cAMP is mediated by a positive control element. *EMBO J*., **10**, 585–592.

Broach,J.R. and Deschenes,R.J. (1990) The function of *RAS* genes in *Saccharomyces cerevisiae*. *Adv. Cancer Res*., **54**, 79–138.

Cameron,S., Levin,L., Zoller,M. and Wigler,M. (1988) cAMP-independent control of sporulation, glycogen metabolism and heat shock resistance in *S.cerevisiae*. *Cell*, **53**, 555–566.

Cannon,J. and Tatchell,K. (1987) Characterization of *Saccharomyces cerevisiae* genes encoding subunits of cAMP-dependent protein kinase. *Mol. Cell. Biol.*, **7**, 2653–2663.

Casadaban,M.J., Martinez-Arias,A., Shapiro,S.K. and Chou,J. (1983) β-Galactosidase gene fusions for analyzing gene expression in *Escherichia coli* and yeast. *Methods Enzymol*., **100**, 293–308.

Christ,C. and Tye,B. (1991) Functional domains of the yeast transcription/replication factor MCM1. *Genes Dev*., **5**, 751–763.

Cuevo,R.S., Garrett,S. and Horowitz,J.M. (1997) Detection and functional characterization of p180, a novel cell cycle regulated yeast transcription factor that binds retinoblastoma control elements. *J. Biol. Chem.*, **272**, 3813–3822.

Engleberg,D., Zandi,E., Parker,C.S. and Karin,M. (1994) The yeast and mammalian Ras pathways control transcription of heat shock genes independently of heat shock transcription factors. *Mol. Cell. Biol.*, **14**, 4929–4937.

Estruch,F. and Carlson,M. (1993) Two homologous zinc finger genes identified by multicopy suppression in a SNF1 protein kinase mutant of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **13**, 3872–3881.

Finley,R.L.,Jr, Chen,S., Ma,J., Byrne,P. and West,R.W. (1990) Opposing regulatory functions of positive and negative control elements in UAS control transcription of the yeast *GAL* genes. *Mol. Cell. Biol.*, **10**, 5663–5670.

Francois,J.M., Thompson-Jaeger,S., Skroch,J., Zellenka,U., Spevak,W. and Tatchell,K. (1992) *GAC1* may encode a regulatory subunit for protein phosphatase type 1 in *S.cerevisiae*. *EMBO J*., **11**, 87–96.

Garrett,S. and Broach,J.R. (1989) Loss of Ras activity in *Saccharomyces cerevisiae* is suppressed by disruptions of a new kinase gene, *YAK1*, whose product may act downstream of the cAMP-dependent protein kinase. *Genes Dev*., **3**, 1336–1348.

Garrett,S., Menold,M.M. and Broach,J.R. (1991) The *Saccharomyces cerevisiae YAK1* gene encodes a protein kinase that is induced by arrest early in the cell cycle. *Mol. Cell. Biol.*, **11**, 4045–4052.

Gimeno,C.J., Ljungdahl,P.O., Styles,C.A. and Fink,G.R. (1992) Unipolar cell divisions in the yeast *S.cerevisiae* lead to filamentous growth: regulation by starvation and RAS. *Cell*, **68**, 1077–1090.

Gorner,W., Durchschlag,E., Martinez-Pastor,M.T., Estruch,F., Ammerer,G., Hamilton,B., Ruis,H. and Schuller,C. (1998) Nuclear localization of the C_2H_2 zinc finger protein Msn2p is regulated by stress and protein kinase A activity. *Genes Dev*., **12**, 586–597.

Hardy,T.A., Huang,D. and Roach,P.J. (1994) Interactions between cAMP dependent and SNF1 protein kinases in the control of glycogen accumulation in *Saccharomyces cerevisiae*. *J. Biol. Chem.*, **269**, 27907–27913.

Hartley,A.D., Ward,M.P. and Garrett,S. (1994) The Yak1 protein kinase of *Saccharomyces cerevisiae* moderates thermotolerance and inhibits growth by an Sch9 protein kinase-independent mechanism. *Genetics*, **136**, 465–474.

Hubbard,E.J.A., Yang,X. and Carlson,M. (1992) Relationship of the cAMP-dependent protein kinase pathway to the SNF1 protein kinase and invertase expression in *Saccharomyces cerevisiae*. *Genetics*, **130**, 71–80.

Johnston,G.C., Pringle,J.R. and Hartwell,L. (1977) Coordination of growth with cell division in the yeast *Saccharomyces cerevisiae. Exp. Cell Res.*, **105**, 79–98.

Kaiser,C., Michaelis,S. and Mitchell,A. (1994) *Methods in Yeast Genetics. A Laboratory Course Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Klein,C. and Struhl,K. (1994) Protein kinase A mediates growth-regulated expression of yeast ribosomal protein genes by modulating RAP1 transcriptional activity. *Mol. Cell. Biol.*, **14**, 1920–1928.

Mager,W.H. and De Kruijff,A.J.J. (1995) Stress-induced transcriptional activation. *Microbiol. Rev*., **59**, 506–531.

Mai,B. and Breeden,L. (1997) Xbp1, a stress-induced transcriptional repressor of the *Saccharomyces cerevisiae* Swi4/Mbp1 family. *Mol. Cell. Biol.*, **17**, 6491–6501.

Marchler,G., Schuller,C., Adam,G. and Ruis,H. (1993) A *Saccharomyces cerevisiae* UAS element controlled by protein kinase A activates transcription in response to a variety of stress conditions. *EMBO J*., **12**, 1997–2003.

Markwardt,D.D., Garrett,J.M., Eberhardy,S. and Heideman,W. (1995) Activation of the Ras/cyclic AMP pathway in the yeast *Saccharomyces cerevisiae* does not prevent G1 arrest in response to nitrogen starvation. *J. Bacteriol*., **177**, 6761–6765.

Martinez-Pastor,M.T., Marchler,G., Schuller,C., Marchler-Bauer,A., Ruis,H. and Estruch,F. (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.

Neuman-Silberberg,F.S., Bhattacharya,S. and Broach,J.R. (1995) Nutrient availability and the *RAS*/cyclic AMP pathway both induce expression of ribosomal protein genes in *Saccharomyces cerevisiae* by different mechanisms. *Mol. Cell. Biol.*, **15**, 3187–3196.

Olson,M.V. (1991) Genome structure and organization in *Saccharomyces cerevisiae*. In Broach,J.R., Jones,E. and Pringle (eds), *The Molecular and Cellular Biology of the Yeast Saccharomyces.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 1–40.

Perkins,D.D. (1949) Biochemical mutants of the smut fungus *Ustilago maydis*. *Genetics*, **34**, 607–629.

Ruis,H. and Schuller,C. (1995) Stress signaling in yeast. *BioEssays*, **17**, 959–965.

Russell,P., Moreno,S. and Reed,S.I. (1989) Conservation of mitotic controls in fission and budding yeasts. *Cell*, **57**, 295–303.

Schmitt,A.P. and McEntee,K. (1996) Msn2p, a zinc finger DNA-binding protein, is the transcriptional activator of the multistress response in *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA*, **93**, 5777–5782.

Schuller,C., Brewster,J.L., Alexander,M.R., Gustin,M.C. and Ruis,H. (1994) The HOG pathway controls osmotic regulation of transcription via the stress response element of the *Saccharomyces cerevisiae CTT1* gene. *EMBO J.*, **13**, 4382–4389.
Sikorski,R.S. and Hieter,P. (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics*, **122**, 19–27.
Thompson-Jaeger,S., Francois,J., Gaughran,J.P. and Tatchell,K. (1991) Deletion of *SNF1* affects the nutrient response of yeast and resembles mutations which activate the adenylate cyclase pathway. *Genetics*, **129**, 697–706.
Timblin,B.K. and Bergman,L.W. (1997) Elevated expression of stress response genes resulting from deletion of the *PHO85* gene. *Mol. Microbiol.*, **26**, 981–990.
Varela,J.C.S., Praekelt,U.M., Meacock,P.A., Planta,R.J. and Mager,W.H. (1995) The *Saccharomyces cerevisiae HSP12* gene is activated by the high-osmolarity glycerol pathway and negatively regulated by protein kinase A. *Mol. Cell. Biol.*, **15**, 6232–6245.
Ward,M.P. and Garrett,S. (1994) Suppression of a yeast cyclic AMP-dependent protein kinase defect by overexpression of *SOK1*, a yeast gene exhibitiing sequence similarity to a developmentally regulated mouse gene. *Mol. Cell. Biol.*, **14**, 5619–5627.
Ward,M.P., Gimeno,C.J., Fink,G.R. and Garrett,S. (1995) *SOK2* may regulate cyclic AMP-dependent protein kinase-stimulated growth and pseudohyphal development by repressing transcription. *Mol. Cell. Biol.*, **15**, 6854–6863.
Werner-Washburne,M., Braun,E., Johnston,G.C. and Singer,R.A. (1993) Stationary phase in the yeast *Saccharomyces cerevisiae*. *Microbiol. Rev.*, **57**, 383–401.
Woodcock,D.M., Crowther,P.J., Doherty,J., Jefferson,S., DeCruz,E., Noyer-Weidner,M., Smith,S.S., Michael,M.Z. and Graham,M.W. (1989) Quantitative evaluation of *Escherichia coli* host strains for tolerance to cytosine methylation in plasmid and phage recombinants. *Nucleic Acids Res.*, **17**, 3469–3478.
Wu,C., Clos,J., Giorgi,G., Haroun,R.I., Kim,S.J., Rabindran,S.K., Westwood,J.T., Wisniewski,J. and Yim,G. (1994) Structure and regulation of heat shock transcription factor. In Morimoto,R., Tissieres,A. and Georgopoulos,C. (eds), *The Biology of Heat Shock Proteins and Molecular Chaperones.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 395–416.

Received March 11, 1998; revised and accepted May 7, 1998