The Yak1 Protein Kinase of *Saccharomyces cerevisiae* Moderates Thermotolerance and Inhibits Growth by an Sch9 Protein Kinase-Independent Mechanism

Alan D. Hartley,* Mary P. Ward*,† and Stephen Garrett*,†

**Section of Cell Growth, Regulation and Oncogenesis and †Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710*

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ABSTRACT

The growth defect associated with the loss of yeast A kinase activity can be alleviated by the overexpression or deletion of two other kinases, Sch9 and Yak1, respectively. Using tests of epistasis, we have shown that Sch9 and Yak1 define separate signaling pathways and must, therefore, suppress the A kinase defect by different mechanisms. Nevertheless, the Yak1 kinase appears to regulate cellular processes that are under A kinase control. For example, acquisition of heat resistance is correlated with Yak1 kinase activity, such that *YAK1*-overexpressing cells are over 200-fold more resistant than isogenic *yak1* strains. These results, for the first time, associate a phenotype, other than suppression of the A kinase growth defect, with the loss of Yak1 activity and argue a broader role for the Yak1 kinase in cell growth.

THE cyclic AMP-dependent protein kinase (A kinase) pathway is required for growth and cell-cycle progression in the budding yeast *Saccharomyces cerevisiae*. In the absence of A kinase activity, cells cease growing and arrest in G_1 in a manner similar to that observed following starvation of cells for essential nutrients (MATSUMOTO *et al.* 1982; JOHNSTON *et al.* 1977). In contrast, mutations yielding elevated A kinase activity prevent cells from arresting in G_1 and cause loss of carbohydrate reserves and sensitivity to heat shock or starvation for nutrients (UNO *et al.* 1982). Thus, A kinase may participate in the cell's decision to enter a quiescent state in G_1 commonly referred to as G_0. Low activity promotes exit from the cell cycle and entry into G_0, and high activity precludes access to G_0.

The combined growth and cell cycle arrest of conditional A kinase mutants is consistent with the notion that A kinase phosphorylation coordinately regulates many cellular processes. For example, mutations that attenuate A kinase activity induce physiological changes normally associated with nutrient depletion, *i.e.*, induction of stress-related proteins, thermotolerance, diminution of general and specific transcription, and, in diploids, induction of sporulation. Conversely, constitutive activation of the pathway induces the breakdown of carbohydrate reserves, sensitizes the cell to heat shock and nutrient deprivation and represses the transcription of several genes (for review, see BROACH and DESCHENES 1990). The precise mechanism by which A kinase phosphorylation influences the cell's decision between growth and quiescence is not known. Biochemical targets have been described in yeast, and include enzymes involved in carbohydrate storage and metabolism, enzymes required for phospholipid metabolism, a growing number of transcriptional factors, as well as functions involved in the synthesis and breakdown of cAMP (BROACH and DESCHENES 1990). Nevertheless, it remains unclear whether A kinase phosphorylation of these known targets can influence the cell's decision to exit the cell cycle or continue proliferation.

Two genes exhibiting significant homology to known protein kinases were identified in genetic screens for growth-related effectors of the A kinase. The *SCH9* gene was isolated as a high-copy suppressor of a temperature-sensitive mutation in the A kinase pathway (TODA *et al.* 1988; DI BLASI *et al.* 1993). Overexpression of *SCH9* was able to alleviate the growth defect of a strain lacking all A kinase activity (*tpk1 tpk2 tpk3*) and, in wild-type cells, resulted in a heat shock-sensitive phenotype exhibited by cells with elevated A kinase levels. In addition, the slow growth defect of *SCH9*-disrupted cells was reversed by activation of the A kinase pathway (TODA *et al.* 1988). These, and other, results prompted TODA *et al.* (1988) to suggest that the functions of the two kinases might be "largely redundant." Although the molecular nature of the redundancy was not explicitly stated, the authors favored a model in which the two kinases regulated functionally similar, growth-specific pathways or processes. Activation of one pathway by overexpression of its cognate kinase was sufficient to compensate, at least in part, for the loss of function of the

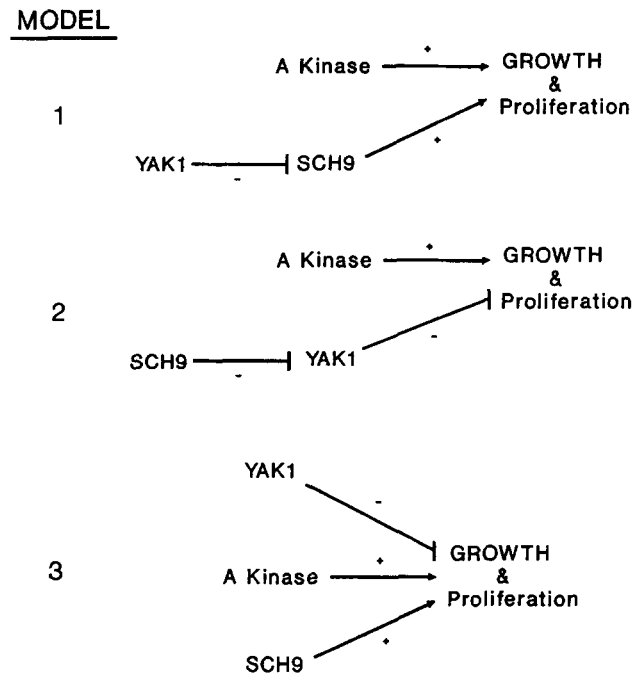

FIGURE 1.—Three models to explain the interaction of three growth-related kinases of yeast.

other pathway. However, the authors also pointed out (TODA *et al.* 1988) that their results could be explained by a model in which the substrate specificities, rather than functions, of the two kinases were similar. According to that scenario, overexpression of one kinase would compensate for the absence of the other through the promiscuous phosphorylation of substrates normally unique to the missing kinase. The latter explanation was supported by the strong sequence similarity between the catalytic domains of the *SCH9* gene product and members of the cAMP-dependent protein kinase family (TODA *et al.* 1988; TAYLOR *et al.* 1990).

The *YAK1* gene was also identified in a genetic screen to identify new components of the A kinase pathway (GARRETT and BROACH 1989). In contrast with the Sch9 kinase, it was the loss of function of the *YAK1* gene product that alleviated the growth defect of strains lacking A kinase activity; *tpk1 tpk2 tpk3 YAK1*$^+$ strains were inviable, but *tpk1 tpk2 tpk3 yak1* strains grew. Recent results (GARRETT *et al.* 1991) suggested that the Yak1 kinase functioned as a negative regulator of growth in a pathway that was parallel to that of the A kinase, with overlapping targets but antagonistic effects.

Since the mechanism(s) by which *SCH9* overexpression and/or Yak1 inactivation suppressed the A kinase growth defect was not understood, we reasoned that it would be instructive to determine the epistatic relationship of these suppressors. At least three models (Figure 1) account for A kinase suppression by the loss and gain of Yak1 and Sch9 function, respectively. [In most cases, the molecular details of suppression by Sch9 (*i.e.*, overlapping function *vs.* overlapping substrate specificity) were not critical to the interpretation of the results.] Two of the models proposed that Yak1 and Sch9 were constituents of a single pathway that modulated the activity of growth-specific substrates. In the first model, the Sch9 protein kinase positively regulated growth by modifying the substrates; Yak1 inhibited growth by negatively regulating Sch9 activity. According to that scenario, derepression of Sch9 kinase activity, by Yak1 inactivation or *SCH9* overexpression, would result in the productive phosphorylation of one or more substrates. In contrast, model 2 posited that the Yak1 kinase functioned as a negative regulator of growth that could be inactivated by Sch9-dependent phosphorylation. In the second model, loss of Yak1 function, by *YAK1* disruption or Sch9 overproduction, would restore growth to an A kinase-deficient strain. Finally, a third model postulated that Sch9 and Yak1 were constituents of separate, convergent signaling pathways.

The results of this manuscript suggest that the Sch9 and Yak1 kinases are constituents of separate transduction pathways, and are inconsistent with a model in which the Sch9 kinase is part of sensing pathway which is redundant with the A kinase pathway. In addition, the results suggest that the Yak1 kinase may play a role in the cell's ability to withstand heat shock. Finally, genetic analyses have established the identity between *YAK1* and a locus (*SRA7*) previously identified (CANNON and TATCHELL 1987) by dominant suppressing alleles of a conditional defect in the A kinase pathway.

MATERIALS AND METHODS

Media and growth conditions: All media, including yeast-rich and minimal media, as well as bacterial media, were prepared as described previously (DESCHENES and BROACH 1987). Except where noted, yeast were heat shocked by growing cultures in liquid to an OD_{600} of 1.0, and then transferring serial dilutions (see figure legends) of each culture to prewarmed agar and placing the agar plates in a shallow water bath set to 55° for the indicated period. The plates were then removed and incubated at 23° for several days. Induction of *GAL10*-mediated gene expression was achieved by consecutive 16-hr subcultures in medium containing raffinose and then galactose as the only fermentable carbon source.

Strains: Yeast strains are listed, with references where appropriate, in Table 1. Bacterial strain MC1066 [Δ(*lac*)*X74 galU galK strA*^{r} *hsdR trpC9830 leuB6 pyrF*::Tn*5*] has been described (CASADABAN *et al.* 1983).

DNA manipulations: Plasmid DNA was prepared from *Escherichia coli* using the alkali lysis method (MANIATIS *et al.* 1982). All enzymes were used according to the instructions of their suppliers (New England Biolabs) and cloning techniques were as described (MANIATIS *et al.* 1982). Yeast transformation was by the lithium acetate method (ITO *et al.* 1983), and other yeast manipulations were performed as described (SHERMAN *et al.* 1986).

TABLE 1

List of yeast strains

Strain	Genotype	Source/Reference
SGP4	*MATα leu2-3,112 trp1 ura3-52 his3 ade8 ras1::HIS3 RAS2-URA3*	GARRETT and BROACH (1989)
JR477	*MATa leu2-3,112 trp1 his3 ura3-52 his3 ade8 ras1::URA3 RAS2^{Val19}*	J. R. BROACH
SGP55	JR477 *yak1::HIS3*	This study
AHY1	SGP4 *yak1::LEU2*	This study
AHY2	SGP4 *sch9::ADE8*	This study
AHY3	SGP4 *sch9::ADE8 yak1::LEU2*	This study
AHY67	SGP4 *GAL10-YAK1*	This study
AHY68	SGP4 *GAL10-yak1^{K398Y}*	This study
AHY54	*MATα ura3-52 his3 leu2-3,112 trp1 ade8*	This study
AHY59	AHY54 *sch9::HIS3*	This study
MWY63	*MATα ura3-52 leu2-3,112 trp1 his3 ade8 tpk1::URA3 tpk2-63*(Ts) *tpk3::TRP1 bcy1::LEU2*	This study
MWY123	MWY63 *yak1::HIS3*	This study
AHY84	MWY63 *sch9::ADE8*	This study
AHY86	MWY123 *sch9::ADE8*	This study
JC257-21C	*MATa leu2 ura3 ino1 met3 tpk1::URA3 SRA7-14*	J. CANNON
KT335	*MATα leu2 ura3 lys2 ras2::LEU2 SRA7-1*	K. TATCHELL

The genomic *YAK1* gene was disrupted by single step gene replacement using pGS136-A (*yak1-2::LEU2*, GARRETT and BROACH 1989) digested with *Sma*I and *Hin*dIII. The genomic *SCH9* gene was disrupted using the plasmid p*sch9::ADE8* (TODA *et al.* 1988) digested with *Pvu*II prior to transplacement into the genome. Gene disruptions were confirmed by Southern analyses. Plasmids YEp(*SCH9*) and p*SCH9-ADE8* were based on the *LEU2* and *ADE8* high copy vectors YEp213 and YEpADE8, respectively (TODA *et al.* 1988). The high copy *TPK1* plasmid was based on the vector YEp13 and has been described (TODA *et al.* 1987). Plasmid pGS159, used to supply *YAK1* on a *LEU2 ADE8*-based centromeric vector, was constructed by digesting pGS100 (GARRETT and BROACH 1989) with *Spe*I and inserting a 4-kb *Xba*1 fragment containing *ADE8* (derived from the vector YEpADE8; TODA *et al.* 1988).

The wild-type and mutant *YAK1* alleles (*YAK1* and *yak1-K398Y*) were placed under control of the galactose-inducible promoter *GAL10* by inserting a 3-kb *Bsp*HI *Hin*dIII *YAK1* fragment into the *Sal*I cloning site of the high copy, expression vector YEp51 (BROACH *et al.* 1983). The *YAK1* plasmid pGS100 was digested with *Bsp*HI, fragment ends were made flush using Klenow, and *Xho*I linkers were ligated on the ends before final digestion with *Xho*I and *Hin*dIII to liberate the *YAK1* coding region. The *Xho*I(*Bsp*HI) to *Hin*dIII *YAK1* DNA fragment was then purified and ligated with *Sal*I- and *Hin*dIII-digested YEp51 DNA to generate the *GAL10-YAK1* fusion plasmid, pGS137. The 2-μm origin of pGS137 was removed by digestion with *Spe*I followed by religation to generate the *YAK1* integrative plasmid, pGS146. A nonfunctional variant, pGS148, of plasmid pGS146 was created by replacing the *Bam*HI/*Hin*dIII fragment of *YAK1* in pGS146 with the corresponding fragment from the *yak1^{K398Y}* phage, mpG6 (GARRETT *et al.* 1991). The *yak1^{K398Y}* allele substitutes a tyrosine for the invariant lysine at residue 398 and has been described (GARRETT *et al.* 1991). The *GAL10-YAK1* fusions plasmids were integrated into the chromosomal *leu2-3,112* locus by digesting the plasmids with *Bst*X1 prior to transformation and selecting for Leu$^+$ transformants.

Mapping *YAK1*: The *YAK1* gene was assigned to chromosome *X* by probing yeast chromosomes transferred to nitrocellulose with a random primer-labeled internal 850-bp *Eco*RV fragment of *YAK1*. Genetic mapping placed *YAK1* on chromosome *X* in the order *CENX PBS2 URA2 YAK1 TPK1* (Table 2). Comparison of the *YAK1* DNA sequence with the GenBank data base revealed that the *RPB4* gene (WOYCHIK and YOUNG 1989) is found upstream of the *YAK1* promoter and the two genes are transcribed divergently.

TABLE 2

Genetic mapping of the *YAK1* locus

Cross	PD[a]	NPD[b]	T[c]	Linkage[d]
yak1::LEU2 × *tpk1::URA3*	57	0	13	9 cM
yak1::LEU2 × *ura2*	71	0	15	8 cM
yak1::LEU2 × *pbs2::URA3*	49	0	11	9 cM

[a] Parental ditype.
[b] Nonparental ditype.
[c] Tetratype.
[d] Determined by the formula of PERKINS (1949).

RESULTS

Mutations in *SCH9* are suppressed by altered A kinase activity, but not by suppressors of the A kinase growth defect: If, as suggested by TODA *et al.* (1988), the *SCH9* product affected a pathway that was largely redundant with the A kinase-regulated pathway, it seemed likely that the slow growth defect of cells lacking Sch9 protein would be rescued by other A kinase suppressors, including disruptions of *YAK1*. Although such tests of epistasis must be interpreted with caution, they have served to order the function of components of several similarly complex signaling pathways in other genetic systems, including bacteria, Drosophila and nematodes (AVERY and WASSERMAN 1992). To test if the loss of Yak1 function could alleviate the slow growth of an *sch9* strain, we constructed isogenic strains with all combinations of the *yak1::LEU2* and *sch9::ADE8* disruptions. As expected (GARRETT and BROACH 1989; TODA *et al.* 1988), the

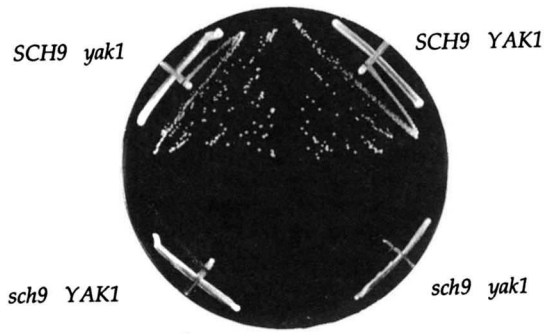

FIGURE 2.—Epistasis between *sch9* and *yak1* mutations. Strains *SCH9 yak1* [AHY1], *SCH9 YAK1* [SGP4], *sch9 yak1* [AHY3] and *sch9 YAK1* [AHY2] were streaked on YEPD medium and incubated at 30° for 2 days.

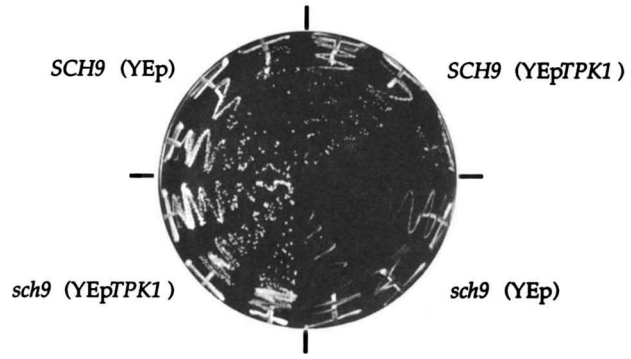

FIGURE 3.—Overexpression of *TPK1* suppresses the slow growth of an *sch9* strain. Strains *SCH9* (YEp) [SGP4/YEp13], *SCH9* (YEp*TPK1*) [SGP4/YEP13-*TPK1*], *sch9* (YEp) [AHY2/YEp13] and *sch9* (YEp*TPK1*) [AHY2/YEp13-*TPK1*] were streaked on minimal medium and incubated at 30° for 2 days.

yak1 disruption had no effect on the growth rate of a wild-type strain, whereas the *sch9* disruption conferred an obvious growth disadvantage (Figure 2). It was also clear (Figure 2) that the growth defect of the Sch9$^-$ strain was not ameliorated by the loss of Yak1 function. Moreover, overexpression of two novel gene-dosage suppressors (*SOK1* and *SOK2*; M. P. WARD and S. GARRETT, unpublished results) of a conditional A kinase defect also failed to alleviate the slow growth of the *sch9::ADE8* strain (data not shown).

In contrast to the failure of these A kinase suppressors to remediate the *sch9* defect, and in agreement with previous results (TODA *et al.* 1988; however, see DI BLASI *et al.* 1993), overexpression of one of the genes encoding the catalytic subunit of the A kinase was able to restore normal growth to the *sch9* mutant [compare *sch9* (Yep*TPK1*) with *sch9* (YEp) in Figure 3]. Thus, while modification of four genes (*SCH9*, *SOK1*, *SOK2* and *YAK1*) restored growth to an A kinase compromised strain, the loss of Sch9 function was compensated for only by elevated levels of A kinase activity (Figure 3 and TODA *et al.* 1988). These results are inconsistent with model 2, in which the compromised growth of the *sch9* deletion results from the derepression of Yak1 kinase activity, but can be accommodated by models in which Yak1 is upstream

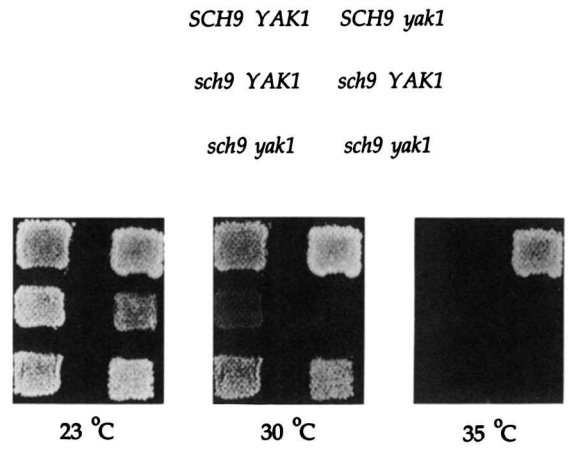

FIGURE 4.—Mutations in *yak1* alleviate the growth defect of an A kinase-deficient strain in the absence of the *SCH9* gene product. Colonies were patched onto minimal medium agar for 3 days at 23°, and then replicated to the same medium and incubated at 23°, 30° and 35° for 3, 2 and 2 days, respectively. Strains were derivatives of the *tpk*(Ts) mutant MWY63 [*tpk1 tpk2-63*(Ts) *tpk3*]. Individual strains were: *SCH9 YAK1* [MWY63], *SCH9 yak1* [MWY123], *sch9 YAK1* [AHY84], *sch9 yak1* [AHY86].

of Sch9 (model 1) or in which each kinase controls a separate pathway or function (model 3).

Deletions of *YAK1* suppress the growth defect associated with attenuated A kinase activity even in strains lacking the *SCH9* gene product: The first model of Figure 1 suggests that Yak1 inactivation suppresses the loss of A kinase function by derepressing Sch9 activity. While that model is purposely vague about the molecular mechanism by which Sch9 activation ameliorates the A kinase deficiency, it does predict that suppression by deletion of *YAK1* would be blocked by the disruption of Sch9 function. To test if Sch9 activity was essential to temperature-independent growth of a *tpk*(Ts) *yak1* strain, we constructed isogenic strains containing various combinations of the various alleles. As expected, disruption of *YAK1* function in the *tpk*(Ts) *SCH9* strain suppressed the conditional growth defect at the nonpermissive temperature of 35° (Figure 4). It was also obvious that disruption of *SCH9* lowered the non-permissive temperature of the *tpk*(Ts) *YAK1* strain from 35° to 30° (Figure 4, compare the growth of the two *sch9 YAK1* strains with the *SCH9 YAK1* strain at the three temperatures). Whether the more severe growth defect of the double mutant was due to overlapping functions or to the synergistic effect of combining two mutations that result in slow growth, the loss of Sch9 function did not completely block suppression by *yak1* since the *tpk*(Ts) *yak1 sch9* strain grew at 30° (contrast this with the growth defect of the *tpk*(Ts) *YAK1 sch9* strain at the same temperature). Thus, suppression of the A kinase growth defect by loss of Yak1 function was not blocked by removing Sch9 activity.

The epistasis results of Figure 4 suggest that the A kinase growth defect is suppressed by Yak1 inactiva-

tion through a mechanism that is partly independent of Sch9 kinase activity. Those results would be consistent with model 1 only if *SCH9* was a member of a duplicated gene family (*i.e.*, *TPK1*, *TPK2*, *TPK3*, TODA *et al.* 1987; *CLN1*, *CLN2*, *CLN3*, RICHARDSON *et al.* 1989). Since disruption of *SCH9* merely compromised cell growth (Figure 2 and TODA *et al.* 1988), it seemed plausible that yeast might contain one or more attenuated "*SCH9*" homologs that were sufficient for survival and through which Yak1 could function. However, we saw no evidence for an *SCH9* homolog when genomic DNA was probed with a DNA fragment encompassing the catalytic domain of *SCH9*, even under conditions of reduced stringency (data not shown).

While these results argue against model 1, they do not allow us to differentiate between model 2, which postulates that Yak1 functions downstream of Sch9, and model 3, in which Yak1 and Sch9 are thought to regulate independent pathways. However, model 2 assumes that the synthetic growth defect of the *tpk*(Ts) *sch9* strain (Figure 4) is due to induction of the negative growth-regulator, Yak1. Strict adherence to that model would predict that disruption of *YAK1* would completely alleviate the synthetic defect, resulting in a phenotype identical to the isogenic *SCH9 yak1* strain. That appears not to be the case, since the *tpk*(Ts) *sch9 yak1* strains (bottom of Figure 4) are unable to survive temperatures above 30°, whereas the *tpk*(Ts) *SCH9 yak1* strain exhibits normal growth at 35° (upper right corner of Figure 4).

Finally, the temperature sensitivity of the *tpk*(Ts) *sch9* strain was partially suppressed at 30° by overexpression of *SOK1* and *SOK2*, suggesting that these novel A kinase suppressors also act independent of Sch9 function (data not shown).

Yak1 protein kinase may protect cells against stress: Cells with elevated A-kinase activity are acutely sensitive to many forms of stress including heat shock and nutrient starvation (BROACH and DESCHENES 1990). The heat shock-sensitive phenotype of the activated A kinase mutants is mimicked by cells overexpressing *SCH9* (TODA *et al.* 1988). In the latter case, sensitivity may be due to the inappropriate phosphorylation of one or more stress-related substrates of the A kinase. Alternatively, Sch9 may play a direct and physiological role in regulating resistance to stress. Although previous results (GARRETT and BROACH 1989) failed to ascribe a stress-related phenotype to the loss of the *YAK1* gene product, THOMPSON-JAEGER *et al.* (1991) recently presented indirect evidence that was consistent with a role for Yak1 in governing resistance to extreme temperatures. Accordingly, we tested if deletion of the *YAK1* gene would affect the heat sensitivity of a strain overproducing the *SCH9* gene product. Isogenic *YAK1* and

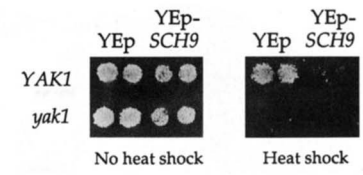

FIGURE 5.—Deletion of *YAK1* confers a heat shock-sensitive phenotype. Duplicate patches were pregrown on ADE minimal medium agar at 30° for several days and then replicated onto the same agar, with or without heat shock, and incubated for several days at 30°. The isogenic *YAK1* [SGP4] and *yak1* [AHY1] strains carried the multicopy vector YEp [YEpADE], or its *SCH9* derivative YEp-*SCH9* [p*SCH9-ADE8*], as indicated.

yak1 strains were transformed with a high copy *SCH9* plasmid, or the original vector as a control, and exposed to 55° for 10 min (Figure 5). As observed previously, *SCH9* overexpression conferred a heat shock-sensitive phenotype to strains containing a wild-type *YAK1* gene (compare the duplicate YEp transformants with the two strains containing the YEp-*SCH9* plasmid in Figure 5). However, the *yak1* deletion strain was also sensitive to heat shock, even in the absence of *SCH9* overexpression. By contrast, neither *SCH9* overexpression nor the loss of Yak1 activity had a measurable (>4-fold) effect on the sensitivity of cells to prolonged nutrient starvation (data not shown).

The role of the Yak1 protein kinase in regulating heat shock resistance was investigated by testing the sensitivity of cells containing various levels of the *YAK1* gene product. Figure 6 shows the survival of several different strains after 0, 10 and 20 min at 55°. Consistent with the results of Figure 5, disruption of *YAK1* rendered the cells at least 20-fold more sensitive to elevated temperatures (compare the *YAK1* strain with the isogenic *yak1* derivative; see also Figure 8); this sensitivity was completely reversed by providing *YAK1* on a low copy vector (YCp*YAK1*). Thus, the presence of the Yak1 protein kinase appeared to provide some protection to cells toward exposure to elevated temperatures. To determine the nature of this protection, we tested if cells overproducing the Yak1 kinase could be further protected against heat shock. Plasmids containing either the wild-type *YAK1* gene or a non-functional variant under the control of the galactose-inducible *GAL10* promoter were introduced into the *YAK1* strain of Figure 6 and shifted to galactose prior to heat shock (see MATERIALS AND METHODS). Under these conditions, the levels of Yak1 protein and activity have been observed to increase by a factor of 50–100-fold (M. M. MENOLD and S. GARRETT, unpublished results). The strain overproducing Yak1 was clearly more heat shock resistant than the isogenic strain from which it was derived (Figure 6, compare cell viabilities of the *YAK1* strain of row 1 with the *YAK1* [*GAL10-YAK1*] strain of row 4 after 20 min; Figure 8). To our surprise, overexpression of the nonfunctional *yak1*^{K398Y} variant (GAR-

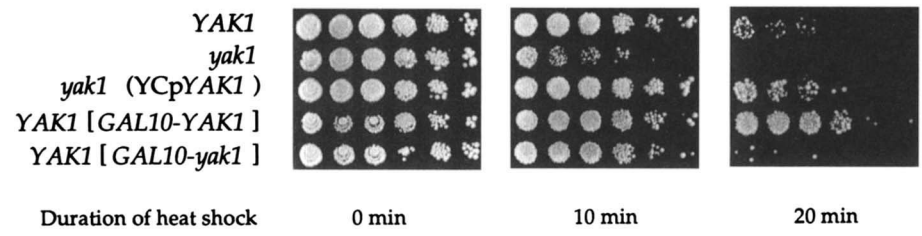

FIGURE 6.—The Yak1 protein kinase may protect cells against stress. Serial dilutions of the cultures were transferred to plates according to the protocol in MATERIALS AND METHODS, exposed to 55° for the indicated times, and then incubated at 30° for several days. The strains were: *YAK1* [SGP4], *yak1* [AHY1], *yak1* (YCp*YAK1*) [AHY1/YCp*YAK1*], *YAK1* [*GAL10-YAK1*] [AHY67/*GAL10-YAK1*], *YAK1* [*GAL10-yak1*] [AHY68/*GAL10-yak1*^{K398Y}]. Dilutions, from left to right, were: 0, 1/2, 1/2, 1/10, 1/10 and 1/10.

RETT *et al.* 1991; M. M. MENOLD and S. GARRETT, unpublished results) had a very weak, but reproducible, adverse effect on heat shock resistance. Although we have not pursued the mechanism of this effect any further, it may occur through the nonfunctional protein product interfering with wild-type Yak1 protein encoded by the chromosomal gene or the phosphorylation of a stress-related substrate of Yak1.

The heat-sensitive phenotype of *yak1* mutants is not alleviated by mutations in *SCH9*: The *YAK1* gene was first identified in a screen for downstream components of the A kinase pathway. Previous results were most consistent with a model in which Yak1 and A kinase were components of parallel, and antagonistic, growth-related pathways. However, we have been unable to rule out the possibility that Yak1 could directly mediate A kinase growth control by serving as a negative regulator of cell growth that, in turn, could be inactivated by phosphorylation by the A kinase (GARRETT *et al.* 1991). The heat shock-sensitive phenotype of the *yak1* deletion strain (*e.g.*, Figure 6) was relatively weak compared to the exquisite sensitivity of the hyperactive *RAS2*^{Val19} mutant or a strain lacking the A kinase regulatory gene, *BCY1* (TODA *et al.* 1985; CANNON and TATCHELL 1987). Thus, the sensitivity associated with elevated A kinase activity cannot be accounted for solely by inactivation of the Yak1 kinase. To determine if the heat shock sensitivity conferred by the *RAS2*^{Val19} mutation could be affected by Yak1 activity, we tested the heat sensitivities of several *RAS2*^{Val19} *YAK1* strains and their isogenic *yak1* derivatives. As shown in Figure 7, the heat shock sensitivity associated with the activated *RAS2*^{Val19} allele was noticeably exacerbated by the disruption of *YAK1*. In contrast to the synergism between these two mutations, we found that disruption of *YAK1* conferred the same 50–100-fold increase in heat shock sensitivity to strains with very low A kinase activity (CAMERON *et al.* 1988) as it had to wild-type strains (data not shown).

The heat shock-sensitive phenotype associated with the loss of Yak1 function is explained in model 1 of Figure 1 by the deregulation of Sch9 activity. This model predicts that the *yak1* phenotype would be alleviated by a corresponding loss of *SCH9* function.

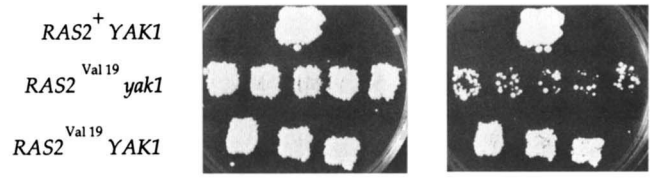

FIGURE 7.—Deletion of *YAK1* exacerbates the heat shock-sensitive phenotype of an activated *RAS2*^{Val19} mutant. Strains *RAS2*$^{+}$ *YAK1* [SGP4], *RAS2*^{Val19} *yak1* [SGP55] and *RAS2*^{Val19} *YAK1* [JR477] were patched onto rich medium (YEPD) agar for 2 days at 30° and then transferred to identical agar plates that were, or were not, briefly exposed to 50° and then placed at 30° for several days.

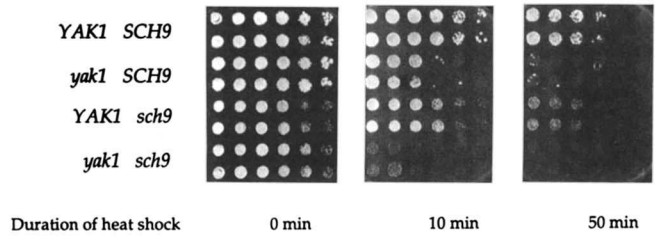

FIGURE 8.—The heat shock-sensitive phenotype of a *yak1* strain is not alleviated by loss of Sch9 function. Serial dilutions of the cultures were transferred to plates according to the protocol in MATERIALS AND METHODS, exposed to 55° for the indicated times and then incubated at 30° for several days. The strains were: *YAK1 SCH9* [SGP4], *yak1 SCH9* [AHY1], *YAK1 sch9* [AHY2] and *yak1 sch9* [AHY3]. Dilutions were as in Figure 7.

To test this hypothesis, the heat shock-sensitive phenotypes of isogenic *YAK1 SCH9*, *yak1 SCH9*, *YAK1 sch9* and *yak1 sch9* strains were compared. Duplicate *YAK1 sch9* deletion strains exhibited a heat shock response that was essentially identical to their *YAK1 SCH9* parents (Figure 8). In addition, the loss of Sch9 function did not appear to suppress the heat shock-sensitive phenotype of the *yak1* deletion strain (compare the survival of the *yak1 sch9* double mutants with that of the *yak1 SCH9* strains after 10 and 50 min). Indeed, the heat shock sensitivity of the double mutant was comparable to, if not greater than, that of cells lacking only Yak1. This result is consistent only with models 2 and 3 of Figure 1.

***YAK1* is allelic with *SRA7* and maps on chromosome *X*:** The *YAK1* gene was assigned to chromosome *X* by hybridization to electrophoretically separated

yeast chromosomes. Subsequent genetic mapping placed *YAK1* on the left arm of chromosome *X*, tightly linked to *TPK1* in the order *CENX . . . PBS2 . . . URA2 . . . YAK1 . . . TPK1* (Table 2).

Genetic analyses have identified several loci which can be mutated to alleviate the growth defect of a Ras-deficient strain (GARRETT and BROACH 1989; CANNON *et al.* 1986). One of these, *SRA7*, was previously linked to *TPK1* on chromosome *X* (CANNON and TATCHELL 1987). Despite the proximity of *YAK1* and *SRA7*, the identity of the two loci seemed unlikely given the recessive and dominant nature of the respective suppressing alleles. To investigate the relation between these two suppressors, the *SRA7-1* and *SRA7-14* alleles (K. TATCHELL, personal communication; J. CANNON, personal communication) were retested, by diploid analyses, for their ability to suppress several different defects in the A kinase pathway. In all cases, the *SRA7-1* and *SRA-14* alleles were recessive. To test if *yak1* and *SRA7-14* (hereafter referred to as *sra7-14*) were allelic, a *MAT***a** *ras*(Ts) *yak1* strain was mated with a *MATα ras*(Ts) *sra7-14* strain of the opposite mating type. The growth of the diploid at the nonpermissive temperature was taken as evidence that the *yak1* and *sra7-14* mutations were in the same gene. In addition, the *sra7-14* mutant was complemented by the wild-type allele of *YAK1* carried on a low copy plasmid; further evidence that *yak1* and *sra7-14* define a single complementation group. Finally, we failed to observe recombination (in 40 tetrads) between the *yak1::LEU2* and *sra7-1* alleles, suggesting the genes were identical or very tightly linked.

DISCUSSION

The Yak1 and Sch9 kinases define independent signalling pathways that can compensate for the loss of yeast A kinase function: The *SCH9* and *YAK1* genes were originally identified as suppressors of the loss of A kinase function. The evidence presented here suggests that the products of these two genes do not comprise a single, linear pathway and, therefore, suppress the A kinase defect by different mechanisms.

The three models presented in Figure 1 attempt to explain suppression of the A kinase defect by gain and loss of function mutations in *SCH9* and *YAK1*, respectively. Of these models, two are inconsistent with one or more of our results. In particular, model 1 is at odds with the observation that the conditional growth of a temperature-sensitive A kinase mutant is alleviated by disrupting *YAK1* (or overexpressing *SOK1* or *SOK2*), even in a strain lacking the *SCH9* product (Figure 4). According to model 1, the growth defect associated with the loss of A kinase function should be affected by Yak1 activity only in an Sch9-proficient strain. Although the conditional defect is not completely remediated by the *yak1* deletion, even nominal suppression must be explained by an Sch9-independent mechanism. Our epistasis results could be reconciled with model 1 if *SCH9* was a member of a family of functionally redundant protein kinase genes. According to that scenario, A kinase suppression by Yak1 inactivation would remain intact as a result of the derepression of one or more Sch9 isozymes. However, several observations suggest that is not the case. First, *S. cerevisiae* does not appear to contain a gene that shares significant structural homology with the catalytic portion of *SCH9* since repeated probing at reduced stringency failed to identify a cross-hybridizing band. Second, we have been unable to identify a functional homolog of the *SCH9* kinase in several genetic screens directed at restoring wild-type growth to an *SCH9*-deficient strain (A. D. HARTLEY and S. GARRETT, unpublished results). Finally, the presence of an *SCH9* functional homolog is inconsistent with our observation that deletion of *YAK1* (as well as *SOK1* and *SOK2* overexpression) does not alleviate the slow growth defect of a strain lacking the *SCH9* product. It seems unlikely that derepression of an *SCH9* homolog would suppress the loss of the A kinase without also compensating for the loss of Sch9.

The second model proposed to account for the interaction between Sch9 and Yak1 suggests that the Yak1 kinase functions as a negative regulator of growth that is normally inactivated by Sch9-dependent phosphorylation. This model is particularly attractive since the slow growth of an *sch9* strain is alleviated by activation of the A kinase (TODA *et al.* 1988), and Yak1 is phosphorylated *in vivo* and *in vitro* by several cellular kinases (GARRETT *et al.* 1991). However, model 2 predicts that the slow growth phenotype associated with deletion of *SCH9* would be suppressed by overexpression of the A kinase *or* lesions that removed the *YAK1* product. It is clear from our results that the *sch9* defect is relieved only by activated A kinase. The simplest interpretation of this result is that the slow growth of the *SCH9* strain is not due to unregulated Yak1 kinase activity. Once again, this conclusion rests on the assumption that the *YAK1* gene product is not a redundant member of an overlapping family of Yak1 proteins. However, biochemical [DNA hybridization (data not shown); immunoprecipitation (GARRETT *et al.* 1991)] and genetic [suppressor studies and synthetic lethal analysis (M. M. MENOLD and S. GARRETT, unpublished results)] experiments have failed to provide any support for the notion that *YAK1* is duplicated. We also do not think the lack of suppression is due to weak suppression since deletion of *YAK1* alleviates the growth arrest of strains lacking all A kinase activity (GARRETT and BROACH 1989; CANNON and TATCHELL 1987). Finally, the *sch9* growth defect was also refractory to suppression by two new genes isolated as gene-dosage suppressors of an A kinase-

deficient strain, further support for the independence of the pathways regulated by Sch9, Yak1 and the A kinase.

By eliminating models 1 and 2, our results argue strongly for the third model of Figure 1, in which the Sch9 and Yak1 kinases regulate cell growth through independent pathways. Our data could be accounted for by more complicated versions of models 1 and 2. However, we see no reason to invoke such changes when all of the available data (TODA *et al.* 1988; DENIS and AUDINO 1991; DI BLASI *et al.* 1993; GARRETT and BROACH 1989; GARRETT *et al.* 1991) can be adequately explained by a simpler model (model 3).

The models of Figure 1 are purposely vague about the molecular details of A kinase suppression. Based on previous data, TODA *et al.* (1988) proposed a model in which the *SCH9* product might affect a growth regulatory pathway that was partially redundant with the A kinase pathway. Activation of either pathway would presumably compensate for the loss of some essential function under the control of the other. Although we have not extensively addressed the mechanism of the reciprocal suppression, our results are inconsistent with one prediction of that model. If, for example, the Sch9 and A kinase pathways were partially redundant, suppressors of a defect in one pathway should also suppress the growth defect of the other. However, disruption of *YAK1* had no effect on the slow growth of the *sch9* strain. Because the *yak1* deletion is able to confer growth to a strain completely deficient in A kinase activity (GARRETT and BROACH 1989; CANNON and TATCHELL 1987), we do not think this lack of suppression is due simply to "weak" suppression. The lack of effect also cannot be explained by placing Yak1 activity upstream of *sch9* since the temperature-resistant growth of a *tpk*(Ts) *yak1* strain is maintained on deletion of *SCH9* (Figure 4). Accordingly, we think the collective results fit best with the alternate possibility put forward by TODA *et al.* (1988) for Sch9 function. Namely, the *SCH9* gene encodes a highly divergent form of the A kinase. This need not imply that Sch9 activity is regulated by cAMP, but rather that the substrate specificities of the two kinases are similar enough to allow significant phosphorylation of the substrates of one pathway when the kinase of the other pathway is overproduced.

The Yak1 kinase contributes to thermotolerance: Acquisition of thermotolerance is a complicated process that is influenced by multiple environmental signals and genetic loci (THOMPSON-JAEGER *et al.* 1991; TOH-E *et al.* 1993; ELLIOT and FUTCHER 1993; ROWLEY *et al.* 1993; WERNER-WASHBURNE *et al.* 1993). A kinase activity is but one of many cellular functions that has been implicated in this process (SHIN *et al.* 1987). Cells exhibiting elevated levels of A kinase are exquisitely sensitive to high temperatures (CANNON and TATCHELL 1987), whereas attenuated A kinase activity renders an otherwise wild-type strain more resistant to heat (CAMERON *et al.* 1988). Despite the remarkable correlation between A kinase activity and sensitivity to elevated temperatures, the molecular mechanism of A kinase phosphorylation-dependent thermotolerance is not understood. A gene (*SKO1*) with homology to the bZIP family of transcriptional activators was recently isolated as a high copy suppressor of the heat shock-sensitive phenotype of cells overexpressing *TPK2*, however its role in heat tolerance is not clear (NEHLIN *et al.* 1992).

The observation that the Yak1 kinase may also affect thermotolerance provides another example of a growth-related process (ELLIOT and FUTCHER 1993; WERNER-WASHBURNE *et al.* 1993) that is affected by both A kinase and Yak1 (GARRETT *et al.* 1991; GARRETT and BROACH 1989; THOMPSON-JAEGER *et al.* 1991). Although the absolute effect of altering Yak1 kinase activity is not as great as that elicited by modification of the A kinase, the protective effect of the *YAK1* product (Figures 5 and 6) correlates exactly with its relative abundance (GARRETT and BROACH 1989; BROACH and DESCHENES 1990). Moreover, the limited effect of Yak1 alteration on thermotolerance is consistent with the observation that deletions of *YAK1* only partially alleviate the growth defect of an A kinase-deficient strain (GARRETT and BROACH 1989; THOMPSON-JAEGER *et al.* 1991), and, in contrast to A kinase, have no apparent effect on glycogen metabolism (GARRETT and BROACH 1989; THOMPSON-JAEGER *et al.* 1991). Thus, A kinase activity must regulate other pathways and processes, some of which also impinge upon cell growth and resistance to stress (BROACH and DESCHENES 1990). While the molecular mechanism of Yak1-dependent thermotolerance is not understood, several observations may provide some insight into the role of Sch9 and the A kinase. First, the heat shock sensitivity of a *yak1* strain was not relieved by mutations that removed Sch9 activity. Thus, the heat sensitivity conferred by Yak1 inactivation is not due to the derepression of Sch9 activity, providing further argument against model 1. Second, the growth-specific interactions between Yak1 and the A kinase have previously been described in two models (GARRETT and BROACH 1989; GARRETT *et al.* 1991). In one, Yak1 could directly mediate A kinase growth control and heat resistance by serving as a negative regulator of cell growth/heat sensitivity that could be inactivated by A kinase-dependent phosphorylation. Alternatively, Yak1 and A kinase could be components of parallel, interdependent pathways that regulate both growth and thermotolerance. In either case, it is clear that the effect of the A kinase on thermotolerance cannot be explained solely by its regulation of Yak1 activity. If it were, strains completely lacking

Yak1 activity would exhibit a heat shock sensitivity identical to strains containing fully activated A kinase activity (*Ras2*^{Val19}, *bcy1*) and the heat shock sensitivity of the *yak1* strain would not be exacerbated by increased A kinase activity. These, and other (THOMPSON-JAEGER *et al.* 1991; GARRETT and BROACH 1989) results clearly suggest otherwise. We would argue, therefore, that the Yak1 protein, as part of the A kinase pathway or as a component of a parallel, interdependent pathway, is one of many growth-regulatory proteins that affects resistance to stress.

Finally, we have shown that mutations in *YAK1* and *SRA7*, isolated in a selection for suppressors of an A kinase growth defect (CANNON *et al.* 1986), are allelic. While we do not fully understand the original classification of the *SRA7* mutations as dominant suppressors, it is fairly clear that these suppressors are identical and it is the *loss* of function of Yak1 that restores growth to an A kinase-deficient strain.

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