Published in final edited form as:

J Mol Biol. 2007 April 20; 368(1): 30–43.

Mutual Interdependence of *MSI1* (*CAC3*) and *YAK1* in *Saccharomyces cerevisiae*

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Summary

The *MSI1* (*CAC3*) gene of *Saccharomyces cerevisiae* has been implicated in diverse cellular functions including suppression of the *RAS*/cAMP/Protein Kinase A signaling pathway, chromatin assembly and transcriptional co-repression. Seeking to identify the molecular mechanisms by which Msi1p carries out these distinct activities, a novel genetic interaction was uncovered with *YAK1*, which encodes a kinase that antagonizes the *RAS*/cAMP pathway. *MSI1* was capable of efficiently suppressing the heat shock sensitivity caused by deletion of *yak1*. Surprisingly, the *YAK1* gene is required for Msi1p to associate with Cac1p in the yeast two-hybrid system. A new activity of Msi1p was identified: the ability to activate transcription of a reporter gene when tethered near the promoter, but only in the absence of fermentable carbon sources. This transcriptional activation function was substantially diminished by the loss of *YAK1*. Furthermore, *MSI1* influences *YAK1* function; overexpression of *YAK1* decreased the growth rate, but only in the presence of a functional *MSI1* gene. Finally, it is shown that *YAK1* antagonizes nuclear accumulation of Msi1p in nonfermenting cells. Taken together, these data demonstrate a novel interaction between Msi1p and Yak1p in which each protein influences the activity of the other.

Keywords

Signal transduction; chromatin assembly; ras; nonfermentable carbon source; kinase

Introduction

Growth of the budding yeast *Saccharomyces cerevisiae* is coordinated by multiple signal transduction pathways that are sensitive to nutrient availability. Among these, the *RAS*/cAMP pathway is a major mechanism by which yeast respond to environmental glucose. Glucose-dependent activation of a Ras protein stimulates adenylyl cyclase, leading to the accumulation of cAMP and activation of Protein Kinase A (PKA).¹ Activation of the *RAS*/cAMP pathway leads to a broad suite of responses that includes increases in glycolytic and growth rates and a concomitant decrease in storage carbohydrate concentrations and resistance to environmental stresses. This pathway is essential as these yeasts must retain at least partial activity for the

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survival and growth. Despite considerable investigation, the molecular mechanisms by which the *RAS/cAMP* pathway regulates growth in response to carbon source remain to be elucidated. In this paper, we present new data that expands our knowledge of the interdependent regulation of this pathway by the products of the *MSI1* and *YAK1* genes.

YAK1 was initially identified as a gene whose disruption suppressed the lethal phenotype associated with either the loss of *RAS* function or the loss of the catalytic subunit of PKA.² Yak1p is a kinase, which acts as a negative regulator of overall growth and activates many of the same processes that are affected by PKA, including heat shock resistance³ and pseudohyphal growth.⁴ Yak1p can be phosphorylated by PKA directly *in vitro*⁵ and is transcriptionally activated indirectly by PKA⁶ indicating that Yak1p functions downstream of PKA. In cells with decreased *RAS/cAMP* pathway activity, excess copies of *YAK1* leads to growth arrest⁵ confirming the role of this gene as a growth inhibitor.

In addition to being affected by PKA, Yak1p has also been implicated as a regulator of PKA function in what is thought to be an autoregulatory loop. The presence of glucose is sensed by yeast cells and leads to activation of PKA, whereas growth on nonfermentable carbon sources such as glycerol or ethanol do not activate the kinase.¹ Griffioen *et al.* demonstrated that the phosphorylation and subcellular localization of Bcy1p (the regulatory subunit of PKA) is regulated by the carbon source available to the cell. Bcy1p is mostly nuclear in glucose-grown cells but found in both the nucleus and cytoplasm in ethanol-grown cells in a *YAK1*- and *ZDS1*-dependent process. The localization is a direct result of Yak1p-dependent phosphorylation of Bcy1p, where the phosphoprotein is either actively exported from the nucleus or retained in the cytoplasm by associating with Zds1p.⁷ Surprisingly, phosphorylation by Yak1p and relocalization of the regulatory subunit does not seem to have a substantial effect on many PKA functions but is hypothesized to redirect PKA to a different set of substrates.⁸ Furthermore, the subcellular localization of the Yak1 protein itself is carbon source-dependent, as it accumulates in the nucleus in the absence of glucose.⁹ Yak1p is an essential participant in at least one other nutritional signaling pathway beyond the *RAS/cAMP* pathway. The TOR pathway is conserved from yeast to humans and is active in well-fed cells but becomes blocked by either amino-acid starvation or the drug rapamycin. Inhibition of this pathway in yeast leads to a decrease in translation initiation and cell-cycle arrest¹⁰. Blocking the TOR pathway leads to the nuclear accumulation of Yak1p¹¹ and activates Yak1p to phosphorylate the Crf1p co-repressor allowing its nuclear retention as well. The Crf1 protein has recently been shown to interact with the Fhl1p transcription factor and inhibits the transcription of ribosomal protein genes.¹² Viewed together, these data clearly demonstrate that the Yak1p kinase plays an important role in nutritional signaling through both the *RAS/cAMP* and TOR pathways.

The *MSI1* gene from yeast was originally identified as a high-copy suppressor of activated *RAS/cAMP* signaling pathway.¹³ Overexpression of this gene was found to efficiently suppress heat shock sensitivity and defective sporulation phenotypes associated with the constitutively activated *RAS2^{G19V}* allele.¹³ All eukaryotic genomes examined to date contain at least one *MSI1* homolog, but to date no homolog has been identified in prokaryotes.¹⁴ Most genes in this family encode proteins with seven WD40 repeat domains that are thought to form a flat disk consisting of seven β -propeller folds which have been proposed to mediate a variety of protein-protein interactions.¹⁵ The yeast Msi1p also contains a 57-residue amino-terminal extension not found in most other members of the family. The human genome encodes two Msi1p homologs, RbAp46 and RbAp48, and the overexpression of either of these proteins can substitute for Msi1p and suppress an activated *RAS/cAMP* pathway in yeast.¹⁶ More recently, it has been demonstrated that overexpression of Msi1p does not alter the quantity, subcellular localization or phosphorylation state of Ras2p.¹⁷ The ability of Msi1p to suppress the *RAS/cAMP* pathway is fully dependent on the presence of Bcy1p, yet surprisingly does not change cAMP concentrations or PKA activity.¹⁸ Furthermore, this suppression is dependent upon the

physical interaction of Msi1p with the TOR pathway-regulated kinase Npr1p.¹⁷ The precise mechanism(s) by which Msi1p suppresses the RAS/cAMP pathway in yeast remains unclear.

Considering that the human Ras proteins are key players in oncogenesis,¹⁹ proteins that suppress their activity are of great interest. In yeast, Ras proteins activate PKA by stimulating cAMP synthesis.¹ However, in higher eukaryotes, Ras proteins do not signal through cAMP but rather work through multiple effectors including serine/threonine kinases, phosphoinositide 3-kinases and phospholipases.¹⁹ Thus, Msi1p homologs would not necessarily be expected to suppress Ras signal transduction in animals. However, the *C. elegans* homolog of Msi1p (lin-53) has been shown to functionally antagonize the Ras signaling pathway *in vivo*.^{20, 21} Overexpression of RbAp48 in human cells correlates with the dephosphorylation of Akt, which is downstream of a phosphoinositide 3-kinase and Ras.²² Furthermore, overexpression of RbAp48 has recently been shown to induce p53-mediated apoptosis in mammalian exocrine cells.²³ Therefore, Msi1p homologs may indeed down-regulate Ras signaling in higher organisms as well as yeast.

Surprisingly, Msi1p has activities beyond the suppression of RAS/cAMP signaling. Msi1p/Cac3p was isolated as one of three subunits of the Chromatin Assembly Factor-I (CAF-I), along with Cac1p and Cac2p.²⁴ The CAF-I complex is conserved from yeast to humans and assembles histones H3 and H4 onto newly replicated DNA.²⁴ In vertebrates, lack of CAF-I activity causes checkpoint activation, S phase arrest and eventually leads to programmed cell death.²⁵⁻²⁷ In contrast, CAF-I function is not essential for viability in *S. cerevisiae*,^{24, 28} but its absence leads to significantly abnormal chromatin and the misregulation of many functions. In yeast, the change in chromatin structure leads to a variety of phenotypes, including increased sensitivity to UV radiation,²⁴ defects in transcriptional silencing,^{24, 28} retrotransposition,²⁹ and kinetochore function.³⁰ Many of the phenotypes associated with the loss of CAF-I function are relatively mild. The additional mutation of the *HIR* genes, *ASF1*, *MEC1*, *APC5* or the *SAS* genes lead to a synergistic enhancement of many of these phenotypes.²⁹⁻³⁴ Importantly, deletion of either *CAC1* or *CAC2* does not impair the ability of Msi1p to suppress the activated RAS/cAMP pathway,^{17, 18} indicating that Msi1p has at least two separable functions.

Yet a third function was demonstrated by Kennedy *et al.* who showed that Msi1p and the histone deacetylase Rpd3p are essential for the ability of the retinoblastoma susceptibility gene product (pRB) to repress transcription in yeast.³⁵ In higher eukaryotes, pRB is a well-characterized tumor suppressor protein that helps maintain the quiescent cell state. pRB physically interacts with the E2F family of transcription factors and represses transcription from the associated promoters in G₀ phase.³⁶ The requirement of Msi1p as a co-repressor is clearly conserved in *Drosophila*, as the Msi1p homolog p55 binds to pRB homologs, localizes to regulated promoters and is needed for the correct regulation of a subset of pRB-regulated genes.³⁷ Similarly, RbAp48 has been shown to bind pRB *in vitro* and *in vivo*¹⁶ and to co-purify with multiple histone deacetylases.^{38, 39} Microinjection of anti-RbAp48 antibodies into murine cells increases the activity of an E2F-driven reporter gene, consistent with the idea that RbAp48 is acting as a co-repressor with pRB.³⁹ Taken together, these data suggest a model in which pRB acts by binding to Msi1p/RbAp48, which in turn associates with one or more histone deacetylases to repress transcription.³⁵ Significantly, the ability of Msi1p to act as a co-repressor for pRB is independent of Cac2p,³⁵ suggesting that this is likely a third function for Msi1p.

The mechanism(s) by which Msi1p suppresses RAS/cAMP signaling and the molecular basis for the multiple functions of this protein remain obscure. To better understand Msi1p function, we have investigated the interaction of *MSI1* with *YAK1*. In this paper, we present new data

that shows a mutual dependence between these two genes for many, but not all, of their phenotypes.

Results

MSI1 suppresses the *yak1Δ* phenotype

Overexpression of *MSI1* suppresses dominant *RAS2* mutations that constitutively activate the *RAS/cAMP* pathway but does not suppress activation of the pathway by deletion of *BCY1*. This observation has been interpreted to mean that Msi1p acts between Ras and PKA.¹³ However, the more recent observation by Zhu *et al.* that *MSI1* overexpression does not change cAMP concentration or PKA activity and that the ability of *MSI1* to suppress this pathway is dependent upon an intact *BCY1* gene¹⁸ indicates that the effect of *MSI1* upon the *RAS/cAMP* pathway is more complex. To better understand how *MSI1* affects the *RAS/cAMP* pathway, we sought to determine how *MSI1* and *YAK1* interact. Deletion of the *yak1* gene causes a variety of phenotypes that are highly similar to an activated *RAS/cAMP* pathway, including sensitivity to transient heat shock.³ Additionally, loss of *yak1* synergizes with the constitutively activated *RAS2^{G19V}* allele, causing enhanced sensitivity.³ We measured the ability of *MSI1* to suppress the heat shock sensitivity of *yak1Δ* mutants alone or in combination with an activated *RAS2* allele. We found that excess copies of Msi1p efficiently suppressed the heat shock sensitivity of either *RAS2^{G19V}* or *yak1Δ* mutants, as well as the hypersensitivity of cells carrying both of these genetic alterations (Fig. 1). Overexpression of Msi1p has been proposed to suppress the *RAS/cAMP* pathway by sequestering the Npr1p kinase.¹⁷ In agreement with that model, deletion of *NPR1* is also able to suppress the heat shock sensitivity caused by an activated *RAS2* allele or deletion of *YAK1* (Fig. 1). Considering that *YAK1* has been shown to act downstream of PKA,^{3, 6} our data indicate that Msi1p can suppress the *RAS/cAMP* pathway at points beyond PKA.

Having established this connection between *MSI1* and *YAK1* in the regulation of the *RAS/cAMP* pathway, we investigated whether *YAK1* is important for other known functions of Msi1p, beginning with its ability to physically associate with Cac1p and Cac2p to form CAF-I.²⁴ To investigate this function, we utilized the previous observation that Msi1p and Cac1p interact in the yeast two-hybrid system,¹⁷ enabling efficient transcription of the *HIS3* reporter gene and allowing the cells to grow in the absence of histidine and in the presence of the His3p competitive inhibitor 3-aminotriazole (3-AT). Considering that neither *CAC1* nor *CAC2* is needed for *MSI1* to suppress heat shock sensitivity caused by *RAS2^{G19V}*,^{17, 18} we hypothesized that loss of *yak1* or activation of *RAS2* would not affect the formation of CAF-I. Surprisingly, we found that an intact *YAK1* gene was essential for Msi1p/Cac1p two-hybrid association (Fig. 2). Loss of *YAK1* does not simply interfere with the two-hybrid reporter system as a fragment of the murine p53 protein and SV40 large T antigen still interacted efficiently in the *yak1Δ* strain. Additionally, the *yak1Δ* modification does not interfere with all Msi1p associations by simply destabilizing the protein, as Msi1p was still able to bind efficiently to Npr1p (Fig. 2). Furthermore, Cac1p and Cac2p bind to each other in the two-hybrid system and this binding was independent of both the *MSI1* and *YAK1* genes. A robust two-hybrid interaction was not detected between Msi1p and Cac2p (data not shown) which is consistent with the idea that the two smaller subunits of CAF-I (*i.e.*, Cac2p and Msi1p) bind only to the largest subunit (Cac1p) and do not directly bind to each other. Since loss of *YAK1* leads to the activation of the *RAS/cAMP* pathway, we next examined if the loss of Msi1p/Cac1p was due to the activation of this pathway or was specific to *yak1Δ*. The activated *RAS2^{G19V}* allele had no effect on the association of Msi1p and Cac1p. Overexpression of the *PDE2* gene encoding the high-affinity phosphodiesterase capable of degrading cAMP suppresses the *RAS/cAMP* pathway⁴⁰ but also had no effect on the Msi1p/Cac1p interaction (Fig. 2). Therefore, loss of the *YAK1* gene can prevent only Msi1p from associating with the CAF-I complex and need not lead to the general dissociation of the complex. Although the

effect that *MSI1* has on the *RAS*/cAMP pathway has been established for more than fifteen years,¹³ this is the first indication that the Yak1 kinase from this pathway can have an effect on the chromatin assembly complex.

Carbon-Source Regulated One-Hybrid Activity of Msi1p

Knowing that the *RAS*/cAMP pathway is principally activated by the presence of glucose,¹ we attempted to extend these observations by altering the carbon source present and assessing its effect on the two-hybrid interaction. To our surprise, we found that Msi1p alone activated transcription of the *HIS3* reporter gene (and thus resistance to 3-AT) when the cells were grown using glycerol as a carbon source (Fig. 3a). We refer to this phenomenon as the carbon-source regulated one-hybrid activity of Msi1p. Other proteins fused to the Gal4p DNA binding domain (DB), including Act1p from *S. cerevisiae*, lamin C from *Homo sapiens*, a p53 fragment from *Mus musculus* or RscS from *Vibrio fischeri* did not show a one-hybrid activity under identical conditions (data not shown). Neither DB-Cac1p nor DB-Cac2p showed a one-hybrid activity (data not shown) indicating that the transcriptional activation is not due to the recruitment of CAF-I to the promoter. Msi1p showed a regulated one-hybrid activity in either the PJ694 or HF7c reporter strain (data not shown).

To characterize this carbon-source regulated one-hybrid activity, fifteen different carbon sources were tested for their ability to induce one-hybrid activity when they were the sole carbon source in the media. All carbon sources either fully induced the one-hybrid activity or were fully noninducing; no intermediate inductions were observed. We found that the one-hybrid activity of Msi1p was robustly induced by ten different carbon sources, all of which are metabolized nonfermentatively (Fig. 3b). They include the two- and three-carbon molecules glycerol, acetate, ethanol, pyruvate and lactate, which are generally catabolized via the Krebs cycle and thus consumed nonfermentatively. The monosaccharide ribose and the disaccharides cellobiose and trehalose are also inducing carbon sources. Ribose cannot be catabolized by fermentation.⁴¹ Both trehalose and cellobiose are glucose dimers, joined by different linkages. Despite the fact that the constituent monosaccharide supports fermentative growth, trehalose⁴² and cellobiose⁴³ are consumed nonfermentatively by several yeast species, a phenomenon known as the Kluyver effect. We confirmed that this specific strain of *S. cerevisiae* metabolizes ribose, trehalose and cellobiose strictly nonfermentatively by showing that respiration-deficient (ρ^0) mutants⁴⁴ failed to grow when these sugars were the sole carbon source (data not shown). Finally, both myristate (a saturated fatty acid) and oleate (an unsaturated fatty acid) induced a one-hybrid response.

Noninducing carbon sources include the monosaccharides glucose and fructose, the disaccharides maltose and sucrose, and the trisaccharide raffinose. All of these carbon sources support alcoholic fermentation in *Saccharomyces cerevisiae*. Exposing cells to both 2% glucose and 2% glycerol simultaneously did not induce one-hybrid activity (data not shown) presumably because the cells attempt to ferment the glucose first but can't grow on this media without activation of the reporter gene. Providing a sub-optimal amount of glucose (0.2% instead of the usual 2%) also did not induce a one-hybrid activity (data not shown), suggesting that the one-hybrid activity did not result from simple nutrient limitation or slower growth. Other stressful conditions, including elevated temperature, high osmolarity, limiting concentrations of nitrogen or the inclusion of the TOR-inactivating drug rapamycin, or histone deacetylase inhibitors trichostatin A or sodium butyrate did not induce the one-hybrid activity in glucose nor block it in glycerol (data not shown). Under the conditions tested thus far, the correlation between one-hybrid activity of Msi1p and nonfermentative growth is perfect (Fig. 3b).

The carbon-source regulated one-hybrid activity was unaffected by the deletion of *CAC1*, *NPR1* or *ZDS1*, the *RAS2*^{G19V} mutation or overexpression of either *PDE2*, *NPR1* or *YAK1* (Fig.

3c and data not shown). However, when the *YAK1* gene was deleted, we observed a reproducible diminishment of the regulated one-hybrid activity (Fig. 3a). The strength of the one-hybrid activity was reduced approximately 100-fold and the resulting colonies were smaller than the wildtype controls. The *npr1Δ yak1Δ* double mutant had no additional effect on the one-hybrid activity (data not shown). The effect of *YAK1* is specific to Msi1p, because the deletion of *YAK1* had no effect on activation of the reporter gene by an intact Gal4p (Fig. 3a). Therefore, the presence of the Yak1 kinase is essential for full induction of the regulated one-hybrid activity of Msi1p.

YAK1 is Dispensable for Co-repression with pRB

The human tumor suppressor protein pRB is one of the best-understood negative regulators of cell cycle progression in mammals. pRB indirectly associates with DNA via additional proteins and acts as a transcriptional repressor during the G₀/G₁ phases of the cell cycle. At least three distinct mechanisms of repression have been demonstrated, including the recruitment of histone deacetylases.³⁶ Some reporter strains of *Saccharomyces cerevisiae* show leaky transcription of the *HIS3* reporter leading to a baseline level of 3-AT resistance; pRB fused to the Gal4 DB (DB-pRB) can repress this leaky transcription.³⁵ Significantly, full repression by pRB requires an intact *MSI1* gene.³⁵ Presumably, this reflects the demonstrated ability of pRB to associate with the human Msi1p homologs RbAp46 and RbAp48,¹⁶ which in turn associate with at least one histone deacetylase.⁴⁵ The co-repressor phenotype seems to be less dramatic in our hands than the one published by Kennedy *et al.*, but we did find a reproducible difference in resistance that is dependent upon *MSI1*. We then sought to determine if *YAK1* influenced the ability of Msi1p to act as a co-repressor for pRB. Deletion of the *YAK1* gene had no effect on transcriptional repression by pRB either in the absence or presence of *MSI1* (Fig. 4). Additionally, growing these cells on glycerol instead of glucose had no effect on the ability of Msi1p to act as a co-repressor of transcription with pRB (data not shown). In sharp contrast to other activities of Msi1p, the corepressor activity is not affected by *YAK1*.

YAK1 overexpression decreases growth rate in an *MSI1*-dependent fashion

YAK1 has been described as a negative regulator of growth in *Saccharomyces cerevisiae*³ and overexpression of the kinase has been shown to cause full growth arrest if signaling through the *RAS*/cAMP pathway is diminished.⁵ We sought evidence that excess copies of *YAK1* might lead to more subtle and quantifiable growth defects in the presence of wildtype *RAS*/cAMP signaling. Yeast cells carrying the plasmid pRS426-*ADHI-YAK1* (a high-copy plasmid that has the *YAK1* coding region under the control of the strong *ADHI* promoter⁴) had an exponential phase doubling time in glucose that was nearly twice as long as cells carrying a control plasmid (Fig. 5). When grown using glycerol as the carbon source, overexpression of *YAK1* still caused the maximal growth rate to slow nearly two-fold (data not shown). However, in the absence of the *MSI1* gene, the overexpression of *YAK1* had no effect on the doubling time of the culture compared to the wildtype yeast cells. Deletion of the *CAC1* gene did not affect the ability of *YAK1* to decrease the growth rate (Fig. 5), indicating that this phenotype is specific to *MSI1* and not due to general chromatin defects that can lead to a modest activation of checkpoints during the cell cycle.⁴⁶ Similarly, deletion of *NPR1* had no effect on the ability of *YAK1* to increase the doubling time. The lag times for these strains showed no reproducible differences (data not shown). This is the first reported indication that the product of the *MSI1* gene is required for a function of Yak1p.

Truncation of Msi1p blocks Cac1p Binding and the Regulated One-Hybrid Activity

We sought mutations in the *MSI1* gene that might be able to genetically separate some of these functions. We began by taking advantage of an existing restriction site to generate *msi1-8*, a mutant that truncates the protein after amino acid 281. This mutation removes three of the

seven WD40 domains present in Msi1p and is similar to the naturally occurring *cac3-1/msi1-1* allele that truncates the protein after amino acid 292, which appears to have fully lost the ability function in chromatin assembly.²⁹ The ability of *msi1-1* to support other functions is unknown. Immunoblotting indicated that DB-*msi1-8* fusion protein was approximately as abundant as the DB-Msi1 fusion protein (data not shown), indicating that this variant is reasonably stable *in vivo*. As predicted, Msi1-8p failed to productively interact with Cac1p in the two-hybrid system (Fig. 6a). In contrast, both Msi1-8p and full-length Msi1p bound Npr1p equally well (Fig. 6a) demonstrating that the N-terminal extension and the first four WD40 repeats are sufficient for Npr1p binding. Considering that suppression of the RAS/cAMP pathway is believed to require the binding of Npr1p and is independent of Cac1p, we hypothesized that overexpression of the *msi1-8* allele would restore heat-shock resistance to cells with an activated RAS/cAMP pathway. Indeed, overexpression of this allele suppressed an activated RAS/cAMP pathway (Fig. 6b, compare with Fig. 1). Thus, this allele effectively separates the ability of Msi1p to bind Cac1p and assemble chromatin from its ability to bind Npr1p and suppress the RAS/cAMP pathway.

We next investigated the ability of the Msi1-8p truncated protein to support the carbon-source regulated one-hybrid activity. This protein had completely lost the ability to activate transcription when the cells were grown on a nonfermentable carbon source and had a much more severe phenotype than deletion of *yak1* (Fig. 6c). These data indicate that the carboxy-terminal 130 residues are required both for binding Cac1p to associate with the CAF-I complex and for a second function that allows the carbon source regulated one-hybrid activity. Furthermore, the phenotypes resulting from the truncated *msi1* allele are qualitatively similar to those of *yak1Δ*; that is, both mutations block Cac1p binding and decrease the regulated one-hybrid activity without affecting the binding of Npr1p or suppression of the RAS/cAMP pathway. The most likely explanation for this convergence is that Yak1p affects Msi1p via the last three WD40 repeats of Msi1p.

Yak1 Affects the Localization, but not the Mobility or Steady State Accumulation, of Msi1p

We hypothesized that the regulated one-hybrid activity may be simply explained by a quantitative change in Msi1p levels in response to nonfermentative conditions. We tagged the endogenous *MSI1* gene with the myc₁₃ epitope⁴⁷ in strains with and without an intact *YAK1* gene. These strains were grown in either glucose or glycerol, lysed and the fusion protein was separated by SDS-PAGE and detected by immunoblotting. The immunoblots revealed that the Msi1 protein isolated from respiring cells consistently accumulated to a higher steady-state level compared to fermenting cells (Fig. 7). Quantitation of the immunoblots showed approximately 3-fold more Msi1 protein in cells grown in the nonfermentable carbon sources of glycerol, acetate or ethanol than cells grown in either glucose or sucrose (Fig. 7 and data not shown). The elevated accumulation of Msi1p caused by nonfermentable carbon sources was independent of either *YAK1* or *NPRI*. Considering that the levels of *MSI1* mRNA do not appreciably change as yeast cells shift from fermentative to aerobic growth,^{48, 49} it appears that the Msi1 protein is regulated translationally or post-translationally based on the carbon-source that the cells are utilizing. Additionally, no clear change in mobility that could be caused by a phosphorylation of Msi1-myc₁₃p was identified (Fig. 7). Treatment of the protein lysate with either potato acid phosphatase or lambda phosphatase had no effect on the mobility of Msi1-myc₁₃p (data not shown). Considering that at least one of the mammalian homologs of Msi1p is phosphorylated on a tyrosine residue in response to a mitogenic signal⁵⁰ and that Yak1p has been reported to be an active tyrosine kinase,⁵¹ we also sought evidence that Msi1p might be phosphorylated on a tyrosine residue. Immunoprecipitated Msi1-myc₁₃p was analyzed by western blotting with an anti-phosphotyrosine serum. Regardless of growth conditions or *YAK1* status, no evidence of tyrosine phosphorylation on Msi1p could be detected

(data not shown). These data are consistent with the idea that Yak1 does not directly phosphorylate Msi1p in response to metabolic changes.

Previous work has shown that Yak1p is either important or essential for the subcellular localization of several proteins involved in nutritional regulation^{7, 9, 11, 12} so we investigated the possibility that Yak1p could influence the subcellular localization of Msi1p. Previous work has shown that GFP-tagged Msi1p expressed from a highly induced *GAL* promoter on a high-copy plasmid is located in both the nucleus and the cytoplasm of wildtype cells.¹⁷ To determine the subcellular localization of Msi1p under more physiologically relevant conditions, we used indirect immunofluorescence to examine yeast whose endogenous *MSI1* gene is tagged with the myc epitope. When these cells are grown on the fermentable carbon source glucose, Msi1p is found throughout the cell, with no obvious accumulation of Msi1p in the nucleus of the cell. Under these physiological conditions, the subcellular location of Msi1p is independent of *YAK1*. However, when this strain was grown on the nonfermentable carbon source glycerol, a notable shift in localization occurred, with approximately 32% of the cells showing an accumulation of Msi1 in the nucleus (Fig. 8). In these nonfermenting conditions, *YAK1* showed a substantial effect on Msi1p nuclear accumulation. When *YAK1* was deleted, approximately 57% of the glycerol-grown cells showed a distinct nuclear localization of Msi1p. We found a striking reversal of this trend when Msi1p localization was assayed in a strain that overexpressed *YAK1* - in fact, in this strain, only 13% of the cells exhibited a distinct nuclear accumulation of Msi1p, resulting in a localization pattern not substantially different from that observed when cells were grown on glucose. The percentage of glycerol-grown cells showing nuclear accumulation of Msi1p was significantly different ($p < 0.05$) in all pairwise comparisons among the wildtype, *yak1Δ* and *YAK1*-overexpressed cells. Deletion of the *npr1* gene did not affect the subcellular localization pattern of Msi1p in either carbon source (data not shown). Thus we conclude that the subcellular localization is a third characteristic of Msi1p that is significantly influenced by Yak1p and the carbon source available to the cells.

Discussion

This work establishes a new phenotypic connection between the *YAK1* and *MSI1* genes of *Saccharomyces cerevisiae*. Although the association of each of these genes with the *RAS*/cAMP pathway has been established for some time,^{2, 13} our experiments revealed that their interaction is complex and extends well beyond this one pathway. Table 2 summarizes our finding that the Yak1 kinase is important for a subset of the multiple functions of Msi1p and that Msi1p is important for Yak1p function as well.

We have identified *yak1Δ* as a genetic condition that interrupts the two-hybrid interaction between Msi1p and Cac1p in the CAF-I complex without resulting in the apparent complete dissolution of the complex. This suggests a potential feedback loop to regulate *RAS*/cAMP signaling. The Yak1 kinase antagonizes *RAS*/cAMP signaling,³ thus the loss of Yak1p activity should have the effect of increasing *RAS*/cAMP signaling. We have shown that deletion of *YAK1* releases Msi1p from CAF-I, perhaps facilitating the ability of Msi1p to associate with Npr1p and decrease *RAS*/cAMP signaling.¹⁷

In the course of this work, we have uncovered a surprising and previously unknown ability of Msi1p to activate transcription. It is not unusual to find that a “bait” fusion protein intended for use in the two-hybrid system shows direct activation of transcription instead.⁵² Msi1p, however, activates transcription of the reporter only in the absence of fermentation, and the *YAK1* gene influences this activity. This regulatory pattern suggests that we have discovered a novel function of Msi1p that may be physiologically relevant. Thus far, we have only been able to measure the carbon source-regulated one-hybrid activity using the DB-Msi1p fusion protein and the artificial *HIS3* reporter gene. Neither Msi1p nor other proteins in this family

have been proposed to bind DNA directly, but are widely believed to associate with histones.¹⁴ The *bone fida* target(s) of the ability of Msi1p to activate transcription remain to be identified.

Yak1p has a substantial effect on the subcellular localization of Msi1p, promoting cytoplasmic accumulation under nonfermentative growth conditions. As such, Msi1p joins Crf1p¹² and Bcy1p⁷ as proteins whose nuclear accumulation is influenced by Yak1p in response to nutrient availability. Strikingly, the means by which Yak1p influences the subcellular localization of these three proteins are distinctly different. For both Msi1p and Bcy1p, Yak1p antagonizes nuclear accumulation but it does so in differing physiological contexts: Msi1p accumulates in the nucleus when the cells are grown in glycerol, while Bcy1p accumulates in the nucleus when the cells are grown in glucose.⁷ Yak1p directly phosphorylates both Crf1p, increasing its nuclear accumulation,¹² and Bcy1p, decreasing its nuclear accumulation in cooperation with Zds1p.⁷ However, Zds1p does not have an effect on the physical association of Msi1p and Cac1p (Fig. 2) or on the regulated one-hybrid activity, and Yak1p does not appear to phosphorylate Msi1p. Therefore, Yak1p appears to influence the localization of Msi1p by a mechanism different than those used for Bcy1p and Crf1p.

Growth on nonfermentable carbon sources has at least three effects on Msi1p: total protein accumulation increases, a cryptic ability to activate transcription when tethered to a promoter is revealed and nuclear localization of Msi1p increases. Only the last two of these effects are influenced by *YAK1*. It is tempting to speculate that the carbon-source regulated one-hybrid activity of Msi1p may be partially controlled by the protein's quantity and/or subcellular localization. The fact that Msi1p accumulated in the nucleus in approximately three times as many cells when grown under nonfermentative conditions correlates with the protein's activation of transcription under these same nutritional conditions. However, we discovered the transcriptional activation function using the Gal4 DB-Msi1p fusion protein, whose DNA-binding domain contains a strong, constitutive nuclear localization signal.⁵³ Thus we expect that this fusion protein is likely found in the nucleus regardless of the carbon source. Furthermore, deletion of *YAK1* modestly decreased the transcriptional activation function but increased the nuclear accumulation of Msi1p. Taken together, these findings indicate that Yak1p must have effects on Msi1p beyond influencing nuclear accumulation and that nuclear accumulation alone cannot explain the ability of Msi1p to activate transcription.

Importantly, Yak1p does not influence all of the functions of Msi1p. Loss of *YAK1* had no effect on the increased accumulation of Msi1p in nonfermentable carbon sources, the ability of Msi1p to act as a transcriptional co-repressor for pRB or its ability to associate with Npr1p. Thus, the Yak1 kinase is essential for only a subset of the known functions of *MSI1*.

Additionally, we have established that Msi1p can have a sizeable influence on *YAK1* phenotypes. Loss of *YAK1* causes an increase in heat shock sensitivity of cells with or without an activated *RAS* allele³ which was fully suppressed by *MSI1* overexpression. We have also found that elevated concentrations of Yak1p significantly increased the doubling time of a logarithmic yeast culture, but this phenotype is fully dependent on an intact *MSI1* gene. The ability of excess copies of *YAK1* to decrease growth rate can likely be accounted for by the intimate involvement of the *RAS*/cAMP pathway with cell cycle control¹ and quiescence.⁵⁴ What is surprising, however, is our finding that *MSI1* is required for this function of *YAK1* even though deletion of *MSI1* alone has no effect on growth rate. It is possible that nuclear accumulation of Msi1p helps to decrease the cellular growth rate, either due to growth under nonfermentable conditions or due to overexpression of *YAK1*. The molecular basis of this requirement is not yet clear but is under active investigation.

Materials and Methods

Yeast Strains and Plasmids

Plasmids YEplac195,⁵⁵ YEplac195-*PDE2*,⁵⁶ pRS426-ADH-*YAK1*,⁴ pML9 (*npr1::LEU2*),⁵⁷ pM2741 (*zds1::URA3*),⁵⁸ pDB and pDB-RB³⁵ have been previously described and were gifts from their respective laboratories. Plasmids pGAL4, pDBD-p53 and pAD-Tag were obtained from Stratagene and pGAD424 was obtained from Clontech. Plasmids YEp55-*MSI1*, pGBT9-*MSI1*, pGAD1-*NPR1*₅₆₁₋₆₀₅ and pGAD1-*CAC1* (which contains amino acids 119-606 of *CAC1*) have been described previously.¹⁷ The pGBT9-*msi1*-8 and YEp55-*msi1*-8 plasmids were made by cutting either pGBT9-*MSI1* or YEp55-*MSI1* with *Bgl*III and religating the large fragment, resulting in a deletion of the last 141 amino acids. The *CAC2* gene was amplified by PCR from a W303a strain using primers that added an *Eco*RI and *Bam*HI sites. After digestion with these two enzymes, this gene was ligated into pGBT9 to make pGBT9-*CAC2*.

Strains used in this study are listed in isogenic groups in Table 1. Strains were grown in standard laboratory complete media with the appropriate amino acid dropouts and the indicated carbon source.⁴⁴ The *npr1::TRP1* and *yak1::TRP1* deletion alleles and the *MSI1-myc13::HIS3MX6* allele were made by PCR epitope tagging as described⁴⁷ and were verified by PCR analysis of the relevant locus. Auxotrophic markers were swapped as described.⁵⁹ In some cases, the *MSI1* or *YAK1* gene was deleted by PCR amplifying the *yak1::KanMX4* or *msi1::KanMX4* allele from the deletion collection⁶⁰ and transforming the PCR product into the appropriate strain. All genomic modifications were confirmed by PCR of the locus.

Phenotypic Analyses

Determinations of heat shock survivability were performed essentially as described.¹⁷ Briefly, cells were grown in synthetic medium and 2% galactose for three days before being washed and incubated at 55°C for ninety minutes. A control aliquot of the same cells was not heat shocked to determine the number of cells initially present. After cooling to room temperature, serial dilutions were plated and fraction of cells surviving was determined. Averages of at least six trials for each strain are shown on the graphs with standard deviations indicated by the error bars.

One- and two-hybrid assays were conducted by growing the indicated yeast transformants or integrated strains on selective plates overnight followed by ten-fold serial dilutions to plates containing the specified media. Plates were incubated two to five days at 30°C before being photographed. For unknown reasons, the *Msi1p/Npr1p* was inefficient in the PJ69-4 background and thus the HF7c background was used to assay these interactions.

Growth dynamics were determined by monitoring the OD₆₀₀ of the indicated yeast strains. Cultures were diluted into SC media with 2% glucose⁴⁴ and grown at 30°C with shaking at 225 rpm. Doubling time and the length of lag phase were calculated by using the MicroFit program⁶¹ and were determined independently at least three times for each strain.

Immunoblotting

Actively growing cultures in rich media were collected by centrifugation, washed in water and resuspended in cold Lysis Buffer with protease and phosphatase inhibitors (10 mM Tris-Cl pH8.0, 150 mM NaCl, 10% glycerol, 0.05% Tween-20, 5 mM EDTA, 1 mM DTT, 1 mM PMSF, 50 mM NaF, 0.2 mM Na₃VO₄). The cells were lysed by vortexing with glass beads for five minutes at room temperature before unlysed cells were removed by centrifugation. Total protein concentration was estimated by measuring the absorbance at 280nm and equal quantities of protein were resolved by SDS-PAGE. The myc epitope was detected by incubating

the blots with a 1:40,000 dilution of the 9E10 antibody (Santa Cruz Biotechnology) followed by a 1:40,000 dilution of anti-mouse conjugated to horseradish peroxidase and detected with SuperSignal substrate (Pierce).

Indirect Immunofluorescence

Yeast strains were cultured in synthetic media lacking uracil to exponential phase before being harvested by filtration and fixed in buffer (0.1 M KH_2PO_4 , 0.5 M MgCl_2 , 40 mM KOH) with formaldehyde (1:10 dilution) for 5 minutes. Cells were washed with phosphate buffered saline (PBS), briefly sonicated, washed again in PBS, and then washed in sorbitol citrate buffer (0.1 M K_2HPO_4 , 33 mM citric acid, 1.2 M sorbitol, 2 mM DTT). Cells walls were removed by digestion with a 1:10 volume of glusulase (Perkin Elmer), a 1:100 volume of a 20 mg/ml concentration of zymolyase 20T (ICN), and 1 mM DTT for 2 hours at 30°C. Cells were washed four times with sorbitol citrate buffer. Cells were fixed to polylysine-coated slides by placing the cell suspension in wells for 3 minutes and aspirating off the suspension until they were dry. The slides were placed in 100% methanol for 3 minutes, 100% acetone for 30 seconds, and then air dried. Fixed cells were then rehydrated and blocked by incubation with 2% nonfat dry milk in PBS-Tween solution (1x PBS, 0.2% Tween 20) overnight at 4°C. Primary monoclonal antibody 9E10 was diluted 1:200 in blocking solution, cleared for 2 minutes by centrifugation in a microcentrifuge, and incubated on the cells for 2 hours. The wells were washed with 2% nonfat dry milk PBS-Tween solution quickly four times, followed by three 5 minute washes. Secondary antibody (Cy3-conjugated anti-mouse immunoglobulin G; Jackson Immunochemicals) was diluted 1:200 in blocking solution, cleared, and incubated on the wells for 2 hours. Cells were washed again and mounting solution (10% 1x PBS in glycerol with 0.02 $\mu\text{g/ml}$ DAPI [4', 6'-diamidino-2-phenylindole] and 1 mg/ml of phenylenediamine) was added to the wells. The negative control for integrated myc-tagged Msi1 protein was the wild-type strain yJB195. At least three independent samples were analyzed for each growth condition and more than 300 cells were counted per sample. Statistical significance of the data was determined by ANOVA analysis. Immunofluorescence was done using a BX51 Olympus Fluorescence Microscope (Olympus, Inc.) using an Apoplan 100X objective. Images were captured using a SPOT RX digital camera. All images presented were captured and processed in parallel by identical means.

Acknowledgements

The authors thank Sara Harley, Scott Halkyard, Alis Heidar, Jason Karpus, Devin Miller, Bethany Stark and Jon Visick for excellent technical assistance and helpful comments on the manuscript. We thank D. Kesler for statistical analysis of localization data. We thank Judy Berman, Joseph Heitman, Brian Kennedy, Joe Mymryk, David Stillman and Johan Thevelein for providing strains and plasmids. This work was supported by National Institutes of Health grant 1R15GM67262 to SDJ. MEM. was supported by grant number 1R15CA098257-01 from the National Cancer Institute. Its content are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute.

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Figure 1.

MSII overexpression suppresses heat shock sensitivity caused by activation of the *RAS*/cAMP pathway. Stationary phase cells with the indicated relevant genotypes were incubated at 55°C for ninety minutes before being serially diluted and plated. The number of surviving cells was normalized to an aliquot that was not heat shocked to determine the fraction of cells that survived treatment. Averages of six trials are shown and the standard deviations are shown by the error bars. The yeast strains used were yJB195 (wildtype), yJB2235 (*RAS2^{G19V}*), ySJ795 (*yak1Δ*), ySJ939 (*RAS2^{G19V} yak1Δ*), ySJ126 (YE_{p55}-*MSII*), yJB2320 (*RAS2^{G19V} YE_{p55}-MSII*), ySJ799 (*yak1Δ YE_{p55}-MSII*), ySJ958 (*RAS2^{G19V} yak1Δ YE_{p55}-MSII*), yJB3712 (*npr1Δ*), yJB3723 (*RAS2^{G19V} npr1Δ*), ySJ1142 (*yak1Δ npr1Δ*) and ySJ1141 (*RAS2^{G19V} yak1Δ npr1Δ*).

Figure 2.

YAK1 is needed for the association of Msi1p with Cac1p but not with Npr1p. Yeast strains were serially diluted ten-fold and plated on either nonselective media (left) or media that selects for a positive two-hybrid interaction (right). The strains used were ySJ973 (pGBT9-*MSI1*, pGAD1), ySJ520 (pGBT9-*MSI1*, pGAD1-*CAC1*), ySJ533 (pGBT9-*MSI1*, pGAD1-*CAC1*, *URA3::RAS2^{G19V}*), ySJ522 (pGBT9-*MSI1*, pGAD1-*CAC1*, YEplac195-*PDE2*), ySJ942 (pGBT9-*MSI1*, pGAD1-*CAC1*, *yak1::URA3*), ySJ957 (pGBT9-*MSI1*, pGAD1-*CAC1*, *zds1::URA3*), ySJ960 (pGBT9-*MSI1*, pGAD1-*CAC1*, pRS426-*YAK1*), ySJ761 (pDBD-p53, pAD-TAg, *yak1::URA3*), ySJ84 (pGBT9-*MSI1*, pGAD1), yJB3438 (pGBT9-*MSI1*, pGAD1-*NPR1₅₆₁₋₆₀₅*), and ySJ1015 (pGBT9-*MSI1*, pGAD1-*NPR1₅₆₁₋₆₀₅*, *yak1::KanMX6*).

Figure 3.

Msi1p activates the transcription of a reporter gene when grown under nonfermentative conditions. (a) Yeast strains were serially diluted ten-fold and plated on synthetic media lacking leucine and tryptophan to select for the plasmids. Plates contained 2% of either glucose or glycerol for a carbon source, as indicated. Plates labeled "+ 3-AT" lacked histidine and contained 20mM 3-AT. The strains used were ySJ559 (pGBT9, pGAD1), ySJ542 (pGBT9-*MSII*, pGAD1), ySJ752 (pGBT9-*MSII*, pGAD1, *yak1::URA3*), ySJ753 (pGBT9, pGAL4), and ySJ754 (pGBT9, pGAL4, *yak1::URA3*). (b) Growing cells on a 2% concentration of any of the ten listed "inducing" carbon sources activated the one-hybrid activity of Msi1p; growing cells on any of the five listed "noninducing" carbon sources did not. (c) Yeast strains were serially diluted ten-fold and plated on SC -leu -trp -ura -his + 20mM 3-AT containing 2% glycerol. The strains used were ySJ973 (pGBT9-*MSII*, pGAD1, YEplac195), ySJ552 (pGBT9-*MSII*, pGAD1, *cac1::URA3*), ySJ1013 (pGBT9-*MSII*, pGAD1, *URA3::RAS2^{G19V}*), ySJ1014 (pGBT9-*MSII*, pGAD1, YEplac195-*PDE2*), ySJ572 (pGBT9-*MSII*, pGAD1, *npr1::URA3*), and ySJ775 (pGBT9-*MSII*, pGAD1, pRS426-*YAK1*).

Figure 4.

YAK1 is not required for Msi1p to act as a co-repressor with pRB. Yeast strains were serially diluted ten-fold and plated on SDC -leu -his +10mM 3-AT. The strains used were ySJ577 (pDB), ySJ579 (pDB, *msi1::hisG*), ySJ918 (pDB, *yak1::TRP1*), ySJ920 (pDB, *msi1::hisG*, *yak1::TRP1*), ySJ578 (pDB-pRB), ySJ580 (pDB-pRB, *msi1::hisG*), ySJ919 (pDB-pRB, *yak1::TRP1*), and ySJ921 (pDB-pRB, *msi1::hisG*, *yak1::TRP1*).

Figure 5.

YAK1 overexpression decreases the growth rate only in the presence of *MSH1*. Average doubling times are shown with standard deviations indicated by the error bars. Minimal doubling times during logarithmic growth were calculated using the MicroFit program.⁶¹ The strains used were ySJ977 (wildtype), ySJ978 (*YAK1* overexpression), ySJ979 (*msi1*Δ), ySJ980 (*msi1*Δ, *YAK1* overexpression), ySJ1078 (*cac1*Δ), and ySJ1079 (*cac1*Δ, *YAK1* overexpression).

Figure 6.

Characterization of the *msi1-8* mutation. (a) The *msi1-8* mutation blocks association with Cac1p but not with Npr1p. Yeast strains were serially diluted ten-fold and plated on SDC -leu -trp -his +20mM 3-AT and allowed to grow at 30°C for two days. The strains used were ySJ83 (pGBT9, pGAD1-*NPR1*₅₆₁₋₆₀₅), ySJ501 (pGBT9, pGAD1-*CAC1*), yJB3438 (pGBT9-*MSI1*, pGAD1-*NPR1*₅₆₁₋₆₀₅), yJB3197 (pGBT9-*MSI1*, pGAD1-*CAC1*), ySJ493 (pGBT9-*msi1-8*, pGAD1-*NPR1*₅₆₁₋₆₀₅), and ySJ495 (pGBT9-*msi1-8*, pGAD1-*CAC1*). (b) *msi1-8* suppresses the heat shock sensitivity caused by activation of the RAS/cAMP pathway. Stationary phase cells with the indicated relevant genotypes were incubated at 55°C for ninety minutes before being serially diluted and plated. The number of surviving cells was normalized to an aliquot that was not heat shocked to determine the fraction of cells that survived treatment. Averages of six trials are shown and the standard deviations are indicated by the error bars. The yeast strains used were ySJ965 (YEp55-*msi1-8*), ySJ966 (*RAS2*^{G19V} YEp55-*msi1-8*), ySJ967

(*yak1Δ* YEp55-*msi1-8*) and ySJ968 (*RAS2^{G19V}* *yak1Δ* YEp55-*msi1-8*). (c) The *msi1-8* mutation dramatically decreases the regulated one-hybrid activity of Msi1p. Yeast strains were serially diluted ten-fold and plated on SC -leu -trp -his +20mM 3-AT containing 2% glycerol and allowed to grow at 30°C for one week. The strains used were ySJ559 (pGBT9, pGAD1), ySJ542 (pGBT9-*MSI1*, pGAD1), ySJ561 (pGBT9-*msi1-8*, pGAD1) and ySJ752 (pGBT9-*MSI1*, pGAD1, *yak1::URA3*).

Figure 7.

Msi1p accumulates in nonfermenting cells. Cells of the indicated genotype were grown in rich media supplemented with either glucose or glycerol. Equal amounts of total protein were separated by SDS-PAGE and Msi1p tagged with 13 tandem copies of the myc epitope was detected by immunoblotting. The arrow indicates full-length, tagged Msi1p; the asterisk indicates a nonspecific band. Yeast strains used were yJB195 (wt), ySJ605 (*MSI1-myc₁₃-HIS3MX6*), ySJ797 (*MSI1-myc₁₃-HIS3MX6 yak1::TRP1*) and ySJ806 (*MSI1-myc₁₃-HIS3MX6 npr1::TRP*).

Figure 8.

Yak1p decreases the nuclear accumulation of Msi1p in nonfermenting cells. (A) Quantitation of the percentage of cells in which Msi1p accumulates in the nucleus as a function of growth condition and *YAK1* status. Black bars correspond to the nuclear accumulation of Msi1p in cells grown in glucose and gray bars correspond to cells grown in glycerol. Error bars represent one standard deviation. (B) Representative indirect immunofluorescent images. The images in the left columns show DAPI staining and the right columns show Msi1p-myc₁₃ localization in the same cells. The indicated strains were grown to early log phase with the noted carbon source. Yeast strains used were yJB195 (wt), ySJ605 (*MSI1-myc₁₃-HIS3MX6*), ySJ797 (*MSI1-myc₁₃-HIS3MX6 yak1::TRP1*) and ySJ956 (*MSI1-myc₁₃-HIS3MX6, YAK1* overexpression).

Table 1

Yeast Strains Used

Isogenic Group and Strain	Relevant Genotype	Source or Reference
W303	MATa ura3-1 ade2-1 his3-11 leu2-3,112 can1-100 trp1-1	
yJB195	wildtype	17
yJB2235	URA3::RAS2 ^{G19V}	17
yJB2320	URA3::RAS2 ^{G19V} YE55-MSII	17
yJB3712	npr1::LEU2	17
yJB3723	URA3::RAS2 ^{G19V} npr1Δ	17
ySJ126	YE55-MSII	this study
ySJ605	MSII-myc ₁₃ ::HIS3MX6	this study
ySJ795	yak1::TRP1	this study
ySJ797	MSII-myc ₁₃ ::HIS3MX6 yak1::TRP1	this study
ySJ799	yak1::TRP1 YE55-MSII	this study
ySJ806	MSII-myc ₁₃ ::HIS3MX6 npr1::TRP1	this study
ySJ939	yak1::TRP1 URA3::RAS2 ^{G19V}	this study
ySJ956	MSII-myc ₁₃ ::HIS3MX6 pRS426-ADH-YAK1	this study
ySJ958	yak1::TRP1 URA3::RAS2 ^{G19V} YE55-MSII	this study
ySJ965	YE55- <i>msi1</i> -8	this study
ySJ966	URA3::RAS2 ^{G19V} YE55- <i>msi1</i> -8	this study
ySJ967	yak1::TRP1 YE55- <i>msi1</i> -8	this study
ySJ968	yak1::TRP1 URA3::RAS2 ^{G19V} YE55- <i>msi1</i> -8	this study
ySJ977	YEplac195	this study
ySJ978	pRS426-ADH-YAK1	this study
ySJ979	<i>msi1</i> :: <i>hisG</i> YEplac195	this study
ySJ980	<i>msi1</i> :: <i>hisG</i> pRS426-ADH-YAK1	this study
ySJ1078	<i>cac1</i> :: <i>HIS3</i> YEplac195	this study
ySJ1079	<i>cac1</i> :: <i>HIS3</i> pRS426-ADH-YAK1	this study
ySJ1141	URA3::RAS2 ^{G19V} yak1::TRP1 npr1::LEU2	this study
ySJ1142	yak1::TRP1 npr1::LEU2	this study
PI69-4	MATa trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ GAL2-ADE2	62
	LYS2::GAL1-HIS3 <i>met2</i> ::GAL7-lacZ	
ySJ520	pGBT9-MSII pGAD-CAC1 YEplac195	this study
ySJ522	pGBT9-MSII pGAD-CAC1 YEplac195-PDE2	this study
ySJ533	URA3::RAS2 ^{G19V} pGBT9-MSII pGAD-CAC1	this study
ySJ542	pGBT9-MSII pGAD1	this study
ySJ552	pGBT9-MSII pGAD1 <i>cac1</i> ::URA3	this study
ySJ559	pGBT9 pGAD1	this study
ySJ561	pGBT9- <i>msi1</i> -8 pGAD1	this study
ySJ572	pGBT9-MSII pGAD1 npr1::URA3	this study
ySJ752	yak1::URA3 pGBT9-MSII pGAD1	this study
ySJ753	pGBT9 pGAL4	this study
ySJ754	yak1::URA3 pGBT9 pGAL4	this study
ySJ761	yak1::URA3 pDBD-p53 pAD-TAg	this study
ySJ775	pGBT9-MSII pGAD1 pRS426-YAK1	this study
ySJ942	yak1::URA3 pGBT9-MSII pGAD-CAC1	this study
ySJ957	<i>zds1</i> ::URA3 pGBT9-MSII pGAD-CAC1	this study
ySJ960	pGBT9-MSII pGAD-CAC1 pRS426-YAK1	this study
ySJ973	pGBT9-MSII pGAD1 YEplac195	this study
ySJ973	pGBT9-MSII pGAD1 YEplac195	this study
ySJ1013	pGBT9-MSII pGAD1 URA3::RAS2 ^{G19V}	this study
ySJ1014	pGBT9-MSII pGAD1 YEplac195-PDE2	this study
ySJ1057	pGBT9-CAC2 pGAD424	this study
ySJ1060	pGBT9-CAC2 pGAD-CAC1	this study
ySJ1061	<i>msi1</i> :: <i>KanMX4</i> pGBT9-CAC2 pGAD-CAC1	this study
ySJ1062	yak1::URA3 pGBT9-CAC2 pGAD-CAC1	this study
HF7c	MATa ura3-52 his3-200 ade2-101 lys2-801 trp1-901 leu2-3,112 gal4-542 gal80-538 LYS2::GAL1-HIS3 URA3::GAL4 _{3×17mers} -CYC1 _{TATA} -lacZ	63
yJB3438	pGBT9-MSII pGAD1-NPR1 ₅₆₁₋₆₀₅	17
yJB3197	pGBT9-MSII pGAD1-CAC1	17
ySJ83	pGBT9 pGAD1-NPR1 ₅₆₁₋₆₀₅	this study
ySJ84	pGBT9-MSII pGAD1	this study
ySJ493	pGBT9- <i>msi1</i> -8 pGAD1-NPR1 ₅₆₁₋₆₀₅	this study
ySJ495	pGBT9- <i>msi1</i> -8 pGAD1-CAC1	this study
ySJ501	pGBT9 pGAD1-CAC1	this study
ySJ1015	yak1:: <i>KanMX6</i> pGBT9-MSII pGAD1-NPR1 ₅₆₁₋₆₀₅	this study
MaV103	ura3-52 leu2-3,112 trp1-901 his3Δ200 ade2-1 gal4Δ gal80Δ can1 ^R <i>cyh2</i> ^R GAL1::HIS3@LYS2 GAL1::lacZ SPAL10::URA3	64
ySJ512	wildtype	35
ySJ513	<i>msi1</i> :: <i>hisG</i>	35
ySJ577	pDB	this study

Isogenic Group and Strain	Relevant Genotype	Source or Reference
ySJ578	pDB-RB	this study
ySJ579	<i>msi1::hisG</i> pDB	this study
ySJ580	<i>msi1::hisG</i> pDB-RB	this study
ySJ918	<i>yak1::TRP1</i> pDB	this study
ySJ919	<i>yak1::TRP1</i> pDB-RB	this study
ySJ920	<i>msi1::hisG yak1::TRP1</i> pDB	this study
ySJ921	<i>msi1::hisG yak1::TRP1</i> pDB-RB	this study

Table 2

Summary of Phenotypes

<u><i>MSI1</i> Phenotype</u>	<u><i>YAK1</i> Effect</u>
Physically associates with Cac1p	<i>YAK1</i> is required
Carbon source regulated one-hybrid activity	<i>YAK1</i> is required
Carbon source regulated nuclear localization	<i>YAK1</i> antagonizes
Carbon source regulated protein accumulation	No effect
Co-repressor with pRB	No effect
Physically associates with Npr1p	No effect
<u><i>YAK1</i> Phenotype</u>	<u><i>MSI1</i> Effect</u>
Loss causes heat shock sensitivity	<i>MSI1</i> antagonizes
Overexpression decreases growth rate	<i>MSI1</i> is required