The Hsp90 Molecular Chaperone Complex Regulates Maltose Induction and Stability of the Saccharomyces MAL Gene Transcription Activator Mal63p*

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Induction of the Saccharomyces MAL structural genes encoding maltose permease and maltase requires the MAL activator, a DNA-binding transcription activator. Genetic analysis of MAL activator mutations suggested that protein folding and stability play an important role in MAL activator regulation and led us to explore the role of the Hsp90 molecular chaperone complex in the regulation of the MAL activator. Strains carrying mutations in genes encoding components of the Hsp90 chaperone complex, hsc82Δ hsp82-T101I and hsc82Δ cpr7Δ, are defective for maltase induction and exhibit significantly reduced growth rates on media containing a limiting concentration of maltose (0.05%). This growth defect is suppressed by providing maltose in excess. Using epitope-tagged alleles of the MAL63 MAL activator, we showed that Mal63p levels are drastically reduced following depletion of cellular Hsp90. Overexpression (~3-fold) of Mal63p in the hsc82Δ hsp82-T101I and hsc82Δ cpr7Δ strains suppresses their Mal⁺ growth phenotype, suggesting that Mal63p levels are limiting for maltose utilization in strains with abrogated Hsp90 activity. Consistent with this, the half-life of Mal63p is significantly shorter in the hsc82Δ cpr7Δ strain (reduced about 6-fold) and modestly affected in the Hsp90-ts strain (reduced about 2-fold). Most importantly, triple hemagglutinin-tagged Mal63p protein is found in association with Hsp90 as demonstrated by co-immunoprecipitation. Taken together, these results identify the inducible MAL activator as a client protein of the Hsp90 molecular chaperone complex and point to a critical role for chaperone function in alternate carbon source utilization in Saccharomyces cerevisiae.

In Saccharomyces cerevisiae, maltose fermentation requires maltose permease, a proton symporter that transports maltose across the plasma membrane; maltase, an α-glucosidase that hydrolyzes maltose to produce glucose; and the MAL activator, a DNA-binding transcription activator (reviewed in Refs. 1 and 2). Maltose-induced expression of maltase and maltose permease requires the MAL activator and maltose permease, and strains lacking either gene are noninducible (3). Wang et al. (4) showed that intracellular maltose is sufficient to stimulate induction, thereby demonstrating that the role of the permease in induction is simply to provide sufficient intracellular inducer to activate the maltose sensor. The maltose sensor has not been identified, but the MAL activator is a candidate.

The genes encoding maltose permease, maltase, and the MAL activator are clustered in a complex MAL locus. S. cerevisiae yeast strains can carry anywhere from one to five unlinked copies of a MAL locus, named MAL1–4 and MAL6, which map to sites near the telomere of different chromosomes (5). The presence of any one of these five loci is sufficient for maltose fermentation. All of the five MAL loci are highly homologous both structurally and functionally. The genes encoding maltose permease and maltase share a bi-directional promoter that contains the DNA-binding site of the MAL activator, thereby providing for the coordinate expression of these maltose utilization enzymes (2, 6).

MAL63 encodes an inducible allele of the MAL activator at MAL6 (3, 5). Mal63p is 470 residues in length and contains a six-cysteine zinc finger DNA-binding domain in the N-terminal ~60–100 residues, a single transcription activation domain in approximately residues 60–250, and a C-terminal regulatory domain to the C-terminal ~200 residues and demonstrated that this region is a negative regulator of MAL activator function. mal64 is a nonfunctional homologue of MAL63 but can be activated to a constitutive MAL activator by mutation (11). These MAL64-C mutations are nonsense mutations at codons 282 and 307 (8). The sequence of the MAL activator constitutive mutants MAL23-C and MAL43-C of the MAL2 and MAL4 loci, respectively, reported in Gibson et al. (8) contain multiple sequence alterations located largely in the C-terminal regulatory domain. Danzi et al. (9) used in vitro mutagenesis to localize those residues in the MAL activator that are important for negative regulation. They identified clustered alterations in three regions (residues 250–307, 343–357, and 419–461), and the introduction of multiple alterations in any one of these
Hsp90 Chaperone Regulates the Saccharomyces MAL Activator

| Strain | Genotype | Source |
|--------|----------|--------|
| W303   | MATa leu2-3,112 ura3-1 trp1-1 his3-11,15 can1-100 GAL SUC2 | S. Lindquist Ref. 20 |
| hsc82Δ | Isogenic to W303 except hsc82::LEU2 | Ref. 26 |
| S153   | MATa leu2-3,112 ura3-1 trp1-1 ade2-1 can1-100 GAL SUC hsc82::LEU2 hsp82::T1011 | Ref. 26 |
| cpr7Δ  | Isogenic to W303 except cpr7::HIS3 | Ref. 26 |
| hsc82Δ cpr7Δ | Isogenic to W303 except hsc82::LEU2 cpr7::TRP1 | Ref. 26 |
| 5CG2   | MATa ura3-32 lys2-801 ade2-101 trp1-63 his3-200 leu2-1 hsc82::URA3 hsp82::GAL1-HSP82::LEU2 | S. Lindquist |

regions fully relieves negative regulation by this C-terminal domain. They also found that other sites throughout the protein could modulate the constitutive phenotype of mutations in these regions. But the C-terminal region also plays a positive role in MAL activator induction. Charged cluster to alanine scanning mutagenesis of this C-terminal negative regulatory domain of Mal63p produced a series of noninducible alleles that alter residues in regions adjacent to or overlapping two of the three negative regulatory regions defined by Danzi et al. (9, 10).

Together, this genetic analysis suggests that conformational changes involving complex protein-protein interactions regulate MAL activator activity. All efforts to identify interactions between different domains of Mal63p using two-hybrid analysis were unsuccessful, and Hu et al. (2) proposed that the interactions were likely to be intermolecular. The well documented role for the Hsp90 molecular chaperone complex in the induction binding and regulation of other transcription activators such as the steroid hormone receptors raised the possibility that the Hsp90 chaperone complex could be a candidate for this MAL activator-interacting protein(s) and thus may be involved in the maltose stimulation of the MAL activator. The results reported here explore this possibility.

Hsp90 is a highly conserved, abundantly expressed, essential protein in eukaryotes that is localized to the cytoplasm and nucleus (specific references may be found in Refs. 12–20). Hsp90 is the key component of a large complex of proteins, many of them molecular chaperones, that function together assisting certain proteins to achieve an activated conformation in response to external or physiological signals. Despite its abundance, Hsp90 is unlikely to be required for de novo folding of the bulk of newly synthesized proteins. Instead it appears to play a role in the maturation of a specific set of newly synthesized proteins, so-called client or substrate proteins, and in the refolding and assembly of misfolded proteins that accumulate in cells following exposure to mild heat shock or other stresses. Therefore, Hsp90 has both stress-related and housekeeping functions and, as such, plays an essential role in processes controlling cell growth and differentiation in unstressed cells.

Many of the components of the Hsp90 chaperone complex are conserved both in structure and function from S. cerevisiae to mammals. In Saccharomyces this includes the following genes: HSC82 and HSP82 encode Hsp90; the SSA and SSB family of proteins encode Hsp70 isoforms (21, 22); YDJ1 encodes Hsp40 (23); STI1 encodes Hop/p60 protein (24); SSA1 encodes p23 (22, 25); CPR6 and CPR7 encode cyclophilins (26); CDC37 encodes p50CHC1 (27); AHA1 and HCH1 encode hAha1 (28); and SSE1 encodes p23, p50 Cdc37, and Aha1 modulate the ATPase activity of Hsp90. The immunophilins, which include cyclophilins and FBPK proteins, have peptidyl proline isomerase activity and tetratricopeptide repeat domains and work to modulate client protein maturation and activation. Saccharomyces has been used for the study of mammalian steroid hormone receptor activation (particularly glucocorticoid and androgen receptors) and Src protein kinase maturation. Neither of these proteins is a natural substrate of the yeast Hsp90 chaperone complex, and although much important information has been obtained, it would be valuable to identify and characterize endogenous yeast substrates. To date the only Saccharomyces proteins identified as substrates of the Hsp90 chaperone complex are Ste11 protein kinase, Gen2 kinase, and the home-regulated transcription activator Hap3 (30–32). Here we investigate the role of Hsp90 chaperone complex in MAL gene regulation. We find that in strains carrying mutations in components of the Hsp90 complex maltose-dependent MAL gene induction is defective. Depletion of Hsp90 causes the rapid loss of Mal63p MAL activator protein, and in Hsp90 chaperone mutant strains, Mal63p half-life is reduced up to 5-fold. Most significantly, Mal63 MAL activator immunoprecipitates with His-tagged Hsp90 from native cell extracts. Thus, the Saccharomyces MAL activator is shown to be a novel signal transducing protein client of the Hsp90 chaperone complex, further demonstrating the integration of chaperone function into non-stress cellular metabolism.

**MATERIALS AND METHODS**

**Yeast Strains and Plasmids**—The Saccharomyces strains used in this study are listed in Table 1. Strain W303 carries naturally occurring defective copies of the MAL1 and MAL3 loci (33). Both loci contain functional maltose permease and maltase genes, referred to as MAL11 (also known as AGT1) and MAL12, respectively, at MAL1 and MAL31 and MAL33, respectively, at MAL3. Sequences homologous to the MAL63 MAL activator gene are found at both MAL1 and MAL3, referred to as mal13 and mal33, respectively, but these genes are non-functional. Thus, strain W303 does not ferment maltose. Plasmid-borne copies of MAL63 complement the defective chromosomal copies of the MAL activator genes. Plasmid pMAL63 was constructed by subcloning the BamHI-Sall fragment carrying MAL63 on its native promoter into the Escherichia coli yeast/shuttle vector YCP50 as described by Gibson et al. (8).

The following series of plasmids were constructed using vectors described by Mumberg et al. (34). Plasmid p414GPD of this series was used to construct p414GPD-MAL63/FLAG. It is a CEN vector containing the TRP1 selection marker and the promoter of TDH1, encoding glyceraldehyde-3-phosphate dehydrogenase, which is a highly expressed constitutive promoter. The MAL63 coding region was amplified by PCR using an upstream primer that inserts a BamHI site and the sequence encoding the FLAG epitope at the 5’ end of the MAL63 open reading frame (primer B1; Table II) and a downstream primer that inserts a Sall site immediately following the MAL63 termination codon (primer B5; Table II). The amplified BamHI-Sall fragment was inserted into the BamHI and Sall sites in the multiple cloning sequence of p414GPD creating a GPD promoter-MAL63/FLAG fusion gene and plasmid p414GPD-MAL63/FLAG.

The FLAG tag sequence in plasmid p414GPD-MAL63/FLAG was...
were grown in the appropriate selective minimal medium to mid-log
titer-containing three copies of the sequences encoding the HA epitope was
using primer KM-N (Table II) as the 5
promoter. The full
gene and the promoter of the
frozen cells were defrosted and resuspended in 1 ml of 50 mM HEPES
A
water bath for 20 min, after which an additional 50
/H9262
added to the cell suspension, and the samples were vortexed at a
3
/H11032
MAL63
MAL63
MAL63/HA3 as follows. The fragment encoding the 5
/H11032
ing a GPD promoter-
epitope was inserted into the NotI site in the proper orientation, creat-
fragment containing three copies of the sequences encoding the HA
epitope was inserted into the NotI site in the proper orientation, creat-
ing a GPD promoter-MAL63/HA3 fusion gene and plasmid
p414GPD-MAL63/HA3.
Plasmid p414GPD from the Mumberg et al.
Vector plasmid p416TEF is another from the Mumberg
et al.
(34) series. It is a CEN plasmid and contains URA3 as the selectable marker but is otherwise the same as p414. The Sac1-Kpn1 fragment containing the entire GPD promoter-MAL63/HA3 tagged fusion gene was released from p414GPD-MAL63/HA3 by diges-
tion with Sac1 and Kpn1 and inserted into Sac1-Kpn1-digested plasmid
p416 to create plasmid p416GPD-MAL63/HA3.
Vector plasmid p416TEF is another from the Mumberg et al. (34)
series. It is a CEN plasmid and contains URA3 as the selectable marker but is otherwise the same as p414. The Sac1-Kpn1 fragment containing the entire GPD promoter-MAL63/HA3 tagged fusion gene was released from p414GPD-MAL63/HA3 by diges-
tion with Sac1 and Kpn1 and inserted into Sac1-Kpn1-digested plasmid
p416 to create plasmid p416GPD-MAL63/HA3.
Preparation of Cell Extracts and Immunoblot Analysis—The strains
were grown in the appropriate selective minimal medium to mid-log
phase (A600 of 0.2–0.5). An aliquot of the culture, volume in milliliters
approximately equal to 15 divided by A600 was harvested by filtration,
washed with 50 mM KPO4 buffer, pH 7.4, plus 2% sodium azide, and
frozen while still on the filter paper at –80 °C for at least 20 min. The frozen
cells were defrosted and resuspended in 1 ml of 50 mM HEPES
buffer, pH 7.5, containing a mixture of protease inhibitors (Roche Ap-
plied Science; complete, mini, EDTA-free protease Inhibitor tablets
(catalogue number 1636170) plus Sigma yeast protease inhibitor mix-
ture (catalogue number P8215)), pelleted by centrifugation, and resus-
pended in 300 μl of SB buffer. SB buffer is prepared by dissolving one
Table II
List of primers

| Primer    | Primer sequence                                                                 |
|-----------|-------------------------------------------------------------------------------|
| B11       | 3'-GGGCGATCCCGGCTATTTGTTGTAAGGATTGCACTGGCAAAACAGCTT3' |
| B5        | 3'-GGGCGATCCGCAACGGCGGCTGGAACAAAT3'                                           |
| KM-N      | 5'-GGGATCCCAAATGGCGGCGGCCATTGCGAAACAGCTTGC-3'                                 |
| MB-4      | 5'-CCAAATGGGCGCGGCCACCTTTTACCC-3'                                            |
| MB-5      | 5'-CGCAATACCACGGCAAACAGC-3'                                                   |

The abbreviations used are: HA, hemagglutinin; PGK, phosphoglyc-
erol kinase.

| Primer    | Primer sequence                                                                 |
|-----------|-------------------------------------------------------------------------------|
| B11       | 3'-GGGCGATCCCGGCTATTTGTTGTAAGGATTGCACTGGCAAAACAGCTT3' |
| B5        | 3'-GGGCGATCCGCAACGGCGGCTGGAACAAAT3'                                           |
| KM-N      | 5'-GGGATCCCAAATGGCGGCGGCCATTGCGAAACAGCTTGC-3'                                 |
| MB-4      | 5'-CCAAATGGGCGCGGCCACCTTTTACCC-3'                                            |
| MB-5      | 5'-CGCAATACCACGGCAAACAGC-3'                                                   |

 replacing a triple HA tag sequence to create plasmid p414GPD-
MAL63/HA3 as follows. The fragment encoding the 5’ half of the
MAL63 open reading frame was amplified using a 5’ primer (KM-N; Table II) that inserts a NotI site between codons 2 and 3 of MAL63 and a
3’ primer complementary to a sequence just downstream of the EcoRI
site at codons 215/216 of MAL63. This amplified product was digested
with BamHI and EcoRI and used to replace the BamHI-EcoRI fragment
containing the 5’ end of the tagged MAL63 gene in p414GPD-MAL635-
FLAG, thereby removing the FLAG sequence. A 115-base pair NotI
fragment containing three copies of the sequences encoding the HA
epitope was inserted into the NotI site in the proper orientation, creat-
ing a GPD promoter-MAL63/HA3 fusion gene and plasmid
p414GPD-MAL63/HA3.

Plasmid p414GPD from the Mumberg et al. (34) series contains
URA3 as the selectable marker but is otherwise the same as p414. The
Sac1-Kpn1 fragment containing the entire GPD promoter-MAL63/HA3 tagged fusion gene was released from p414GPD-MAL63/HA3 by diges-
tion with Sac1 and Kpn1 and inserted into Sac1-Kpn1-digested plasmid
p416 to create plasmid p416GPD-MAL63/HA3.

Maltase Assay—The cells were grown to mid-log, harvested by cen-
trifugation, and resuspended in 0.5 ml of potassium phosphate buffer,
and an equal volume of glass beads was added. The extracts were
pre pared by vortexing the cell suspension three times for 1 min each, keeping the mixture cooled on ice. Maltase activity was measured in
whole cell extracts as described in Dubin et al. (35). Activity is ex-
pressed as nmol of p-nitrophenol-a-gluco pyranoside hydrolyzed per
min/mg of protein. Protein concentration of the cell extracts was meas-
ured using the Bio-Rad protein assay dye reagent. The assay values are
the averages of results from three independent transfo rmants assayed
in duplicate. Variation is <20%.

RESULTS
Maltase Induction and Maltose Utilization Are Defective in Strains Carrying Mutations in Components of the Hsp90 Chaperone Complex—We investigated the effects of Hsp90 chaper-
one mutations using an isogenic strain series derived from strain W303 carrying mutations in the genes encoding the two
differentially expressed Hsp90 isoforms, HSC82 and HSP82, or
in the gene encoding the constitutively expressed cyclophilin isoform,
CPR7. Strain hsc82Δ (hsc82Δ HSP82) lacks the gene
encoding the constitutive Hsp90 isoform, HSC82. Strain S153
(hsc82Δ hsp82-T101I) contains a null mutation in both HSC82 and
HSP82 but carries a plasmid-borne copy of the tempera-
ture-sensitive allele hsp82-T101I expressed from the high level
constitutive glyceraldehyde-3-phosphate dehydrogenase gene
promoter. The hsp82-T101I product exhibits reduced activity
even at the permissive temperature (24 °C) but is inactivated
further at higher temperatures (36). In strains expressing only
hsp82-T101I, no growth is observed on media containing glu-
cose as the sole carbon source at temperatures above –35 °C,
indicating full loss of Hsp90 activity. Strains carrying a null
mutation in CPR7 and null mutations in both CPR7 and
HSC82 were also studied. Duina et al. (26) found that growth on
glucose-containing medium at 30 °C was slowed in the
cpr7Δ strain, unaffected in the hsc82Δ strain, but reduced in the
cpr7Δ hsc82Δ double null strain.
Hsp90 Chaperone Regulates the Saccharomyces MAL Activator

Strain W303 carries two copies of the genes encoding maltose permease (MAL11, also called AGT1), and MAL31) and maltase (MAL12 and MAL32) but does not ferment maltose because it lacks a functional MAL activator gene. To study maltose utilization in this strain series MAL63, the MAL activator gene from the MAL6 locus was introduced into the Hsp90 mutant strains by transformation with the CEN plasmid pMAL63. The ability of these strains to induce maltase expression was determined. Transformants were grown in selective medium under uninduced conditions at 24 °C and mid-log phase and induced with maltose at either 24 or 35 °C. Maltase activity was assayed at time 0 and at 4 h after the addition of maltose. The assays were carried out in duplicate on at least three independent transformants.

The wild-type strain is able to induce to similar levels at both 24 and 35 °C. Loss of HSC82 alone causes a modest decrease in the rate of induction at 24 °C, ~2-fold, but induction at 35 °C is reduced to about 25% of the wild-type strain. Maltase induction is significantly decreased in the Hsp90 temperature-sensitive strain (hsc82Δ hsp82Δ-T101I) at both the permissive and the nonpermissive temperatures. Deletion of one of the two immunophilin genes, crp7, slightly decreases maltase induction, about 2-fold at 24 °C and about 4-fold at and 35 °C. This is comparable with the effect of the hsc82Δ mutation alone. Double deletion of both crp7 and hsc82 enhances the maltase induction defect seen in the single deletion strains and decreases induction even further, about 6-fold at 24 °C and 12-fold at 35 °C. This synergistic effect between the hsc82Δ and crp7Δ deletions is consistent with the previously reported repression of the yeast heat shock factor HSF observed by Duina et al. (37).

The maltose induction defect in the Hsp90 chaperone mutant strains can also be observed as a reduced ability to grow on a low concentration (0.05%) of maltose. Fig. 2 compares the growth rate of wild-type and Hsp90 chaperone mutant strains on 0.05% maltose and 0.05% glucose at 30 °C. Growth on limiting glucose was not affected for any of the strains, but the Hsp90-ts and the hsc82Δ crp7Δ double null strain exhibited a significant growth defect on 0.05% maltose. We also found that these maltose growth defects were suppressed by increasing the maltose concentration in the medium (Fig. 3). Increased inducer concentration has similarly been found to improve the activation of other known Hsp90 chaperone client proteins including the human androgen receptor and the Saccharomyces heme-responsive transcription activator Hap1 (25, 38). The results reported in Figs. 1–3 indicate genetically that the Hsp90 molecular chaperone complex is involved in MAL activator-dependent MAL gene induction and maltose utilization but do not identify the specific client protein.

Mal63 MAL Activator Is Destabilized in Hsp90 Depleted Cells—One hallmark of Hsp90 chaperone client proteins is that they become very sensitive to degradation under conditions that interfere with chaperone complex formation or interaction. We used strain 5CG2, which expresses HSP82 from the GAL1 promoter as the sole source of Hsp90, to deplete Hsp90 and monitor the effect of loss of Hsp90 protein on Mal63p levels. Plasmid p414GPD-MAL63/FLAG carries MAL63 tagged with an N-terminal FLAG epitope tag expressed from the high level gyceraldehyde-3-phosphate dehydrogenase promoter. Mal63/FLAG MAL activator is functional and able to induce wild-type levels of the MAL structural genes (data not shown). Plasmid p414GPD-MAL63/FLAG was transformed into strain 5CG2. When grown on galactose both Hsp90 (Hsp82p) and the FLAG-tagged MAL activator Mal63/FLAG protein are easily detected (Fig. 4). Following 7.5 h of growth on glucose medium, which blocks the continued expression of GAL1 promoter-HSP82, Mal63p is nearly fully depleted, and Mal63/FLAG protein levels are barely detectable. In contrast, PGK levels are apparently unaffected. These results strongly suggest that Mal63p/FLAG MAL activator may depend on the Hsp90 chaperone for stability and function.

Mal63 MAL Activator Binds to Hsp90 in Vivo—Hsp90 client proteins can be found bound to the Hsp90 chaperone complex. To determine whether Mal63p MAL activator binds to the Hsp90 chaperone complex, His6-Mal63 and triple hemagglutinin-tagged Mal63 were co-expressed in the wild-type strain W303. His6-Mal63 was purified from native cell extracts using Ni2+ resin, and the bound proteins were analyzed by Western blotting. A low level of triple hemagglutinin-tagged Mal63 protein is observed in the control strain lacking His6-Hsp82 (Fig. 5), likely because of weak binding of the cysteine-rich DNA-binding domain of Mal63 MAL activator to the resin. Nevertheless, Fig. 5 shows a significant enhancement of the amount of Mal63/HAP3 protein that co-purifies with His6-Hsp82 when both proteins are co-expressed. We attempted the reciprocal experiment, i.e. immunoprecipitating triple hemagglutinin-tagged Mal63p and testing for co-isolation of Hsp90, but we were unable to effectively purify Mal63/HAP3 protein.

Overproduction of Mal63 MAL Activator Suppresses the Maltose Growth Defect in Hsp90 Mutant Strains—Hsp90 chaperone complex reportedly plays various roles in regulation of client proteins. It is proposed to stabilize client proteins, protecting them from degradation by the proteasome pathway. It also is suggested to maintain client proteins in an activation-competent state poised to respond to the regulating signal.
Finally, in response to the appropriate signal, client proteins are released from the Hsp90 chaperone complex and proper folding stimulated by the chaperone to enable the client protein to achieve the activated conformation. The results in Fig. 4 indicate that Hsp90 stabilizes the **MAL** activator protein and protects it from degradation. We postulated that in strains with defective Hsp90 chaperone complex **MAL** activator protein may be destabilized, and if so, we should expect to find that overproduction of Mal63 protein should relieve the defects observed in strains with mutant Hsp90 chaperone complex. We tested this as follows. The open reading frame of the triple HA-tagged allele of **MAL63** was fused to the high strength **GPD** promoter or the lower strength **TEF1** promoter using the plasmid vector series developed by Mumberg et al. (34). Plasmids pTEF-MAL63/HA3 and pGPD-MAL63/HA3 were transformed into strains W303 (**HSC82** **HSP82** **CPR7**), hsc82Δ (**hsc82** **HSP82** **CPR7**), and hsc82ΔΔcap7Δ (**hsc82**ΔMAL63/ΔHSP82 Δcap7Δ) transformed with plasmid pMAL63 was determined by serial dilution on selective minimal medium containing either 0.05% maltose or 0.05% glucose. The plates were grown at room temperature (RT, −21 °C) or 35 °C for 3 days.

**FIG. 4.** Stability of Mal63 **MAL** activator in cells depleted of Hsp90. Strain 5CG2 containing an integrated GAL1 promoter-**HSP82** gene was transformed with plasmid p414GPD-MAL63/FLAG or the empty vector p414GPD and grown to mid-logarithmic phase in selective minimal galactose-containing medium. The cells bearing the FLAG-tagged MAL63 plasmid were harvested, washed, and grown for an additional 7.5 h in either galactose-containing (**gal**) or glucose-containing (**glc**) medium, as indicated. The cells were harvested and flash frozen, and the protein extracts were prepared for Western analysis with the indicated antibodies. PGK was used as a loading control.
HA3 protein expression in each strain. The level of Mal63/HA3 protein was quantitated using the ECF detection system as described under “Materials and Methods” and normalized to the level of the PGK signal in the same lane. Fig. 6B confirms that the expression levels of Mal63/HA3 from the GPD promoter are 2.5–3-fold higher in each of the strains tested compared with expression from the TEF1 promoter.

The half-life of Mal63/HA3 in the Hsp90 chaperone mutant strains was determined. Transformants of the wild-type, Hsp90-ts, and hsc82Δ/hsp82-T101I CPR7 and hsc82Δ CPR7 CPR7/cpr7Δ were transformed with either p416GPD-MAL63/HA3 or p416TEF-MAL63/HA3. These transformants were characterized as follows. A, growth of the transformant strains on 0.05% maltose was compared using serial dilution. The plates were grown for 3 days at room temperature (~21 °C). B, the transformed strains were grown to early log phase at room temperature (~21 °C) in selective minimal medium containing 2% maltose. Total cell extracts were prepared for Western analysis using anti-HA antibody. Quantitation of Mal63/HA3p expression levels was carried out as described under “Materials and Methods” using PGK levels to normalize for variation in loading. The relative level of Mal63/HA3p was determined in at least three independent transformants. The results presented are the average of at least three experiments. C, transformant strains were grown to early log phase in selective minimal medium containing 2% maltose at room temperature (~21 °C). At time 0, cycloheximide (CHX) was added to a final concentration of 30 μg/ml, and the total cell extracts were prepared at time 0 and at the indicated time points for 6 h. Western analysis was carried out using anti-HA antibody. The relative level of Mal63/HA3p at each time point was quantified as described under “Materials and Methods” and normalized to the level of PGK. The Mal63/HA3p half-life was calculated from at least three independent transformants, and the average values are presented.

**DISCUSSION**

The results reported here demonstrate that the *Saccharomyces* MAL activator Mal63p is a client protein of the Hsp90 molecular chaperone complex. We show that the growth rate on low concentrations of maltose (0.05%) is significantly slowed in strains carrying Hsp90 chaperone mutations and that induction of the MAL structural gene encoding maltase is defective. Mal63p levels are drastically reduced in strains depleted of Hsp90, and the rate of Mal63/HA3p degradation is significantly higher in strains carrying mutations in Hsp90 chaperone complex components. Most significantly, Mal63/HA3p binds to the Hsp90 complex in vivo.

In the case of steroid hormone receptors, stabilization and hormone binding competence are achieved by association with Hsp90. The Hsp90 chaperone complex appears to play a similar role in stabilizing the MAL activator protein. Similar decreases in protein stability are obtained with other known Hsp90 complex client proteins, like human glucocorticoid receptor and Src.
kinase (12, 20, 39, 40). Genetic analysis of MAL activator constitutive mutations demonstrated that complex folding patterns and intramolecular protein–protein interactions regulate MAL activator activity (9). Additionally, overproduction of some of these noninducible mal63 mutant proteins suppresses the mutant phenotype and restores maltose inducibility. In Fig. 6 we demonstrate that overexpression of the MAL activator can overcome the maltose utilization defects in Hsp90 chaperone mutant strains. This suggests the possibility that these previously isolated Mal63 mutant proteins may be reduced in their ability to interact with the Hsp90 chaperone complex and thus might exhibit higher rates of degradation than wild-type Mal63p. We are currently exploring this possibility.

Two types of defective Hsp90 chaperone strains were tested, one expressing a temperature-sensitive Hsp90 allele and another lacking both the constitutively expressed HSC82 and CPR7 genes. Maltose utilization and maltase induction are defective in both types of mutants, indicating that not only is HSP90 required for maltase induction but that other components of the Hsp90 chaperone complex are also required, specifically the Cpr7 cyclophilin. Moreover, it is interesting to note that although the Hsp90-ts strain exhibited a more significant growth defect than the cpr7Δ hac8Δ22 double null strain, we found that Mal63/HA3 MAL activator degraded much more rapidly in the double null strain. This result suggests that Hsp90 itself functions to optimize induction and that this is in addition to its role in stabilizing the MAL activator as a component of the Hsp90 chaperone complex. This function could be to maintain the MAL activator in a maltose-binding competent conformation and/or stimulate the conformational changes required for maltose induction. We are currently testing other Hsp90 temperature-sensitive alleles to determine the severity of the maltose growth defect and the half-life of Mal63 MAL activator.

Wang et al. (4) reported that constitutive expression of maltose permease, the maltose transport protein, suppresses the ability of strain YPH500 to ferment maltose and enables it to osse permease, the maltose transport protein, suppresses the mal1 gene. This suggests that the very high concentration of intracellular maltose that results in constitutive expression of maltose permease and mal1 allele carries the same Hsp90 temperature-sensitive allele to determine the severity of the maltose growth defect and MAL63 MAL activator.

Taken together, these results are consistent with the following model of MAL activator regulation. We propose that, in the absence of maltose, MAL activator protein is bound to Hsp90 chaperone complex and is protected from degradation. The addition of maltose stimulates the release of MAL activator from the complex in an active conformation, allowing it to bind to MAL gene promoters and activate transcription. The Hsp90 chaperone complex is clearly involved in the stabilization of the MAL activator. A role in maintaining an induction-competent state and in the process of achieving the activated conformation is suggested but has not been demonstrated.

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