Multimerization of Hsp42p, a Novel Heat Shock Protein of *Saccharomyces cerevisiae*, Is Dependent on a Conserved Carboxyl-terminal Sequence*

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Rap1p is a transcriptional regulator of *Saccharomyces cerevisiae*, which plays roles in both transcriptional activation and silencing. To identify proteins involved in Rap1p-dependent regulation of transcription, we used the two-hybrid system to screen for Rap1p-interacting proteins. Two of the clones isolated from this screen encode a truncated protein with homology to small heat shock proteins (HSPs). Here we present an analysis of this novel *S. cerevisiae* HSP, which we name Hsp42p. Expression of HSP42 is regulated by a range of stress conditions similar to *S. cerevisiae* HSP26, with which Hsp42p shares most homology. However, HSP42 expression is more sensitive to increased salt concentration and to starvation and, in contrast to HSP26, is expressed in unstressed cells. Hsp42p interacts with itself in the two-hybrid assay. This interaction is dependent on a hydrophobic region which is conserved among small HSPs. Using bacterially expressed Hsp42p fusion proteins, we demonstrate that this is a direct interaction. Fractionation of yeast protein extracts by size demonstrates that all of the Hsp42p in these extracts is present in complexes with a molecular mass of greater than 200 kDa, suggesting that Hsp42p exists in high molecular mass complexes.

The repressor/activator protein (Rap1p) of *Saccharomyces cerevisiae* plays an important role in transcriptional silencing at both HM loci and telomeres (1–3). Rap1p is also able to activate gene expression and is essential for viability (4), presumably because of its role in the activation of glycolytic and ribosomal protein genes (5–7). We were interested in identifying other proteins involved in these processes, in an attempt to gain further insight into the different functions of Rap1p. We have previously used the two-hybrid system to identify proteins which play a role in the silencing functions of Rap1p. Sir3p and Sir4p interact with the carboxyl terminus of Rap1p in a two-hybrid assay (8), and the silencing protein, Rif1p, was identified in a similar way (9). We decided, therefore, to extend this search for Rap1p-interacting proteins. Of the clones identified by this screen, two encoded the same truncated protein, with homology to small heat shock proteins.

When eukaryotic cells are exposed to conditions of stress, such as increased temperature, the expression of proteins known as heat shock proteins (HSPs) is induced. HSPs can be divided into four classes; the hsp90 and hsp70 families, the GroEL-related HSPs, and the small HSPs, which are typically up to 40 kDa in size (10). Some of these HSPs, such as hsp70, are highly conserved between organisms as divergent as mammals, yeast and bacteria (11, 12). However, the small HSPs, share far less sequence similarity between species, with the main region of homology being a hydrophobic stretch of about 35 amino acids, located near the carboxyl terminus of the protein (13, 14). The number of small HSPs identified in different species varies greatly. For example, in many species of plants such as the soybean, more than 20 small HSPs have been identified (14), whereas in *Drosophila* six small HSPs are known (15) and in humans only one has been identified (16). The function of small heat shock proteins remains unclear. However, in *Dictyostelium* a mutation that abolishes induction of small HSP gene expression causes reduced stress tolerance, suggesting that these proteins do indeed play a role in stress resistance (17).

In *S. cerevisiae*, the major small HSP is Hsp26p, the expression of which is rapidly induced when cells are transferred to higher temperatures. Hsp26p is one of the major polypeptides produced on heat shock (18–21). To date, one other small HSP has been identified from *S. cerevisiae*, a 12-kDa protein with no homology to Hsp26p (22). In addition to heat shock, HSP26 expression is induced under other conditions of stress, such as increased salt concentration and starvation (20, 23). However, no phenotype has been observed on disruption of HSP26 (18, 19), suggesting that the function of *Hsp26* in stress tolerance may overlap with the functions of other HSPs. The identification of other *S. cerevisiae* HSPs may, therefore, provide a greater insight into the function of the small HSPs in yeast.

Here we present the identification and analysis of a novel small HSP of *S. cerevisiae*. This HSP (Hsp42p) is most similar to *S. cerevisiae* Hsp26p. HSP42 expression is up-regulated by all stress conditions tested. In contrast to HSP26, HSP42 is expressed at a relatively high level in cells growing exponentially at 25 °C. By sucrose gradient fractionation, it has been demonstrated that Hsp26p is present in large complexes (21). We show that Hsp42p is present in high molecular mass complexes, which are heterogeneous in size. Our results also demonstrate that Hsp42p interacts with itself and that this interaction is direct. The interaction of Hsp42p with itself is dependent on the carboxyl-terminal region of the protein including the conserved hydrophobic region.

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1 The abbreviations used are: HSP, heat shock protein; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; HA, hemagglutinin.
Plasmids—The LexA/RAP1 fusions are as described (8). The GAD fusion library screened with LexA/RAP1 was created in pGAD424 (24) by P. James (Wisconsin University). orf721 was subcloned into pRS423 (25) to create pK721. The 3’ region of HSP42 was created by PCR and subcloned into pK721, creating pD215. LexA/HSP42(22–353) was created by subcloning an Msd to BamHI fragment from pK721 into the LexA fusion plasmid pBTM116 (24). LexA/HSP42(22–332) was created by donning an Msd to Accl fragment of HSP42 into pBTM116. LexA/HSP42(148–375) was created by transferring HSP42 sequences from the shorter GAD fusion into pBTM116. LexA/HSP42(182–375) was generated by PCR. HA-tagged Hsp42p was expressed from within pRS423 (pD238), in which the 3’ region of the gene was generated by PCR, to include a unique NotI site 5′ of the termination codon. A NotI fragment encoding three copies of the HA peptide (YPYDVPDYA), recognized by the I2CA5 monoclonal antibody (BABCO), was ligated into this construct. His-tagged bacterial expression constructs were generated within pQE40 (Qiagen) and T7 epitope-tagged constructs in PET21a (Invitrogen). The HSP42 disruption was created by replacing sequences between an Xhol site (406 base pairs 5′ to the ATG) and an EcoRV site (at codon 80) with the HIS3 gene. HSP26 was disrupted by replacing a BglII to NruI fragment containing the entire coding sequence with a 2.2-kilobase pair LEU2 fragment. Disruption constructs were integrated using standard techniques (26). Sequencing was carried out using the dyeoxy chain termination method, using Sequenase (Amersham).

Northern Analysis—Cell pellets from 5-ml cultures were resuspended in 0.2 ml of extraction buffer (0.5 M NaCl, 0.2 M Tris-HCl, pH 7.6, 10 mM EDTA, 1% SDS). 0.4 g of acid-washed glass beads (0.45–0.5 mm) and 0.2 ml of phenol/chloroform/isoamyl alcohol and precipitated with ethanol. 10 μg of RNA (per lane) were electrophoresed through 1% agarose and transferred to nylon membranes (Hybond). Membranes were hybridized with random-primed DNA probes in 0.5 M sodium phosphate, pH 7.2, 7% SDS, 1 mM EDTA, 2× SSPE, and 42°C, washed in 2× SSC, 1% SDS at 65°C, and exposed to Kodak X-AR5 film.

Western Blotting—Proteins were fractionated on 12% polyacrylamide and electroblotted onto nitrocellulose membranes. Membranes were blocked in PBS, 5% nonfat milk, 0.1% Tween 20 and incubated with the appropriate antibody (anti-HA from BABCO, or anti-T7 from Invitrogen) in PBS, 1% nonfat milk, 0.1% Tween 20, for 1 h at room temperature. After washing in PBS, 0.1% Tween 20 four times, membranes were incubated with peroxidase-conjugated mouse IgG-specific antibody for 1 h. Following further washing, blots were developed using ECL (Amersham).

Size Separation of Yeast Protein Extracts by FPLC—The cell pellet from a 1-liter culture (A600 > 1.0) of W303-1B with pD238 was resuspended in 5 ml lysis buffer (20 mM HEPES, pH 6.8, 150 mM KOAc, 2 mM MgOAc, 2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride). 1 ml of glass beads was added, and cells were vortexed for 1 min and left on ice for 1 min. This was repeated eight times. Cell debris was removed by centrifugation at 7000 rpm for 15 min at 4°C. The supernatant was centrifuged at 50,000 rpm for 30 min at 4°C and filtered through a 0.22-μm filter. A mg of protein was loaded onto a Superose 12 column that had been calibrated with proteins of known size. 40 fractions of 0.5 ml were collected, and 5 μl of each fraction were analyzed by SDS-PAGE and Western blotting.

Expression of Bacterial Fusion Proteins—Proteins were expressed from within pQE40 in M15(pREP4) cells (Qiagen). PET21a fusions were expressed in BL21 cells (Invitrogen). A saturated culture was diluted 1:10 and grown at 37°C for 90 min. Protein production was induced by the addition of isopropyl-1-thio-β-D-galactopyranoside to 2 mM. After 4 h, cells were collected by centrifugation, lysed by sonication in 500 μl of NETN (100 mM NaCl, 10 mM Tris, pH 8, 1 mM EDTA, 0.5% Nonidet P-40), and insoluble proteins were removed by centrifugation. Histidine-tagged fusions were partially purified on nickel-agarose beads. 50 μl of nickel-agarose, with protein from 0.5 ml of culture, was incubated with 20 μl of bacterial extract containing T7-tagged Hsp42p at 4°C in 500 μl of NETN for 1 h. The beads were washed with 4 ml of NETN and boiled in 2× SDS-PAGE loading buffer. Proteins were analyzed by SDS-PAGE and Western blotting.

Screening of GAD Fusion Libraries—The pGAD3-based library was screened with LexA/RAP1 on CTY10–5D as described previously (8, 24). The pGAD424 library was transformed into L40 cells (27), transformants were grown in SC-Leu, collected by centrifugation, and frozen in −80°C with 15% glycerol at −70°C. After spreading onto SC-Leu plates and incubation at 30°C for 2 days, they were replica-plated onto YPD plates that had been spread with 100 μl of an overnight culture of HLY655 containing the LexA fusion. After mating overnight, cells were replica-plated to SC-Leu-Trp-His, to select for both plasmids (diploids) and activation of the LexA operator-HIS3 reporter (28). His⁺ colonies were picked after 2 or 3 days and tested for their ability to activate the LexA operator-lacZ reporter on a filter assay as described previously (8). GAD plasmids were recovered from His⁺, LacZ⁻ diploids.

RESULTS

Overexpression of orf721 Causes LexA/RAP1(635–827) to Become an Activator—The Rap1p protein has been shown to play important roles in both transcriptional activation and silencing (1). To identify other proteins involved in these processes which may interact with Rap1p, we screened a two-hybrid library with a construct containing RAP1 sequences fused to LexA. The fusion protein produced contained amino acids 635–827 of Rap1p. This region has previously been shown to contain a domain involved in transcriptional silencing at the mating-type loci HML and HMR, and can interact with the silencing factors Sir3p and Sir4p (8, 29). In addition, most of the Rap1p activation domain, which has been mapped to amino acids 630–695, is contained within this region (30). However, the LexA/RAP1(635–827) fusion does not activate transcription of reporter constructs containing multiple LexA operators.

CTY10–5D was co-transformed with LexA/RAP1(635–827) and a GAD fusion library. Transformants were then assayed for β-galactosidase activity on nitrocellulose filters. Plasmids were isolated from positive colonies and assayed for the ability to confer the activating phenotype on re-transformation into CTY10–5D.

Plasmids that activated the LexA operator-lacZ reporter in the presence of LexA/RAP1(635–827), but not LexA/famin or LexA alone, were further tested for their ability to derepress a TRP1 reporter gene at HMR (31). At the silent mating-type loci, Rap1p functions to recruit silencing factors such as Sir3p. Proteins which interact with Rap1p and play a role in silencing at the HM loci might be expected to derepress these loci when fused to the Gal4p activation domain, as this activation domain would be recruited to the silent loci. Of the clones that activated the LexA operator-lacZ reporter in a LexA/RAP1(635–827)-dependent manner, and derepressed hmr·lacZ/TRP1, two contained overlapping inserts. Sequence analysis demonstrated that in neither case was an in-frame fusion with GAD sequences present. Thus, these clones appeared to encode proteins capable of activating independently of GAD. Further analysis revealed that they contained the same DNA sequences, cloned in opposite orientations, and that activation was dependent on an open reading frame of 353 amino acids. This open reading frame contained no translational stop codon, but was fused either to the GAD sequences, in reverse orientation, or to the termination sequence (Fig. 1A).

To determine whether the protein encoded by this open reading frame (orf721) was responsible for activation of the LexA operator-lacZ reporter, it was cloned into the 2-μm vector pRS423 (25). In this plasmid (pK721) the 3′ junction resulted in the addition of a single codon (His) after amino acid 353 of...
orf721 Encodes a Small Heat Shock Protein—Comparison of the predicted amino acid sequence of orf721 to GenBank™ and EMBL data bases revealed that amino acids 305–339 of orf721 have homology with a conserved hydrophobic region found at the carboxyl terminus of many small heat shock proteins (Fig. 2). This region also shows homology to α-crystallin (19). The protein most similar to that encoded by orf721 is S. cerevisiae Hsp26p (Fig. 2A), sharing 46% identity (66% similarity) with amino acids 305–339 of orf721 (Fig. 2A). This conserved region is within an extended region of weaker homology between the carboxyl termini of Hsp42p and Hsp26p. As shown in Fig. 2B, within the conserved region (amino acids 305–339 of orf721) amino acids at several positions are absolutely conserved between divergent species (13, 16, 19, 32). The observed homology suggests that orf721 encodes a small HSP, of predicted molecular mass 42.8 kDa. We, therefore, name this gene HSP42 and the truncated protein, encoded by orf721, hsp42t.

Regulation of HSP42 mRNA Expression—To determine whether expression of HSP42 mRNA is sensitive to conditions of stress, such as increased temperature or salt concentration, Northern analysis was undertaken. Expression of HSP26 and actin mRNAs was also analyzed for comparison. RNA was isolated from W303-1B cells grown at either 25°C or at 25°C and shifted to 30, 37, or 39.5°C for 20 to 60 min. Incubation of cells at 37°C for 20 min resulted in an increase in the amount of HSP42 mRNA. The increase in HSP42 mRNA levels was even more dramatic when cells were transferred from 25°C to 39.5°C (Fig. 3). Little change in the level of HSP42 mRNA was observed in cells transferred to 30°C. When the same blot was re-hybridized with an HSP26-specific probe, a similar pattern of induction was observed (Fig. 3). However, there was no detectable level of HSP26 expression at 25°C, whereas HSP42 was expressed at a relatively high level in cells grown at 25°C. Actin mRNA expression was unaffected by temperature change. Thus, the levels of HSP42 and HSP26 mRNAs appear to be regulated similarly by heat shock, confirming that HSP42 is a heat shock-responsive gene.

HSP26 mRNA levels are increased when cells are transferred to medium containing high concentrations of NaCl (23). Cells were grown at 25°C in rich medium (YPD) and then transferred to YPD containing 0.7 m NaCl for 20 min to 3 h, after which the RNA was isolated. As shown in Fig. 4A, the level of HSP42 mRNA starts to increase within 20 min of the addition of NaCl and continues to increase for at least 1 h. The observed increase in HSP26 mRNA appears to be less rapid (Fig. 4A). To compare more directly the relative increases in HSP42 and HSP26 mRNA levels, RNA isolated from cells grown at 25°C, cells shocked at 39.5°C for 20 min, and cells incubated in 0.7 m NaCl for 40 min was electrophoresed in adjacent lanes (Fig. 4B, lanes 1–3). In comparison to HSP42, the increase in HSP26 mRNA expression is greater on heat shock than on addition of NaCl. Up-regulation of HSP42 expression appears to be more sensitive, than HSP26 expression, to increased salt concentration, whereas HSP42 expression is more sensitive to increased temperature. However, these differences are relatively subtle and may simply reflect differences in the rates of induction of expression.
As cells are transferred to sporulation medium or move into stationary phase, HSP26 mRNA expression is up-regulated (20). As shown in Fig. 4B (lanes 4–8), when cells were transferred to YPAc, the mRNAs for both HSP26 and HSP42 were up-regulated, although the increase in HSP42 mRNA was more obvious. Similarly, when cells were grown to high density, both RNAs were up-regulated (Fig. 4B, lanes 9–11). In this case, HSP26 expression increased more dramatically. Thus, expression of the HSP42 gene responds to the same range of stress conditions as HSP26, although there are clear differences in the relative increases in expression levels of the two genes.

Phenotypic Analysis of Cells Lacking a Functional HSP42 Gene—To determine whether expression of Hsp42p was necessary for stress tolerance, we disrupted the HSP42 gene in W303-1B cells. This mutation replaced 405 base pairs of the promoter and the first 243 base pairs of coding sequence with the HIS3 gene. Using a probe containing the entire HSP42 coding sequence, no expression of HSP42 mRNA was detectable by Northern analysis (data not shown). A deletion of the entire HSP26 coding sequence was also created. Wild-type and mutant cells were subjected to a range of stress conditions, and viability was assessed. No significant differences in the tolerance of elevated temperature were observed between wild-type cells and cells lacking the HSP42 gene or both HSP42 and HSP26 (data not shown). Incubation of wild-type and hsp42hsp26 cells in YPD containing 1 M NaCl greatly reduced the viability of the cultures. However, when cells were plated onto YPD plates after 3 or 4 h in 1 M NaCl, no significant differences in the viability of wild-type and mutant cultures were observed.

When equal numbers of cells that had been stored at 4°C for 5 months on YPD were plated onto fresh YPD plates, cells lacking HSP42 showed a slightly reduced viability compared to wild-type W303-1B cells. This replication replaced 405 base pairs of the promoter and the first 243 base pairs of coding sequence with the HIS3 gene. Using a probe containing the entire HSP42 coding sequence, no expression of HSP42 mRNA was detectable by Northern analysis (data not shown). A deletion of the entire HSP26 coding sequence was also created. Wild-type and mutant cells were subjected to a range of stress conditions, and viability was assessed. No significant differences in the tolerance of elevated temperature were observed between wild-type cells and cells lacking the HSP42 gene or both HSP42 and HSP26 (data not shown). Incubation of wild-type and hsp42hsp26 cells in YPD containing 1 M NaCl greatly reduced the viability of the cultures. However, when cells were plated onto YPD plates after 3 or 4 h in 1 M NaCl, no significant differences in the viability of wild-type and mutant cultures were observed.

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nal Domain—In an attempt to identify proteins which interact with Hsp42p, we screened a two-hybrid (GAD fusion) library with a LexA/HSP42 construct encoding amino acids 22–353 of Hsp42p. LexA/HSP42(22–353) was transformed into HLY655 cells, which contain integrated LexA operator-\(\alpha\)lacZ and LexA operator-HIS3 reporters. The GAD library was transformed into L40 cells (L40 differs from HLY655 only in its mating type). Cells containing the GAD library were then mated with LEXA/HSP42-containing cells. Colonies able to grow on medium lacking leucine, tryptophan, and histidine were selected. GAD

To determine whether the interaction observed in the two-hybrid assay is direct, Hsp42 fusion proteins were expressed in E. coli. As shown in Fig. 6A, two His-tagged, DHFRS/HSP42 fusions (within pQE40), encoding amino acids 50–375 or 148–375 of Hsp42p (6H-hsp(50–375) and 6H-hsp(148–375); Fig. 6A) were created. A third construct expressed amino acids 148–375 of Hsp42p, tagged with an epitope recognized by a T7-specific antibody (T7-hsp(148–375); Fig. 6A). His-tagged fusion proteins were isolated on nickel-agarose. Crude bacterial extract containing the T7-tagged Hsp42p was incubated with either of the nickel-agarose-bound His-tagged Hsp42p fusions or with 6xHis-DHFRS. The nickel-agarose was washed extensively and bound proteins were separated by SDS-PAGE, Western blot, and incubated with a T7-specific monoclonal antibody. As shown in Fig. 6B, a T7-reactive protein was present in the lanes containing proteins isolated in the presence of both the His-tagged Hsp42p fusion proteins, expressed from 6H-hsp(50–375) and 6H-hsp(148–375). A small amount of T7-tagged Hsp42p was retained on the 6xHis-DHFRS (pQE40) nickel-agarose; however, this was probably the result of non-specific binding to the nickel-agarose. Thus, the bacterially produced T7-tagged Hsp42p was capable of interacting specifically with His-tagged Hsp42p in the absence of other yeast proteins, strongly suggesting that this interaction is direct.

**S. cerevisiae Hsp42p**

![Fig. 4. Induction of HSP42 expression under conditions of stress.](image)

Northern analysis was carried out as in Fig. 3, with: A, RNA from cells incubated in 0.7 M NaCl for 20 min to 3 h (lanes 2–6); and B, RNA from cells grown at 25 °C, cells heat-shocked at 39.5 °C for 20 min, and cells incubated in 0.7 M NaCl for 40 min electrophoresed in lanes 1–3. RNA was analyzed from cells incubated in sporulation medium (YPAc; lanes 5–8) and from cultures grown to stationary phase for 2 or 6 h (Stat; lanes 10 and 11).
Hsp42p was detected in the fractions containing proteins below 66 kDa, where monomeric Hsp42p would be expected to be found. Hsp42p was present exclusively in fractions 7–14, suggesting that it is present in complexes ranging upwards from 200 kDa in size (Fig. 7). Given the apparent molecular mass of HA-tagged Hsp42p (approximately 52 kDa), the size range in which Hsp42p eluted from the Superdex 200 column suggests that, if these complexes consist of Hsp42p alone, it is at least tetrameric. The broad range over which Hsp42p eluted suggests that the Hsp42p complexes are heterogeneous in size.

**Discussion**

We have isolated a novel S. cerevisiae gene that encodes a small HSP. Hsp42p shares a high degree of homology with other HSPs over a conserved hydrophobic region present in many small HSPs (19).

Analysis of the regulation of HSP42 mRNA expression demonstrates that this gene is responsive to conditions of stress. HSP42 expression is up-regulated by increases in temperature and salt concentration, as well as by conditions of limiting growth and overgrowth of cell cultures. Interestingly, although HSP26 expression is also up-regulated under all these conditions, there are differences in the responses of these two genes to the various conditions of stress. This may reflect slightly differing functions of these two proteins. Thus, Hsp42p may play a more important role in the response to increased salt concentration, whereas Hsp26p may be required for tolerance of high temperatures.

Several small HSPs, including Hsp26p, have been shown to aggregate within cells (21, 33–35). When a GAD fusion library was screened with LexA/HSP42, the majority of the interacting clones isolated encoded in-frame fusions of the Gal4p activation
domain with Hsp42p. Thus, as with other HSPs, Hsp42p appears to interact with itself. Using bacterial fusion proteins, we have demonstrated that this Hsp42p-Hsp42p interaction is direct. Analysis of the high molecular mass complexes containing Hsp26p, by sucrose gradient and SDS-PAGE fractionation, has demonstrated that Hsp26p is the predominant protein within these complexes (21). However, the presence of less abundant or otherwise undetectable proteins cannot be ruled out. The demonstration that the interaction of Hsp42p with itself is direct lends weight to the idea that small HSPs form high molecular mass complexes by homo-multimerization. The interaction of Hsp42p with itself appears to be dependent on the conserved hydrophobic region. Therefore, it seems likely that the aggregation of Hsp26p within the cell is via a direct interaction dependent on the analogous region of Hsp26p. An additional possibility is that Hsp42p and Hsp26p interact with each other. To test this, we created a LexA/HSP26 fusion. Although a fusion protein of the expected size was produced at high levels, no interaction with Gsp/HSP42 fusions was observed (data not shown). We were unable to test the interaction of Hsp26p with a LexA/HSP26 fusion because Gsp/HSP26 fusions were toxic to the cells. In this context, it is of interest that HSP42 is expressed at relatively high levels in unstimulated cells whereas HSP26 expression is undetectable. Thus, Hsp42p may function in both stressed and unstimulated cells.

Disruption of the HSP26 gene does not result in any detectable phenotype, even under conditions of stress. As Hsp42p is the protein most similar to Hsp26p identified so far in S. cerevisiae, it was possible that disruption of both genes would result in a discernible phenotype. However, we were unable to detect any significant difference in viability between cultures of wild-type and a hsp42hsp26 double mutant. Thus, as with other HSPs, Hsp42p appears to be able to form long highmolecularmass complexes by homo-multimerization. The effects observed on Rap1p-dependent silencing may only be obvious because of the sensitivity of this system. It is of interest, in this regard, that recent work has demonstrated a possible mechanistic link between stress, aging, and silencing in yeast (36). Specifically, both stress resistance and life-span are regulated, at least in part, by the putative Sir protein silencing complex. Given the clearly-established role of Rap1p in HM locus and telomeric silencing, it is not unreasonable to speculate that the effect of Hsp42p on Rap1p described here may have consequences for both stress response and life span in yeast.

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