Expression of hsp16 in response to nucleotide depletion is regulated via the spc1 MAPK pathway in Schizosaccharomyces pombe

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ABSTRACT

A universal response to elevated temperature and other forms of physiological stress is the induction of heat shock proteins (HSPs). Hsp16 in Schizosaccharomyces pombe encodes a polypeptide of predicted molecular weight 16 kDa that belongs to the HSP20/α-crystallin family whose members range in size from 12 to 43 kDa. Heat shock treatment increases expression of the hsp16 gene by 64-fold in wild-type cells and 141-fold in cdc22-M45 (ribonucleotide reductase) mutant cells. Hsp16 expression is mediated by the spc1 MAPK signaling pathway through the transcription factor atf1 and in addition through the HSF pathway. Nucleotide depletion or DNA damage occurs in cdc22-M45 mutant cells, or during hydroxyurea or camptothecin treatment, is sufficient to activate hsp16 expression through atf1. Our findings suggest a novel role for small HSPs in the stress response following nucleotide depletion and DNA damage. This extends the types of damage that are sensed by the spc1 MAPK pathway via atf1.

INTRODUCTION

A universal response to elevated temperature and other forms of stress is the induction of heat shock proteins (HSPs). HSPs are classified into seven major families according to their size, structure and function. These include HSP100, HSP90, HSP70, HSP60, HSP40, HSP33 and the small HSPs related to α-crystallin (1). The conserved α-crystallin family includes: vertebrate hsp27 (hsp25), induced by a variety of environmental stresses; D.melanogaster hsp22, hsp23, hsp26, hsp27; the C.elegans hsp16 multigene family; in fungi, HSP26 (S.cerevisiae) and hsp30 (Neurospora crassa and Aspergillus nidulans); and in plants four classes of hsp20, plus α-crystallin A and B chains.

The large HSPs have been implicated in major physiological processes such as cell division, transcription, protein folding, transport and membrane function (12–15). To date, however, there is no experimental evidence that small HSPs are essential for normal cellular function. The Hsp20/α-crystallin family act as molecular chaperones in vitro protecting other proteins against heat-induced denaturation and aggregation (15–20). They can form large oligomeric complexes (17,21–23) and have a role in thermostolerance in mammalian cells and Drosophila (24–26) but not in yeast cells (8). In mammalian cells, small HSPs bind specifically to cytoskeletal elements such as actin and to intermediate filaments such as desmin, vimentin and glial fibrillary acidic protein (24,27–29). It has also been reported that small HSPs modulate apoptosis through the Fas/Apo1 receptor (30) and are involved in cell growth and differentiation (31).

Hsp26 in S.cerevisiae functions as a molecular chaperone in vivo (21). It accumulates to high levels after heat shock, during the transition to sporulation and even under other stresses such as increased salt concentration and starvation (32,33). However, Hsp26 does not appear to be required for cell viability under these conditions (8,34). This suggests that the function of Hsp26 in stress response overlaps the functions of other HSPs. To date the regulation and function of small HSPs in yeast remains elusive.

In this paper, we report the isolation of S.pombe hsp16, a member of the small HSP family. We show that hsp16 expression is induced by a number of environmental stimuli including heat shock. In addition, expression of hsp16 is responsive to deoxyribonucleotide depletion or DNA damage and this response is dependent on the spc1 MAPK pathway and the atf1 transcription factor.
MATERIALS AND METHODS

Strains and media

Schizosaccharomyces pombe strains were derived from wild-type 972 h– or 975 h+ (35) (Table 1). Strains were grown in YEA complex medium (yeast extract medium containing adenine) or Edinburgh minimal medium containing nutritional supplements as necessary (36).

Cloning of hsp16 gene

Standard molecular biological and genetic techniques were used (36,37). In an attempt to identify genes whose expression was dependent on cell cycle stage, a differential hybridization screen was mounted using a fission yeast λ genomic library which was replica-blotted and probed with radioactive cDNA probes. The probes were reverse transcribed from RNA isolated from various cdc mutant strains following arrest at 36°C for 4 h (cdc10-129, G1; cdc22-M45, S; cdc25-22, G2). One λ clone, 15-66, was found to give a very strong signal with the cdc22-M45 probe but not with the others. The hybridizing region was subcloned and sequenced yielding hsp16 (38,39).

hsp16 has been independently sequenced by the fission yeast genome project (Sanger database) and has also recently been characterized by Danjoh and Fujiyama (6).

Construction of ∆hsp16

The entire hsp16 open reading frame (ORF) was replaced with the ura4+ gene by one-step gene replacement (37). Stable

### Table 1. Schizosaccharomyces pombe strains used in this study

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ura4+ haplotypes were selected and exact gene replacement was confirmed by PCR, northern blot hybridization and western blotting. The strain was extensively out-crossed to ensure that no background mutations were present.

**Construction of hsp20::ura4+**

Hsp20 was identified by similarity to hsp16 within the fission yeast genome project. The hsp20 gene from –172 to +591 bp relative to the 420 bp ORF was amplified by PCR from S.pombe genomic DNA using high fidelity Taq polymerase (Roche Molecular Biochemicals). The PCR product was subcloned into pCR2.1-Topo (Invitrogen) and the ura4+ gene subcloned into the blunted BstEII site at +52 bp in the hsp20 ORF to generate pCR2.1-Topo-hsp20::ura4+. The hsp20::ura4+ cassette in the recombinant vector was then PCR amplified with high fidelity Taq polymerase (Roche Molecular Biochemicals) and this PCR product used to replace hsp20 in a haploid strain (ura4-D18 h+) (37). Stable ura4+ haplotypes were selected and exact gene replacement was confirmed by PCR.

**Expression of hsp16**

Total RNA was prepared as described (37) and 5 µg of each sample was resolved on a formaldehyde gel. Hybridization probes were labeled using the Rediprime II random prime labeling system ([32P]dCTP; Amersham Pharmacia Biotech). An NdeI–SalI hsp16 fragment was used to detect the hsp16 mRNA and a BamHI fragment of rDNA (plasmid provided by M. Yanagida, Kyoto University, Japan) was used to detect ribosomal RNA as a control. Signals were quantitated directly using a PhosphorImager (Molecular Dynamics) and expressed relative to 18S rRNA levels, which served as an internal loading control. Two independent RNA extractions were prepared and analyzed.

**Quantitation of green fluorescent protein (GFP)**

A very strong correlation exists between the amount of GFP in a cell and the total fluorescence (40,41). This was therefore used to quantitate protein expression.

Wild-type and mutant yeast strains were grown with shaking at 25°C in YEA to a density of 2–5 × 10^6 cells/ml and then shifted to the restrictive temperature of 36°C for 4 h. At various times 10 ml of liquid culture was collected into ice-cold water to a final cell density of 1 × 10^7 cells/ml and kept on ice. Hsp16–GFP levels were quantitated using a Luminescence Spectrometer LS50B (Perkin Elmer) and normalized relative to fluorescence levels in a wild-type cell not expressing GFP. Assays were performed in triplicate and independently repeated three times. Control experiments showed that the GFP fluorescence was stable for at least 6 h while samples were kept at 0°C. The washing and analysis procedure alone did not induce expression of this gene.

**Production of GST–hsp16 fusion protein**

To prepare the protein product of the hsp16 gene, the coding region of hsp16 was fused to the IPTG-inducible glutathione S-transferase (GST) in pGEX-2T (Amersham Pharmacia Biotech). Following expression in *Escherichia coli* with 1 mM IPTG at 37°C the GST–hsp16 fusion protein was purified on a glutathione–agarose column and eluted with 10 mM reduced glutathione in 50 mM Tris–HCl pH 8.0 according to the manufacturer’s instructions (Amersham Pharmacia Biotech).

**Immunoochemical analysis**

To generate polyclonal antibodies against the hsp16 protein, GST–hsp16 protein was separated on a 12% SDS–polyacrylamide gel, excised, eluted and mixed with Titer Max Gold Adjuvant (Cedarlane) according to the manufacturer’s instructions. Following a second injection at day 28, serum was collected on day 40 and used as a source of antibody for western blot analysis and immunofluorescence experiments.

**Fluorescence microscopy**

All fluorescent images were taken with a Leica fluorescence microscope equipped with a high performance CCD camera (Sensicam) and Slidebook software (Intelligent Imaging System). Cells were collected using Whatman 934-AH glass microfibre filters (Fisher Scientific) and fixed in 100% methanol at –20°C for at least 20 min. Immunofluorescence was carried out as described in Sawin and Nurse (42). The primary antibody used was the rabbit polyclonal GST–hsp16 antisera generated in the laboratory (1:5000) and the secondary antibody used was Alexa™ goat anti-rabbit IgG (H + L) conjugate (1:250) (Molecular Probes). Stained cells were counterstained with 1 µg/ml DAPI.

**Protein extraction**

For native protein extracts, cells were harvested by centrifugation, washed once with ice-cold stop buffer (150 mM NaCl, 50 mM NaF, 10 mM EDTA, 1 mM NaN3 pH 8.0) and immediately frozen at –70°C. The cell pellet was resuspended in 200 µl lysis buffer (50 mM Tris–HCl pH 8.0, 150 mM NaCl, 5 mM EDTA, 1% Nonidet P-40, 1 mM DTT, 10% glycerol, 50 mM NaF, 1 mM Na3VO4, 1 mM PMSF and 20 µg/ml each leupeptin, pepstatin and aproprinin) and glass beads added to the meniscus. Cells were broken open by vortexing with glass beads and centrifuged to prepare a cleared whole-cell extract. Protein concentration was determined using the Bio-Rad protein assay. Extracts (20 µg) were separated by 12% SDS–PAGE, electroblotted to a PVDF membrane (Santa Cruz) and detected by immunoblotting with polyclonal anti-GST–hsp16 antibody (1:1000). Immunoreactive bands were revealed with HRP-conjugated secondary goat anti-rabbit IgG antibody (1:2000) (Santa Cruz) and the luminol-based ECL detection kit (Santa Cruz).

**Yeast two-hybrid screen**

The full-length hsp16 orf was fused to the 3' end of the lexA DNA-binding domain in pEG202 (43) by generating a BamHI/NotI hsp16 fragment by PCR and cloning into the BamHI/NotI sites, to yield the bait plasmid. The λ ACT *S.pombe* cDNA library (obtained from ATCC 87289) was fused to the Gal4 transcriptional activation domain and cloned into the Xhol site with subsequent conversion to plasmid form using cre-lox site-specific recombination (44). The two-hybrid experiments were performed with *S.cerevisiae* strain Y1003 (MATa/ MATαURA3::lexAop-lacZ/I8lex-ADE2::URA3 ura3-1/jura3-1 leu2-2/leu3-2 his3-11/1his3-11 trp1-1/1trp1-1 ade2-1/1ade2 can1-100/can1-100) (45). Approximately 750 000 cDNA clones were screened. Following confirmation of the assay, positive clones were sequenced.
RESULTS

**hsp16 expression is strongly induced in a cdc22-M45 mutant background**

A λ plaque was identified that displays a very strong signal when hybridized to labeled cDNA from cdc22-M45 arrested cells but not from probes made from cdc10-129 or cdc25-22 arrested cells. The λ clone (designated 15–66) was nick-translated and used to hybridize to northern blots of RNA derived from the same three arrested cell populations. A mRNA of ~750 bp was found to account for the differential signal (39, data not shown). The region coding for this transcript was subcloned, sequenced and found to be hsp16. The clone was of interest since it was clearly responsive to more than just heat shock because that had been kept constant for all strains. It was subsequently found to be somewhat elevated, relative to heat shock response alone, in cdc17-117 (DNA ligase). This response was lower than seen for cdc22-M45 (38,39).

To examine this quantitatively we generated a number of reagents and further characterized the system (Figs 1 and 2). Expression of the hsp16 transcript was induced in response to heat shock treatment and this response was further elevated in a cdc22-M45 mutant background (Fig. 1A and B). Western blotting showed that the gene product, hsp16, is responsive to heat shock treatment (Fig. 1C, lanes 1 and 2) and is further upregulated in a cdc22-M45 mutant background both at 25 and 36°C (Fig. 1C, lanes 5 and 6). Hsp16 expression is clearly regulated by heat shock, in contrast to previously published results (6).

A fusion construct, placing hsp16–GFP under the control of the native promoter of hsp16, was constructed by homologous recombination to generate a single copy chromosomal fusion (Fig. 2A). Expression of the hsp16–GFP behaved in a fashion similar to that of hsp16 alone in a wild-type background at 36°C (Fig. 2B). The presence of the GFP tag does not appear to affect expression or protein levels. The phenotype of wild-type cells expressing hsp16 tagged with GFP was indistinguishable from that of wild-type cells alone.

The hsp16–GFP fusion construct enabled us to easily and quantitatively study the normal regulation of hsp16 in a wide variety of contexts (Fig. 2C and D). The expression of hsp16–GFP was increased after heat shock treatment from 25 to 36°C at various times and by an additional 2–3-fold at 36°C in a cdc22-M45 background compared to wild-type. We also examined the heat shock response of hsp16–GFP in a shift from 30 to 37°C for various times. Expression under these conditions is similar to that in a treatment from 25 to 36°C (Fig. 2D).

**hsp16 transcription is mediated in part by the spc1 MAPK pathway via atf1**

Comparison to earlier work. Previous reports (6) have suggested that hsp16 does not respond to heat shock and that its expression is dependent on the activity of the ras1 pathway with expression being reduced 15-fold in a ras1 deletion background. However, in this investigation, northern blotting showed that heat shock alone is sufficient to elevate transcript levels ~64-fold in a wild-type background (Table 2). Our data also clearly show that in both the ras1 and rasval17 backgrounds, hsp16 is transcribed at levels similar to that seen in wild-type.

Effect of cdc22-M45. hsp16 expression was found to be elevated at both the restrictive and permissive temperatures in the cdc22-M45 mutant background (Fig. 1A and B; Table 2). The cdc22-M45 mutation inactivates deoxyribonucleotide production and is known to activate the DNA replication checkpoint. We therefore examined the dependence of induction on the checkpoint by analyzing hsp16 expression in the rad1-1 and radl-1 cdc22-M45 mutant strains. Transcript levels in the rad1-1 cdc22-M45 mutant strain are similar to that seen in the cdc22-M45 mutant alone (Table 2). Curiously, at high temperature there is also increased expression in a rad1-1 mutant background alone. This suggests the possibility that heat shock alone elicits DNA damage. This damage rather than the cell cycle block per se appears to cause hsp16 activation since by removing the checkpoint the rad1-1 cdc22-M45 mutant strain proceeds through mitosis at 36°C.

Dependence on MAP kinase pathway. hsp27-mediated inhibition of actin polymerization is regulated in part by the p38 MAPK (46,47) corresponding to fission yeast spc1 (48–50).
Therefore, we examined hsp16 expression in a \( \Delta \text{spc1} \) mutant background to inactivate the pathway (48) and in a \( \Delta \text{pyp1} \) mutant background, an inhibitor of spc1 MAPK, to activate it (49). At 36°C, the transcript level for hsp16 was decreased by 2-fold in the \( \Delta \text{spc1} \) mutant background compared to wild-type (Table 2). Interestingly, increased activity of spc1 MAPK as in the \( \Delta \text{pyp1} \) mutant background has no additive effect on hsp16 expression in an otherwise wild-type strain (Table 2).

We next tested the effect of spc1 deletion on the cdc22-M45 response. At the reduced restrictive temperature of 30°C, cdc22-M45 \( \Delta \text{spc1} \) arrested with an additive morphological phenotype yielding very elongated cells. However, hsp16 expression at 36°C in the cdc22-M45 \( \Delta \text{spc1} \) mutant strain remained similar to that seen in wild-type (Table 2). This suggests that the spc1 pathway is necessary to respond to nucleotide depletion or to a DNA synthesis block as occurs in the cdc22-M45 mutant strain. This is reinforced by the observation that increased activity of spc1 (as occurs in \( \Delta \text{pyp1} \) mutant strain) has an additive effect on hsp16 expression in the \( \Delta \text{pyp1} \) cdc22-M45 mutant strain (Table 2).

Dependence on atf1. Conjugation, meiosis and osmotic stress response are affected by spc1 at least in part through atf1 whose expression and activity are stimulated by spc1 MAPK (51). hsp16 expression levels in a \( \Delta \text{atf1} \) mutant background are similar to that in wild-type cells (Table 2). However, in a \( \Delta \text{atf1} \) cdc22-M45 mutant background, hsp16 expression levels are greatly reduced compared to a cdc22-M45 mutant alone and comparable to or somewhat higher than in a \( \Delta \text{spc1} \) mutant alone (Table 2). These results suggest that atf1 is an important part of the response pathway for this type of replicational stress and that it is sufficient to account for all spc1-dependent activation of hsp16 but not for its response to heat shock.

Search for synthetic interaction. We decided to generate double mutants with \( \Delta \text{hsp16} \) and cdc22-M45, \( \Delta \text{spc1} \), \( \Delta \text{atf1} \) to see if a hsp16 deletion could interact genetically with these mutations. \( \Delta \text{hsp16} \) cdc22-M45, \( \Delta \text{hsp16} \) \( \Delta \text{spc1} \), \( \Delta \text{hsp16} \) atf1 double mutants were indistinguishable from the single mutants alone upon heat shock at 36°C (data not shown).

During the course of this study, we found another small HSP, hsp20 (accession no. AL02378I in the S.pombe Sanger database) that has significant homology within the C-terminal region of hsp16. Since this might provide a redundant function, we disrupted hsp20 and constructed a double mutant of \( \Delta \text{hsp16} \) and \( \Delta \text{hsp20} \). The \( \Delta \text{hsp16} \Delta \text{hsp20} \) double mutant did not exhibit a visible phenotype (data not shown).
Table 2. Quantitation of relative level of hsp16 transcript in various genetic backgrounds at 25 and 36°C

<table>
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<tr>
<th>Strains</th>
<th>Relative level at 25°C compared to wild-type level</th>
<th>Relative level at 36°C compared to wild-type level at 25°C</th>
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<tr>
<td>wild-type</td>
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<tr>
<td>Δhsp16</td>
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<td>8.7 ± 0.2</td>
<td>158 ± 4.0</td>
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Relative level of hsp16 transcript was obtained by densitometric analysis of a more lightly exposed autoradiograph of Figure 1 and two other independent northern experiments. The values presented were first normalized to the ribosomal DNA for each RNA sample and then to the levels in the wild-type background. These results suggest that elevated expression of hsp16 is dependent on the cell cycle arrest, or direct signaling through the rad1 pathway. Neither occurs in the rad1-1 mutant background since the checkpoint is abolished and these cells continue to divide. The Δspc1 mutation had little effect on the level of hsp16–GFP in the presence of hydroxyurea or camptothecin treatment (Fig. 3). These experiments were all performed at 25°C and differences in cell size cannot account for these differences.

Hsp16–GFP accumulation in response to heat shock is partly dependent upon the spc1 MAPK pathway (Tables 2 and 3). The question arises as to whether atf1 participates in the hydroxyurea or camptothecin responses. Hsp16–GFP expression was completely abolished in a Δatf1 mutant background in the presence of hydroxyurea or camptothecin (Fig. 3). In a Δatf1 mutant strain under these conditions the hsp16 gene appears to be negatively regulated.

Localization of hsp16 protein

Hsp16–GFP protein was localized in the cytoplasm and the nucleus in rich media (Fig. 4A, left panel). In stationary phase, hsp16–GFP localization changed and was distributed to one or sometimes two sharply defined structures close to, but not within, the nucleus as judged by DAPI (Fig. 4A, middle panel). The same pattern of expression was seen in cells upon nitrogen starvation in spores (Fig. 4A, right panel) and in cells
following heat shock treatment (data not shown). Spheroplasted cells lysed with 1% Triton X-100 showed that the 

hsp16–GFP protein was not membrane bound since it was not dispersed by detergent and therefore it is likely to be an inclu-

sion body aggregate. Following hydroxyurea or camptothecin treatment the distribution was similar to that seen in stationary 

phase (data not shown).

Immunolocalization using antibodies against hsp16 also showed hsp16 to localize to the cytoplasm and the nucleus in both wild-type and in the cdc22-M45 mutant background. This signal was absent in exponentially growing Δhsp16 mutant cells at 25°C (Fig. 4B). Cells were examined following the various treatments described earlier in the paper and in all cases the localization was the same.

Two-hybrid screen using hsp16

To identify potential hsp16 interacting proteins we performed a yeast two-hybrid screen using full-length hsp16 as bait. A total of 750 000 transformants were screened. The screen produced 41 reproducible interactions with eight different targets (Table 4). One of the clones repeatedly isolated in the screen was hsp16 indicating that the protein interacts with itself. We found that the hsp16–hsp16 interaction produces 224 U β-galactosidase activity. We did not pursue these targets further at this time.

DISCUSSION

We found the hsp16 gene to be responsive to heat shock treatment with a 64-fold induction of the transcript at 36°C. We have also examined hsp16 protein levels using both a reporter fusion construct, hsp16–GFP, as well as a polyclonal antibody
against hsp16. The protein levels are elevated at 36°C with
∼5-fold increase in the steady-state level of protein in a wild-
type background. This contradicts a recent publication by
Danjoh and Fujiyama (6) who reported that hsp16 is not heat
response, based on northern blot analysis. Close
inspection of these data (6; Fig. 4) shows a relatively constant,
but highly expressed, level of hsp16 signal at the permissive
temperature and upon heat shock conditions, hence their
conclusion. However, the control lane (30°C) shows a substantial
deficit of cdc2 transcript relative to the heat shocked samples.
Since cdc2 is not heat shock responsive (57), if one normalized
the data to cdc2 levels, this blot may suggest a very strong
expression of hsp16 at 30°C and reduced levels at 37°C. There
is no indication of the reproducibility of this result. The high
level of expression before heat shock treatment shown in this
publication is in direct contradiction to all of our findings. We
have no simple explanation for this but are confident, based on
our protein data as well, that hsp16 has a strong heat shock
response. It is possible, depending on how cells were harvested
and held prior to RNA isolation, that a MAPK-dependent
stress response was induced and this accounts for the expres-
sion in all strains used in their northern blots.

In support of our conclusion two potential heat shock
consensus sites are located at positions −157 to −170 (AGAAa-
tTCGATTTCtcGcGAa) and −529 to −543 (CGAAttTTCtcGcGTAA) from
consensus sites are located at positions –157 to –170 (AGAAa-
ntTCGATTTCtcGcGAa) and −529 to −543 (CGAAttTTCtcGcGTAA) from

Table 4. Results of cDNA protein interactions with hsp16 protein

<table>
<thead>
<tr>
<th>Bait</th>
<th>Target ORF name</th>
<th>Target gene name</th>
<th>Start-end domain amino acid</th>
<th>Number of clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>LexA-hsp16</td>
<td>SPAC19A5.10</td>
<td>Hypothetical zfp</td>
<td>5–254</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>SPBC3E7.02C</td>
<td>Hsp16</td>
<td>5–143</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>SPCP31B10.06 (SPAC513.01C)</td>
<td>Efl1=Efl2 (elongation factor 2)</td>
<td>543–842</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>SPBC365.06</td>
<td>Pnt3</td>
<td>2–117</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>SPAC630.14C</td>
<td>Tup1</td>
<td>141–586</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>SPBC1734.06</td>
<td>Putative DNA repair and recombination protein</td>
<td>16–387</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>SPCC417.08</td>
<td>Ef-3 (putative elongation factor 3)</td>
<td>836–1047</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>SPBC8E4.07C</td>
<td>Hypothetical serine/threonine repeat containing protein</td>
<td>219–1283</td>
<td>1</td>
</tr>
</tbody>
</table>

All the clones isolated included the C-termini and were deleted from the N-terminus to varying degrees, reflecting the nature of the cDNA library.

Role of the spc1 MAPK pathway

The spc1 MAPK pathway is known to play a role in adaptation
to adverse external stimuli including heat stress through atf1
(61–64). We provide the first example of a heat shock gene
being regulated in part by the spc1 MAPK pathway via atf1.
The heat shock factor pathway presumably accounts for the
remainer of hsp16 induction and the two pathways appear to
be additive. It is interesting that both the spc1 and atf1
promoters have heat shock element consensus sites. In other
systems it has been shown that the spc1 MAPK homolog, p38
in mammalian cells (65) and HOG1 in budding yeast (66) are
involved in the regulation of a small HSP but for different
environmental stresses such as oxidative stress and osmolarity.
The wis1 MAPKK is not involved in the regulation of hsp16 in
response to heat shock. Earlier studies have shown that activa-
tion of wis1 is weak and transient after heat shock (67). It
seems likely that another MAPKK dedicated to heat shock
stimuli might interact with spc1 although no candidate gene
suggests itself. This model would be similar to that found in
vertebrates where p38 is phosphorylated by two different
MAPKKs, SEK1 and MKK3/6 (68,69).

Relationship to DNA replication

Our data demonstrate a novel role for a small heat shock gene
in response to nucleotide depletion or potential DNA damage.
Ribonucleotide reductase is an essential enzyme for DNA
precursor metabolism. Failure to regulate dNTP levels can lead
to genetic abnormalities or cell death (70). Our findings
strongly suggest that the spc1 pathway responds to this stim-
ulus via atf1 and stimulates hsp16 induction under these condi-
tions.

We were able to generalize the cdc22 response to other types
of DNA synthesis block such as hydroxyurea or camptothecin
treatment. The induction of hsp16 in response to these agents
was not blocked by the AspC1 mutation. However, a Satf1
mutation appears to completely abolish hsp16 expression in
the presence of these two drugs. This contrasts with the response
to a cdc22 block.
Treatments that interfere with DNA synthesis arrest the cell cycle by activation of the checkpoint pathway dependent upon rad1. It is interesting that releasing the block by inactivating the checkpoint (rad1-1) does not affect the transcript level for hsp16. It does however cause a reduction in the accumulation of the hsp16 protein, presumably by affecting translation or stability. This contrasts with the response of the small subunit of ribonucleotide reductase encoded by suc22+, which requires the rad1+ gene for induction in response to DNA damage but not in response to heat shock (71). We found that the hsp16 transcript was also induced by heat shock in a rad1-1 mutant background. This is similar to levels as in a cdc22-M45 mutant background, which suggests that the possibility that heat shock itself is damaging to DNA. This damage rather than cell cycle progress leads to hsp16 activation since a rad1-1 cdc22-M45 mutant proceeds through mitosis at 36°C, yet the transcript is still induced.

Two-hybrid screen

The two-hybrid screen isolated a number of targets that potentially interact with hsp16 and one of the targets was hsp16 itself. Another target that was isolated as frequently as hsp16 was a novel hypothetical zinc finger protein (accession no. SPAC19A8.10) that has no apparent homolog in S.cerevisiae. Zinc finger proteins are components of transcription factors involved in mediating protein–protein interactions.

In S.cerevisiae one of the genes that was found to negatively regulate the DNA damage response of ribonucleotide reductase was the tup1 transcription factor (72). We isolated tup12, the tup1 homolog of S.cerevisiae as a potential target of hsp16. The recent identification of tup11 and tup12 proteins in another pathway through the spc1 MAPK pathway suggesting the possibility of the spc1 MAPK pathway and the HSF pathway regulate hsp16 expression in response to heat shock treatment both downstream of atf1 and this is independent of the heat shock factor (HSF) pathway. In response to heat shock treatment both the spc1 MAPK pathway and the HSF pathway regulate hsp16 expression. However, when cells are treated with hydroxyurea or camptothecin, hsp16 expression is regulated by atf1 but not through the spc1 MAPK pathway suggesting the possibility of another pathway.

REFERENCES


