Regulation of transcription by *Saccharomyces cerevisiae* 14-3-3 proteins

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14-3-3 proteins form a family of highly conserved eukaryotic proteins involved in a wide variety of cellular processes, including signalling, apoptosis, cell-cycle control and transcriptional regulation. More than 150 binding partners have been found for these proteins. The yeast *Saccharomyces cerevisiae* has two genes encoding 14-3-3 proteins, *BMH1* and *BMH2*. A *bmh1 bmh2* double mutant is unviable in most laboratory strains. Previously, we constructed a temperature-sensitive *bmh2* mutant and showed that mutations in *RTG3* and *SIN4*, both encoding transcriptional regulators, can suppress the temperature-sensitive phenotype of this mutant, suggesting an inhibitory role of the 14-3-3 proteins in Rtg3-dependent transcription [van Heusden and Steensma (2001) *Yeast* 18, 1479–1491]. In the present paper, we report a genome-wide transcription analysis of a temperature-sensitive *bmh2* mutant. Steady-state mRNA levels of 60 open reading frames were

increased more than 2.0-fold in the *bmh2* mutant, whereas those of 78 open reading frames were decreased more than 2.0-fold. In agreement with our genetic experiments, six genes known to be regulated by Rtg3 showed elevated mRNA levels in the mutant. In addition, several genes with other cellular functions, including those involved in gluconeogenesis, ergosterol biosynthesis and stress response, had altered mRNA levels in the mutant. Our data show that the yeast 14-3-3 proteins negatively regulate Rtg3-dependent transcription, stimulate the transcription of genes involved in ergosterol metabolism and in stress response and are involved in transcription regulation of multiple other genes.

Key words: 14-3-3 proteins, *BMH2*, ergosterol, microarray, *RTG3*, *Saccharomyces cerevisiae*.

INTRODUCTION

The 14-3-3 proteins form a family of highly conserved acidic dimeric proteins that are present, often in multiple isoforms, in all eukaryotic organisms investigated (reviewed in [1–5]). They bind to more than 150 different proteins and play a role in the regulation of many cellular processes, including signalling, cell-cycle control, apoptosis, exocytosis, cytoskeletal rearrangements, regulation of enzymes and transcription. Although the exact function of the 14-3-3 proteins is still not completely understood, three main mechanisms appear to be important. First, 14-3-3 proteins positively or negatively regulate the activity of enzymes; secondly, 14-3-3 proteins may act as localization anchors, controlling the subcellular localization of proteins; and thirdly, 14-3-3 proteins can function as adaptor molecules or scaffolds, thus stimulating protein–protein interactions. Binding motifs have been identified in a number of proteins that bind to the 14-3-3 proteins. Many, but not all, of these binding motifs contain a phosphorylated serine residue [6–10].

The yeast *Saccharomyces cerevisiae* has two genes, *BMH1* and *BMH2*, encoding 14-3-3 proteins [11–14]. A *bmh1 bmh2* disruption is lethal in most, but not all, laboratory strains, and the lethal *bmh1 bmh2* disruption can be complemented by at least four of the *Arabidopsis* isoforms and by a human and a *Dictyostelium* isoform [15,16]. As in higher eukaryotes, the *S. cerevisiae* 14-3-3 proteins are involved in many cellular processes, and many different binding partners have been identified [14]. These include the protein kinases Ste20p [17] and Yak1p [18], the protein phosphatase regulator Reg1p [19], the filament-forming protein Fin1p [20–22], the Mks1 protein [23], and the transcription factors Rtg3p [24], Msn2p and Msn4p [25]. Recently, it has been shown that the yeast 14-3-3 proteins bind to cruciform DNA [26].

In a previous study, we constructed a temperature-sensitive *bmh2* mutant by the disruption of both *BMH* genes and the introduction of a mutated *bmh2* allele [24]. We used this mutant to identify extragenic suppressor mutations bypassing the requirement of active 14-3-3 proteins. Recessive mutations in *RTG3* and *SIN4* resulted in growth at the restrictive temperature. *RTG3* encodes a basic helix–loop–helix transcription factor involved in the expression of *CIT2* and other genes in respiratory-deficient yeast cells (retrograde signalling) [27]. The Rtg3 protein forms a heterodimer with another basic helix–loop–helix transcription factor (Rtg1p) and binds to the core binding site 5′-GTCAC-3′ (R box) [27]. The expression of Rtg1- and Rtg3-regulated genes is negatively influenced by the target of rapamycin (TOR) signalling pathway [28]. *SIN4* encodes a global transcriptional regulator, which can stimulate or repress the expression of several genes and which is a component of the RNA polymerase II complex [29–31]. We showed that the yeast 14-3-3 proteins bind to the Rtg3 protein. Our genetic and biochemical studies suggested that the Rtg3 protein is inactivated by the 14-3-3 proteins. Recently, it was shown that the yeast 14-3-3 proteins also bind to the Mks1 protein [23], another regulator of retrograde signalling [32]. These studies indicate that the 14-3-3 proteins may have a major role in Rtg3-regulated gene expression. It has also been shown that the activity of the Msn2 and Msn4 transcription factors is influenced by 14-3-3 proteins. The 14-3-3 proteins sequester the phosphorylated forms of the Msn proteins into the cytoplasm [25].

In the present study, we investigated further the role of 14-3-3 proteins in the regulation of transcription. To this end, we investigated the effect of mutation of the *BMH* genes on the steady-state mRNA levels in *S. cerevisiae* at a genome-wide scale. As deletion of both *BMH* genes is lethal in most laboratory strains, we used a strain with the temperature-sensitive *bmh2* allele, which

Abbreviations used: Cy3, cyanine 3; Cy5, cyanine 5; ORF, open reading frame; TOR, target of rapamycin.

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Table 1 Yeast strains

Strain	Genotype	Source/reference
CEN-PK113-7D	<i>MATa</i>	P. Kötter (Göttingen, Germany)
CEN-PK113-13D	<i>MATa ura3-52</i>	P. Kötter (Göttingen, Germany)
GG3093	<i>MATa ura3-52 bmh2(Ts)</i>	Present study
GG3094	<i>MATa bmh1::kanMX</i>	Present study
GG3096	<i>MATa bmh1::kanMX bmh2(Ts)</i>	Present study

partly complements the *bmh1 bmh2* double disruption. We showed that in this *bmh* mutant, Rtg3-regulated genes have elevated mRNA levels, indicating that the 14-3-3 proteins inhibit the transcription of these genes. In addition, genes involved in gluconeogenesis were activated, and many genes involved in ergosterol synthesis and stress response were down-regulated in the mutant, showing a regulatory role of the 14-3-3 proteins in the transcription of these genes.

MATERIALS AND METHODS

Strains and culture media

The *S. cerevisiae* strains are listed in Table 1. *Escherichia coli* (strain XL1-blue) and yeast were cultured as described previously [12].

Construction of *bmh2(Ts)* strain GG3096

BMH2 was replaced by *URA3* in the *MATa* strain CEN-PK113-13D as described previously [12]. Subsequently, the *bmh2::URA3* allele was replaced by the *bmh2(Ts)* allele using a DNA fragment obtained by PCR on plasmid YCplac22[*bmh2(Ts)*] [24] and selection for 5-fluoro-orotic acid resistance, yielding strain GG3093. *BMH1* was deleted in the *MATa* strain CEN-PK113-7D by replacing the coding region by the *kanMX* cassette as described by Güldener et al. [33], yielding strain GG3094. Strains GG3093 and GG3094 were crossed and the resulting diploid was sporulated. After dissection of the asci, *MATa* haploids were selected having the *bmh1::kanMX*, *bmh2(Ts)* and *URA3* alleles. One of these haploids, strain GG3096, was analysed further, e.g. the correct integration of the *bmh2(Ts)* allele was confirmed by sequencing, and used in this study.

Chemostat cultivation

Steady-state chemostat cultures were grown in laboratory fermentors (Applikon) of 1 litre working volume, essentially as described in [34]. The cultures were fed with a defined mineral medium containing glucose as the growth-limiting nutrient at a dilution rate of 0.1 h^{-1} at a temperature of 30°C . The pH was kept at 5.0 ± 0.2 by addition of 2 M KOH, the airflow was $0.6\text{--}0.8 \text{ l} \cdot \text{min}^{-1}$. Culture purity was checked by phase-contrast microscopy. For each strain, two independent cultures were run. Steady-state cells were harvested after 9–12 volume changes by pouring samples of approx. 100 ml of culture into a beaker containing approx. 500 ml of liquid nitrogen. The mixture was stirred vigorously, allowing instant freezing of the sample. Frozen samples were broken into pieces and stored at -80°C .

Microarray analysis

Pieces of frozen culture containing approx. 2×10^9 cells were thawed on ice and cells were harvested by centrifugation. Total RNA was isolated using the RNeasy midi kit (Qiagen) after

disruption of the cells in the FastPrep instrument (Bio101). Cy3 (cyanine 3)- and Cy5 (cyanine 5)-labelled cDNAs were made using the Fluorescent direct label kit from Agilent Technologies (Stockport, Cheshire, U.K.). The labelled cDNAs were hybridized to Agilent yeast oligonucleotide microarrays containing 10807 60-mer oligonucleotide probes representing 6256 known ORFs (open reading frames) from the S288C strain of *S. cerevisiae*, according to the instructions from Agilent Technologies. The microarrays were scanned using an Agilent Technologies dual-laser microarray scanner, and the data were extracted using Agilent Feature extraction software. Four microarrays were used: (i) hybridized to Cy5-labelled cDNA from culture 1 of CEN-PK113-7D and Cy3-labelled cDNA from culture 2 of CEN-PK113-7D; (ii) hybridized to Cy5-labelled cDNA from culture 1 of CEN-PK113-7D and Cy3-labelled cDNA from culture 1 of GG3096; (iii) hybridized to Cy5-labelled cDNA from culture 1 of CEN-PK113-7D and Cy3-labelled cDNA from culture 2 of GG3096; and (iv) hybridized to Cy5-labelled cDNA from culture 1 of GG3096 and Cy3-labelled cDNA from culture 1 of CEN-PK113-7D. cDNA labelling, microarray hybridization, scanning and data extraction were performed by ServiceXS, Leiden, The Netherlands. The data are presented as the mean of the data obtained from microarrays 2, 3 and 4. The data are excluded if $\log(\text{Cy3/Cy5})$ obtained from microarray 1 is >0.2 or <-0.2 .

RESULTS AND DISCUSSION

Construction of a *bmh2* mutant

In order to study the role of 14-3-3 proteins in transcription regulation, we investigated the effect of mutation of the *BMH* genes on the genome-wide transcription profile. Such studies are complicated by the fact that deletion of both *BMH* genes is lethal in most laboratory strains [12,13]. In our previous study, we constructed a temperature-sensitive *bmh2* mutant by deleting both *BMH* genes and introduction of a plasmid containing a temperature-sensitive *bmh2* allele [24]. In this allele, a single point mutation resulted in the replacement of the serine residue at position 189 by a proline residue. This mutant allele partly complements the lethal *bmh1 bmh2* double disruption, allowing growth at 22°C and 30°C , but not at 37°C . To analyse the effect of this *bmh* mutation on the genome-wide transcription, we preferred to use a mutant lacking auxotrophic markers and plasmids. Therefore we constructed a new mutant (GG3096) in the CEN-PK background in which the *BMH1* gene has been deleted and the temperature-sensitive *bmh2* allele is integrated at the *BMH2* locus. GG3096 has been constructed in the CEN-PK113-7D background, a strain used by several laboratories for transcriptome analyses [35]. At 22°C and 30°C , GG3096 grows slower than the wild-type strain CEN-PK113-7D (growth rates at 30°C : GG3096, 0.13 h^{-1} ; CEN-PK113-7D, 0.24 h^{-1}). At 37°C , GG3096 grows very poorly, although slightly better than our original temperature-sensitive *bmh2* strain. Similar to our original mutant, GG3096 is sensitive to $0.02 \mu\text{g/ml}$ rapamycin, and forms chains of cells and cells with irregular buds at 22°C (results not shown).

Microarray experiments

To allow optimal comparison of the expression profile of the mutant GG3096 with that of the wild-type CEN-PK113-7D, both strains were grown in duplicate in glucose-limited chemostat cultures at 30°C , as it is known that important cultivation conditions, such as dissolved oxygen, metabolite concentrations and pH, change over time in shake-flask cultures. To exclude effects of

different growth rates [34], we grew both strains at the same dilution rate of 0.1 h^{-1} . RNA was extracted from each steady-state culture, labelled with Cy3 or Cy5 and hybridized to commercial oligonucleotide microarrays (Agilent Technologies) representing 6256 *S. cerevisiae* ORFs. The resulting data set is shown in Table S1 (available at <http://www.BiochemJ.org/bj/382/bj3820867add.htm>), and is deposited at the GEO-NCBI database under accession numbers GSM13009 to GSM13012. As a control, a hybridization was performed using Cy3- and Cy5-labelled cDNA from two independently grown cultures of CEN-PK113-7D. Only 2% of the ORFs, mainly with a low expression, had a Cy3/Cy5 ratio lower than 0.62 or higher than 1.6. These ORFs were excluded from further analysis. As expected, *BMH1* was not expressed in the mutant. The steady-state mRNA levels of 60 ORFs were increased at least 2.0-fold in the *bmh2*(Ts) mutant. The largest increase (6.8-fold) was found for *PCK1*, encoding phosphoenolpyruvate carboxykinase, involved in gluconeogenesis (Table 2). The steady-state mRNA levels of 78 ORFs were decreased at least 2.0-fold in the mutant. The largest decrease (8.9-fold) was found for *SPS100*, involved in spore wall assembly (see Table 4). Similar results could be obtained by other methods. For at least two genes, *PCK1* and *CIT1*, identical results were obtained by Northern blot analysis (Figure 1). The ratio of *PCK1* mRNA levels in the mutant relative to those of wild-type was 7.3 for the Northern blot compared with 6.8 for the microarrays (Table 2). For *CIT1*, these values were 2.1 compared with 1.7 (see Table S1 at <http://www.BiochemJ.org/bj/382/bj3820867add.htm>). Previously, using a β -galactosidase assay, we showed that the expression of *CIT2* was 3.3-fold higher in the *bmh2*(Ts) mutant (37 °C) than in the wild-type (referred to in [24]), while the microarrays gave a ratio of 3.8 (Table 2).

Classification of affected genes

The ORFs with a more than 2.0-fold increase in steady-state mRNA levels in the mutant were catalogued according to the functional category defined at the MIPS yeast genome database (<http://mips.gsf.de/genre/proj/yeast/index.jsp>) and are shown in Table 2. The largest groups of ORFs with increased mRNA levels belong to the 'metabolism' (20 ORFs), 'unclassified proteins' (15 ORFs), 'energy' (eight ORFs) and 'transcription' (eight ORFs) functional categories. On the other hand, many of the other functional categories did not contain ORFs with more than 2.0-fold increased mRNA levels (Table 3). After correction for the number of ORFs in each category, the most obvious effects were found for the 'energy' (3.1% of the ORFs in this category), 'metabolism' (1.9%) and 'cell rescue, defence and virulence' (1.8%) functional categories (Table 3).

The ORFs with a more than 2.0-fold decrease in mRNA level in the mutant were catalogued and are shown in Table 4. The largest groups of ORFs with a more than 2.0-fold reduced mRNA level in the *bmh2* mutant GG3096 belong to the 'unclassified proteins' (27 ORFs), the 'cell rescue, defence and virulence' (18 ORFs) and the 'metabolism' (16 ORFs) functional categories (Tables 3 and 4). After correction for the number of ORFs in each category, the most obvious effects were found for the 'cell rescue, defence and virulence' (6.4% of the ORFs in this category), 'energy' (2.7%) and 'transport facilitation' (2.2%) functional categories (Table 3). A number of other functional categories did not contain ORFs with more than 2.0-fold reduced mRNA levels.

Effect on Rtg3-regulated genes

Genetic evidence from our previous study suggested an inhibitory role of the 14-3-3 proteins on the Rtg3-dependent transcription [24]. Rtg3 is a basic helix–loop–helix transcription factor involved

in the expression of *CIT2* and other genes in yeast cells with mitochondrial dysfunction (retrograde signalling) [27]. We showed a physical interaction between the 14-3-3 proteins and the Rtg3 transcription factor. Recently, it was shown that the 14-3-3 proteins also bind to the hyperphosphorylated form of the Mks1 protein [23]. This protein is a negative regulator of retrograde signalling by inactivating Rtg2, a positive regulator of retrograde signalling. The inactive form of Rtg3 is sequestered in the cytoplasm [23]. The expression of six genes involved in glyoxylate, the first steps of gluconeogenesis and glutamate metabolism is known to be regulated by Rtg3 [37]. As shown in Table 5, the steady-state mRNA levels of these six genes are increased between 1.6- and 4.3-fold in GG3096. These data indicate that in agreement with our genetic observations and the observations by Liu et al. [23], the yeast 14-3-3 proteins have an inhibitory effect on the expression of Rtg3-regulated genes. Probably, many more genes are regulated by Rtg3, as the R box sequence is present in the upstream region of many ORFs. The expression of *DIP5*, encoding a glutamate–aspartate transporter, is also increased in the *bmh2*(Ts) mutant (4.9-fold), as well as in respiratory deficient cells [38]. As this gene is involved in glutamate metabolism and it contains two R boxes (in the reverse orientation) in its upstream region, *DIP5* is a possible candidate. An *RTG3* mutation can suppress the temperature-sensitive phenotype of the *bmh2*(Ts) mutant. Therefore the increased expression of the Rtg3-regulated genes is most likely, at least partly, to be responsible for the temperature-sensitivity of this mutant. On the other hand, the abnormal morphology of the *bmh2*(Ts) mutant is not influenced by the *RTG3* mutation [24].

Effect on genes involved in gluconeogenesis

The most prominent increase (6.8-fold) in mRNA levels in the *bmh2*(Ts) mutant was observed for *PCK1*, encoding phosphoenolpyruvate carboxykinase involved in gluconeogenesis. Two other genes involved in gluconeogenesis also showed an at least 2.0-fold increased mRNA level, i.e. *MDH2* (2.1-fold) and *TPI* (2.0-fold). These data suggest an inhibitory effect of the yeast 14-3-3 protein on gluconeogenesis.

Effect on genes involved in sterol metabolism

Steady-state mRNA levels of many other genes involved in metabolism are reduced after mutation of the *BMH* genes (Table 4). This is especially the case for a number of genes involved in ergosterol metabolism: *HES1* (4.8-fold), *ERG11* (2.7-fold), *ERG1* (2.3-fold) and *ERG28* (2.1-fold). In addition, mRNA levels of *ERG25* and *HMG1* were reduced 1.8-fold. These data suggest a stimulatory effect of the yeast 14-3-3 proteins on ergosterol synthesis. Recently, cluster analysis of many data sets of yeast genome-wide expression analyses revealed a set of overlapping transcriptional modules [39]. One of these modules with 27 ORFs (module 67 in [39]) contains many genes involved in ergosterol synthesis. As shown in Figure 2(B), almost all ORFs in this module had decreased mRNA levels. The mRNA levels of five ORFs in this module (out of 27 ORFs) were decreased more than 2.0-fold, including *YPL272c* (5.4-fold), *HES1* (4.8-fold), *ERG11* (2.7-fold), *ERG1* (2.3-fold) and *ERG28* (2.1-fold) (Figure 2B). In contrast, in the total data set, ORFs with decreased and increased mRNA levels were present in almost equal amounts (Figure 2A). These data support a stimulatory role of the 14-3-3 proteins in the transcription of the ORFs in this module.

Effect on genes encoding stress-related proteins

Mutation of the *BMH* genes has a very prominent effect on genes in the 'cell rescue, defence and virulence' category as the mRNA

Table 2 ORFs with a more than 2-fold increased mRNA level in GG3096 relative to CEN-PK113-7D

Some ORFs are catalogued in more than one category. The data are the means $\pm$ S.D. of the mutant/wild-type ratio obtained from microarrays 2, 3 and 4 (see the Materials and methods section). If an ORF is represented twice on each microarray, the data are calculated from six data points. If an ORF is represented once on each microarray, the data are calculated from three data points.

ORF	Gene	Biological process/molecular function	Fold induction (<i>n</i>)
Metabolism (1073 entries)			
YKR097W	<i>PCK1</i>	Gluconeogenesis/phosphoenolpyruvate carboxykinase (ATP)	6.8 ± 0.7 (3)
YPL265W	<i>DIP5</i>	Amino acid transport/amino acid transporter	4.9 ± 1.1 (6)
YOR303W	<i>CPA1</i>	Arginine biosynthesis/carbamoyl-phosphate synthase	4.0 ± 0.6 (6)
YCR005C	<i>CIT2</i>	Glutamate biosynthesis/citrate synthase	3.8 ± 0.4 (6)
YJL218W	<i>YJL218W</i>	Unknown/unknown	3.2 ± 1.3 (6)
YNL117W	<i>MLS1</i>	Glyoxylate cycle/malate synthase	3.0 ± 0.3 (6)
YPL135W	<i>ISU1</i>	Iron homeostasis/unknown	3.0 ± 0.4 (6)
YER065C	<i>ICL1</i>	Not yet annotated/isocitrate lyase	3.0 ± 0.2 (6)
YNR016C	<i>ACC1</i>	Nuclear membrane organization/acetyl-CoA carboxylase	3.0 ± 0.2 (3)
YBR069C	<i>TAT1</i>	Transport/amino acid permease	2.3 ± 0.6 (6)
YER062C	<i>HOR2</i>	Response to osmotic stress/glycerol-1-phosphatase	2.3 ± 0.3 (6)
YOL126C	<i>MDH2</i>	Gluconeogenesis/malic enzyme	2.1 ± 0.2 (6)
YIR019C	<i>MUC1</i>	Pseudohyphal growth/not yet annotated	2.1 ± 0.4 (3)
YJR109C	<i>CPA2</i>	Arginine biosynthesis/carbamoyl-phosphate synthase	2.0 ± 0.4 (6)
YDR050C	<i>TP11</i>	Gluconeogenesis/triosephosphate isomerase	2.0 ± 0.4 (3)
YOL007C	<i>YOL007C</i>	Not yet annotated/not yet annotated	2.0 ± 0.3 (6)
YPL075W	<i>GCR1</i>	Positive regulation of glycolysis/transcriptional activator	2.0 ± 0.3 (6)
YER024W	<i>YAT2</i>	Not yet annotated/carnitine O-acetyltransferase	2.0 ± 0.2 (6)
YOR317W	<i>FAA1</i>	Not yet annotated/long-chain-fatty-acid-CoA-ligase	2.0 ± 0.1 (6)
YIL053W	<i>RHR2</i>	Glycerol metabolism/not yet annotated	2.0 ± 0.2 (6)
Energy (255 entries)			
YKR097W	<i>PCK1</i>	Gluconeogenesis/phosphoenolpyruvate carboxykinase	6.8 ± 0.7 (3)
YCR005C	<i>CIT2</i>	Glutamate biosynthesis/citrate synthase	3.8 ± 0.4 (6)
YNL117W	<i>MLS1</i>	Glyoxylate cycle/malate synthase	3.0 ± 0.3 (6)
YER065C	<i>ICL1</i>	Not yet annotated/isocitrate lyase	3.0 ± 0.2 (6)
YEL039C	<i>CYC7</i>	Not yet annotated/not yet annotated	2.1 ± 0.4 (6)
YEL071W	<i>DLD3</i>	Lactate metabolism/b-lactate dehydrogenase (cytochrome)	2.1 ± 0.2 (3)
YOL126C	<i>MDH2</i>	Gluconeogenesis/malic enzyme	2.1 ± 0.2 (6)
YDR050C	<i>TP11</i>	Gluconeogenesis/triosephosphate isomerase	2.0 ± 0.4 (3)
Cell cycle and DNA processing (671 entries)			
YOR028C	<i>CIN5</i>	Regulation of transcription/transcription factor	5.6 ± 2.0 (3)
YNL289W	<i>PCL1</i>	Cell cycle/cyclin-dependent protein kinase, regulator	3.1 ± 0.5 (6)
YPL256C	<i>CLN2</i>	Cell cycle/cyclin-dependent protein kinase, regulator	2.2 ± 0.3 (6)
YDR055W	<i>PST1</i>	Unknown/unknown	2.1 ± 0.4 (6)
YER024W	<i>YAT2</i>	Not yet annotated/carnitine O-acetyltransferase	2.0 ± 0.2 (6)
Transcription (836 entries)			
YOR028C	<i>CIN5</i>	Regulation of transcription/transcription factor	5.6 ± 2.0 (3)
YDR259C	<i>YAP6</i>	Transcription/transcription factor	2.4 ± 0.2 (6)
YNL030W	<i>HHF2</i>	Chromatin assembly/disassembly/DNA binding	2.4 ± 0.5 (6)
YBR009C	<i>HHF1</i>	Chromatin assembly/disassembly/DNA binding	2.4 ± 0.2 (3)
YNL031C	<i>HHT2</i>	Chromatin assembly/disassembly/DNA binding	2.2 ± 0.4 (3)
YBR010W	<i>HHT1</i>	Chromatin assembly/disassembly/DNA binding	2.2 ± 0.4 (6)
YPL075W	<i>GCR1</i>	Positive regulation of glycolysis/transcriptional activator	2.0 ± 0.3 (6)
YJL089W	<i>SIP4</i>	Not yet annotated/transcription factor	2.0 ± 0.3 (6)
Protein fate (folding, modification, destination) (614 entries)			
YIL015W	<i>BAR1</i>	Pheromone catabolism/aspartic-type endopeptidase	2.0 ± 0.1 (6)
Cellular transport and transport mechanisms (522 entries)			
YKR093W	<i>PTR2</i>	Transport/not yet annotated	2.3 ± 0.3 (6)
YBR069C	<i>TAT1</i>	Transport/amino acid permease	2.3 ± 0.6 (6)
Cell rescue, defence and virulence (283 entries)			
YPL163C	<i>SVS1</i>	Not yet annotated/unknown	3.0 ± 0.4 (3)
YMR095C	<i>SN01</i>	Vitamin B ₆ metabolism/imidazoleglycerol-phosphate synthase	2.3 ± 0.2 (6)
YMR096W	<i>SNZ1</i>	Vitamin B ₆ metabolism/unknown	2.3 ± 0.3 (6)
YER062C	<i>HOR2</i>	Response to osmotic stress/glycerol-1-phosphatase	2.3 ± 0.3 (6)
YEL039C	<i>CYC7</i>	Not yet annotated/not yet annotated	2.1 ± 0.4 (6)
Cell fate (485 entries)			
YPL187W	<i>MF(ALPHA)1</i>	Pheromone response/pheromone	5.5 ± 1.4 (6)
YNL180C	<i>RHO5</i>	Rho protein signal transduction/Rho small GTPase	2.9 ± 0.3 (6)
YJL116C	<i>NCA3</i>	Mitochondrion organization and biogenesis/unknown	2.3 ± 0.4 (6)
YPL256C	<i>CLN2</i>	Cell cycle/cyclin-dependent protein kinase, regulator	2.2 ± 0.3 (6)
YDR055W	<i>PST1</i>	Unknown/unknown	2.1 ± 0.4 (6)
YIL140W	<i>AXL2</i>	Axial budding/unknown	2.1 ± 0.2 (6)
YIL015W	<i>BAR1</i>	Pheromone catabolism/aspartic-type endopeptidase	2.0 ± 0.1 (6)
Transposable elements, viral and plasmid proteins (118 entries)			
YIL082W	<i>YIL082W</i>	Unknown/unknown	2.4 ± 0.6 (6)
Control of cellular organization (426 entries)			
YPL256C	<i>CLN2</i>	Cell cycle/cyclin-dependent protein kinase, regulator	2.2 ± 0.3 (6)
YOL007C	<i>YOL007C</i>	Not yet annotated/not yet annotated	2.0 ± 0.3 (6)

Table 2 (contd.)

ORF	Gene	Biological process/molecular function	Fold induction (<i>n</i>)
Transport facilitation (318 entries)			
YPL265W	<i>DIP5</i>	Amino acid transport/amino acid transporter	4.9 ± 1.1 (6)
YKR093W	<i>PTR2</i>	Transport/not yet annotated	2.3 ± 0.3 (6)
YBR069C	<i>TAT1</i>	Transport/amino acid permease	2.3 ± 0.6 (6)
YPL058C	<i>PDR12</i>	Transport/xenobiotic-transporting ATPase	2.2 ± 0.1 (3)
YER024W	<i>YAT2</i>	Not yet annotated/carnitine O-acetyltransferase	2.0 ± 0.2 (6)
Classification not yet clear-cut (118 entries)			
YOL164W	<i>YOL164W</i>	Unknown/unknown	5.4 ± 0.9 (6)
Unclassified proteins (2456 entries)			
YNL300W	<i>YNL300W</i>	Unknown/unknown	3.2 ± 0.1 (3)
YJL108C	<i>PRM10</i>	Mating/unknown	2.8 ± 0.3 (6)
YGR066C	<i>YGR066C</i>	Unknown/unknown	2.4 ± 0.7 (3)
YLR053C	<i>YLR053C</i>	Unknown/unknown	2.4 ± 0.5 (6)
YMR122W-A	<i>YMR122W-A</i>	Unknown/unknown	2.2 ± 0.2 (3)
YOL084W	<i>PHM7</i>	Unknown/unknown	2.2 ± 0.5 (3)
YKL153W	<i>YKL153W</i>	Unknown/unknown	2.1 ± 0.7 (6)
YFL012W-A	<i>YFL012W-A</i>	Unknown/unknown	2.1 ± 1.7 (6)
YDR222W	<i>YDR222W</i>	Unknown/unknown	2.1 ± 0.1 (6)
YKR013W	<i>PRY2</i>	Unknown/unknown	2.0 ± 0.2 (6)
YBR071W	<i>YBR071W</i>	Unknown/unknown	2.0 ± 0.1 (3)
YDR034W-B	<i>YDR034W-B</i>	Unknown/unknown	2.0 ± 0.2 (3)
YLR194C	<i>YLR194C</i>	Unknown/unknown	2.0 ± 0.1 (6)
YNL058C	<i>YNL058C</i>	Unknown/unknown	2.0 ± 0.3 (3)
YLR414C	<i>YLR414C</i>	Unknown/unknown	2.0 ± 0.3 (6)
Not in a category			
YGR189C	<i>CRH1</i>	Unknown/unknown	2.4 ± 0.2 (6)

Figure 1 Northern blot analysis of the effect of the *bmh*(Ts) mutation on steady-state mRNA levels of *PCK1* and *CIT1*

Total RNA from GG3096 (*bmh2*) or CEN-PK113-7D (wt, wild-type) (6.5 µg) was used for Northern blot analysis with the *PCK1* or *CIT1* ORF as probes (upper panels). Ethidium bromide staining of the ribosomal RNAs is shown in the lower panels. RNA was quantified using the Quantity One software (Bio-Rad).

levels of 18 genes, which is 6.4 % of the genes in this category, are reduced more than 2.0-fold. Many of the genes encode proteins belonging to the Pau-protein family (*PAU4*, 4.0-fold; *PAU7*, 4.0-fold; *YOL161c*, 3.7-fold; *PAU6*, 3.6-fold; *PAU3*, 3.5-fold; *PAU1*, 3.5-fold; *PAU5*, 3.4-fold; *YHL046c*, 3.1-fold and *PAU2*, 2.4-fold). Also many 'unclassified proteins' with a more than 2.0-fold reduction in mRNA levels have similarity to Pau proteins (*YGL261c*, 4.7-fold; *DAN2*, 4.4-fold; *YGR294w*,

Table 3 Classification in functional categories of ORFs with a more than 2.0-fold increase or decrease in mRNA levels in GG3096 relative to CEN-PK113-7D

The number of ORFs in a category relative to the total number of ORFs in that category is given as a percentage in parentheses.

MIPS functional category	Number of ORFs with > 2.0-fold increased mRNA level in GG3096	Number of ORFs with > 2.0-fold decreased mRNA level in GG3096
Metabolism	20 (1.9)	16 (1.5)
Energy	8 (3.1)	8 (3.1)
Cell cycle and DNA processing	5 (0.7)	2 (0.3)
Transcription	8 (1.0)	0 (0.0)
Protein synthesis	0 (0.0)	0 (0.0)
Protein fate	1 (0.2)	3 (0.5)
Cellular transport and transport mechanisms	2 (0.4)	3 (0.6)
Cellular communication/signal transduction	0 (0.0)	0 (0.0)
Cell rescue, defence and virulence	5 (1.8)	18 (6.4)
Regulation of/interaction with cellular environment	0 (0.0)	6 (3.0)
Cell fate	7 (1.4)	1 (0.2)
Transposable elements/viral and plasmid proteins	1 (0.8)	0 (0.0)
Control of cellular organization	2 (0.5)	2 (0.5)
Subcellular localization	0 (0.0)	0 (0.0)
Protein activity regulation	0 (0.0)	0 (0.0)
Protein with binding function/cofactor requirement	0 (0.0)	0 (0.0)
Transport facilitation	5 (1.6)	10 (3.1)
Classification not yet clear-cut	1 (0.8)	0 (0.0)
Unclassified proteins	15 (0.6)	27 (1.1)
Not in a category	1	3

Table 4 ORFs with a more than 2.0-fold reduced mRNA level in GG3036 relative to CEN-PK113-7D

Some ORFs are catalogued in more than one category. The data are the means $\pm$ S.D. of the mutant/wild-type ratio obtained from microarrays 2, 3 and 4 (see the Materials and methods section). If an ORF is represented twice on each microarray, the data are calculated from six data points. If an ORF is represented once on each microarray, the data are calculated from three data points.

ORF	Gene	Biological process/molecular function	Fold repression
Metabolism (1073 entries)			
YOR237W	<i>HES1</i>	Sterol metabolism/unknown	4.8 $\pm$ 0.9 (6)
YGR289C	<i>MAL11</i>	α -Glucoside transport/ α -glucoside:hydrogen symporter	4.2 $\pm$ 0.2 (6)
YIL162W	<i>SUC2</i>	Sucrose catabolism/ β -fructofuranosidase	4.2 $\pm$ 0.9 (6)
YML123C	<i>PHO84</i>	Phosphate transport/inorganic phosphate transporter	3.5 $\pm$ 1.4 (3)
YJL216C	<i>YJL216C</i>	Not yet annotated/ α -glucosidase	3.2 $\pm$ 0.6 (6)
YGR292W	<i>MAL12</i>	Maltose catabolism/ α -glucosidase	3.0 $\pm$ 0.3 (6)
YBR299W	<i>MAL32</i>	Maltose catabolism/ α -glucosidase	2.8 $\pm$ 0.4 (6)
YHR007C	<i>ERG11</i>	Ergosterol biosynthesis/lanosterol 14 α -demethylase	2.7 $\pm$ 0.2 (6)
YDR453C	<i>TSA2</i>	Regulation of redox homeostasis/thioredoxin peroxidase	2.4 $\pm$ 0.1 (3)
YIR030C	<i>DCG1</i>	Unknown/not yet annotated	2.3 $\pm$ 0.3 (3)
YER054C	<i>GIP2</i>	Unknown/protein phosphatase regulator	2.3 $\pm$ 0.2 (6)
YGR175C	<i>ERG1</i>	Ergosterol biosynthesis/squalene mono-oxygenase	2.3 $\pm$ 0.1 (3)
YER044C	<i>ERG28</i>	Ergosterol biosynthesis/unknown	2.1 $\pm$ 0.3 (6)
YHL032C	<i>GUT1</i>	Not yet annotated/glycerol kinase	2.0 $\pm$ 0.5 (6)
YFR053C	<i>HXK1</i>	Fructose metabolism/hexokinase	2.0 $\pm$ 0.2 (3)
YMR081C	<i>ISF1</i>	Unknown/unknown	2.0 $\pm$ 0.4 (6)
Energy (255 entries)			
YPL171C	<i>OYE3</i>	Not yet annotated/NADPH dehydrogenase	3.9 $\pm$ 0.7 (6)
YJL216C	<i>YJL216C</i>	Not yet annotated/ α -glucosidase	3.2 $\pm$ 0.6 (6)
YMR244W	<i>YMR244W</i>	Unknown/unknown	3.0 $\pm$ 1.6 (6)
YBR299W	<i>MAL32</i>	Maltose catabolism/ α -glucosidase	2.8 $\pm$ 0.4 (6)
YHR179W	<i>OYE2</i>	Not yet annotated/NADPH dehydrogenase	2.5 $\pm$ 0.1 (3)
YER054C	<i>GIP2</i>	Unknown/protein phosphatase regulator	2.3 $\pm$ 0.2 (6)
YER073W	<i>ALD5</i>	Metabolism/aldehyde dehydrogenase	2.0 $\pm$ 0.1 (6)
YFR053C	<i>HXK1</i>	Fructose metabolism/hexokinase	2.0 $\pm$ 0.2 (3)
Cell cycle and DNA processing (671 entries)			
YGL229C	<i>SAP4</i>	Cell cycle/protein serine/threonine phosphatase	2.2 $\pm$ 0.2 (3)
YGR049W	<i>SCM4</i>	Cell cycle/not yet annotated	2.0 $\pm$ 0.2 (6)
Protein fate (folding, modification, destination) (614 entries)			
YNR069C	<i>YNR069C</i>	Unknown/unknown	2.6 $\pm$ 1.0 (3)
YBR072W	<i>HSP26</i>	Stress response/heat-shock protein	2.5 $\pm$ 0.4 (6)
YLR327C	<i>YLR327C</i>	Unknown/unknown	2.2 $\pm$ 0.1 (3)
Cellular transport and transport mechanisms (522 entries)			
YNL142W	<i>MEP2</i>	Pseudohyphal growth/ammonium transporter	2.9 $\pm$ 0.5 (6)
YPR124W	<i>CTR1</i>	Transport/not yet annotated	2.6 $\pm$ 0.3 (6)
YMR319C	<i>FET4</i>	Low-affinity iron transport/iron transporter	2.0 $\pm$ 0.6 (6)
Cell rescue, defence and virulence (283 entries)			
YBL075C	<i>SSA3</i>	Stress response/heat-shock protein	5.8 $\pm$ 1.3 (6)
YCR021C	<i>HSP30</i>	Stress response/heat-shock protein	4.1 $\pm$ 0.4 (6)
YAR020C	<i>PAU7</i>	Unknown/unknown	4.0 $\pm$ 0.3 (6)
YLR461W	<i>PAU4</i>	Unknown/unknown	4.0 $\pm$ 0.5 (6)
YOL161C	<i>YOL161C</i>	Unknown/unknown	3.7 $\pm$ 0.3 (3)
YNR076W	<i>PAU6</i>	Unknown/not yet annotated	3.6 $\pm$ 0.4 (6)
YCR104W	<i>PAU3</i>	Unknown/unknown	3.5 $\pm$ 0.5 (6)
YJL223C	<i>PAU1</i>	Unknown/unknown	3.5 $\pm$ 0.6 (3)
YOR009W	<i>TIR4</i>	Unknown/unknown	3.4 $\pm$ 0.5 (6)
YFL020C	<i>PAU5</i>	Unknown/unknown	3.4 $\pm$ 0.3 (3)
YGR234W	<i>YHB1</i>	Stress response/unknown	3.2 $\pm$ 0.6 (6)
YHL046C	<i>YHL046C</i>	Unknown/unknown	3.1 $\pm$ 0.3 (3)
YHR007C	<i>ERG11</i>	Ergosterol biosynthesis/lanosterol 14 α -demethylase	2.7 $\pm$ 0.2 (6)
YBR072W	<i>HSP26</i>	Stress response/heat-shock protein	2.5 $\pm$ 0.4 (6)
YEL049W	<i>PAU2</i>	Unknown/unknown	2.4 $\pm$ 1.3 (6)
YDR453C	<i>TSA2</i>	Regulation of redox homeostasis/thioredoxin peroxidase	2.4 $\pm$ 0.1 (3)
YNL065W	<i>AQR1</i>	Unknown/unknown	2.2 $\pm$ 0.6 (3)
YBR054W	<i>YRO2</i>	Unknown/not yet annotated	2.1 $\pm$ 0.3 (6)
Regulation of/interaction with cellular environment (201 entries)			
YCR021C	<i>HSP30</i>	Stress response/heat-shock protein	4.1 $\pm$ 0.4 (6)
YML123C	<i>PHO84</i>	Phosphate transport/inorganic phosphate transporter	3.5 $\pm$ 1.4 (3)
YPR124W	<i>CTR1</i>	Transport/not yet annotated	2.6 $\pm$ 0.3 (6)
YMR319C	<i>FET4</i>	Low-affinity iron transport/iron transporter	2.0 $\pm$ 0.6 (6)
YNL144C	<i>YNL144C</i>	Unknown/unknown	2.0 $\pm$ 0.1 (6)
YBR295W	<i>PCA1</i>	Not yet annotated/H ⁺ /K ⁺ -exchanging ATPase	2.0 $\pm$ 0.3 (3)
Cell fate (485 entries)			
YHR139C	<i>SPS100</i>	Spore wall assembly/unknown	8.9 $\pm$ 1.8 (3)
Control of cellular organization (426 entries)			
YIR030C	<i>DCG1</i>	Unknown/not yet annotated	2.3 $\pm$ 0.3 (3)
YER073W	<i>ALD5</i>	Metabolism/aldehyde dehydrogenase	2.0 $\pm$ 0.1 (6)

Table 4 (contd.)

ORF	Gene	Biological process/molecular function	Fold repression
Transport facilitation (318 entries)			
YPR192W	<i>AQY1</i>	Water transport/water channel	4.8 ± 0.7 (6)
YGR289C	<i>MAL11</i>	Transport/general α -glucoside:hydrogen symporter	4.2 ± 0.2 (6)
YML123C	<i>PHO84</i>	Phosphate transport/inorganic phosphate transporter	3.5 ± 1.4 (3)
YNL142W	<i>MEP2</i>	Pseudohyphal growth/ammonium transporter	2.9 ± 0.5 (6)
YPR124W	<i>CTR1</i>	Transport/not yet annotated	2.6 ± 0.3 (6)
YNL065W	<i>AQR1</i>	Unknown/unknown	2.2 ± 0.6 (3)
YMR319C	<i>FET4</i>	Low-affinity iron transport/iron transporter	2.0 ± 0.6 (6)
YLL053C	<i>YLL053C</i>	Unknown/unknown	2.0 ± 0.3 (6)
YNL144C	<i>YNL144C</i>	Unknown/unknown	2.0 ± 0.1 (6)
YBR295W	<i>PCA1</i>	Not yet annotated/H ⁺ /K ⁺ -exchanging ATPase	2.0 ± 0.3 (3)
Unclassified proteins (2456 entries)			
YDL218W	<i>YDL218W</i>	Unknown/unknown	6.4 ± 1.1 (3)
YPL272C	<i>YPL272C</i>	Unknown/unknown	5.4 ± 0.8 (6)
YAL068C	<i>YAL068C</i>	Unknown/unknown	5.0 ± 1.0 (6)
YGL261C	<i>YGL261C</i>	Unknown/unknown	4.7 ± 0.3 (3)
YPL282C	<i>YPL282C</i>	Unknown/unknown	4.3 ± 0.5 (96)
YGR294W	<i>YGR294W</i>	Unknown/unknown	4.2 ± 0.4 (6)
YMR325W	<i>YMR325W</i>	Unknown/unknown	4.1 ± 0.3 (6)
YOR394W	<i>YOR394W</i>	Unknown/unknown	3.8 ± 0.2 (3)
YIR041W	<i>YIR041W</i>	Unknown/unknown	3.6 ± 0.2 (6)
YJL105W	<i>SET4</i>	Unknown/unknown	3.6 ± 0.3 (6)
YKL224C	<i>YKL224C</i>	Unknown/unknown	3.6 ± 0.4 (6)
YIL176C	<i>YIL176C</i>	Unknown/unknown	3.5 ± 0.5 (6)
YLL064C	<i>YLL064C</i>	Unknown/unknown	3.5 ± 0.6 (3)
YDR542W	<i>YDR542W</i>	Unknown/unknown	3.3 ± 0.6 (3)
YIL057C	<i>YIL057C</i>	Unknown/unknown	3.3 ± 0.9 (6)
YBL108C-A	<i>YBL108C-A</i>	Unknown/unknown	3.2 ± 0.6 (6)
YGR236C	<i>SPG1</i>	Unknown/unknown	3.0 ± 0.3 (6)
YNR068C	<i>YNR068C</i>	Unknown/unknown	3.0 ± 0.5 (3)
YHR087W	<i>YHR087W</i>	Unknown/unknown	2.7 ± 0.5 (6)
YDR521W	<i>YDR521W</i>	Unknown/unknown	2.7 ± 1.1 (3)
YIL037C	<i>PRM2</i>	Mating/unknown	2.4 ± 0.6 (3)
YPL201C	<i>YPL201C</i>	Unknown/unknown	2.4 ± 0.2 (3)
YGR146C	<i>YGR146C</i>	Unknown/unknown	2.2 ± 0.6 (6)
YNR034W-A	<i>YNR034W-A</i>	Unknown/unknown	2.1 ± 0.5 (3)
YJL144W	<i>YJL144W</i>	Unknown/unknown	2.1 ± 0.1 (6)
YHR126C	<i>YHR126C</i>	Unknown/unknown	2.1 ± 0.3 (3)
YOL131W	<i>YOL131W</i>	Unknown/unknown	2.0 ± 0.3 (3)
Not in a category (number of entries unknown)			
YLR037C	<i>DAN2</i>	Unknown/unknown	4.4 ± 0.6 (3)
YBR301W	<i>DAN3</i>	Unknown/unknown	3.8 ± 0.5 (6)
YNL134C	<i>YNL134C</i>	Unknown/unknown	3.5 ± 0.3 (6)

Table 5 The effects of *bmh2*(Ts) mutation on the transcription of *Rtg3*-regulated genes

The data are the means ± S.D. of the mutant/wild-type ratio obtained from microarrays 2, 3 and 4 (see Table 2).

Gene	Fold increase in steady-state mRNA levels in GG3096 (<i>n</i>)
<i>ACO1</i>	1.7 ± 0.3 (6)
<i>CIT1</i>	1.7 ± 0.3 (6)
<i>CIT2</i>	3.8 ± 0.2 (6)
<i>DLG3</i>	2.1 ± 0.2 (3)
<i>IDH1</i>	1.9 ± 0.3 (3)
<i>IDH2</i>	1.7 ± 0.3 (6)

4.2-fold; *YMR325w*, 4.1-fold; *YOR394w*, 3.8-fold; *YIR041w*, 3.7-fold; *YKL224c*, 3.6-fold; *YIL176c*, 3.5-fold; *YLL064c*, 3.5-fold and *YDR542w*, 3.3-fold). These observations suggest a stimulatory role of the 14-3-3 proteins on the expression of these genes. In addition to genes encoding Pau proteins, many other genes

encoding stress-related proteins are affected, both positively and negatively, by mutation of the *BMH* genes. Reduced expression of stress-related genes may contribute to the temperature-sensitive phenotype of the *bmh2*(Ts) mutant.

Effect on Msn2- and Msn4-regulated genes

14-3-3 proteins are known to regulate the Msn2 and Msn4 transcription factors by sequestering the phosphorylated forms of these proteins to the cytoplasm [25]. Phosphorylation of the Msn transcription factors is regulated by the RAS-protein kinase A as well as the TOR signalling pathways [25,40–43]. The Msn2 and Msn4 transcription factors bind to a stress-response element in the promoters of many stress-related genes. Thus it is conceivable that in the *bmh2*(Ts) mutant, the expression of genes having a stress-response element in their promoters is altered. A computer search revealed 81 genes having a promoter containing at least two stress-response elements [44]. Out of these 81 genes, only three showed a more than 2.0-fold increase in mRNA levels, i.e. *YNR014w* (2.7-fold), *CYC7* (2.1-fold) and *MDH2* (2.1-fold) and

Figure 2 Effect of the *bmh2*(Ts) mutation on steady-state mRNA levels

(A) Log ratio of mRNA levels in GG3096 relative to CEN-PK113-7D detected by the 10807 oligonucleotide probes on the microarrays. (B) Log ratio of mRNA levels in GG3096 relative to CEN-PK113-7D of ORFs classified in module 67 by Ihmels et al. [39]. (C) Log ratio of mRNA levels in GG3096 relative to CEN-PK113-7D of ORFs with at least two stress-response elements in their promoter regions [44]. The ORFs are arranged according to ascending mutant/wild-type ratios.

three showed a more than 2.0-fold reduction in expression, i.e. *SPS100* (8.9-fold), *PAU6* (3.6-fold) and *HXK1* (2.0-fold) (Figure 2C). These results indicate that decreased 14-3-3 protein activity did not have a clear effect on the expression of Msn2- and Msn4-regulated genes. On the other hand, it is certainly possible that under the growth conditions used for our experiments, the stress-response signal transduction pathway is not activated, and that the activity of the Msn2 and Msn4 transcription factors is independent from the 14-3-3 proteins.

Conclusions

Our data are consistent with a role of the yeast 14-3-3 proteins in the regulation of the transcription of genes involved in different processes. First, 14-3-3 proteins have an inhibitory effect on the transcription of genes involved in the retrograde response by inhibition of the Rtg3 transcription factor. The *bmh2*(Ts) mutation has a major positive effect (6.8-fold increase) on the expression of *PCK1*, involved in gluconeogenesis, and a major negative effect on the expression of genes involved in ergosterol synthesis (up to 4.8-fold decreased expression of *HES1*). In addition, the expression of many stress-related genes is affected, both positively and negatively. Despite the 14-3-3 proteins binding to the Msn2 and Msn4 transcription factors, the *bmh2*(Ts) mutation does not have a clear effect on the steady-state mRNA levels of genes regulated by these transcription factors. The mRNA level of a number of genes encoding transporter proteins is decreased. These observations indicate that 14-3-3 proteins regulate transcription at multiple levels. However, the molecular mechanisms of the 14-3-3-protein-dependent regulation of transcription, other than that of the Rtg3-dependent transcription, remain to be established.

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