In fission yeast, nutrient starvation induces physiological, biochemical, and morphological changes that enable survival. Collectively these changes are referred to as stationary phase. We have used a green fluorescent protein random insertional mutagenesis system to isolate two novel stress-response proteins required in stationary phase. Ish1 is a nuclear envelope protein that is present throughout the cell cycle and whose expression is increased in response to stresses such as glucose and nitrogen starvation, as well as osmotic stress. Expression of Ish1 is regulated by the Spc1 MAPK pathway through the Atf1 transcription factor. Although overexpression of Ish1 is lethal, cells lacking ish1 exhibit reduced viability in stationary phase. Bis1 is a novel interacting partner of Ish1. Bis1 is the Schizosaccharomyces pombe member of the ES2 nuclear protein family found in Mus musculus, Drosophila melanogaster, Homo sapiens, and Arabidopsis thaliana. Overexpression of Bis1 results in a cell elongation phenotype, whereas bis1 cells exhibit a reduced viability in stationary phase similar to that seen in ish1 cells.

The regulation of cellular growth and proliferation in response to environmental stress is important for development as well as for maintenance of cell viability. Nutritional limitation causes cells to arrest cell growth and enter stationary phase (1–3). This is a metabolically quiescent state where expression of genes required for survival is induced, whereas expression of cell cycle genes is repressed (4, 5). Fission yeast cells enter stationary phase from G2 upon glucose starvation and from G1 upon nitrogen starvation (6).

In eukaryotic organisms the mitogen-activated protein kinase (MAPK)1 pathways are ubiquitous for sensing and responding to environmental stresses (7). In fission yeast the stress-activated MAPK, Spc1, like the mammalian p38 kinase is activated by the Wis1 MAPK kinase, which is in turn activated by two MAPK kinase kinases, Wak1 (also known as Wik1 and Wis4) or Win1 (7–12). Attenuation of Spc1 activity is accomplished by the actions of two tyrosine phosphatases, Pyp1 and Pyp2 (11, 12). Pyp2 is regulated at the transcriptional level by the Atf1 transcription factor and participates in a down-regulation of the Spc1 MAPK via a negative feedback loop (8). Inactivation of Pyp1 activates the Spc1 pathway.

One Spc1-regulated transcription factor, Atf1 (12, 13), is essential for the response of cells to nitrogen starvation, osmotic stress, conjugation, meiosis, entry into stationary phase, and even DNA damage (13–16). Atf1 is highly homologous to mammalian ATF-2, which is itself involved in stress response (8).

The completion of the fission yeast genome sequencing project has revealed the existence of a large number of open reading frames (ORFs) of no known function or apparent homologues. To provide information regarding the localization of some of these gene products in the cell, we have developed a GFP random insertional mutagenesis system (17). This system utilizes GFP-ura4 PCR-generated cassettes randomly integrated into the genome. GFP in-frame fusion integrants are expressed under the control of native promoters allowing us to examine expression levels and intracellular localization of their protein products under a variety of growth conditions. The affected genes from cells displaying specific intracellular GFP localizations are isolated by inverse PCR and are sequenced (17, 18).

To search for novel genes expressed in stationary phase, we screened for GFP in-frame fusions that had increased expression under glucose limiting conditions. We report the isolation of a novel stress protein named Ish1+ (induced in stationary phase) localized to the nuclear envelope and the plasma membrane. Ish1 expression is elevated in response to a number of environmental stimuli including glucose starvation and osmotic stress and is regulated by the Spc1 MAPK pathway through Atf1. We also report the isolation of a novel nuclear protein, Bis1 (binds to ish1), isolated by two-hybrid screening, and we show that it specifically interacts with Ish1 in vivo. Bis1 is the fission yeast homologue of the strongly conserved ES2 family of proteins known from Homo sapiens, Drosophila melanogaster, Arabidopsis thaliana, and Caenorhabditis elegans. They are of unknown function although we show that both ish1 and bis1 contribute to viability in stationary phase.

**EXPERIMENTAL PROCEDURES**

**Strains and Media**—Strains (Table I) were grown in yeast extract medium (YE) or Edinburgh minimal medium (EMM) supplemented as required (19, 20). Double mutants were identified in nonparental diploid tetrads, and genotypes were confirmed by out-crossing as well as by PCR.
Cloning of the ish1 Gene—Standard genetic methods and molecular biology techniques were used (1, 37). The ish1 gene was isolated using our GFP insertional mutagenesis screen (17). ish1 was previously reported as an ORF upstream of the kba1 gene (21) and by the fission yeast genome project (Sanger data base).

Overexpression of Ish1 and Ish1-GFP Proteins Using the nmt1 Promoter—The ish1 gene was PCR-amplified using High Fidelity Taq polymerase (Roche Molecular Biochemicals) with genomic DNA as template and primers sporf9 and sporf12 (Table II). The 2052-bp fragment was cloned into pREP1 under the control of the thiamine-repressible nmt1 promoter (22, 23), resulting in pLT1-1. pLT1-1 was transformed into Schizosaccharomyces pombe strains, and transformants were selected under repressing conditions (15 μg/ml thiamine).

pREP1-GFP was constructed by removing the NdeI restriction site residing in the GFP(S65T) open reading frame using a silent mutation created by overlap extension PCR (24). The resulting fragment with BamHI and XmaI sites added by PCR was subcloned into pREP1.

The ish1 ORF was PCR-amplified using primers sporf9 and sporf10 (Table II) that incorporated AscI and SalI restriction sites, and the product was cloned into pREP1-GFP, resulting in pREP-lish1-GFP and is referred to as pLT1-2.

pLT1-2 was digested with BgII and BamHI to excise a fragment encoding Ish1 amino acids 581–684. The remaining vector backbone was gel-purified and then self-ligated to create pREP1-lish1AC104-GFP and is referred to as pLT1-3.

An N-terminal deletion of Ish1 comprising amino acids 270–684 was made using primers sporf11 and sporf10 (Table II) that incorporate NdeI and SalI restriction sites. The product AN278ish1 was then cloned into pREP1-GFP to create pREP1-AN278ish1-GFP and is referred to as pLT1-4.

Construction of the ish1 and ish1-GFP Expression Vectors Using the ish1 Native Promoter—The entire ish1 gene from –351 to +2052 bp was PCR-amplified using primers sporf5 with sporf12 or sporf10 (Table II) incorporating PstI and SalI restriction sites. pREP1 was digested with PstI and SalI to excise the 1200-bp fragment encoding the nmt1 promoter sequence. The ish1 PCR products were cloned into the gel-purified vector backbones pREP1 or pREP1-GFP to create pLT2-1 or pLT2-2, respectively.

Construction of ish1 Disruption—PCR-based gene targeting (25–27) was used to replace a 1934-bp fragment of the ish1 coding region with the ura4+ gene leaving 115 bp of the C-terminal ORF of ish1 (Fig. 1A). The PCR primers used, sporf1 and sporf2 (Table II), included 80 bp of flanking sequence homologous to the ish1 sequence in the genome. Gene replacement was confirmed by PCR using primers sporf3 and sporf4 (Table II) and Southern blot analysis. Sporulation of the diploid and extensive out-crossing (6 times) was performed to ensure that no background mutations were present.

Construction of bis1 Disruption—Bis1 was identified as a two-hybrid target interacting with Ish1. The bis1 gene was amplified from S. pombe cDNA using primers lankol1 and lankol2 (Table II) with High Fidelity Taq polymerase (Roche Molecular Biochemicals). The PCR product was subcloned into pGEM-T (Promega) to create pGEM-T-bis1. pGEM-T-bis1 was digested with XbaI, and the ends were filled in using the Klenow fragment of DNA polymerase, and then the plasmid was digested with CiaI. The ura4+ gene was excised from pZA25 using Smal and CiaI restriction enzymes and subcloned into the XbaI (blunt-ended)/CiaI site of pGEM-T-bis1 to generate pGEM-T-bis1ura4+. The bis1::ura4+ cassette in this recombinant vector was then PCR-amplified using High Fidelity Taq polymerase (Roche Molecular Biochemicals) and used to replace bis1 in a haploid strain (ura4-D18 h+) by one-step gene disruption (20) (Fig. 1B). Stable ura4+ haploids were selected, and exact gene replacement was confirmed by PCR. The strain was out-crossed extensively to ensure that no background mutations were present.

Overexpression of Bis1 and Bis1-YFP Proteins Using the nmt1 Promoter—The bis1 ORF was PCR-amplified from genomic DNA using primers lan1 and lan2 or lan1 and lan3 (Table II), incorporating NdeI and SalI restriction sites, with High Fidelity Taq polymerase (Roche Molecular Biochemicals). The PCR products were cloned into pREP2 or pREP2-YFP containing the ura4-selectable marker (23), resulting in pREP2-bis1 and pREP-bis1-YFP referred to as pLT3-1 and pLT3-2, respectively. pLT3-1 and pLT3-2 were transformed into cells, and positive transformants were selected in the presence of 15 μg/ml thiamine. The YFP gene from pEYFP (Invitrogen) was excised from the vector by digesting with EcoRI and blunt-ended using the Klenow fragment of DNA polymerase. The pREP2 vector was digested with SalI, and the

### Table I

| Strains | Genotype | Source |
|---------|----------|--------|
| Q250    | h+ wild type | Lab collection |
| Q688    | h+ leu1-32 | Lab collection |
| Q411    | h+ ura4-D18 | Lab collection |
| Q1668   | h+ ura4-D18 leu1-32 | Lab collection |
| Q1667   | h+ ura4-D18 leu1-32 ade1-D25 ade6-216 his31+ | This study |
| Q1814   | h+ his1-26 | This study |
| Q1951   | h+ ish1-GFP ura4-D18 | This study |
| Q1894   | h+ bis1 ura4-D18 | This study |
| Q1935   | h+ ish1-GFP bis1 ura4-D18 | This study |
| Q1751   | h+ ish1-GFP spc1 ura4-D18 leu1-32 | This study |
| Q1622   | h+ af1 ura4-D18 leu1-32 his3-D1 | This study |
| Q1610   | h+ spc1 ura4-D18 leu1-32 | This study |
| Q1699   | h+ spc1 ura4-D18 | This study |
| Q910    | h+ pyr1 ura4-D18 | S. Ottile |
| Q1757   | h+ ish1-GFP pyr1 ura4-D18 leu1-32 | This study |
| Q1785   | h+ ish1-GFP af1 ura4-D18 leu1-32 | This study |
| Q1752   | h+ ish1-GFP/S65T ura4 spc1 ura4-D18 leu1-32 | This study |
| Q1756   | h+ ish1-GFP/S65T ura4 pyr1 ura4-D18 leu1-32 | This study |
| Q1757   | h+ ish1-GFP/S65T ura4 af1 ura4-D18 leu1-32 | This study |
| Q1777   | h+ ish1-GFP/S65T ura4 af1 ura4-D18 leu1-32 | This study |
| Q1799   | h+ leu1-32 plT1-2 (nmt1ish1-GFP leu2) | This study |
| Q1799   | h+ leu1-32 plT1-3 (nmt1ish1C104-GFP leu2) | This study |
| Q1795   | h+ leu1-32 plT1-4 (nmt1ish1C104-GFP leu2) | This study |
| Q1798   | h+ leu1-32 plT1-5 (ish1-GFP leu2) | This study |
| Q1807   | h+ leu1-32 plT1-1 (nmt1ish1 leu2) | This study |
| Q1808   | h+ ish1-GFP leu1-32 ura4-D18 plT1-1 (nmt1ish1 leu2) | This study |
| Q1809   | h+ spc1 ura4 leu1-32 plT1-1 (nmt1ish1 leu2) | This study |
| Q1836   | h+ ura4-D18 plT3-1 (nmt2bisia1 ura4) | This study |
| Q1837   | h+ ura4-D18 plT3-2 (nmt2bisia1-YFP ura4) | This study |
| Q1841   | h+ ura4-D18 leu1-32 plT1-2 (nmt1ish1-GFP leu2) | This study |
| Q1847   | h+ ura4-D18 leu1-32 plT1-3 (nmt1ish1C104-GFP leu2) | This study |
| Q1926   | h+ bis1 ura4-D18 leu1-32 plT1-2 (nmt1ish1-GFP leu2) | This study |
| Q1852   | h+ ish1-GFP leu1-32 ura4-D18 plT3-2 (nmt1bisia1-YFP leu2) | This study |
| Q1852   | h+ ish1-GFP leu1-32 ura4-D18 plT3-2 (nmt1bisia1-YFP leu2) | This study |

Ish1 and Bis1 Proteins of S. pombe

10563
Adjuvant (Cedarlane) as described in the manufacturer against Bis1, the GST-Bis1 fragment was separated on a 12% SDS-polycrylamide gel, excised, eluted, and mixed with Titer Max Gold Adjuvant (Cedarlane) as described in the manufacturer’s instructions. The rabbit was boost at day 28 and day 40. Serum was collected on day 50 and used as a source of antibody for Western blot analysis and immunofluorescence experiments.

Fluorescence Microscopy—A Leica fluorescence microscope equipped with a high performance CCD camera (Senicam) and Slidebook software (Intelligent Imaging System) was used for all imaging. Cells were collected onto Whatman 934-AH glass microscope filters (Fisher) and fixed with 100% ice-cold methanol at −20 °C for 20 min. The immunofluorescence protocol used is described in Savin and Nurse (28). Rabbit GST-Bis1 polyclonal antiserum generated in the lab (1:5000) was used with Alexa™ 488 goat anti-rabbit IgG (H + L) conjugate (1:250) (Molecular Probes). Stained cells were counterstained with 1 μM/ml DAPI.

Protein Lysates—Protein extracts (29) (20 μg or 50 μg, Bio-Rad protein assay) were separated by 7.5% or 10% SDS-PAGE, electrophoeted to a polyvinylidene difluoride membrane (Santa Cruz Biotechnology), and detected by polyclonal anti-GST-Bis1 antibody or monoclonal anti-GFP antibody (1:1000) (Roche Molecular Biochemicals). Immunoreactive bands were detected with a horseradish peroxidase-conjugated secondary goat anti-rabbit IgG antibody (Santa Cruz Biotechnology) or goat anti-mouse IgG antibody (1:2000) and the luminol-based ECL detection kit (Santa Cruz Biotechnology). Protein loading was monitored by Coomassie Blue staining of gels.

Immunoprecipitation—Harvested cells in HB buffer (25 mM MOPS, pH 7.2, 60 mM β-glycerophosphate, 15 mM p-nitrophenyl phosphate, 15 mM MgCl₂, 15 mM EGTA, 1 mM dithiothreitol, 0.1 mM sodium vanadate, 1% Triton X-100) (20), supplemented with complete protease inhibitor mixture (Roche Molecular Biochemicals), were broken by vortexing with glass beads and centrifuged to prepare a cleared whole-cell extract. Cell extracts (300 μg, Bio-Rad protein assay) were incubated with 10 μl of polyclonal anti-GST-Bis1 antibody in a 500-μl volume in HB buffer at 4 °C for 16 h, and 60 μl of protein G-Sepharose beads (Amersham Bio-Sciences) were added for 1 h at 4 °C. Beads were washed extensively with HB buffer, resuspended in 2× SDS loading buffer, and analyzed by SDS-PAGE. For immunoblot analysis, 25 μg of total protein was loaded for detection of proteins in the total yeast lysate; 10% of total immunoprecipitated material was loaded for detection of the immunoprecipitated protein, and 90% was loaded for detection of the communoprecipitated proteins. The proteins were subjected to immunoblot analysis as described above.

Viability in Stationary Phase—Cells were grown to stationary phase in YEA or EMM medium, and incubation was continued for 6 days. A portion of each culture was removed at day 0, 1, 2, 4, and 6 and plated on YEA or EMM after appropriate dilution to determine cell viability. Samples were taken in duplicate over a 6-day period, and the experiment was independently repeated twice.

Yeast Two-hybrid Screen—DNA encoding amino acids 34–684 of ish1 (ΔN33ish1) was PCR-amplified with primers ish1lexA1 and ish1lexA2 (Table II) incorporating BamHI and NotI restriction sites using High Fidelity Taq polymerase (Roche Molecular Biochemicals). The ΔN33ish1 ORF was fused to the 3’ end of the LexA DNA-binding domain in pEG202 (30) by cloning into the BamHI and NotI sites of pEG202 to construct pEG202-Nish1lexA. An S. pombe cDNA library (obtained from ATCC 87289) was screened (31). The two-hybrid experiments were performed with Saccharomyces cerevisiae strain Y1003 (MATa URA3::lexAop-lacZ/SlexA::ADE2::URA3 ura3-I3-1 ade2-1/ade2-1 can1-100/100-100) (32). Approximately, 3,000,000 cDNA clones were screened against the Ish1 bait.

DNA encoding amino acids 91–384 of bis1 was PCR-amplified with primers bis1lexA1(DN90) and bis1lexA3 (Table II) with High Fidelity
Taq polymerase incorporating BamHI and NotI restriction sites. pEG202-ΔN90bis1lexA was constructed as outlined above. Approximately 2,000,000 cDNA clones were screened using this bait.

RESULTS

Isolation of ish1 Using the GFP Insertional Mutagenesis System—An insertional mutagenesis cassette containing GFP on the 5‘ terminus was used to isolate in-frame insertional mutants that expressed GFP (17). A GFP fusion, lt13-26, was isolated based on the localization of the GFP to the nuclear envelope and the plasma membrane under glucose starvation conditions. In zygotic asci the GFP localized only to the nuclear envelope (17). GFP expression increased by 5-fold following glucose and nitrogen starvation as well as during hyper- and hypo-osmotic stress (data not shown).

lt13-26 is expressed throughout the cell cycle in growing cultures (Fig. 2A). Sequencing revealed that lt13-26 was a single copy chromosomal fusion with an 873-bp 3‘-truncation of the ish1 gene and expression remaining under the control of the ish1 native promoter. Cells expressing lt13-26 were indistinguishable from wild type cells with respect to growth and morphology. We will refer to the original integrant, lt13-26, as ish1ΔC291-GFP from this point.

The predicted full-length ish1 gene product was a 684-amino acid protein of unknown function and a calculated molecular mass of 76 kDa (GenBank™ accession number AL07867.1, locus SPBC365.12c). Computer searches revealed a late embryogenesis abundant (LEA) motif from amino acids 227–285 and 366–452 (ProDom data base). LEA motif proteins are induced in plants by a variety of stresses including dessication, heat stress, and osmotic stress (33, 34). The LEA motif has also been identified in LEA-1, F25H58.5, and K08H10.2, proteins of unknown function in C. elegans. Ish1 exhibits 21–23% overall identity to these proteins (Proteome data base). Additionally, searches revealed a similarity to the C terminus of S. pombe SPAC23C4.05c (identity = 44:159 (27%), positives = 74:159 (45%)) as well as the N terminus of S. cerevisiae probable membrane protein YML128c (also known as Gin3p or Msc1p) (35) (identity = 46:362 (19%), positives = 87:362 (36%)). Ish1, SPAC23C4.05c, and YML128c have one putative transmembrane domain near their N termini.

Overexpression of Ish1 Is Lethal—In the presence of thiamine to repress the nmt1 promoter, cells containing ish1-GFP, ΔN278ish1-GFP, or ish1ΔC104-GFP expression constructs displayed normal growth and morphology (Fig. 2B). In
Ish1 and Bis1 Proteins of S. pombe

**FIG. 3. Ish1 expression is regulated by the Spc1 MAPK pathway.** A, expression of *ish1ΔC291-GFP* integrant in wild type or various mutant strains grown in YEA to mid-log at 30 °C was analyzed by Western blotting for protein expression using a monoclonal anti-GFP antibody (20 μg protein per lane). B, analysis of full-length *ish1-GFP* integrant. Wild type cells containing *ish1-GFP* (*Q1798*) integrated into the genome were grown in EMM to mid-log at 30 °C and analyzed by Western blotting as above (50 μg of protein per lane). C, expression of *ish1ΔC291-GFP* integrant in wild type or various mutant strains grown in YEA to mid-log at 30 °C and then treated with 1.2 M KCl for 4 h. D and E, Ish1 is responsive to 1.2 M KCl treatment and heat shock. Ish1 expression is shown in response to 1.2 M KCl treatment for times indicated (D) or shifted to 36 °C for the times indicated (E).

In the absence of thiamine, overexpression of *ish1*, *ish1-GFP*, and *ish1ΔC104-GFP* was toxic, arresting cell proliferation (Fig. 2B). Microscopic examination showed no obvious morphological changes, and arrest appears to be in G2 based on morphological criteria. Examination of the cells 15 h after induction showed similar localization of Ish1-GFP to that seen in the original integrant, *ish1ΔC291-GFP* (Fig. 2A).

Overexpression of ΔN278ish1-GFP was not toxic. ΔN278ish1-GFP appears to be retained in the endoplasmic reticulum (Fig. 2C). This suggests that the N-terminal region is necessary for nuclear envelope and plasma membrane targeting and/or localization of Ish1. Deletion of the N-terminal sequence renders the protein incapable of normal localization thus sparing the toxic effects observed with the full-length protein. We speculate that Ish1 at high concentration somehow interferes with membrane function or the function of some protein in the membrane and that the observed toxicity is the result. Deletion of the N terminus of Ish1 renders the protein incapable of being correctly targeted, and the toxicity is thus not observed.

**Ish1 Expression Is Regulated by the Spc1 MAPK Pathway via Atf1**—Expression of *ish1ΔC291-GFP* is under the control of its native promoter; therefore, it was used to examine the expression of the Ish1 protein in various mutant backgrounds. Western blotting performed using a monoclonal anti-GFP antibody shows that Ish1ΔC291-GFP has a molecular mass of ~90 kDa, whereas Ish1-GFP migrates at ~120 kDa (Fig. 3, A and B). The other bands in the Western blot are the result of nonspecific binding of the GFP antibody, which are detected in wild type cells not expressing GFP.

Ish1ΔC291-GFP expression was examined in a Δspc1 mutant strain to inactivate the MAPK pathway (14) and in a Δpyp1 mutant strain, an inhibitor of Spc1, to activate it (12). Ish1ΔC291-GFP protein expression is almost undetectable in a Δspc1 mutant strain (Fig. 3A). However, increased activity of Spc1 as occurs in a Δpyp1 mutant strain resulted in an ~10-fold increase in Ish1 expression (Fig. 3A).

Because expression and activity of Atf1 are regulated by Spc1 MAPK (13), we examined Ish1 expression in a Δatf1 mutant background. There is no detectable level of Ish1 protein in the Δatf1 mutant background (Fig. 3A) showing that Ish1 is regulated by the Spc1 MAPK pathway via Atf1. In a Δatf1 Δpyp1 mutant background, Ish1 is detectable but very low, suggesting that when Spc1 is hyperactivated a second transcription factor might make a small contribution.

The response to osmotic stress occurs at least in part through *atf1* (13). Under 1.2 M KCl stress, Ish1 expression in a Δpyp1 mutant background is similar to that in wild type cells (Fig. 3C). However, in a Δatf1 and Δpyp1 mutant background Ish1 protein expression levels are very low but still present compared with wild type. In response to osmotic stress, expression is primarily through Atf1; however, low level Atf1-independent expression is also present.

Ish1 was isolated on the basis of its overexpression in response to glucose starvation. Glucose starvation results in both the activation of the Spc1 MAPK pathway and a reduction in activity of the cyclic AMP pathway (36–40). We examined whether the cyclic AMP-Pka1 pathway regulated Ish1 expression. Ish1ΔC291-GFP expression in a Δpka1 (catalytic subunit of cAMP-dependent protein kinase) mutant background was similar to wild type cells following glucose starvation. This suggests that the Pka1 pathway does not regulate Ish1 expression (data not shown).

We sought to determine whether the toxicity observed with Ish1 overexpression could be rescued by deletion of elements in the Spc1 MAPK pathway. Δspc1, Δpyp1, or Δatf1 mutations
could not rescue the toxic effect caused by constitutive overexpression of Ish1 (data not shown).

**Time Course Experiment during Stress Response—**Ish1 expression in response to KCl treatment increases within the initial 20 min of treatment and stays relatively constant for at least 4 h (Fig. 3D). A similar time course of expression was observed in response to sorbitol treatment (data not shown).

Because all stress conditions examined appear to increase expression of Ish1 in response to KCl treatment increases within the initial 20 min of treatment and stays relatively constant for at least 4 h (Fig. 3D). A similar time course of expression was observed in response to sorbitol treatment (data not shown). Ish1 and Bis1 show reduced viability in stationary phase. Cells were grown to stationary phase in YEA medium, and incubation was continued for 6 days. A portion of each culture was plated on YEA plates. The number of viable colonies forming cells was determined over a 6-day incubation period. Wild type (Q250), Δish1 (Q1951), Δbis1 (Q1894), Δish1 Δbis1 (Q1935), and Δspc1 (Q1699) mutant cells were used.

**Characterization of Ish1 Protein—**To characterize the expression level of endogenous Ish1, antibodies were generated against a GST-Bis1 fusion protein expressed in E. coli. By using our GST-Bis1 polyclonal antibody, we were unable to detect native Ish1 protein in cell extracts or following strong overexpression of native Ish1 protein (Fig. 5B). However, this antibody is able to detect a predominant band at ~80 kDa which corresponds to the full-length Ish1-YFP fusion protein (Fig. 5B). The ability to detect Ish1 following overexpression of Ish1-YFP suggests that the native protein is stabilized by YFP. Other smaller bands were detected by Western blot analysis, suggesting that the Ish1 protein is most likely susceptible to rapid proteolytic cleavage or degradation in vivo. Choosing different times of expression did not markedly affect this result.

**Ish1 Interacts with Bis1 in Vivo—**To confirm that Ish1 and Bis1 interact in vivo, Ish1-GFP and Bis1-YFP were expressed under the control of the thiamine-repressible nmt1 promoter in wild type cells. Soluble proteins were prepared from cells that had been expressing these fusion proteins for no longer than yeast two-hybrid screen using ΔN33ish1 as bait in which the potential transmembrane domain has been deleted (see “Experimental Procedure”; Fig. 5A). A total of 3 million transformants were screened resulting in eight independent interactions representing only three different target genes (Table III).

One of the targets was a fragment of Ish1, amino acids 384–580. This region of Ish1 may be important for dimerization or oligomerization. Another target isolated was Rps6, a ribosomal protein repressed by ammonium starvation and regulated by the Scp1 MAPK and the Pka1 pathways (41). The third target, of which two different isolates were obtained, was Bis1. All of the targets induced expression of the β-galactosidase reporter gene (Table III).

To determine the region of Ish1 important for protein-protein interactions we generated ΔN278ish1, Ish1ΔC104, and ish1-(384–580) constructs as baits (see “Experimental Procedures”; Fig. 5A). The Ish1-Ish1 interaction is largely unaffected by the N-terminal deletion. Although the interaction with the C-terminal deletion construct remains strong, it is reduced 2-fold compared with ΔN33 (Table III). The results indicate that the central region of the protein from amino acid 384–580 mediates the majority of the Ish1-Ish1 interactions. In contrast, Bis1 shows a somewhat increased binding affinity for the Ish1ΔC104 or Ish1-(384–580) baits and slightly reduced binding affinity for ΔN33ish1 or ΔN278ish1 baits. These results indicate that Bis1 interact with different regions of the Ish1 protein. The main region of contact encompasses amino acids 384–580.

**Bis1 Is a Homologue of the ES2 Protein—**The Bis1 (GenBank™ accession number AL022243.3, locus SPCC364.02c) protein is 384 amino acids in length with a predicted molecular mass of 42.7 kDa. Bis1 is homologous to the ES2 nuclear protein family (ProDomain Data base) for which no function has been identified. The Bis1 protein shows extensive homology with ES2 family members from C. elegans (42), Drosophila (43), H. sapiens (44, 45), and A. thaliana (Proteome data base) (Fig. 5E). There is no homologue in S. cerevisiae.

Inspection of the Bis1 amino acid sequence revealed seven potential consensus phosphorylation sites for MAPKs (Ser-Pro/Thr-Pro) in the C-terminal region of the protein, namely Thr-199 (MYTP), Thr-318 (YSTP), Thr-330 (NLTP), Ser-344 (LRSP), Ser-350 (GSSP), Thr-371 (TPP), and Thr-380 (AOTP). The identification of potential MAPK phosphorylation sites is particularly important because the ES2 homologue in C. elegans interacts with the MAPK, MKP1, in a two-hybrid screen (46). We examined the possibility that Bis1 interacts with Spc1 by two-hybrid analysis. We found no interaction.

**Isolation of bis1 Using a Two-hybrid Library Screen—**To identify potential Ish1 interacting partners, we performed a
Fig. 5. Ish1 interacts with Bis1. A, deletion constructs of Ish1 in pEG202 as indicated. The positions of the first and last amino acids of each construct are shown relative to Ish1.

B, Bis1 is undetectable in the absence of the YFP tag. \( \text{bis}1 \) mutant cells, wild type cells at 30°C and shifted to 36°C for 4 h, or cells producing high levels of Bis1 (\( nmt2: \text{bis}1 \)), and (\( nmt2: \text{bis}1\text{-YFP} \)) were analyzed for Bis1 expression levels at 30°C. Following 18 h of growth, cell lysates were prepared. A portion of the extract (20 µg) was separated by SDS-PAGE and used for Western blotting. The Bis1 protein was detected using our anti-GST-Bis1 antibody.

C and D, Ish1 and Bis1 proteins interact in vivo. Extracts were prepared from wild type cells cotransformed with plasmids overexpressing Ish1-GFP and Bis1-YFP (Q1841) or Ish1 \( C104 \)-GFP and Bis1-YFP (Q1847) under the control of \( nmt1 \) promoter. Cells were grown for 15 h in EMM at 30°C, and native extracts were prepared following centrifugation and bead lysis. Bis1 was immunoprecipitated from extracts using anti-Bis1-GST antibody. Bound proteins were eluted and subjected to immunoblot analysis with anti-GFP (C) or anti-Bis-GST (D) antibody. Lanes 1 and 6, \( \text{bis}1 \) mutant cells; lanes 2 and 4, \( nmt1: \text{ish}1\text{-GFP} \) and \( nmt2: \text{bis}1\text{-YFP} \); and lanes 3 and 5, \( nmt1: \text{ish}1\text{AC}104\text{-GFP} \) and \( nmt2: \text{bis}1\text{-YFP} \). As a control 25 µg of \( \text{bis}1 \) mutant cell lysates was probed directly for the presence of Bis1-YFP or Ish1-GFP. E, Bis1 is a member of a conserved ES2 coiled nuclear protein family. ClustalW alignment of conserved domains of \( S. \text{pombe} \) Bis1 (CAA18284), \( H. \text{sapiens} \) DGSI (NP_073210), \( C. \text{elegans} \) F42H10.7 (P34420), \( D. \text{melanogaster} \) DES2 (AAF46375), and \( A. \text{thaliana} \) F17A17.13 (AAF21189). Shaded boxes enclose regions with sequence identity to \( S. \text{pombe} \) Bis1.
TABLE III
Two-hybrid interactions of Ish1

| DNA binding domain fusion | Activation domain fusion | LacZ expression |
|---------------------------|--------------------------|-----------------|
| ΔN3Ish1                   | Vector                   | 0.12 ± 0.0      |
| ΔN3Ish1                   | Ish1-(384–580 aa)        | 2444 ± 500      |
| ΔN3Ish1                   | ΔN3Bis1                  | 69 ± 5.7        |
| ΔN3Ish1                   | Bis1                     | 88.5 ± 5.8      |
| ΔN3Ish1                   | ΔN1Ips6                  | 590 ± 31        |
| ΔN278Ish1                 | Vector                   | 1.6 ± 0.0       |
| ΔN278Ish1                 | Ish1-(384–580 aa)        | 2167 ± 40       |
| ΔN278Ish1                 | ΔN3Bis1                  | 47.3 ± 2.6      |
| ΔN278Ish1                 | Bis1                     | 63.6 ± 5.6      |
| Ish1ΔC104                 | Vector                   | 0.61 ± 0.11     |
| Ish1ΔC104                 | Ish1-(384–580 aa)        | 1164 ± 105      |
| Ish1ΔC104                 | ΔN3Bis1                  | 142 ± 11.4      |
| Ish1ΔC104                 | Bis1                     | 108 ± 7.8       |
| Ish1-(384–580 aa)         | Vector                   | 0.03 ± 0.03     |
| Ish1-(384–580 aa)         | ΔN3Bis1                  | 170 ± 7.2       |
| Ish1-(384–580 aa)         | Bis1                     | 187 ± 16.7      |

15 h. Bis1 was immunoprecipitated using our GST-Bis1 polyclonal antibody and the presence of Ish1-GFP in the precipitates was assessed using an anti-GFP monoclonal antibody. An immunoprecipitated Bis1-YFP was able to coprecipitate Ish1-GFP or Ish1ΔC104-GFP (Fig. 5C). Our anti-GST-Bis1 polyclonal antibody immunoprecipitated Bis1-YFP (Fig. 5D). Approximately 30% of Bis1-YFP was immunoprecipitated from the yeast lysate, and ~1% of the total Ish1-GFP, a much more abundant protein, coimmunoprecipitated with the Bis1-YFP.

bis1 Contributes to Viability in Stationary Phase—A null allele was constructed by replacing the bis1 gene with urad1 in a haploid strain (Fig. 1B). The bis1 mutant is viable, and bis1 is nonessential for growth. We were unable to demonstrate a visible phenotype for the Δbis1 mutant under any of the conditions tested for ish1. However, Δbis1 mutant cells do exhibit a reduction in cell viability in stationary phase (Fig. 4). Similarly to ish1 mutant cells, bis1 mutant cells display 100% plating efficiency during logarithmic growth but not from stationary phase. This result suggests that bis1 contributes to stationary phase viability as does ish1.

Localization of Bis1 Protein—We examined the phenotype and localization of Bis1-YFP expressed under the control of the nmt2 promoter in a wild type background. Overexpression of Bis1-YFP causes growth inhibition producing elongated cells exhibiting a cell cycle phenotype (Fig. 6A). Overexpression of the full-length native Bis1 protein exhibited a similar cell morphology (data not shown) suggesting that this phenotype is not a result of the tag. Bis1-YFP localizes to the nucleus as shown by DAPI staining (Fig. 6B). Interestingly, during mitosis Bis1-YFP appears to be associated with the mitotic spindle microtubules and possibly chromatin as the cells progress through mitosis (Fig. 6C).

We examined whether overexpression of Bis1 is able to rescue the growth inhibition associated with Ish1 overexpression and found that Bis1 was not able to suppress the lethality.

Some Overlap in Localization of Bis1 and Ish1 Proteins—When Bis1-YFP and Ish1-GFP are co-overexpressed in wild type cells, they display partial overlap in their localization at the nuclear envelope (Fig. 6D and E). To determine whether the localization of Bis1-YFP or Ish1-GFP is dependent upon one another, their localization was examined in null mutant cells of each partner. The localization of Bis1-YFP in the Δish1 mutant background and Ish1-GFP in Δbis1 mutant background is similar to that seen in wild type backgrounds (data not shown).

Search for Synthetic Interaction between ish1 and bis1—We generated a double mutant of Δish1 with Δbis1 to see if ish1 genetically interacts with bis1. The Δish1 Δbis1 double mutant did not exhibit a visible phenotype. We examined the Δish1 Δbis1 double mutant strain extensively under similar conditions to those used to test the Δish1 mutant strain. In all cases there is no visible phenotype. Intriguingly, the Δish1 Δbis1 double mutant strain behaves similarly to the Δish1 or Δbis1 mutant strains and has no additive effect upon the level of survivability in stationary phase (Fig. 4).

Two-hybrid Screen Using Bis1 Bait—We performed a yeast
two-hybrid screen using a Bis1 construct where 90 N-terminal amino acids (ΔN90bis1) have been deleted. ΔN90bis1 was used because the full-length DNA encoding for bis1 transactivated on its own. A total of 2 million transformants were screened. The screen produced at least 71 reproducible interactions with Bis1 because the full-length DNA encoding for amino acids (two-hybrid screen using a Bis1 construct where 90 N-terminal SPAC6F6.12 PhoX domain containing protein 6–401; 137–401; 150–401; 164–401; 189–401 10 29% to Snx4p (S. cerevisiae) SPAC644.04 Pct1 1–303; 12–303 8 30% to Gpd1p (S. cerevisiae) SPAC6B12.15 Cpc2 7–314; 19–314; 25–314; 71–314 6 64% to RACK (mammalian) SBPC211.04c Mis5, Mcm6 2–892 5 42% to MCM4 (H. sapiens) SBPC16HS.11c Skh1 26–646; 262–646 5 36% to SKH1 (human) SPAC22G3.11 Rpn6 8–421; 44–421 4 49% to Rpn1p (S. cerevisiae) SPAC30G10.1c Putative ribonuclease PH-like 4–242 4 39% to Skp1p (S. cerevisiae) SPBC1105.04c Abp1, Cbp1 3–522 4 25% to CENP-Bp (human) SPBC9B6.05c Lsm3 2–93 4 49% to Lsm3p (S. cerevisiae) SPCC162.08C Putative nuclear pore complex-associated protein (coiled-coil protein) 422–1837 3 SBPC1604.08c Importin α subunit 1–539 3 54% to KPN6 (H. sapiens) SBPC19G12.10c Cyp1 199–1002 3 54% to Pcr1p (S. cerevisiae) SBPC14F7.03c Kap123 431–1067 2 31% to Kap123p (S. cerevisiae) SPAC27F1.04c Nufl, centromere protein 10–441; 25–441 2 24% to Nuf2p (S. cerevisiae) SPAC1687.05p Protein contains SAP domain 434–727 1 24% to Nf1p (S. cerevisiae) SPBC1604.06c Protein of unknown function 3–485 1 35% to YPR144C (S. cerevisiae) SPBC839.10 Protein of unknown function 24–695 1 22% to Snm71p (S. cerevisiae) (SPBC24E9.10) SBPC1198.11C Rebl transcription factor 1–504 1 27% to Reblp (S. cerevisiae) SPAC22F3.13 Coiled-coil protein, integral membrane protein 551–899 1 SPCC1672.10 β-Transducin, putative chromosome assembly factor 4–430 1 SPBC365.19c Putative chromatin assembly factor subunit 14–544 1 27% to Cac1p (S. cerevisiae) (SPBC29A10.02c) SPBC211.02c Cwt3, Syf1 231–790 1 24% to Cw3p (S. cerevisiae) all clones isolated included the termini and were deleted from the N terminus to varying degrees, reflecting the nature of the cDNA library.

**DISCUSSION**

**Ish1 Is a Novel Stress-response Protein**—We used GFP insertional mutagenesis to isolate genes up-regulated following glucose starvation, and we identified Ish1, a novel stress-responsive protein. The Ish1-GFP fusion protein localizes predominantly to the nuclear envelope as well as to the plasma membrane throughout the cell cycle making it a useful fluorescent marker for monitoring changes that occur in the nuclear membrane during mitosis and meiosis. Expression of Ish1 is strongly induced in response to a variety of stresses including nitrogen starvation and osmotic stress. Ish1 exhibits limited similarity to S. cerevisiae YML128c. Ish1 displays limited similarity to S. cerevisiae YML128c, YML128c and the fact that both are responsive to glucose starvation, and we identified Ish1, a novel stress-responsive protein. Ish1 displays limited similarity to S. cerevisiae YML128c, YML128c. Ish1 exhibits limited similarity to S. cerevisiae YML128c, YML128c. Ish1 exhibits limited similarity to S. cerevisiae YML128c. Ish1 exhibits limited similarity to S. cerevisiae YML128c. Ish1 exhibits limited similarity to S. cerevisiae YML128c. Ish1 exhibits limited similarity to S. cerevisiae YML128c. Ish1 exhibits limited similarity to S. cerevisiae YML128c. Ish1 exhibits limited similarity to S. cerevisiae YML128c. Ish1 exhibits limited similarity to S. cerevisiae YML128c.
interact in vivo. The relative band intensities for the commu-
noprecipitation of the two proteins suggest a stoichiometry of
Bis1-Ish1 interaction in the complex of ~1:1 or 1:2. Ish1-GFP
and Bis1-YFP proteins show some overlapping localization on
the nuclear envelope. However, cellular localization of the bulk
of Ish1 or Bis1 is not interdependent. We presume that the
interaction occurs mostly when Bis1 contacts the nuclear en-
velope and that this represents only a portion of total Bis1
protein. It is of interest that immunoprecipitation from cell
lysates recovered ~30% of total Bis1 protein. This fraction of
the protein appears to be associated with Ish1. We do not know
whether this percentage is representative of the status in vivo.

The two-hybrid data shows that Ish1 can homodimerize. We
do not know whether the Bis1 interaction is to such a dimer (or
multimer) or whether Ish1 and Bis1 compete in binding Ish1.
Ish1 is stress-responsive and may function to bind and hold out
inculation proteins such as Bis1 or recruit Bis1 to the
membrane in times of stress. Bis1 interacts with different
regions of the Ish1 protein; however, the main region of contact
encompasses amino acids 384–580 of Ish1 as shown by the
two-hybrid data. Sequence analysis identified a strong coiled-
coil region (Coils version 2.1) from amino acids 384 to 580 in
Ish1. This region of the protein also contains an LEA domain
that is found in LEA proteins in higher plants. Interestingly,
LEA proteins are induced under similar stress conditions to
Ish1. We postulate that the LEA domains are involved in
protein-protein interactions.

As discussed previously, ish1 plays a role in stationary phase
viability. The interaction of ish1 with bis1 suggests that bis1
plays a similar role. The ∆bis1 mutant cells show a reduction in
cell viability similar to ∆ish1 mutant cells alone. The ∆ish1
∆bis1 double mutant, however, was indistinguishable from the
single mutant in the reduction of cell viability in stationary
phase. They may both play a role in stationary phase viability,
but their effects are not additive, suggesting they may be mem-
bers of the same pathway.

Possible Roles of Bis1—Bis1 is the S. pombe homologue of the
ES2 family of nuclear proteins that have been found in various
organisms from A. thaliana to H. sapiens. Notably there does
not appear to be an ES2 homologue in S. cerevisiae. Similar to
the results in mouse, Bis1-YFP localizes to the nucleus (43). At
present no function has been identified for the ES2 family of
proteins. Our findings of an effect on stationary phase viability
are the first suggestion of a phenotype. The C. elegans
homologue, F42H10.7, has been shown to interact with Mpk1 in a
two-hybrid screen (46). Mpk1 has regions of similarity to the
kinase domains of Spc1, Spm1, and Spk1 in fission yeast.
Coupled with the existence of several putative MAPK phosphory-
lation sites in the Bis1 sequence, this suggested that Bis1
activity could be regulated by a MAPK. Although we could not
show that Bis1 interacts with Spc1, we have not ruled out the
possibility of Bis1 interacting with Spm1 or Spk1.

In an effort to gain insight into the function of Bis1, we
performed a two-hybrid screen. As might be expected from the
Bis1 localization, ~80% of the two-hybrid targets of Bis1 are
nuclear or nuclear envelope proteins. They fall broadly into
several classes as follows: 1) RNA processing/modification; 2)
protein degradation; and 3) centromere/chromatin structure.
Interestingly, we found that Bis1-YFP localizes to the mitotic
spindle microtubules during mitosis starting in anaphase B.
These results are in line with the functions of the third class of
Bis1 targets.

The second class of Bis1 targets is involved in protein degra-
dation. The high turnover of Bis1 implied by these results
may explain why our antibody was unable to detect endogenous
Bis1 protein. However, it was able to detect Bis1-YFP. GFP
tags have been shown to increase protein stability in some cases
(41). It is possible that the YFP tag increases resistance to
proteolytic degradation allowing the proteins to be detected.

Although we have not carried out any direct experimental
promoter analysis on ish1 and bis1, we have identified a number
of elements upstream of the start site that suggests that they
are stress-responsive genes. We have found at least four
putative nitrogen-response elements (48) in both ish1 and bis1.
Presumably, these nitrogen-response elements account for the
stress response of ish1. This is also likely to be the case for bis1.
In addition, we have found two putative Ste11 binding motifs
(48) in ish1. Ste11 is essential in the mating and meiosis-
response pathway (49). Ste11-binding motifs are found in a
number of genes regulated by Ste11, including mat1-P,
mat1-M, mei2 (49), esc1 (50), ste6 (51), and fus1 (52). It is
conceivable that ish1 may also be regulated by Ste11.

In conclusion, we have presented data that identifies a novel
nuclear envelope protein, Ish1, whose expression is mediated
by Atf1 in response to a variety of stresses. We also identified
a novel nuclear protein, Bis1, that interacts with Ish1. Al-
though neither ish1 nor bis1 is essential for viability during
growth, they both play a role in maintaining stationary phase
viability. The mechanism by which Ish1 or Bis1 maintains cell
viability during glucose starvation remains to be determined.

REFERENCES

1. Egel, R. (1989) in Meiosis in Fission Yeast (Nasim, A., Young, P., and Johnson,
F., eds) pp. 31–73, Academic Press, New York
2. Lillie, S. H., and Pringle, J. R. (1988) J. Bacteriol. 143, 1384–1394
3. Johnston, G. C., Singer, R. A., and McFarlane, E. S. (1977) J. Bacteriol. 132,
723–730
4. Bataille, N., Regnacq, M., and Boucherie, H. (1991) Yeast 7, 367–378
5. Werner-Washburne, M., Braun, E., Johnston, G. C., and Singer, R. A. (1993)
Microbiol. Rev. 57, 383–401
6. Costello, G., Rodgers, L., and Beach, D. (1986) Curr. Genet. 11, 119–125
7. Toone, W. M., and Jones, N. (1998) Genes Cells 3, 485–498
8. Bannett, F. (1998) Microbiol. Mol. Biol. Rev. 62, 249–274
9. Degola, G., Shiozaki, K., and Russell, P. (1996) Mol. Cell. Biol. 16, 2870–2877
10. Kuris, T., Okazaki, K., Murakami, H., Stettler, S., Fantes, P., and Okayama, H.
(1996) FEBS Lett. 378, 207–212
11. Miller, J. B. A., Buck, W., and Wilkinson, M. G. (1996) Genes Dev. 9, 2117–2130
12. Shiozaki, K., and Russell, P. (1995) Nature 378, 739–743
13. Shiozaki, K., and Russell, P. (1996) Genes Dev. 10, 2276–2288
14. Kanoh, J., Watanabe, Y., Ohsumi, M., Iino, Y., and Yamamoto, M. (1996) Genes
Cells 1, 291–408
15. Chua, G., Taricani, L., Stangle, W., and Young, P. G. (2000) Methods Enzymol.
3363–3380
16. Takeda, T., Toda, T., Komimani, K., Kohnou, A., Yanagida, M., and Jones, N.
(1995) EMBO J. 14, 6193–6208
17. Taricani, L., Fellhotter, H. E., Weaver, C., and Young, P. G. (2001) Nucleic Acids
Res. 29, 3030–3040
18. Chua, G., Taricani, L., Stangle, W., and Young, P. G. (2000) Nucleic Acids Res.
138, E53
19. Ohman, H., Gerber, A. S., and Hartl, D. L. (1988) Genetics 120, 621–623
20. Alfa, C., Fantes, P., Hyams, J., McLeod, M., and Warbrick, E. (1993) Experi-
ments with Fission Yeast: A Laboratory Course Manual, Cold Spring Harbor
Laboratory Press, Cold Spring Harbor, NY
21. Moreno, S., Klar, A., and Nurse, P. (1991) Methods Enzymol. 194, 795–823
22. Turi, T. G., Mueller, U. W., Sazer, S., and Rose, J. K. (1996) J. Biol. Chem. 271,
9166–9171
23. Baas, G., Schmidt, E., and Maundrell, K. (1993) Genes (Amst.) 123, 131–136
24. Maundrell, K. (1995) Genes (Amst.) 123, 127–130
25. Horton, R. M., Ho, S. N., Pullen, J. K., Hunt, H. D., Cai, Z., and Pease, L. R.
(1993) Methods Enzymol. 217, 279–279
26. Bialek, J., Wu, G. Q., Longine, M. S., Shah, N. G., McKenzie, A., III, Steeber,
A. B., Wach, A., Philppens, P., and Pringle, J. R. (1998) Yeast 14, 943–951
27. Bautin, A., Orzer-Kalogeropoulos, O., Demouel, A., Lacroute, F., and Cullin, C.
(1993) Nucleic Acids Res. 21, 3293–3299
28. Kaur, R., Ingavale, S. S., and Bachhawat, A. K. (1997) Nucleic Acids Res. 25,
1089–1091
29. Srivastava, K. E., and Nurse, P. (1998) J. Cell Biol. 75, 509–516
30. Egel, R. (1989) in Meiosis in Fission Yeast (Nasim, A., Young, P., and Johnson,
F., eds) pp. 31–73, Academic Press, New York
31. Egel, R. (1989) in Meiosis in Fission Yeast (Nasim, A., Young, P., and Johnson,
F., eds) pp. 31–73, Academic Press, New York
Ish1 and Bis1 Proteins of S. pombe

37. Hoffman, C. S., and Winston, F. (1990) Genetics 124, 807–816
38. Maedae, T., Mochizukin, N., and Yamamoto, M. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7814–7818
39. Shiozaki, K., Shiozaki, M., and Russell, P. (1997) Mol. Biol. Cell 8, 409–419
40. Stettler, S., Warbrick, E., Prochnik, S., Mackie, S., and Fantes, P. A. (1996) J. Cell Sci. 109, 1927–1935
41. Bonnet, C., Perret, E., Dumont, X., Pocard, A., Caput, D., and Lenaers, G. (2000) Yeast 6, 23–33
42. Rizzu, P., Lindsay, E. A., Taylor, C., O'Donnell, H., Levy, A., Scamblar, P. J., and Baldini, A. (1996) Mamm. Genome 7, 639–643
43. Lindsay, E. A., Harvey, E. L., Scamblar, P. J., and Baldini, A. (1998) Hum. Mol. Genet. 7, 629–635
44. Gong, W., Emanuel, B. S., Galili, N., Kim, D. H., Roe, B., Driscoll, D. A., and Budarf, M. L. (1997) Hum. Mol. Genet. 6, 267–276
45. Lindsay, E. A., Rizzu, P., Antonacci, R., Jurecic, V., Delmas-Mata, J., Lee, C-C., Kim, U.-J., Scamblar, P. J., and Baldini, A. (1996) Genomics 32, 104–112
46. Walhout, A. J., Sordella, R., Lu, X., Hartley, J. L., Temple, G. F., Brasch, M. A., Thierry-Mieg, N., and Vidal, M. (2000) Science 287, 116–122
47. Gasch, A. P., Spellman, P. T., Kao, C. M., Carmel-Harel, O., Eisen, M. B., Storz, G., Botstein, D., and Brown, P. O. (2000) Mol. Biol. Cell 11, 4241–4257
48. Heinemeyer, T., Wingender, E., Reuter, I., Hermajakob, H., Kel, A. E., Kel, O. V., Ignatieva, E. V., Ananko, E. A., Podkolodnaya, O. A., Kalpakov, F. A., Podkolodny, N. L., and Kelchman, N. A. (1998) Nucleic Acids Res. 26, 364–370
49. Sugimoto, A., Iino, Y., Maeda, T., Watanabe, Y., and Yamamoto, M. (1991) Genes Dev. 5, 1990–1999
50. Benton, R. K., Reid, M. S., and Okayama, H. (1993) EMBO J. 12, 135–143
51. Hughes, D. A., Yabana, N., and Yamamoto, M. (1994) J. Cell Sci. 107, 3635–3642
52. Petersen, J., Weilguny, D., Egel, R., and Nielsen, O. (1995) Mol. Cell. Biol. 15, 3697–3707