MOLECULAR AND CELLULAR BIOLOGY, Nov. 1997, p. 6491–6501 Vol. 17, No. 11
0270-7306/97/$04.00+0

Xbp1, a Stress-Induced Transcriptional Repressor of the *Saccharomyces cerevisiae* Swi4/Mbp1 Family

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Received 23 June 1997/Returned for modification 5 August 1997/Accepted 19 August 1997

We have identified Xbp1 (*Xho*I site-binding protein 1) as a new DNA-binding protein with homology to the DNA-binding domain of the *Saccharomyces cerevisiae* cell cycle regulating transcription factors Swi4 and Mbp1. The DNA recognition sequence was determined by random oligonucleotide selection and confirmed by gel retardation and footprint analyses. The consensus binding site of Xbp1, GcCTCGA(G/A)G(C/A)g(a/g), is a palindromic sequence, with an *Xho*I restriction enzyme recognition site at its center. This Xbp1 binding site is similar to Swi4/Swi6 and Mbp1/Swi6 binding sites but shows a clear difference from these elements in one of the central core bases. There are binding sites for Xbp1 in the G_1 cyclin promoter (*CLN1*), but they are distinct from the Swi4/Swi6 binding sites in *CLN1*, and Xbp1 will not bind to Swi4/Swi6 or Mbp1/Swi6 binding sites. The *XBP1* promoter contains several stress-regulated elements, and its expression is induced by heat shock, high osmolarity, oxidative stress, DNA damage, and glucose starvation. When fused to the LexA DNA-binding domain, Xbp1 acts as transcriptional repressor, defining it as the first repressor in the Swi4/Mbp1 family and the first potential negative regulator of transcription induced by stress. Overexpression of *XBP1* results in a slow-growth phenotype, lengthening of G_1, an increase in cell volume, and a repression of G_1 cyclin expression. These observations suggest that Xbp1 may contribute to the repression of specific transcripts and cause a transient cell cycle delay under stress conditions.

G_1/S-specific transcription in the budding yeast *Saccharomyces cerevisiae* is mainly controlled by two heterodimeric transcription factors, Swi4/Swi6 and Mbp1/Swi6 (reviewed in reference 5). The DNA-binding subunits Swi4 and Mbp1 confer the specificity of the complexes, binding to SCBs (Swi4/Swi6 cell cycle box) or MCBs (*Mlu*I cell cycle box), respectively (1, 6, 22). One or both of these elements have been found in the promoters of all of the genes that are known to be expressed at the G_1/S boundary of the cell cycle, including the G_1/S cyclin genes *CLN1*, *CLN2*, *CLB5*, *CLB6*, the gene encoding *HO* endonuclease, many genes which are involved in DNA synthesis, and *SWI4* itself (6, 12, 13, 22, 33, 38, 39). SCBs and MCBs are each sufficient to confer G_1/S-specific transcription from heterologous promoters (6, 11, 26), and eliminating the activities that bind to these promoter elements causes cells to arrest in late G_1 (22, 38, 39). MCB elements are also responsible for the periodic transcription of cell cycle-regulated genes in the fission yeast *Schizosaccharomyces pombe* and are bound by related transcription factor complexes containing Cdc10, Res1, and Res2, with Cdc10 having homology with Swi6 and Res1 and Res2 having homology with Mbp1 (8, 36, 54, 65). Functions similar to those of these yeast transcription factors are performed in animal cells by the E2F/DP1 class of transcription factors; however, little or no structural similarities exist between them (25).

All proteins of the Swi4/Mbp1 family of transcription factors share features like a central ankyrin repeat domain, a C-terminal domain necessary for association with the regulatory protein Swi6, and an N-terminal DNA-binding domain (5). The DNA-binding domain is highly conserved between Swi4, Mbp1, Res1, and Res2 and is composed of a core region folded into a helix-turn-helix (HTH) motif, flanked N terminally by a four-stranded β-sheet and C terminally by a two-stranded β-hairpin motif (64). The regions around the HTH motif are thought to be involved in the DNA binding, although the residues contacting DNA are not known (64). There are three other budding yeast proteins which show homologies to the Swi4/Mbp1 DNA-binding domain: Phd1, Sok2, and ORF647 (5, 14, 57). In addition, Efg1 (EMBL accession no. X71621) from *Candida albicans* and StuA from *Aspergillus nidulans* contain related DNA-binding domains (5, 34).

One group of target genes of the Swi4/Mbp1 family of transcription factors are the G_1 cyclin genes *CLN1* and *CLN2* (38), which are known to be regulated not only during the cell cycle (63) but also in response to environmental factors like elevated temperatures (46). In heat-shocked cells, *CLN1* and *CLN2* are transiently repressed, and this delays the onset of S phase (46). The mediators of this heat shock-induced repression have not been identified, but repression is not dependent on the transcriptional regulation by Swi4 or the activity of cyclic AMP (cAMP)-dependent protein kinase A (PKA) (46). G_1 cyclin expression is also affected by carbon source availability through the Ras/cAMP/PKA pathway (55), but the regulatory proteins involved are also not known. Another signaling pathway which connects environmental changes to the regulation of transcription factors has been recently identified. The PKC-mitogen-activated protein (MAP) kinase pathway is activated by elevated temperatures or by treatment of cells with mating pheromone, resulting in a change of the phosphorylation level of Swi4/Swi6 (27).

In this report, we describe the characterization of ORF647, or Xbp1, a new member of the Swi4/Mbp1 family of DNA-binding proteins, which was identified through its homology to the DNA-binding domain of Swi4 (5). Xbp1 binds to a DNA recognition sequence which has similarities to SCBs and MCBs but is clearly different at a central core base. The specificity of DNA binding was confirmed by gel retardation assays and

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TABLE 1. Oligonucleotides used

No.	Sequence[a]
BL150	GTGACACGGTACACTTAACGGACAACCCCCTG GACTCGAGCTTGGTGAGCGCTAGGAG
BL151	GTGGGAGGAGCAGGCGCGATTGGTGAAGAG GGGACTCGAGCCTCGTTCAGAATGACACG
BL152	CGGGATCCATATGAAATATCGCTTTTAG
BL153	GCGGAATTCTGGAAAAACATATAGTACGG
BL154	GCTCTAGATAACGGATCCAGAGCCAAGAG
BL155	GCTCTAGAATTCTTGTTTTGAGTTTGTTTTAA ATTTG
BL156	CGGGATCCATATGGCAAACAATTACATCGATT TT
BL157	CGGGATCCATATGTGGTCGCATGACTCCGGC
BL158	GGCTCGAGCTACACCGTTGGATCTCTTGGCCC
BL159	GCCGGTGACGACGCTCCTC
BL160	GCCAAGATAGAACCACC
BL161	GGCGATGGATTACGATTACCAAGG
BL162	GTAGCGCTTGCTCTATCTTCGGGC
BL163	CATACAGGAATATCACGAGG
BL164	GATAGCGATATCGAAGACG
BL165	GGGGAGAAGAACTGGATGCG
R76 (BL176)	CAGGTCAGTTCAGCGGATCCTGTCG(N_{26})GAG GCGAATTCAGTGCAACTGCAGC
F (BL168)	GCTGCAGTTGCACTGAATTCGCCTC
R (BL169)	CAGGTCAGTTCAGCGGATCCTGTCG

[a] Oligonucleotides are shown 5′ to 3′; introduced restriction sites are underlined.

footprint analyses. The expression of *XBP1* is nearly undetectable under normal growth conditions but is strongly induced when cells are heat shocked, starved for glucose, or treated with reagents causing osmotic shock, oxidative stress, or DNA damage. Finally, we could demonstrate that Xbp1 acts as a transcriptional repressor when brought to a promoter by fusion with the DNA-binding domain of the bacterial LexA protein. Interestingly, overexpression of *XBP1* leads to a slow-growth phenotype and an increase in cell size, which is accompanied by repression of all three of the G_1 cyclins.

MATERIALS AND METHODS

Yeast strains, growth conditions, cell analysis, and β-galactosidase assays. All of the yeast strains used in this study are derived from the homozygous diploid YPH501 (BY2054) (51) (*MAT***a**/α *ura3-52 ura3-52 lys2-801 lys2-801 ade2-101 ade2-101 trp1-Δ63 trp1-Δ63 his3-Δ200 his3-Δ200 leu2-Δ1 leu2-Δ1*). Transformations and tetrad analysis were performed as previously described (2). For the disruption of *XBP1*, a PCR fragment was used to transplace the Xbp1 open reading frame (ORF) (amino acids [aa] 22 to 577) with the *HIS3* gene (4) in BY2054, resulting in BY2055. Tetrad analysis showed full viability of all spores, and BY2058 (*MAT***a** *XBP1*) and BY2059 (*MAT*α *xbp1::HIS3*) were used as *XBP1* wild-type and disruption strains, respectively. The disruption of *XBP1* was confirmed by PCR using the oligonucleotide primers BL152 and BL153 (Table 1). The strain used for the transcriptional activation experiments was L40 (56). It carries $(lexAop)_8$-*lacZ* integrated at *URA3*.

Cells were grown at 30°C unless otherwise specified, in either YEPD medium (1% yeast extract, 2% peptone, 2% dextrose) or synthetic complete medium supplemented with amino acids as appropriate to select for transformants (2). To induce expression from the *GAL1* promoter, 2% galactose was added to a culture grown in 2% raffinose. Heat shock treatment was achieved by rapidly shifting a culture (optical density [OD] of 0.3) grown at 23 to 39°C. Osmotic shocks were done by adding 4 M NaCl or 4 M sorbitol to a final concentration of 0.3 or 0.4 M, respectively. Oxidative stress, DNA damage induction, and heavy-metal exposure were accomplished by adding diamide (Sigma D3648) to 1.5 mM, hydrogen peroxide to 0.3 to 1%, methyl methanesulfonate (MMS) to 0.01 to 0.1%, or cadmium chloride to 5 μM (all final concentrations). Glucose starvation was achieved by shifting a culture (OD of 0.3) grown in 2% glucose to 0.05% glucose.

DNA content was quantitated by fluorescence-activated cell sorting (FACS) analysis using a Becton Dickinson FACScan and CellQuest software. Cell volume analysis was done with a Coulter Counter (model ZM) with a Sampling Stand (model IIA) and a Channelyzer 256.

Quantitative β-galactosidase assays were performed on cells grown to an OD of 0.5 to 0.7 in appropriate medium, using the permeabilization method described elsewhere (2). The assays were carried out with at least three independent transformants of each plasmid in triplicate.

Plasmids. The sequences of all oligonucleotide primers used in the following constructions are provided in Table 1. The *XBP1* gene (YIL101C on chromosome IX) was obtained by PCR using genomic DNA (strain W303a), Vent DNA polymerase, and the oligonucleotide primers BL152 and BL153, which created a 5′ *Bam*HI/*Nde*I site and a 3′ *Eco*RI site. This DNA (pBD2005), cloned into *Bam*HI- and *Eco*RI-cut pUC18, was used for all further constructs. The fragment for disruption of the *XBP1* ORF with the *HIS3* marker gene was generated by PCR using BL150 and BL151, *Taq* DNA polymerase, and pRS303 as template DNA. The PCR fragment contained a 35-bp sequence on each end homologous to the *XBP1* gene, flanked by an *Xho*I site and sequences homologous to *HIS3*. This fragment was used to replace the genomic *XBP1* locus (4).

The His_6-tagged Xbp1 (pBD2008) was generated by cloning a *Bam*HI-*Kpn*I fragment from pBD2005 into the QE32 vector (Qiagen). The glutathione *S*-transferase (GST)–Xbp1 fusion (pBD2013) was created by ligation of a *Bam*HI-*Eco*RI and an *Eco*RI fragment from pBD2005 into pGEX5x-1 (Pharmacia). pBD2018, containing the complete ORF and the *XBP1* promoter up to −672 bp relative to the ATG, was cloned as a 0.7-kb *Xba*I-*Bam*HI fragment into *Xba*I-*Bam*HI-cleaved pBD2005. This fragment was amplified from genomic DNA (strain W303a) by using Vent DNA polymerase and the oligonucleotide primers BL154 and BL153. To express Xbp1 under control of the inducible *GAL1* promoter, a *Bam*HI-*Eco*RI and an *Eco*RI fragment from pBD2005 were cloned into pYES2 (Invitrogen) to generate pBD2014.

The LexA-Xbp1 fusion (pBD2082), which is expressed under the control of the constitutive *ADH1* promoter, was obtained by cloning a *Bam*HI-*Sal*I fragment from pBD2013 into pBTM116 (generously provided by Rolf Sternglanz). The deletion constructs, all fused to LexA in pBTM116, were generated by PCR using *Taq* DNA polymerase and pBD2005 as template. Oligonucleotide primer pairs used were as follows: for generating pBD2083 (LexA-Xbp1[1-511]), BL158-BL152; for generating pBD2085 (LexA-Xbp1[196-647]), BL157-BL155; and for generating pBD2084 (LexA-Xbp1[298-511]), BL156-BL158. The *CYC1-lacZ* reporter plasmids pBD2086 (=pAJ1; upstream activation sequence [UAS]-*lacZ*), pBD2087 [=pJK1621; (4 × *lexA*)-UAS-*lacZ*], and pBD2089 (=pCK30; UAS-*lexA-lacZ*) were obtained from M. A. Osley and are described elsewhere (20).

Expression of XBP1 in *Escherichia coli*. The His_6-tagged Xbp1 was expressed in *E. coli* BL21(DE3) (Novagen). Cells were grown at 30°C and induced for 90 min at 30°C with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). Extracts were prepared in buffer E (20 mM Tris [pH 7.5], 100 mM NaCl, 1 mM $MgCl_2$, 2% glycerol, 0.1% Nonidet P-40, 1 μg of leupeptin per ml, 1 μg of pepstatin per ml, 1 mM phenylmethylsulfonyl fluoride) by sonification. Clarified extracts were purified by nickel chelate chromatography (Qiagen) using 20 mM imidazole in buffer E for washing and 300 mM imidazole in buffer E for elution. The Gst-Xbp1 fusion protein was also expressed in *E. coli* BL21(DE3). Cells were grown under the same conditions but induced with 0.1 mM IPTG. Extracts were prepared as described for the His-tagged protein and purified by using glutathione-agarose (Sigma) according to the manufacturer's protocol. All protein preparations were dialyzed against buffer E and stored in aliquots at −80°C.

In vitro binding site selection. A 76-base single-stranded oligonucleotide (R76; equivalent to BL167), containing a central region of 26 random bases flanked by 25-base regions with defined sequences (gift from R. N. Eisenman), was annealed to oligonucleotide F (BL168), which is complementary to the 3′ 25 bases of R76. The primer was extended by using the Klenow fragment of *E. coli* DNA polymerase I (Boehringer Mannheim), to yield a mixture of double-stranded DNA fragments. This mixture was incubated with recombinant Xbp1 protein in gel retardation binding buffer for 10 min. In each round of selection and amplification, the fusion protein was alternated, using first the His-Xbp1 and then the GST-Xbp1 fusion. Beads (Ni-nitrilotriacetic acid-agarose and glutathione-agarose, respectively) were added after each round, incubated for 10 min, and then washed three times with binding buffer. The DNA was eluted with elution buffer (5 mM EDTA, 0.5% sodium dodecyl sulfate [SDS], 100 mM sodium acetate, 50 mM Tris [pH 8.0]) at 50°C for 5 min, phenol extracted, and ethanol precipitated in the presence of 20 μg of glycogen (Boehringer Mannheim). The bound fragments were PCR amplified (15 cycles of 95°C for 1 min, 62°C for 1 min, and 72°C for 1 min), using the oligonucleotide primers R and F. The PCR products were purified through a 4% native polyacrylamide gel, electroeluted, and used as starting material for the next round of binding site selection. After two, three, and four rounds of selection-amplification, the PCR products were digested with *Bam*HI and *Eco*RI and cloned into pBluescript (Stratagene). Sequences were obtained by cycle sequencing and analyzed on an automatic sequencer (Applied Biosystems).

Gel retardation assay, DNase I footprinting, and dimethylsulfate (DMS) methylation interference. Gel retardation assays of DNA-protein complexes were conducted as follows. One hundred nanograms to 2 μg of protein was incubated with 25 fmol of ^{32}P labeled DNA in 20 μl of binding buffer (20 mM Tris [pH 7.5], 100 mM NaCl, 1 mM $MgCl_2$, 2% glycerol) and 25 μg of poly(dI-dC) per ml at room temperature for 10 min. ^{32}P-labeled DNA probes were generated by filling in overhangs on annealed oligonucleotides, using the Klenow fragment of *E. coli* DNA polymerase I (Boehringer Mannheim) and [α-^{32}P]dCTP. Competition experiments included a 40- to 200-fold molar excess of unlabeled competitor DNA over labeled probe as specified. Reaction mixes were loaded onto an 8%

(30:0.8) nondenaturating polyacrylamide gel and electrophoresed at 200 V in 0.5× Tris-borate-EDTA at room temperature. The gel was dried onto Whatman 3MM paper and autoradiographed.

The affinity constants of Xbp1 binding to DNA were determined by gel retardation experiments. The DNA concentrations of the oligonucleotides were measured fluorimetrically. A fixed amount of protein was incubated with a range (1 to 300 fmol) of labeled double-stranded oligonucleotides, and the reaction products were separated by gel electrophoresis. The radioactivity in the bound and free DNA was measured with a PhosphorImager (Molecular Dynamics), and the affinity constant K_{eq} was determined with the following equation: K_{eq} (M^{-1}) = (DP [fmol] · V [μl])/(D [fmol]) · (P [fmol] − DP [fmol]) · 10^{-9}, where DP is the amount of DNA-protein complex, V is the reaction volume, D is the amount of DNA used for the binding reaction, and P is the amount of active protein used for the binding reaction (9).

Double-stranded DNA fragments of selected Xbp1 binding sites were isolated as *Sac*I-*Xho*I or *Kpn*I-*Xba*I fragments from the binding site selection clones and end labeled on one site by filling in overhangs with Superscript II reverse transcriptase (Gibco BRL) and [α-^{32}P]dCTP. Fragments were purified by native gel electrophoresis, electroeluted, and incubated with recombinant protein in 20 μl of DNase I buffer (100 mM NaCl, 20 mM Tris [pH 7.5], 5 mM $MgCl_2$, 0.5 mM EDTA, 4% glycerol) for 10 min. The DNA was then digested with 1 U of RNase-free DNase I (Promega) for 1 min at room temperature. The reaction was stopped by adding 75 μl of stop solution (30 mM EDTA, 0.5 mg of proteinase K per ml). The partially digested DNA was phenol-chloroform extracted, ethanol precipitated in the presence of 20 μg of glycogen (Boehringer Mannheim), and electrophoresed in an 8% polyacrylamide–8 M urea gel.

DMS methylation interference assays were performed as described previously (2), using fragments prepared as described for the DNase I footprint. The partially methylated DNA was incubated with recombinant protein in scaled-up gel retardation reactions and subsequently electrophoresed in a nondenaturing polyacrylamide gel. The free and bound DNA fractions were electroeluted, concentrated by precipitation in the presence of 20 μg of glycogen, and cleaved in 100 μl of 1 M piperidine at 90°C for 30 min. The DNA was lyophilized and resuspended in H_2O three times, and the reaction products were separated through 8% polyacrylamide–8 M urea gels.

RNA analyses. Total yeast RNA was extracted from 1×10^8 to 2×10^8 cells, exponentially growing under the conditions described. Cells were disrupted with glass beads in RNA buffer (50 mM Tris [pH 7.4], 100 mM NaCl, 10 mM EDTA) by 3 min of vortexing at maximum level at 4°C. RNA-SDS buffer (1.3% SDS in RNA buffer) was added and then phenol was added, followed by an additional 3 min of vortexing. The supernatant was extracted once with phenol-chloroform and once with chloroform and then ethanol precipitated. The RNA was air dried and dissolved in H_2O to 3 to 5 μg/μl.

(i) Northern (RNA) blotting. Equal amounts of RNA (10 to 30 μg) were separated on 1% agarose-formaldehyde gels, blotted to GeneScreen (Dupont NEN), hybridized, and washed as recommended by the manufacturer. Hybridization was done simultaneously or sequentially to the following random-labeled probes: *XBP1* (2.0-kb PCR-generated fragment obtained by using oligonucleotide primers BL152 and BL153), *ACT1* (1,025-bp PCR-generated fragment obtained by using oligonucleotide primers BL159 and BL160), *STE20* (2,482-bp PCR-generated fragment obtained by using oligonucleotide primers (BL161 and BL162), *CLN1* (1,508-bp PCR-generated fragment obtained by using oligonucleotide primers BL163 and BL164), *CLN2* (Bd1941), and *CLN3* (Bd1852). Quantitation of the Northern blot data was performed with a PhosphorImager (Molecular Dynamics), normalizing to the *ACT1* expression.

(ii) Primer extension analysis. Fifty nanograms of RNA (strain BY2058) was hybridized for 4 h to 10 fmol of [γ-^{32}P]ATP end-labeled oligonucleotide BL165 in annealing buffer [400 mM NaCl, 50 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES; pH 6.5), 5 mM EDTA]. After ethanol precipitation, the RNA was reverse transcribed by using Superscript II reverse transcriptase (Gibco BRL) at 42°C for 1 h. The reaction was digested with RNase A and concentrated by ethanol precipitation in the presence of 10 μg of glycogen, and the reaction products were separated through a 5% polyacrylamide–8 M urea sequencing gel. A sequencing reaction performed in parallel with the same oligonucleotide (BL165) on pBD2018 template DNA was used to map the transcription start site.

Western blot analysis. Protein extracts were prepared by glass bead disruption as described previously (2), using radioimmunoprecipitation assay buffer (150 mM NaCl, 50 mM Tris [pH 7.5], 2 mM EDTA, 0.1% SDS, 1 Triton X-100, 50 mM NaF, 1 μg of leupeptin per ml, 1 μg of pepstatin per ml, 1 mM phenylmethylsulfonyl fluoride). Following electrophoresis of 20 to 40 μg of extract, the proteins were transferred to nitrocellulose (Micron Separations Inc.), and Western blot analysis was performed as described, using a polyclonal antibody against LexA at a dilution of 1:2,000. Enhanced chemiluminescence (Dupont-NEN) was used for detection of immune complexes.

RESULTS

Xbp1 has homology to Swi4/Mbp1. A computer search for proteins that contain homologies to the DNA-binding domain consensus derived from all of the Swi4/Mbp1 family of proteins revealed one novel ORF, YIL101C, on chromosome IX (ORF647) (5). The encoded protein shares, in its central domain, about 40% identity and 77% homology over 39 amino acid residues to the C-terminal half of the DNA-binding domain of the *S. cerevisiae* transcription factors Swi4 and Mbp1. The same degree of homology is found to Mbp1 from *Kluyveromyces lactis* and to the DNA-binding proteins Res1 and Res2 from *S. pombe* (Fig. 1). There was no obvious homology found between Swi4 and Xbp1 outside the DNA-binding domain.

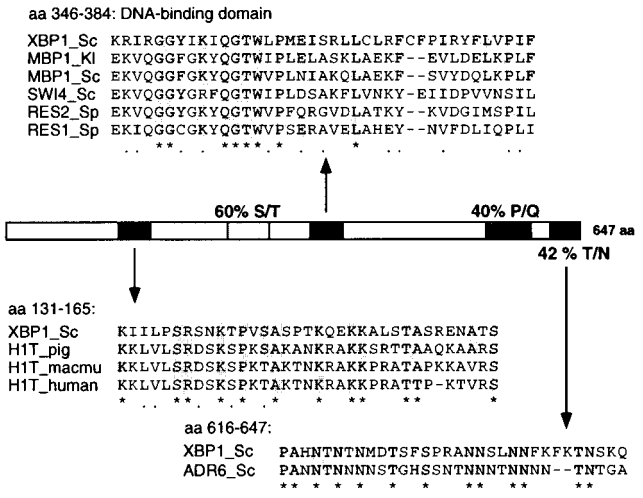

FIG. 1. Sequence similarities of Xbp1 to the Swi4/Mbp1 and histone H1 families. All amino acids which are identical to Xbp1 are shown in grey boxes. The black box depicts the potential DNA-binding domain; the grey and patterned boxes depict parts of Xbp1 which show special sequence features. The sequence searches were done using the BLAST program. ∗, identical in all proteins; •, similar in all proteins. Sc, *S. cerevisiae*; Kl, *K. lactis*; Sp, *S. pombe*; macmu, *M. mulatta.*

In addition, Xbp1 has a 35-residue region that is 40% identical to the conserved C-terminal domain of a family of testis-specific histone H1 proteins and several regions which are highly enriched with certain amino acids, 60% serine or threonine from aa 253 to 296, 40% proline or glutamine from aa 537 to 593, and 42% threonine/asparagine from aa 619 to the C terminus (Fig. 1). The latter region was found to be 42% identical to an N-terminal domain of the transcription regulatory protein Adr6/Swi1.

Determination of the Xbp1 binding site by oligonucleotide selection. The *XBP1* sequence suggests that Xbp1 is a sequence-specific DNA-binding protein. To examine this hypothesis and determine the putative Xbp1 binding site, we used a random oligonucleotide binding-selection strategy (43). Xbp1 was expressed as a fusion to six histidines or to GST in *E. coli* and purified by affinity chromatography. To decrease the influence of either tag on the site selection, we switched between these two differently tagged proteins in each round of binding and selection. The library of oligonucleotides, selected by up to four rounds of Xbp1 binding, was cloned in pBluescript, and 37 independent clones were sequenced. Alignment of the sequences by using the CONSENSUS program (18) allowed us to determine the Xbp1 DNA-binding consensus sequence [GCCTCGA(G/A)G(C/A)G(A/G)] (Fig. 2C). The central core bases, CTCGA, were the most conserved among all of the selected clones. Gel retardation experiments using different selected sites showed that the first C in this core is important for high-affinity binding to DNA (data not shown). More than one-third of the clones overlapped by 1 to 5 bp with the constant part of the oligonucleotide sequence, which explains the first appearance of binding sites after only two

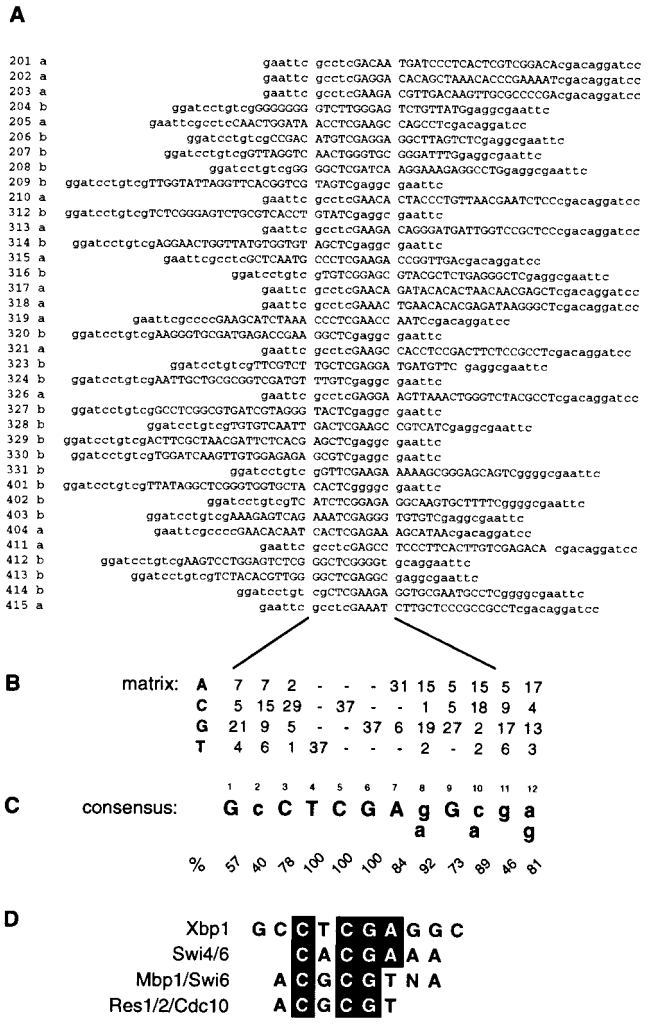

FIG. 2. Determination of the Xbp1 binding site. (A) DNA recovered after two, three, or four rounds of selection by Xbp1 was subcloned and sequenced. The sequences are aligned around the central consensus motif, with binding site clone numbers and orientation (a or b) to the left. Sequence derived from the constant part of the oligonucleotide library is shown in lowercase. (B) Computer-generated matrix of the Xbp1 consensus binding site. Each number indicates how many of the selected oligonucleotides carried that base at the indicated position. The bases of the consensus sequence are highlighted in grey. (C) Delineated Xbp1 consensus binding site. The number below is the percentage of selected oligonucleotides carrying the indicated base(s) at that position. (D) Comparison of the DNA-binding sites of the Swi4/Mbp1 family of transcription factors. Shown are the consensus binding sites of the *S. cerevisiae* Xbp1, Swi4/Swi6, and Mbp1/Swi6 and the *S. pombe* Res1/Res2/Cdc10 proteins, with bases identical to the DNA recognition site of Xbp1 depicted in black boxes.

rounds of selection and amplification. Since the Xbp1 DNA-binding site includes a central palindromic *Xho*I restriction site, the protein was named *Xho*I site-binding protein 1 (Xbp1).

Footprint analyses of Xbp1 binding sites. To verify the DNA-binding site of Xbp1, fragments derived from two of the selected sequences were incubated with bacterially expressed GST-Xbp1 or GST alone and analyzed in DNase I footprint experiments. The region which was protected from DNase I digestion by GST-Xbp1 covered the predicted Xbp1 binding site. The results showed a 20- to 24-bp footprint extending 3 to 5 bp 5′ and 7 to 9 bp 3′ of the predicted Xbp1 binding site (Fig. 3A). Additionally, the footprints on clone 315 showed a reproducible Xbp1-dependent DNase I-hypersensitive site, which is located in the constant part of the selected clone.

To define the bases in the Xbp1 binding sites that are in contact with the protein, we carried out DMS methylation interference assays using the same fragments and bacterially expressed His-tagged Xbp1. As shown in Fig. 3B, methylation of adenines and most of the guanines within the Xbp1 binding site interfered with Xbp1 binding, whereas methylation of a core guanine (position 6 of the consensus sequence) and a central adenine enhanced binding of Xbp1 to DNA. The footprint and methylation interference data are summarized in Fig. 3C. Both methods confirmed that Xbp1 binds the selected oligonucleotides in the region of the predicted consensus binding site.

DNA-binding properties of Xbp1. To confirm the significance of individual bases for Xbp1 binding, we synthesized oligonucleotides carrying the determined consensus sequence or mutations therein (Fig. 4B). Using gel retardation experiments and recombinant Xbp1, we showed that mutations of six

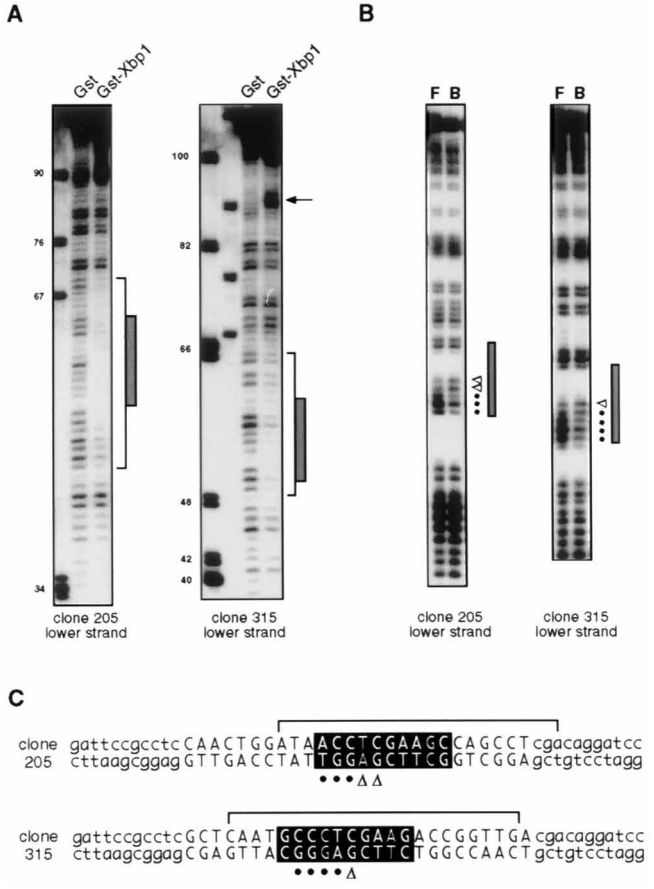

FIG. 3. Importance of specific bases for Xbp1 DNA binding. (A) DNase I footprint analysis with recombinant GST or GST-Xbp1 protein and two selected binding site clones (205 and 315). The DNase I-protected region (bracket) and position of the predicted Xbp1 binding site (grey box) are shown. The arrow refers to the observed DNase I-hypersensitive site. (B) DMS methylation interference footprint analysis using His-tagged Xbp1 and singly end labeled fragments from binding site clones 205 and 315. Dots mark interfering bases; triangles mark bases which increased binding when methylated. Grey boxes show positions of the predicted Xbp1 binding site. Each footprint shows reaction products obtained from free (F) or bound (B) DNA. (C) Combined data from panels A and B illustrated on a double-stranded representation of binding site clones 205 and 315. Each bracket indicates the extent of the DNase I footprint. Reverse-type boxes show positions of the predicted Xbp1 consensus binding site. The dots and triangles are as described for panel B.

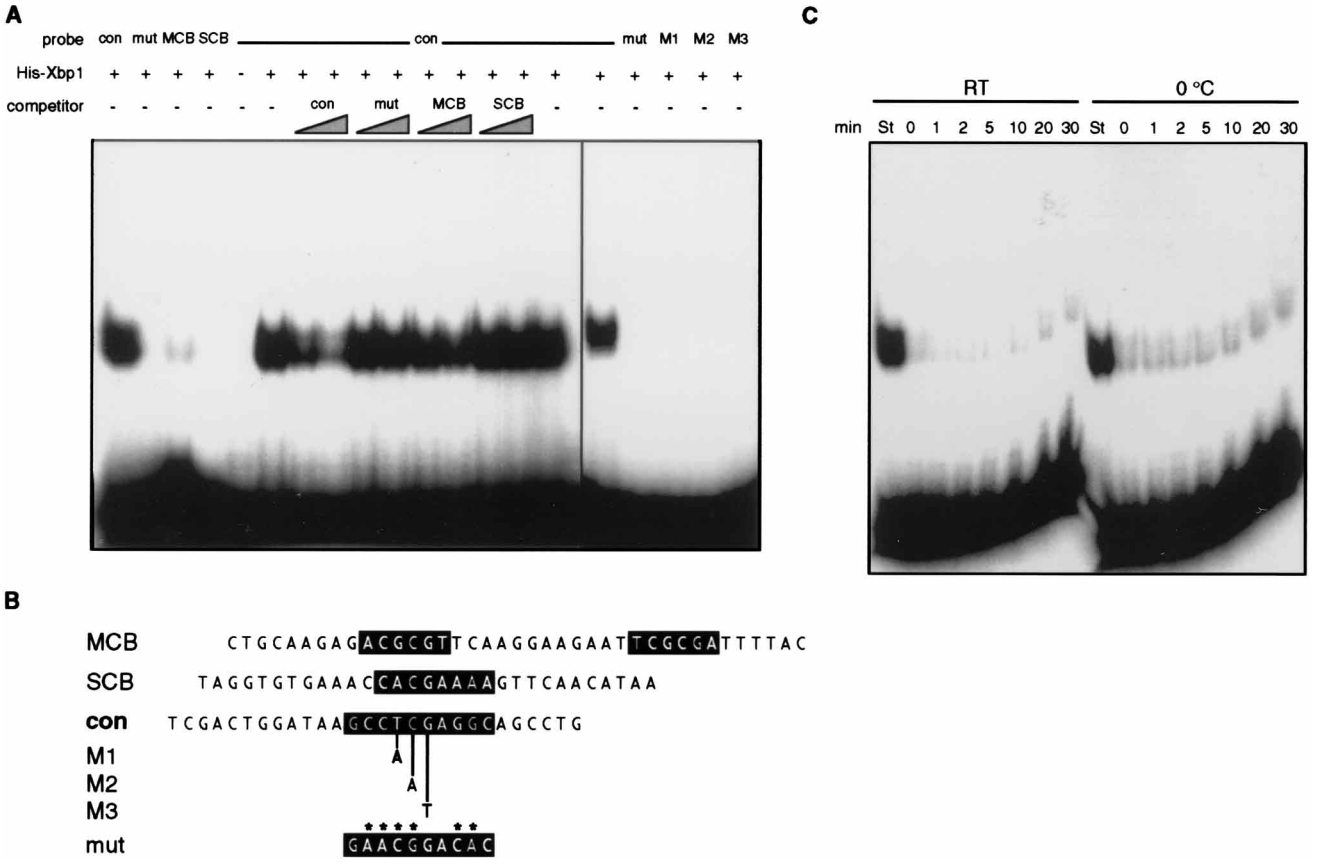

FIG. 4. DNA-binding properties of Xbp1. (A) Gel retardation experiments using recombinant His-tagged Xbp1 and oligonucleotides shown in panel B as probes or competitor DNA. (B) Upper strands (5′ to 3′) of the oligonucleotides used for panel A. For the single-base mutants (M1, M2, and M3), only the altered base is shown. Asterisks mark the six bases changed in the mut (mutant) oligonucleotide. Black boxes in the MCB and SCB oligonucleotides depict the defined binding sites for Mbp1/Swi6 (MCB) and Swi4/Swi6 (SCB). (C) Determination of the off rate of Xbp1. A binding reaction containing the consensus oligonucleotide (con) as probe and recombinant His-tagged Xbp1 was incubated for 10 min and then challenged with cold con oligonucleotide in 200-fold excess. Aliquots were loaded on a band shift gel before (St) and after the addition of competitor at indicated times (0 to 30 min). RT, room temperature.

bases of the consensus binding site or single base mutations of any of the three 100% conserved core bases (TCG) completely abolished binding (Fig. 4A). The specificity of Xbp1 binding to DNA was also shown by using known binding sites for Swi4/Swi6 (SCB) (6) and Mbp1/Swi6 (MCB) (22) in competition assays and as labeled probes. We observed that Xbp1 is able to bind to MCBs, although with a very low efficiency compared to its binding to the consensus sequence. There was no detectable binding to SCBs (Fig. 4A), although some of the bases are common between SCBs and the Xbp1 consensus binding site (Fig. 2).

The weak competition, which was obtained even when the optimal consensus binding site was used, may be explained by a high dynamic exchange rate of Xbp1. Indeed the off rate of Xbp1, as determined by gel retardation, is in the range of seconds (Fig. 4C). The affinity constant of Xbp1 binding to the consensus site was determined to be $3.5 \times 10^8\ M^{-1}$ (see Materials and Methods) (data not shown).

Potential Xbp1 binding sites in the *S. cerevisiae* genome. The promoter of the G1 cyclin gene *CLN1* contains two sequences, 12 bp apart, which include all the crucial core bases of the Xbp1 binding site (Fig. 5B). Using an oligonucleotide (C1) derived from this promoter element as probe or competitor DNA in gel retardation experiments, we showed that recombinant Xbp1 is able to bind to these sequences with the same affinity and specificity as to the Xbp1 consensus binding site (Fig. 5A).

Having defined the binding site of Xbp1, we searched the yeast genome for potential Xbp1 binding sites positioned in promoter sequences (up to 1,100 bp upstream from the ATG). The *Saccharomyces* genome database was searched with the Xbp1 consensus binding sequence and the known binding site from the *CLN1* promoter. Table 2 presents a list of *S. cerevisiae* promoters containing potential binding sites whose sequences are ≥75% identical to that of the selected Xbp1 consensus binding site (top) and promoters which carry ≥83% identical sequences to the Xbp1 binding site from the *CLN1* promoter (bottom). The list includes several transcription factors involved in replication and proteins involved in responses to environmental changes.

Xbp1 functions as a transcriptional repressor. To assess the potential transcriptional activity of Xbp1, we constructed a gene fusion between Xbp1 and LexA, a bacterial DNA-binding protein (7). Together with control protein fusions (LexA-lamin, LexA, LexA-Bicoid, and LexA-Gal4), this construct was assayed for the ability to activate transcription of a *lacZ* reporter gene, in which eight *lexA* operator sequences replace the UAS in a minimal *GAL1* promoter (56). The fusion proteins are expressed, though they appear to be less stable than LexA alone (Fig. 6B). Transcriptional activation was assayed

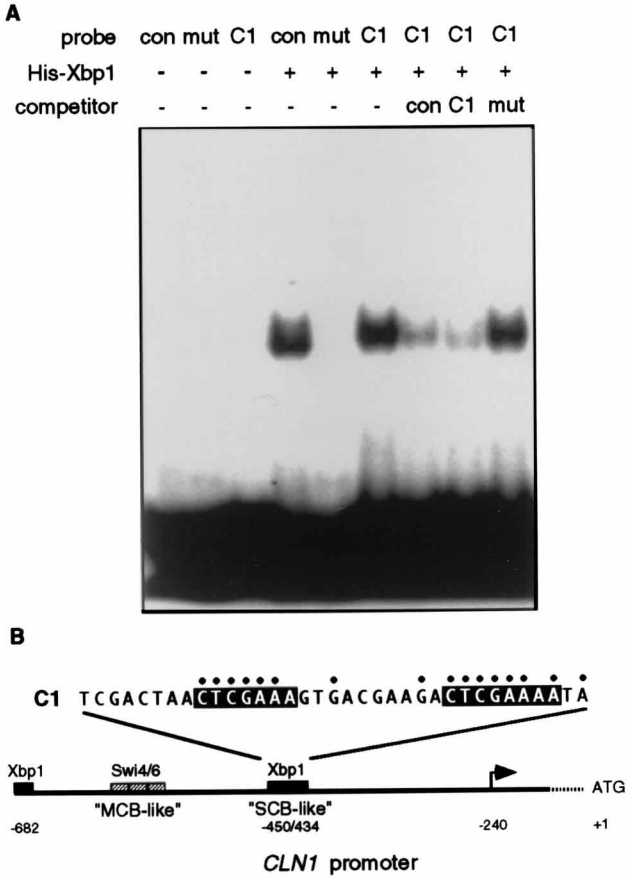

FIG. 5. Xbp1 binds specifically to sequences in the *CLN1* promoter. (A) Gel retardation experiments using recombinant His-tagged Xbp1 and oligonucleotides corresponding to wild-type *CLN1* (C1; B), the consensus Xbp1 binding site (con), or the six-base substitution of it (mut) shown in Fig. 4B as probes or competitor DNA. (B) Schematic representation of the *CLN1* promoter with the binding sites recognized by Swi4/Swi6 (MCBs) and Xbp1. The arrow marks the transcription start site (39). The upper strand of the oligonucleotide C1 derived from the SCB-like sequences (black box) is shown with dots marking bases identical to the Xbp1 consensus binding site.

on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal)-containing plates and in quantitative measurements of the β-galactosidase activity. As shown in Fig. 6A, there is no detectable activation of the reporter gene under conditions where LexA-Gal4 fusions show high activity and both fusions are expressed at about the same level (Fig. 6B).

Since Xbp1 is not a transcriptional activator, we used the LexA-Xbp1 fusion and Xbp1 deletions fused to LexA to see if Xbp1 has properties of a transcriptional repressor. To test this hypothesis, we used a set of *CYC1-lacZ* reporter gene constructs with single or multiple *lexA* binding sites introduced upstream or downstream of the *CYC* UAS (15, 20). These plasmids, as well as plasmids encoding LexA or LexA-Xbp1 fusion proteins, were transformed into cells, and β-galactosidase activity was assayed. In all cases, we observed repression by the LexA-Xbp1 fusions (Fig. 6D). LexA-Xbp1 can repress over threefold from four *lexA* operators upstream of the UAS and nearly fivefold from a single *lexA* operator positioned between the UAS and TATA box of the reporter gene. In contrast, the LexA protein itself gives no repression from upstream of the UAS and only 1.5-fold repression when positioned between the UAS and TATA box, which is comparable to the level of repression observed with the insertion of the *lexA* operator in the absence of any LexA protein (20) (Fig. 6D). Interestingly, all of the LexA-Xbp1 fusion proteins show an expression level significantly lower than that of LexA alone (Fig. 6C). The results shown in Fig. 6 show that Xbp1 can indeed act as a transcriptional repressor when brought to the DNA by fusion with LexA.

Activation of the *XBP1* promoter by several types of stress. Primer extension analysis showed one major and one minor transcriptional start site of the *XBP1* mRNA (Fig. 7A), 38 and 30 bp downstream of a sequence resembling a consensus TATA box. This finding suggests that the promoter of *XBP1* is within the 600-bp region between *XBP1* and *SGA1* (encoding sporulation-specific glucoamylase 1), which is transcribed in the opposite direction. Using a transcription factor database (TRANSFAC), we searched the promoter sequence for poten-

TABLE 2. Identification of promoters with potential Xbp1 binding sites[a]

Position[b]	Sequence	Gene product, function
	GCCTCGAGGcga A a g	Consensus binding site
−1058	GCCTCGAGGgct	CCL1, cyclin C (component of transcription factor IIH)
−1014	GCCTCGAGGAcA	YVH1, nitrogen starvation-induced protein phosphatase
−78	GCCTCGAGGtcA	STV1, vacuolar proton pumping ATPase
−79	GCCTCGAGGAac	NAT1, N-terminal acetyltransferase
−298	GCCTCGAGGAcA	ACH1, acetyl coenzyme A hydrolase
−498	GCCTCGAGGgtt	APM4, clathrin-associated protein
−414	GCCTCGAAGCcA	FHL1, fork head DNA-binding transcription factor
−489	GCCTCGAAGAGt	RRN6, component of CF (ribosomal DNA transcription factor)
−599	GCCTCGAAGtct	SSB1, single-stranded nucleic acid-binding protein
−138	GCCTCGAAGgcA	FAB1, phosphatidylinositol kinase
−465	GCCTCGAAGAcA	CCE1, cruciform cutting endonuclease
−194	GCCTCGAAGCGA	RIB3, DBP synthase (riboflavin biosynthesis)
−202	GCCTCGAAGCcc	GND1, phosphogluconate dehydrogenase
−494	GCCTCGAAGgaG	ILV3, dihydroxy-acid dehydratase
−600	GCCTCGAAGgcc	SEC9, t-SNARE of plasma membrane
−196	GCCTCGAAGttt	LIP5, lipoic acid synthase
−83	GCCTCGAAGtat	ADE6, phosphoribosylformyl glycinamide synthase
−146	GCCTCGAAGgac	VAP1, amino acid permease
−434	GaCTCGAAaAtt	CLN1, G_1 cyclin
−58	GaCTCGAAaAaA	HOG1, MAP kinase
−456	GaCTCGAAaAtG	REB1, RNA polymerase I enhancer-binding protein
−44	GaCTCGAAaAGA	STE50, required for activation of conjugation
−450	aaCTCGAAaGtG	CLN1, G_1 cyclin
−130	aaCTCGAAaGat	MCM3, acts at autonomous replication initiation site
−192	aaCTCGAAaGat	DOA4, ubiquitin isopeptidase
−594	aaCTCGAAaGaA	YAR1, ankyrin repeat protein, required for normal growth at low temp
−265	aaCTCGAAaGaA	HSP82, heat shock protein

[a] Shown are selected *S. cerevisiae* promoter sequences retrieved by a computer search of the *Saccharomyces* genome database by using the FASTA program. All sequences at the top are at least in 9 of 12 bases identical to the selected Xbp1 consensus binding site. The bottom part contains sequences that are at least in 10 of 12 bases identical to the *CLN1* promoter binding sites of Xbp1. Bases identical to the consensus binding site are uppercase; differing bases are lowercase.

[b] Position relative to the ATG.

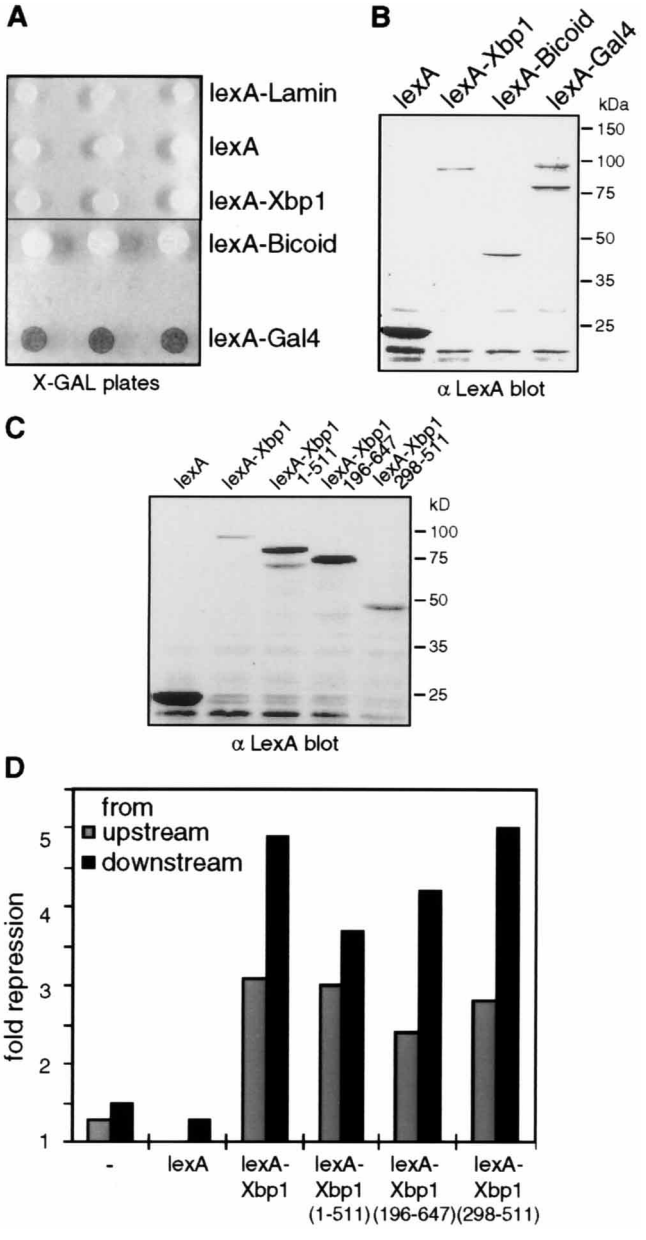

FIG. 6. Xbp1 acts as a transcriptional repressor. (A) Strain L40 [(*lexAop*)$_8$-*lacZ* integrated at *URA3*] was transformed with plasmids expressing the different LexA fusion proteins under the control of the *ADH1* promoter. Three each of the transformants were spotted on appropriate selection plates containing X-Gal to detect β-galactosidase activity. (B) Anti-LexA (α LexA) Western blot analysis using 30 μg of extracts prepared from the constructed strains shown in panel A. The bands below LexA are nonspecific. (C) Anti-LexA Western blot analysis using 30 μg of extracts prepared from the strains shown in panel D. (D) BY2059 (*MATα xbp1::HIS3*) was transformed with the reporter construct pBD2086, pBD2087, or pBD2089 (see Materials and Methods) and with a plasmid expressing none or one of the shown LexA fusion proteins under the control of the *ADH1* promoter. From each combination, at least three independent transformants were taken, and the β-galactosidase activity was determined in triplicate. We calculated the activity ratios pBD2086 (UAS-*lacZ*)/pBD2087 [(4×*lexA*)-UAS-*lacZ*] as repression from upstream of the UAS (grey bars) and pBD2086 (UAS-*lacZ*)/pBD2089 (UAS-*lexA-lacZ*) as repression from downstream of the UAS (black bars). The repression from downstream incorporates steric hindrance effects due to binding of LexA proteins between the UAS and the TATA box. Shown is the averaged fold repression.

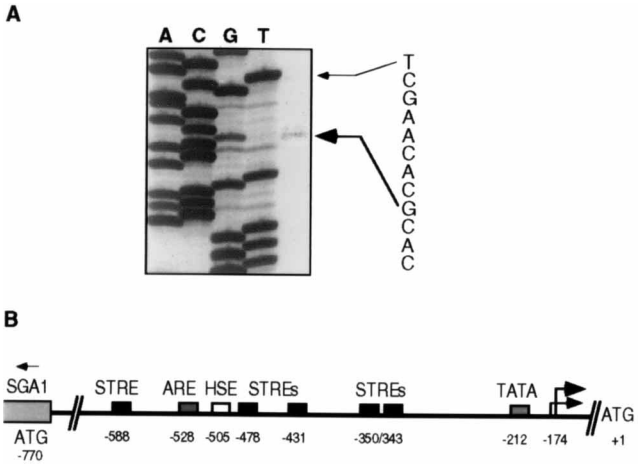

FIG. 7. Structure of the *XBP1* promoter. (A) Primer extension analysis showing one prominent and one minor transcription start site. A sequence reaction done with the same oligonucleotide is shown in the middle. (B) Schematic representation of the *XBP1* promoter with proportional positioning of putative regulation elements ARE, AP1 recognition element; TATA, TATA box). *SGA1* (encoding sporulation-specific glucoamylase 1) is the gene upstream of *XBP1*.

tial binding sites of regulating transcription factors. Interestingly, the search revealed a cluster of binding sites for stress-regulated transcription factors within a 245-bp region (Fig. 7B). There are five stress response elements (STREs) (62), known to be bound and regulated by Msn2 and Msn4 (49, 32), one AP-1 recognition element (23), regulated by yeast AP-1 (yAP-1) and yAP-2 (17), and one heat shock element (HSE) (41), which is the target of heat shock transcription factor (61).

To determine whether *XBP1* expression is stress induced, we isolated RNA from cells exposed to several stress conditions and monitored the expression of *XBP1* by Northern blot hybridization. As shown in Fig. 8A, *XBP1* is almost undetectable in untreated cells but is strongly induced by heat (39°C) or osmotic shock (0.4 M sorbitol or 0.3 M NaCl). *XBP1* is also strongly induced under DNA-damaging conditions (0.01 to 0.1% MMS), whereas exposure of cells to heavy metals (5 μM $CdCl_2$) only weakly induces *XBP1*. Since the transcription activator yAP-1, which binds the ARE, has been shown to be inducible by oxidative stress, we also treated cells with diamide and hydrogen peroxide (59) and found that *XBP1* is greatly induced by addition of diamide (1.5 mM) and moderately induced by 0.3 mM H_2O_2. Nutrient starvation conditions, which are also known to affect the activity of STREs (31) and heat shock factor (53), were tested by shifting cells from 2 to 0.05% glucose. As expected, *XBP1* was strongly induced under this condition as well. Whereas heat and osmotic shocks result in transient induction, glucose starvation and oxidative stress conditions result in a sustained high expression level of *XBP1* for 40 to 180 min (Fig. 8A). Glucose starvation and oxidative stress by diamide lead to the most sustained and highest inductions of *XBP1*; MMS treatment and osmotic stress by sorbitol or NaCl both result in about a four- to sixfold induction of *XBP1* (Fig. 8B). These data make it likely that the stress-regulated promoter elements found in the *XBP1* promoter are active and control the rate of transcription of this gene.

Since Xbp1 binds in vitro to elements in the *CLN1* promoter and functions as a transcriptional repressor, one would predict that the expression levels of *CLN1* in stressed cells would be affected by *XBP1* disruption. To test this hypothesis, *xbp1* cells were exposed to the stress conditions described above and assayed for the expression of *CLN1* (Fig. 8A). Although the

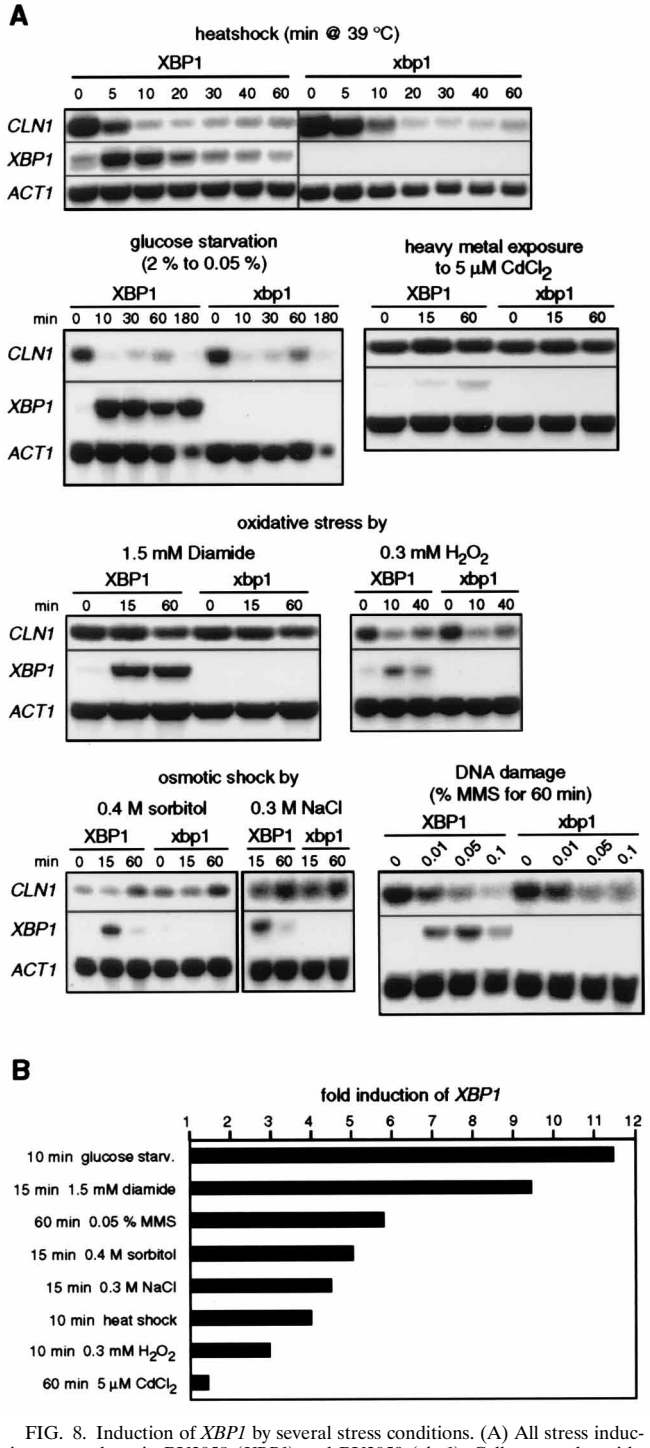

FIG. 8. Induction of *XBP1* by several stress conditions. (A) All stress inductions were done in BY2058 (*XBP1*) and BY2059 (*xbp1*). Cells grown logarithmically in YPD were subjected to the following stress conditions: 39°C heat shock, 1.5 mM diamide or 0.3 mM H_2O_2 oxidative stress, 0.4 M sorbitol or 0.3 M NaCl osmotic shock, DNA damage with 0.01, 0.05, or 0.1% MMS, 2 to 0.05% glucose starvation, and 5 μM $CaCl_2$ heavy-metal exposure. Samples were taken before exposure to stress (0 min) and at the indicated time points thereafter, and RNA was prepared. The expression of *XBP1* and *CLN1* was analyzed by Northern blotting. The RNAs were hybridized simultaneously or sequentially with an *ACT1* probe to verify equal loading. (B) Quantitative evaluation of band intensities shown in panel A was carried out with a PhosphorImager (Molecular Dynamics). *XBP1* expression was normalized to the *ACT1* level. Shown is the maximal fold induction in each stress condition compared to the *XBP1* expression before application of the stress.

induction of *XBP1* correlates with the repression of *CLN1* seen in most stress conditions, Xbp1 is not required for this repression, because the *CLN1* expression levels show no difference between a strain bearing a deletion of *XBP1* and an isogenic wild-type strain (Fig. 8A). Moreover, *CLN1* is not repressed by exposing cells to heavy metals or diamide, the latter being a condition which strongly induces *XBP1*.

Overexpression of *XBP1* leads to slow growth and represses G_1 cyclin expression. Strains carrying a deletion of the *XBP1* gene are viable and show no changes in growth rate, and they have no detectable differences in growth at high or low temperatures or on high-osmolarity medium. There was also no detectable change in cell morphology, DNA content, or cell volume compared to wild-type cells (data not shown). However, when overexpression of *XBP1* was induced in cells carrying the *XBP1* gene under the control of a galactose-inducible promoter, we observed a slow-growth phenotype (Fig. 9A). This was accompanied by an increase in the population of cells in G_1, as judged by FACS analysis (45), and a reproducible 1.5-fold increase in cell volume compared to control cells or cells grown under noninducing conditions (Fig. 9C). The galactose-induced *XBP1* gene is transcribed at a higher level than under the stress conditions that we have tested. Thus, it seemed likely that its target genes would be repressed under these conditions. To test this hypothesis, we analyzed the expression of *CLN1*, whose promoter is bound in vitro by Xbp1 (Fig. 5A), and found a 2.5-fold decrease in its transcription. However, we also observe a similar decrease in *CLN2* and *CLN3* mRNA levels in these cells. As shown in Fig. 9D, there is a severalfold decrease in the transcript levels of all three G_1 cyclins 10 to 20 min after these *pGAL-XBP1* cells are shifted from noninducing to inducing medium, which is not seen in cells carrying the vector alone. The repression of the G_1 cyclins and the induction of the expression of XBP1 correlate very well, but the mechanism of this repression is unclear because *CLN2* has no Xbp1 binding sites in its promoter and *CLN3* has only one potential Xbp1 binding site 415 bp upstream of its ATG.

DISCUSSION

The Swi4/Mbp1 family of heteromeric yeast transcription factors are important for cell cycle-regulated transcription and bind to SCB or MCB elements in promoters of target genes (5). The highly specific DNA-binding subunits, Swi4 and Mbp1 from *S. cerevisiae*, have Swi6 as a common regulatory protein and are evolutionarily conserved in *S. pombe*, with Res1 and Res2 being the DNA-binding partners of Cdc10 (5).

The data shown in this report define Xbp1 as a new member of the Swi4/Mbp1 family of transcription factors. A total of 12 proteins which contain this DNA-binding domain have been identified (5), but interestingly they are all fungal proteins. The 72-kDa Xbp1 protein was found by homology of its central domain to the conserved residues within the DNA-binding domains of Swi4, Mbp1, Res1, and Res2 (5). The DNA-binding domains of these proteins are known to be sufficient for DNA binding and are positioned at the N terminus of each of these proteins (8, 11, 44). Xbp1 differs in that the putative DNA-binding domain is centrally located and contains only the C-terminal half of the DNA-binding domain homology. This central location of the DNA-binding domain is similar to that in the more distantly related proteins Sok2 and Phd1 from *S. cerevisiae* and StuA from *A. nidulans* (14, 34, 57). These proteins are implicated in the regulation of developmental processes like pseudohyphal growth in *S. cerevisiae* or the development of conidiophores in *A. nidulans.*

Since there is no sequence similarity between Xbp1 and the

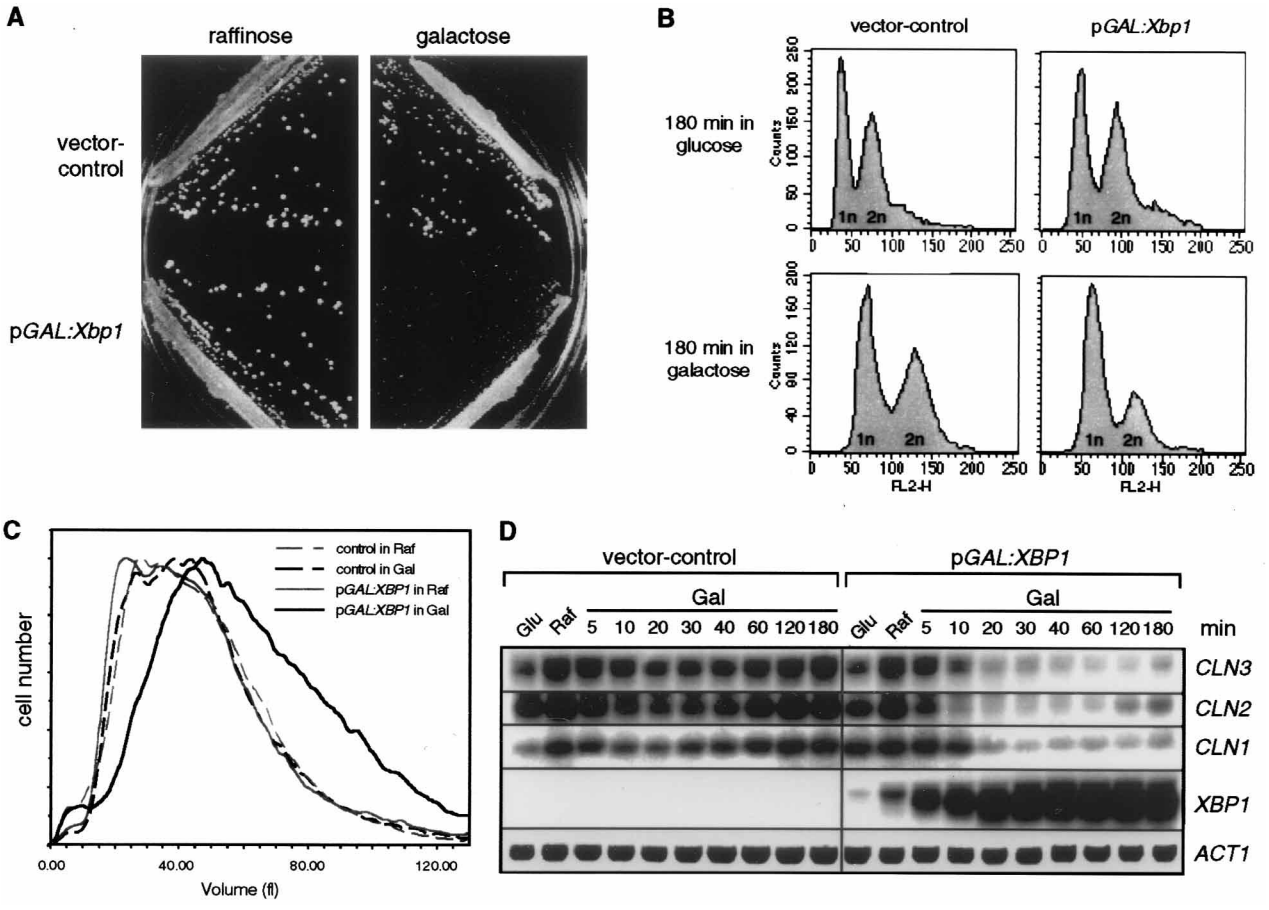

FIG. 9. Overexpression of *XBP1* leads to slow growth and represses G_1 cyclin expression. (A) Strain BY2059 (*xbp1*) was transformed with pBD2014 (p*GAL:XBP1*) or the empty expression vector and plated to single colonies on selection plates containing 2% raffinose or 2% galactose as carbon source. Plates were incubated for 3 days at 30°C. (B) Strains used for panel A were grown in 2% raffinose-containing medium and shifted to 2% glucose or 2% galactose. After 180 min, the DNA content was measured by FACS analysis and analyzed with the CellQuest program. Peaks representing cells with 1n and 2n DNA content are labeled. (C) The same cultures were used to determine the cell volume by using a Coulter Counter. Shown are representative profiles obtained after 180 min of growth in 2% raffinose- or 2% galactose-containing medium. (D) Strains used for panel A were grown in 2% raffinose-containing medium and shifted for 180 min to 2% glucose or 2% galactose. Samples were taken at indicated time points, and RNA was prepared. Lane Raf shows the expression level of the starting culture. The expression of *XBP1*, *CLN1*, *CLN2*, *CLN3*, and *ACT1* was analyzed by Northern blot hybridization. The *ACT1* probe served as a loading control.

Swi4/Mbp1 family outside the putative DNA-binding domain, it appears that a common DNA-binding motif has been combined with various functional domains to create different transcription factors. The ankyrin repeats located in the central domain of several of the Swi4/Mbp1 family members are also not found in Xbp1, Phd1, Sok2, and StuA, and there is no evidence that family members lacking this ankyrin repeat domain need partners to bind DNA (14, 34, 57).

Xbp1 binds to a sequence similar to SCBs and MCBs. In addition to identifying the homology between the Swi4, Mbp1, and Xbp1 proteins, we have found that they bind to a related DNA sequence. The Xbp1 recognition sequence is identical to the binding sites of Swi4/Swi6, Mbp1/Swi6, and Res1/Res2/Cdc10 at positions 3, 5, and 6 (Fig. 2) and can have up to six positions identical to the Swi4/Swi6 binding site. However, the Xbp1 binding site clearly differs from both at position 4, and the consensus MCB and SCB elements differ from each other at this position as well. Thus, this fourth position may be the critical base for distinguishing the binding sites of these proteins. Consistent with this, there is no detectable binding of Xbp1 to SCB elements and only very weak binding to MCB elements. The binding specificity of Swi4/Swi6 and Mbp1/Swi6 has not been analyzed in detail (5). Interestingly, the SCB-like sequences originally proposed to be the cell cycle-regulating elements in *CLN1* (39) are the sites to which Xbp1 binds. These sites are not UAS elements. Rather, it has been shown that Swi4/Swi6 binds to a sequence in *CLN1* which is more closely related to an MCB element (42). This noncanonical Swi4 site indicates that Swi4 may not discriminate between A and G at the fourth position and may be functionally redundant with Mbp1 in some contexts. In contrast, Xbp1 shows a marked preference for T at this position.

Recently the Mbp1 DNA-binding domain has been crystallized and the structure has been solved (64). The part of this structure that is homologous to Xbp1 forms a β-hairpin structure, which lies together with the N-terminal HTH motif on one surface of the molecule. The β-hairpin structure contributes to the formation of a basic channel thought to be involved in binding to DNA, but the residues contacting DNA are not known (64). A secondary structure prediction (10) of this region of Xbp1 shows no HTH motif but does predict the equivalents of all four β-sheets (β1 to β4) that are C terminal to the HTH motif (data not shown). The structural elements of the second half of the Mbp1 DNA-binding domain, β sheets β5 and β6 and helix C, are also conserved in Xbp1 (64). Since the DNA-binding sequence of Xbp1 is related to that of Swi4 and

Mbp1, we predict that most of the sequence-specific contacts made by these proteins are made through the C-terminal half of the DNA-binding domain, the part which is conserved between Xbp1 and the Swi4/Mbp1 family.

Xbp1 also contains a patch of homology to the C-terminal domain of a family of testis-specific histone H1 proteins. This region may contribute to the DNA-binding activity of Xbp1 and could possibly compensate for the missing HTH motif found in the other family members, since this carboxy-terminal domain of the histone H1 variants is sufficient to bind DNA and efficiently condense chromatin (21, 58). This latter property may also provide a clue to the mechanism by which Xbp1 represses transcription.

***XBP1*'s transcription is regulated by stress.** The *XBP1* promoter sequence contains several promoter elements that are known to be induced by different kinds of stress (reviewed in references 30 and 47). Indeed, *XBP1* is transcribed at very low levels in cells growing under normal conditions and is strongly induced by many forms of stress. *XBP1*'s prolonged induction by oxidative stress and glucose starvation conditions and its more transient induction by heat shock and osmotic changes are typical of other known stress-induced genes, like *CUP1*, *HSP12*, *HSP26*, *CTT1*, and *DDR2* (32, 50, 53). However, the expression of *XBP1* is not activated by heavy-metal ions, probably due to the existence of only one HSE, which can mediate gene activation by heavy metals (48), and the lack of a *CUP1* UAS (53).

Some progress has been made in understanding the mechanisms of stress-activated transcription, but little is known about stress-induced repression of transcription. Heat shock causes a conformational change in Hsf1, which enables it to bind HSEs (30). Oxidative stress relocalizes yAP-1 from the cytoplasm to the nucleus and leads to the induction of its target genes (24). When there is an increase in the extracellular osmolarity, the Sln1 and Sho1 osmosensors elicit the activation of the Pbs2/Hog1-MAP kinase pathway. Hog1 then activates the STRE-binding proteins Msn2 and Msn4 by an unknown mechanism, and induction of the STRE-controlled genes ensues (28, 29, 32, 40, 50). The three STREs in the *XBP1* promoter make it a likely target gene for this stress-regulated pathway.

Evidence that Xbp1 is a transcriptional repressor. Unlike the other members of the Swi4/Mbp1 family, which are activators, Xbp1 is most likely to act as a transcriptional repressor. The LexA-Xbp1 fusion, which is expressed at a very modest level, evokes a three- to fivefold repression of transcription. This is quantitatively similar to other well-characterized repressors, i.e., Tup1 (20), Ume6 (19), and Hir1 and Hir2 (52). In addition, when Xbp1 is overproduced, we see repression of two genes, *CLN1* and *CLN3*, that have Xbp1 binding sites in their promoters. However, *CLN2* is also repressed, and its promoter does not contain an obvious Xbp1 binding site. Moreover, repression of *CLN* transcription still occurs in an *xbp1* deletion strain when it is subjected to stress. Therefore, Xbp1 cannot be the only stress-induced repressor of transcription, and in some cases its repression may be indirect.

So far, all of the stress-regulated transcription factors that have been studied are activators (30), and some of their target genes are directly involved in protecting the cell from damaging conditions (47). However, most genes are turned off by heat shock (35), and this is likely to be the case for other stress conditions as well, especially for those gene products that drive progression through the cell cycle. Our finding that Xbp1 can repress transcription suggests that Xbp1 may be the first known example of a stress-induced transcriptional repressor. However, the fact that yeast cells with the *XBP1* gene deleted are viable under stress conditions and can still repress *CLN1* transcription indicates that there must be other factors with similar activity. Thus, we anticipate that Xbp1 may be one of many proteins responsible for actively repressing these genes when conditions are inadequate. It will be interesting to determine the mechanism of this repression and how global these effects are.

Very little is known about the effects of stress on growth control. The expression of the G_1 cyclin genes *CLN1* and *CLN2* is repressed when cells are heat shocked, and this results in a transient cell cycle inhibition, which is not dependent on the transcriptional regulation by Swi4 or the cAMP control of PKA (46). Starvation, which is also considered to be a stress situation, arrests growth and cells enter a nonproliferating stationary phase (60). The availability of nutrients is sensed by the Ras/cAMP/PKA pathway, and increased cAMP levels inhibit the G_1/S transition by repressing the expression of the G_1 cyclin genes *CLN1* and *CLN2*, but the molecular basis for this regulation is not understood (3, 55). G_1 cyclins are rate limiting for the transition to S phase (16, 37), and reduced transcription of these cyclins increases the critical cell size required for the transition to S phase, whereas increased transcription reduces the cell size (55). Interestingly, overexpression of *XBP1*, which may mimic some aspects of a strong and sustained stress, slows growth, lengthens the G_1 interval, and increases cell size (Fig. 9). This may be a result of the correlated reduction in the mRNA levels of *CLN1*, *CLN2*, and *CLN3* (Fig. 9), since these parameters are known to be controlled by the expression levels of the G_1 cyclins (16, 37). It will be of interest to delineate in more detail the relationship between Xbp1 and the expression of the G_1 cyclins and to define additional target genes of Xbp1. In either case, it seems likely that further characterization of *XBP1*'s function will contribute to the identification and characterization of stress-regulated genes and processes which link environmental stress with growth control.

ACKNOWLEDGMENTS

We thank M. A. Osley for plasmids and R. N. Eisenman for site selection oligonucleotides. We also thank G. Stormo for searching the cloned binding sites with the CONSENSUS program. Special thanks are offered to Ingrid Wolf and Christoph Sachsenmeier for critical reading of the manuscript and for numerous constructive suggestions.

This work was supported by NIH grant GM41073 to L.B. and by a Deutsche Forschungsgemeinschaft postdoctoral fellowship to B.M.

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