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The Promoter Region of the Yeast *KAR2* (BiP) Gene Contains a Regulatory Domain That Responds to the Presence of Unfolded Proteins in the Endoplasmic Reticulum

KENJI KOHNO,[1]† KARL NORMINGTON,[1] JOSEPH SAMBROOK,[1] MARY-JANE GETHING,[1,2]* AND KAZUTOSHI MORI[1]

Department of Biochemistry[1] and Howard Hughes Medical Institute,[2] University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75235

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The endoplasmic reticulum (ER) of eukaryotic cells contains an abundant 78,000-Da protein (BiP) that is involved in the translocation, folding, and assembly of secretory and transmembrane proteins. In the yeast *Saccharomyces cerevisiae*, as in mammalian cells, BiP mRNA is synthesized at a high basal rate and is further induced by the presence of increased amounts of unfolded proteins in the ER. However, unlike mammalian BiP, yeast BiP is also induced severalfold by heat shock, albeit in a transient fashion. To identify the regulatory sequences that respond to these stimuli in the yeast *KAR2* gene that encodes BiP, we have cloned a 1.3-kb segment of DNA from the region upstream of the sequences coding for BiP and fused it to a reporter gene, the *Escherichia coli* β-galactosidase gene. Analysis of a series of progressive 5′ truncations as well as internal deletions of the upstream sequence showed that the information required for accurate transcriptional regulation of the *KAR2* gene in *S. cerevisiae* is contained within a ~230-bp *Xho*I-*Dra*I fragment (nucleotides −245 to −9) and that this fragment contains at least two *cis*-acting elements, one (heat shock element [HSE]) responding to heat shock and the other (unfolded protein response element [UPR]) responding to the presence of unfolded proteins in the ER. The HSE and UPR elements are functionally independent of each other but work additively for maximum induction of the yeast *KAR2* gene. Lying between these two elements is a GC-rich region that is similar in sequence to the consensus element for binding of the mammalian transcription factor Sp1 and that is involved in the basal expression of the *KAR2* gene. Finally, we provide evidence suggesting that yeast cells monitor the concentration of free BiP in the ER and adjust the level of transcription of the *KAR2* gene accordingly; this effect is mediated via the UPR element in the *KAR2* promoter.

The endoplasmic reticulum (ER) is not only the portal to the exocytotic pathway but also the organelle in which newly synthesized secretory and transmembrane proteins are folded and assembled into their correct tertiary and quaternary structures. These processes are prerequisites for onward transport of proteins to the Golgi apparatus and other distal organelles of the secretory pathway (for reviews, see references 17, 26, and 51). Unassembled or underglycosylated proteins or mutant polypeptides that fail to fold into the correct conformation are retained in the ER for extended periods of time, often in association with a 78-kDa protein (BiP) that is an abundant component of the organelle (4, 10, 16, 21, 25, 35). BiP is the only member of the stress-70 (hsp70) family of proteins that is located in the ER (for a review, see reference 17). In mammalian cells, this protein was originally described independently as the glucose-regulated protein GRP78 (49, 58) and as the immunoglobulin heavy-chain-binding protein that tenaciously binds heavy chains in the ER of lymphocytes or hybridoma cells that lack immunoglobulin light chains (21, 23, 38). However BiP is now known to associate transiently with a wide range of newly synthesized wild-type secretory and transmembrane proteins (2, 4, 10, 40), and it is thought that BiP promotes productive folding and assembly of nascent polypeptides by stabilizing unfolded or partially folded structures and preventing the formation of inappropriate intra- or interchain interactions (reviewed in reference 17).

Exposure to a variety of environmental and physiological stresses causes mammalian cells to synthesize increased quantities of mRNA encoding BiP (reviewed in reference 32). The augmentation of BiP mRNA is rapid and results from a surge in transcription of the gene. Among the types of stress that lead to activation of the BiP gene are glucose starvation, inhibition of glycosylation with drugs such as tunicamycin, disturbance of intracellular stores of Ca^{2+}, treatment with amino acid analogs, and exposure to heavy metals (32). Because these insults have pleiotropic effects on cellular metabolism, it is not easily possible to pinpoint the specific event that triggers the synthesis of BiP mRNA. However, expression of mutant proteins that are unable to fold correctly in the ER leads to a rapid induction of BiP mRNA in the absence of any other form of stress (30). On the basis of this and other experiments, Kozutsumi et al. (30) proposed that the proximal signal for induction of synthesis of BiP in mammalian cells is the accumulation of unfolded proteins in the ER and that this accumulation is the common result of the various forms of stress listed above. A similar situation obtains in the yeast *Saccharomyces cerevisiae*, in which the BiP protein is the product of the *KAR2* gene (43, 44, 52). The presence of unfolded proteins in the ER induces the synthesis of BiP but not of cytosolic members of the stress-70 family; conversely, the presence of secretory precursors in the cytoplasm induces the synthesis only of cytosolic stress proteins, not of BiP (44). In this study, we have identified a *cis*-acting regulatory domain in the pro-

* Corresponding author.
† Present address: Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565, Japan.

moter region of the yeast *KAR2* gene that responds to events in the ER. This domain is similar in sequence to those described by Lee and coworkers in the upstream regions of the mammalian BiP and *grp94* genes that are involved in induction by calcium ionophores (6, 50). In addition, we have identified a functional heat shock element (HSE) in the regulatory region of the *KAR2* gene that is absent from the mammalian homolog. The presence of this element explains why transcription of yeast BiP, but not mammalian BiP, is induced by heat shock.

MATERIALS AND METHODS

Yeast strains and microbial techniques. SEY6210 (*MATα ura3-52 leu2-3,112 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9*), obtained from S. Emr (California Institute of Technology), was used in all experiments unless specifically noted. PBY408A (*MATα ura3-52 leu2-3,112 his4-519 sec11-7*), RSY12/SEY5536α (*MATα ura3-52 leu2-3,112 sec53-6*), and RDM50-94C/YFP329 (*MATα ura3-52 leu2-3,112 his4 sec62*) were obtained from R. Schekman (University of California, Berkeley). Strain KNH3 (*MATα ura3-1 leu2-3,112 his3-11,15 ade2-1 can1-100 kar2-1*) was isolated from KND1, which was described by Normington et al. (44). Transformation of yeast cells was performed with lithium acetate by the method of Ito et al. (27). Rich broth medium (YPD) and selective medium for transformants are described elsewhere (57).

Plasmid construction. All cloning strategies relied on standard methods (55). An *Eco*RI fragment approximately 1,390 bp in length was purified from YRp7-01, which carries a cloned version of the yeast *KAR2* gene (44), and subcloned into pUC119. The resulting plasmid (pM602) contains the region of the *KAR2* gene that lies between the *Eco*RI sites at nucleotides −1254 and +129. The termini of a *Kpn*I fragment isolated from pM602 were modified by addition of synthetic *Bam*HI linkers (5′-CGGGATCCCG-3′). This *Bam*HI fragment contains sequences of the *KAR2* gene that lie between nucleotides −1254 and +74 and therefore spans the start site of transcription (+1) and contains the region coding for the first 12 amino acids of BiP. The fragment was ligated into pUC119 to generate plasmid pYBK10 (Fig. 1). Two series of deletion mutants were then constructed. The first utilized natural restriction sites in the upstream region of the *KAR2* gene: *Eco*RI (−1254), *Hin*cII (−514), *Pvu*II (−315), *Xho*I (−245), and *Dra*I (−9) (see Fig. 3). The segments of DNA lying between these sites and the synthetic *Bam*HI site at +74 were inserted into plasmid pSEYc102 (a *CEN4-ARS1*-based, single-copy yeast vector containing the *URA3* selectable marker, generously provided by M. Douglas, University of North Carolina, then at the University of Texas Southwestern Medical Center) in frame with the coding sequence of *Escherichia coli* β-galactosidase. The fusion protein encoded by the resulting chimeric genes is predicted to contain 12 amino-terminal residues of BiP joined via three residues (Arg-Asp-Pro) specified by the synthetic *Bam*HI linker to residue 9 of *E. coli* β-galactosidase. The second series of mutants is a set of progressive deletions of nucleotides lying between the *Xho*I (−245) and *Dra*I (−9) sites of the *KAR2* promoter. These were generated by cleaving plasmid pYBK10 (Fig. 1) at the *Xba*I and *Pst*I sites that lie within the polylinker and then treating the DNA with exonuclease III for various lengths of time. After the ends of the DNA were repaired with mung bean nuclease and the Klenow fragment of DNA polymerase I, the heterogeneous population of plasmids was recircularized and used to transform *E. coli*. Thirteen plasmids were selected on the basis of their sizes, and the precise 5′ endpoints of the deletions of the *KAR2* promoter were determined by the dideoxy-chain termination method of DNA sequencing (56). The plasmids were then cleaved at the *Hin*dIII site in the polylinker, treated with the Klenow fragment of DNA polymerase I, and digested with *Bam*HI. The truncated fragments of the *KAR2* promoter were isolated and inserted into plasmids pSEYc102 that had been digested with *Sma*I and *Bam*HI.

To manipulate the promoter and coding regions of the *KAR2* gene separately, we used a construct in which a *Sal*I site had been created immediately upstream of the translational start site (37). The 277-bp *Xho*I-*Sal*I fragment containing the promoter was then cloned into M13mp18 and used as a template for site-directed mutagenesis (31), using two 30-mer oligonucleotides, to loop out nucleotides −167 to −138 (+UPR [unfolded protein response element]) and −142 to −113 (+HSE) respectively. After the sequences of the mutants had been confirmed, the wild-type or mutant promoters were recovered from replicative-form preparations of recombinant M13 bacteriophages by digestion with *Sma*I and *Sal*I and inserted between the *Sma*I and *Bam*HI sites of pSEYc102 (Fig. 1), together with a 21-bp *Sal*I-to-*Bam*HI adaptor fragment (37) that encodes the five N-terminal residues of yeast BiP. This adaptor also ensures that the downstream *lacZ* coding sequences are fused to the BiP sequences in the correct reading frame.

The ~300-bp *Sma*I-*Sal*I fragment containing wild-type *KAR2* promoter sequences was also inserted between the *Sma*I and *Sal*I sites of plasmid pRS313 (a *CEN6-ARSH4* plasmid containing the *HIS3* selectable marker, kindly provided by M. Douglas) (59) and YEp351 (a 2μm-based, multicopy plasmid containing the *LEU2* selectable marker, kindly provided by R. Butow at the University of Texas Southwestern Medical Center) (24) to generate plasmids pRS-WTP and pYEp-WTP, respectively. The ~700-bp *GAL10* promoter was isolated from plasmid pSEYC58-GalP (44) and inserted between the *Sma*I and *Sal*I sites of plasmid pRS315 (a *CEN6-ARSH4* plasmid containing the *LEU2* selectable marker, provided by M. Douglas) (59) to generate plasmid pRS-GALP. Then the 2.5-kb *Sal*I-*Xho*I fragment encompassing the complete BiP coding sequence was isolated from plasmid YEpCup-YB (44). This fragment was inserted into the *Sal*I site of pRS-WTP in the correct and reverse orientations to generate constructs pWTP-BiP and pWTP-PiB, respectively, and also inserted into the *Sal*I sites of pYEp-WTP and pRS-GALP in the correct orientation to generate constructs pmcWTP-BiP and pGALP-BiP, respectively.

Analysis of RNA. Total yeast RNA was isolated as described by Lindquist (33). Electrophoresis of denatured RNA (10 μg) and Northern (RNA) hybridization in 50% formamide were carried out as described previously (55). Three radiolabeled probes were used: a 2.1-kb *Bgl*I fragment of the *lacZ* gene, a 1.1-kb *Eco*RI fragment of the yeast *KAR2* gene that hybridizes specifically to *KAR2* sequences, and a 0.8-kb *Eco*RI fragment of *SSA1*, a yeast gene that encodes a cytosolic member of the stress-70 family and is expressed at high basal levels (64). This probe hybridizes to all four members of the *SSA* gene family. Autoradiographic images were established at −70°C, using intensifying screens.

To determine the site at which transcription of the *KAR2* gene is initiated, primer extension was carried out with only minor modifications of a previously described method (39).

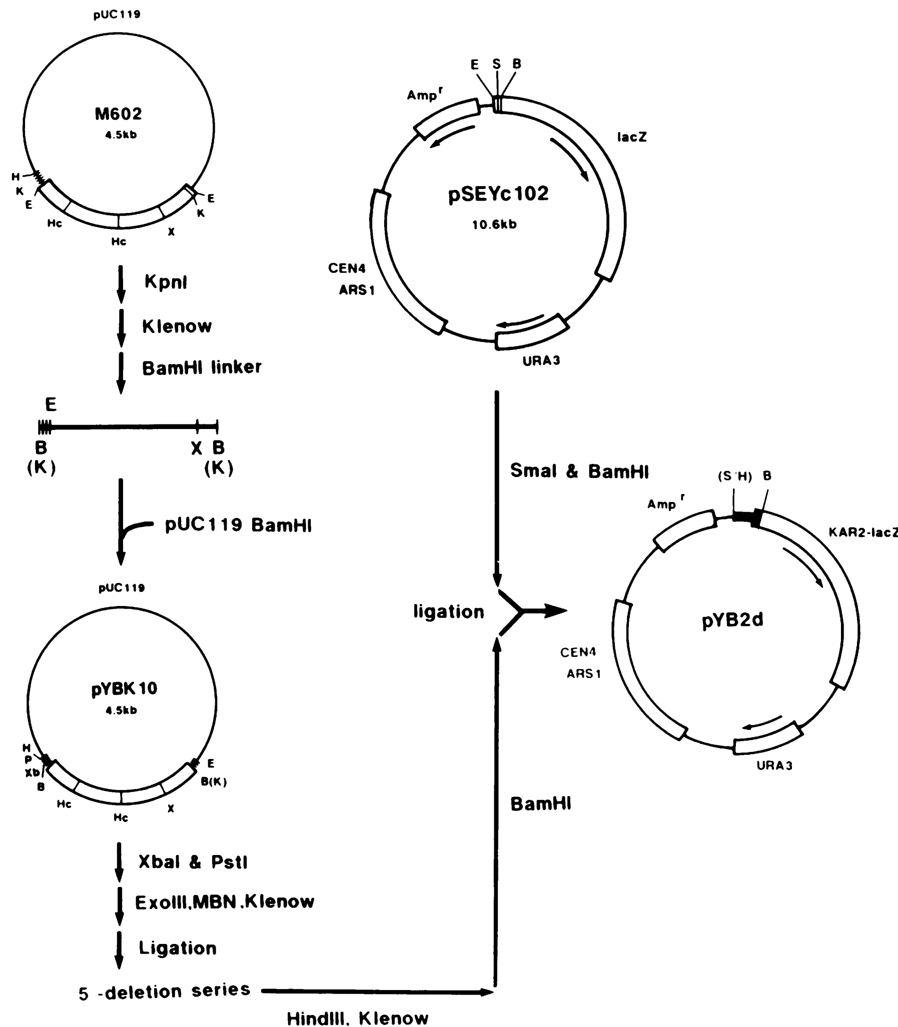


FIG. 1. Construction of plasmids used to identify sequences controlling *KAR2* expression. Plasmids containing 5′-deletion mutants of the *KAR2* promoter region fused to β-galactosidase coding sequences were constructed as described in Materials and Methods. Restriction sites are indicated as follows: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; Hc, *Hin*cII; K, *Kpn*I; P, *Pst*I; S, *Sma*I; X, *Xho*I; Xb, *Xba*I. ExoIII, exonuclease III.

Total RNAs were annealed with a synthetic oligonucleotide primer, complementary to nucleotides (58 to 74) of the *KAR2* gene (see Fig. 3), that had been radiolabeled at its 5′ terminus. The products of primer extension were analyzed by electrophoresis through denaturing polyacrylamide gels, using fragments of M13 mp18 DNA as markers.

Assays of β-galactosidase. Assays of β-galactosidase activity in extracts of yeast cells were carried out as described previously (45, 60). Units of β-galactosidase were defined as $(OD_{420} \times 1{,}000)/(OD_{600} \times t \times V)$, where OD_{420} (optical density at 420 nm) is the reading of the final color, OD_{600} is the turbidity of the culture at the time of harvest, V is the volume of the sample, and t is the number of minutes for which the reaction was incubated. Values are expressed as the mean ± standard error (SE) of duplicate determinations of three independent yeast transformants.

Analysis of BiP expression by immunoblotting. Yeast transformants were cultured at the appropriate temperature in 50 ml of selective medium to mid-log phase (OD_{600} of ~0.7) and harvested by centrifugation at 3,000 rpm for 5 min. Cells were washed with 50 mM Tris-HCl (pH 8.0), resuspended in 300 μl of breakage buffer (50 mM Tris-HCl [pH 8.0] containing 0.1% Triton X-100, 0.05% sodium dodecyl sulfate [SDS], and 1 mM phenylmethylsulfonyl fluoride), and disrupted by vortex mixing with glass beads (0.45 mm) six times for 20 s each time. Extracts were clarified by centrifugation at 12,000 × *g* for 10 min. Aliquots containing 50 μg of total protein were mixed with an equal volume of sample buffer (5 mM Tris-HCl [pH 6.8] containing 20% glycerol, 5% SDS, 0.2 M dithiothreitol, and 0.05% bromophenol blue), boiled for 5 min, separated by SDS–8% polyacrylamide gel electrophoresis, and transferred to a nitrocellulose filter. Yeast BiP protein was detected by using a rabbit polyclonal antiserum raised against a yeast BiP fusion protein expressed in *E. coli* (the generous gift of D. Williams, University of Toronto) and an enhanced chemiluminescence Western immunoblotting kit (Amersham). The chemiluminescent intensities associated with the various protein bands were determined by exposing the filter to X-ray film.

Nucleotide sequence accession number. The GenBank accession number for the sequence reported in this paper is M25394.

RESULTS

Time course of induction of yeast BiP mRNA after exposure of cells to heat or treatment with tunicamycin. We and others have already reported that transcription of the *KAR2* gene is induced in wild-type yeast cells that have been treated with tunicamycin or subjected to heat shock (44, 52). The results shown in Fig. 2 demonstrate differences in the time course of the transcriptional response of the *KAR2* gene to these two forms of stress. In wild-type cells exposed to tunicamycin (1 μg/ml), the concentration of BiP mRNA began to increase 20 min after addition of the drug and, after 90 min of incubation, reached a level 15 times higher than normal. By contrast, when wild-type yeast cells were exposed to elevated temperature (37°C), the concentration of BiP mRNA increased rapidly, reaching a maximum approximately 10 min after the temperature upshift. After 20 min at the elevated temperature, the BiP mRNA level declined, at first rapidly and then more steadily. When the culture was returned to 23°C after 10 min at 37°C, the decline in the concentration of BiP mRNA occurred at a faster rate (data not shown), a result that has previously been reported for *Drosophila* hsp70 mRNA (reviewed in reference 47).

By contrast to BiP, the concentration of *SSA* mRNA(s) encoding the yeast cytosolic stress-70 proteins increased very little during incubation with tunicamycin, confirming previous observations that different cellular compartments behave in an insulated fashion during the stress response (44, 52). These results indicate that the regulatory region of the *KAR2* gene contains an element, which is apparently not present in the regulatory region of the *SSA* genes, that responds to changes caused by exposure of yeast cells to the drug. On the other hand, a temperature upshift from 23 to 37°C led, as expected, to a rapid increase in the concentration of *SSA* mRNA(s). Maximum amounts of *SSA* mRNA were detected 10 min after the upshift, and the concentration of the mRNA then declined with a time course similar to that of the BiP mRNA. The fact that the *KAR2* gene responds to heat in a manner typical of classical heat shock genes such as those of the *SSA* family (reviewed in reference 8) suggests that it may contain a functional HSE in its upstream regulatory region.

Exposure to heat combined with treatment with tunicamycin had additive effects on the induction of BiP mRNA (Fig. 2A). When logarithmically growing cultures of yeast cells were treated with tunicamycin for 1 h at 23°C and then shifted to 37°C for 10 min, the amount of BiP mRNA synthesized was approximately twofold greater than that induced by either treatment alone. This result is consistent with the presence of two regulatory elements in the *KAR2* gene that respond independently to different stressful stimuli.

Sequence of the 5′ regulatory region of the *KAR2* gene. The sequence of 1,292 nucleotides that lie upstream of the coding region of the *KAR2* gene is shown in Fig. 3. The site within this sequence at which transcription of the gene is initiated was mapped by primer extension. Total RNAs were isolated from cultures of wild-type yeast cells exposed to various forms of stress, including heat shock (37°C for 10 min) and treatment with tunicamycin (1 μg/ml for 1.5 h). RNA was also extracted from a *sec53* mutant that had been incubated for 3 h at 30°C and from control cultures of both wild-type and *sec53* cells that had been maintained at 23°C. At 30°C, *sec53* cells are defective in phosphomannomutase (29) and accumulate full-length precursors of secretory proteins that are abnormally glycosylated and malfolded (1, 14). The lengths of the products of primer extension (Fig. 4) demonstrate that under both basal and induced conditions, transcription of the *KAR2* gene begins at an adenine residue (designated as +1 in Fig. 3), 38 nucleotides upstream from the initiating AUG codon. This initiation codon is the first AUG from the 5′ end of the mRNA (Fig. 3).

Located upstream of the initiation site for RNA synthesis are several sequences that match consensus sequences that are signatures for transcriptional control elements. (i) There are TATA sequences at nucleotides −95 and −63. (ii) There is an HSE consensus sequence of 20 nucleotides (−168 to −149) consisting of four 5-bp modular units of nGAAn alternating with nTTCn (34). The 42-bp segment between nucleotides −188 and −147 includes three elements nearly identical to the HSE consensus sequence CnnGAAnnT TCnnG originally defined by Pelham (46). (iii) There is a tract of 16 nucleotides (−148 to −133), 15 of which are G or C residues. Seven of ten contiguous nucleotides in this region are identical to the consensus sequence (GGGGCGGGGC) for binding of the transcription factor Sp1 (28) to the promoter regions of mammalian genes. (iv) There is a 22-bp sequence (nucleotides −131 to −110) that is very similar to sequences found in the promoters of the mammalian glucose-regulated genes (7, 50) and *EUG1*, a yeast ER protein of unknown function (60a). In the promoters of the mammalian glucose-regulated genes, these conserved nucleotides are necessary for the induction of BiP (*grp78*) and *grp94* mRNAs that occurs when cells are exposed to Ca^{2+} ionophores (7, 50). To analyze the relative contributions of these various consensus sequences to basal and stress-induced transcription of the yeast *KAR2* gene, we constructed and assayed a series of deletion mutants of the promoter as described below.

5′-deletion mapping of the basal and stress-responsive elements in the yeast *KAR2* promoter. A 1,328-bp segment of DNA containing the various upstream consensus elements, the initiation sites for transcription and translation, and the codons specifying the first 12 amino acids of BiP was fused in frame to a segment of DNA encoding amino acid residues 9 to 1023 of *E. coli* β-galactosidase (Fig. 1 and Materials and Methods). In addition, a series of constructs with 5′ deletions of the promoter region was generated, either by digestion of the promoter region with appropriate restriction enzymes or by progressive removal of nucleotides lying downstream of the *Xho*I site (−245) by exonuclease III (Fig. 1 and Materials and Methods). All of these constructs were introduced into wild-type yeast cells on the centromere-based, single-copy vector pSEYc102. The amount of β-galactosidase activity produced from each construct was assayed in cultures growing logarithmically at 23°C and in parallel cultures that had been treated with tunicamycin (2 μg/ml) for 3 h. When the full-length construct was tested, the drug-treated culture contained a level of β-galactosidase activity (198 U) that was approximately 2.3-fold greater than that in the control culture (85 U). This level of induction is lower than the approximately 15-fold increase in concentration of *KAR2* mRNA that is observed when wild-type yeast cells are treated with tunicamycin (Fig. 2B). The chief cause of this discrepancy is the relatively high basal level of β-galactosidase activity that is consistently found when reporter constructs are assayed on plasmid vectors. This basal level of enzymatic activity is high enough to essentially obscure the transient heat shock response of the *KAR2* promoter (results not shown). However, when transcription from plasmid vectors was assayed by Northern hybridization rather than by measurement of β-galactosidase activity,

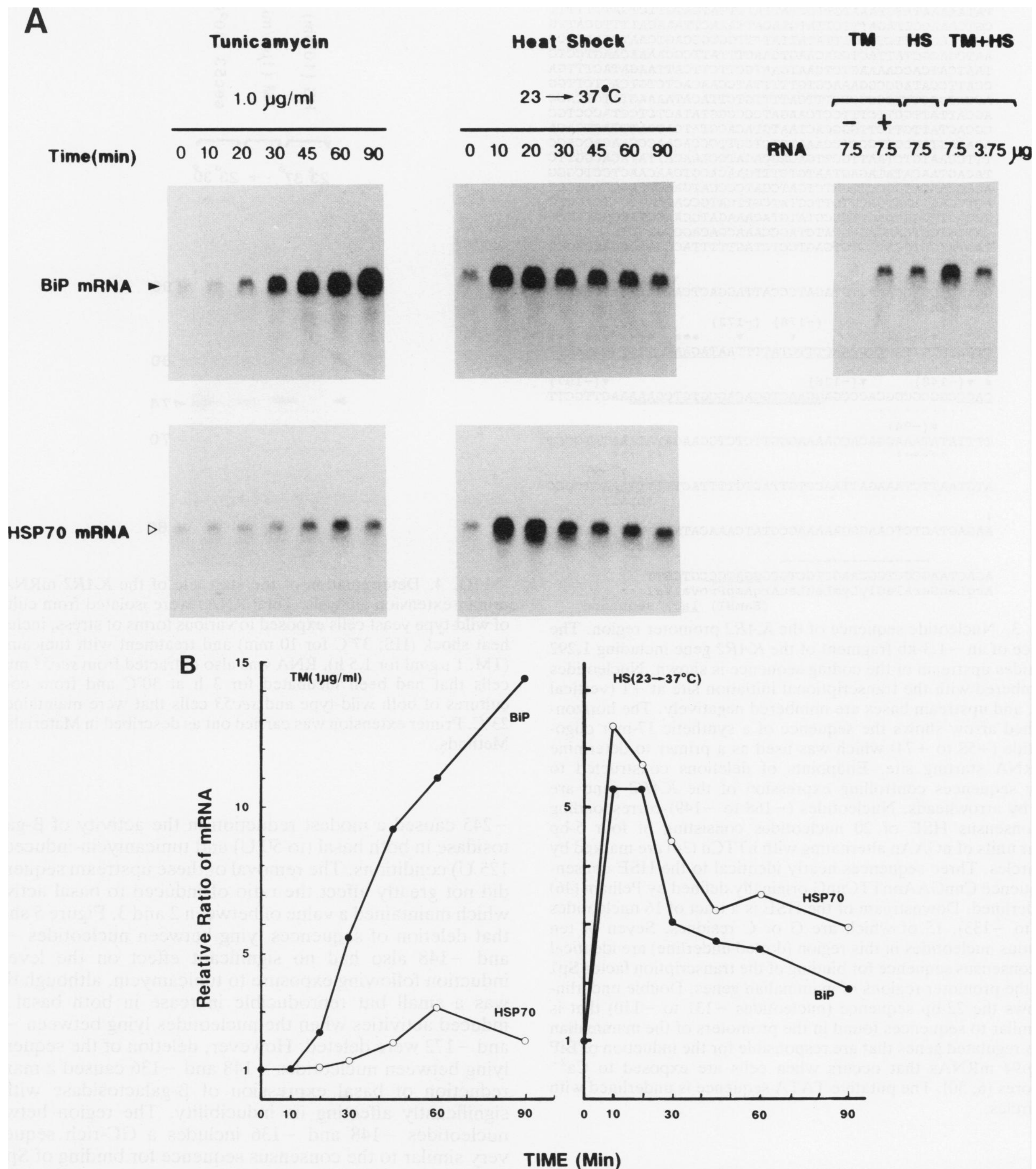


FIG. 2. Induction of the yeast *KAR2* gene by tunicamycin and by heat shock. RNAs extracted from cells grown under normal or stress conditions were separated by agarose gel electrophoresis and transferred to nitrocellulose filters as described in Materials and Methods. Identical filters were hybridized under conditions of high stringency with radiolabeled probes specific for *KAR2* or for a gene (*SSA1*) that encodes a cytoplasmic HSP70 protein (64). (A) Time course of *KAR2* or HSP70 mRNA induction after treatment with tunicamycin or heat shock. The right-hand autoradiogram shows the additive effect of the two treatments. (TM+HS) indicates that cells were incubated in the presence of tunicamycin (1 µg/ml) for 1 h at 23°C (TM) and then heat shocked at 37°C for 10 min (HS). (B) The amounts of radioactive label hybridized to the *KAR2* and HSP70 mRNAs following various times under normal or stress conditions, quantitated by densitometry. The data are presented as the fold induction under stress conditions.

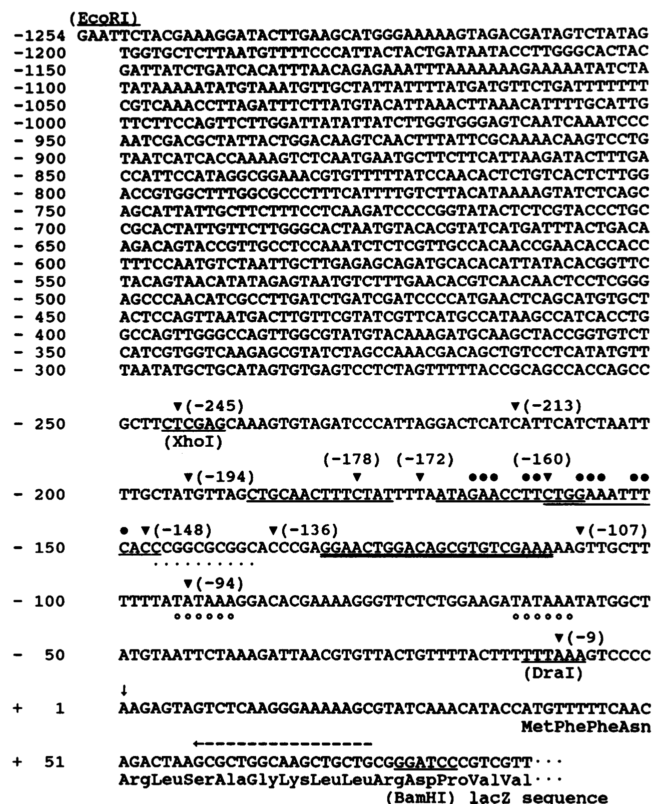


FIG. 3. Nucleotide sequence of the *KAR2* promoter region. The sequence of an ~1.3-kb fragment of the *KAR2* gene including 1,292 nucleotides upstream of the coding sequence is shown. Nucleotides are numbered with the transcriptional initiation site at +1 (vertical arrow), and upstream bases are numbered negatively. The horizontal dashed arrow shows the sequence of a synthetic 17-mer oligonucleotide (+58 to +74) which was used as a primer to determine the mRNA starting site. Endpoints of deletions constructed to identify sequences controlling expression of the *KAR2* gene are shown by arrowheads. Nucleotides (−168 to −149) corresponding to a consensus HSE of 20 nucleotides consisting of four 5-bp modular units of nGAAn alternating with nTTCn (34) are marked by filled circles. Three sequences nearly identical to the HSE consensus sequence CnnGAAnnTTCnnG originally defined by Pelham (46) are underlined. Downstream of the HSE is a tract of 16 nucleotides (−148 to −133), 15 of which are G or C residues. Seven of ten contiguous nucleotides in this region (dotted underline) are identical to the consensus sequence for binding of the transcription factor Sp1 (28) to the promoter regions of mammalian genes. Double underlining shows the 22-bp sequence (nucleotides −131 to −110) that is very similar to sequences found in the promoters of the mammalian glucose-regulated genes that are responsible for the induction of BiP and *grp94* mRNAs that occurs when cells are exposed to Ca^{2+} ionophores (6, 50). The putative TATA sequence is underlined with open circles.

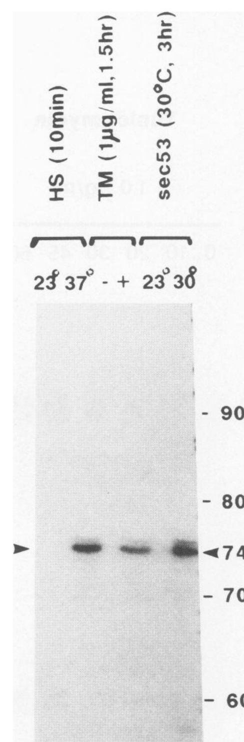
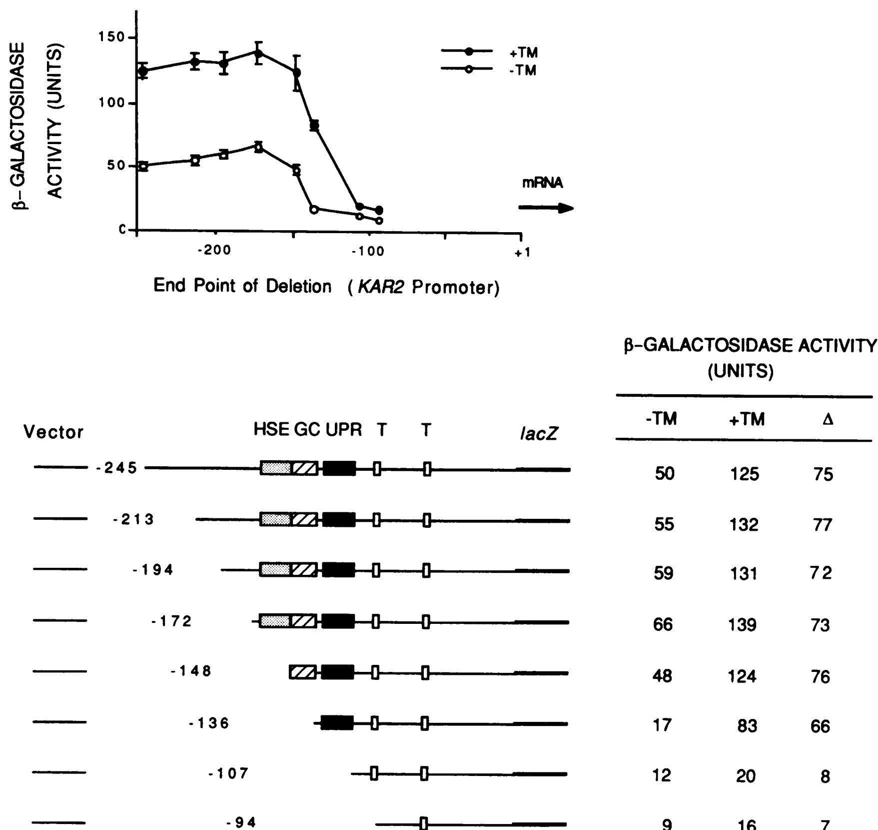


FIG. 4. Determination of the start site of the *KAR2* mRNA by primer extension analysis. Total RNAs were isolated from cultures of wild-type yeast cells exposed to various forms of stress, including heat shock (HS; 37°C for 10 min) and treatment with tunicamycin (TM; 1 µg/ml for 1.5 h). RNA was also extracted from *sec53* mutant cells that had been incubated for 3 h at 30°C and from control cultures of both wild-type and *sec53* cells that were maintained at 23°C. Primer extension was carried out as described in Materials and Methods.

KAR2-mediated induction could readily be detected after exposure of yeast cells to tunicamycin or heat shock (see Fig. 6). These results show that stress-responsive elements of the *KAR2* promoter are contained within ~1,300 bp of DNA that lies upstream of the sequences coding for yeast BiP.

To identify the sequences that are involved in basal and stress-induced transcription, we assayed the ability of the deletion mutants to drive expression of β-galactosidase activity (Fig. 5) or transcription of *lacZ* mRNA (Fig. 6). Deletion of sequences lying between nucleotides −1254 and −245 caused a modest reduction in the activity of β-galactosidase in both basal (to 50 U) and tunicamycin-induced (to 125 U) conditions. The removal of these upstream sequences did not greatly affect the ratio of induced to basal activity, which maintained a value of between 2 and 3. Figure 5 shows that deletion of sequences lying between nucleotides −245 and −148 also had no significant effect on the level of induction following exposure to tunicamycin, although there was a small but reproducible increase in both basal and induced activities when the nucleotides lying between −194 and −172 were deleted. However, deletion of the sequences lying between nucleotides −148 and −136 caused a marked reduction of basal expression of β-galactosidase without significantly affecting its inducibility. The region between nucleotides −148 and −136 includes a GC-rich sequence very similar to the consensus sequence for binding of Sp1 to mammalian promoters (28). However, removal of additional sequences from the promoter (to nucleotide −107) essentially eliminated the induction of activity over the low basal level. These results suggest that the sequences responsible for tunicamycin-induced expression map between nucleotides −136 and −107 of the *KAR2* promoter. This region contains a 22-bp sequence that is homologous to the mammalian consensus sequences required for the induction of BiP (*grp78*) and *grp94* genes by Ca^{2+} ionophores (7, 50). The involvement of these sequences in basal and tunicamycin-induced transcription of the *KAR2* gene was confirmed when



Vector end point	β-GALACTOSIDASE ACTIVITY (UNITS) -TM	+TM	Δ
-245	50	125	75
-213	55	132	77
-194	59	131	72
-172	66	139	73
-148	48	124	76
-136	17	83	66
-107	12	20	8
-94	9	16	7

FIG. 5. Mapping of the tunicamycin-responsive element in the *KAR2* promoter. Wild-type yeast cells were transformed with single-copy plasmids containing 5'-deleted versions of the *KAR2* promoter fused to the *lacZ* gene (see schematic diagrams). β-Galactosidase activity was measured in extracts of cells grown at 23°C for 3 h in the absence or presence of tunicamycin (TM; 2 μg/ml). Values are expressed as the mean ± SE (bar) of duplicate determinations of three independent transformants. The deletion endpoints are inclusive of the base pair indicated. The transcription start site is at +1; the HSE, GC-rich (GC), UPR, and TATAA (T) sequences are boxed.

Northern hybridization was used to compare the effects of the drug on the transcription of the β-galactosidase sequences in the reporter constructs and on the transcription of the chromosomal *KAR2* gene (Fig. 6A). As observed in the experiment shown in Fig. 5, there was a slight increase in the low level of basal transcription of *lacZ* mRNA when sequences lying between nucleotides −194 and −172 were deleted, indicating that there might be a negative regulatory element in that region. Basal transcription of *lacZ* mRNA decreased significantly when the Sp1-like GC-rich sequence was removed by deletion of sequences lying between nucleotides −148 and −136. By contrast, deletion of sequences to −136 had no effect on the high level of induction of transcription of *lacZ* mRNA in cells exposed to tunicamycin. Again, loss of tunicamycin-mediated induction of transcription from the *KAR2* promoter occurred following deletion of the sequences between −136 and −107. As expected, the levels of basal and induced transcription of BiP mRNA from the chromosomal copy of the *KAR2* gene were unaffected by truncation of the promoter sequences in the plasmid constructs.

To map the functional HSE(s) in the *KAR2* promoter, Northern hybridization was also used to compare the effects of heat shock on the transcription of the *lacZ* sequences in the reporter constructs and on the transcription of the chromosomal *SSA* gene(s) (Fig. 6B). Cells growing at 23°C contain low levels of both *lacZ* and *SSA* mRNAs, but both mRNA species were markedly induced 10 min after the temperature was raised to 37°C in cells containing any one of the deletion mutants that retain sequences downstream of nucleotide −172. However, induction of transcription of *lacZ* was essentially eliminated when sequences upstream of nucleotide −160 were deleted, although induction of *SSA* mRNA was still observed. This result demonstrates that the sequences required for induction by heat shock map between nucleotides −172 and −160 of the *KAR2* promoter.

Internal deletion mutants of the yeast *KAR2* promoter. The 5'-deletion analysis revealed that the DNA segment lying between nucleotides −245 and +53 of the *KAR2* gene contains all information required for accurate transcriptional regulation of the yeast *KAR2* gene and that the sequences lying between nucleotides −172 and −107 include three elements important for transcriptional regulation of the yeast *KAR2* gene. Internal deletion mutants were then constructed and analyzed to confirm that the accuracy of the mapping of the regulatory elements by 5' deletion had not been prejudiced by removal of upstream sequences. Wild-type yeast cells were transformed with single-copy (pSEYc102-based) plasmids carrying the coding sequence of β-galactosidase fused in frame to (i) a wild-type promoter (WT promoter) containing nucleotides −245 to +53 from the wild-type *KAR2* gene, (ii) a mutant (+UPR) promoter that retains the tunicamycin-responsive element that maps between nucleotides −136 and −107 but lacks 30 nucleotides (−167 to

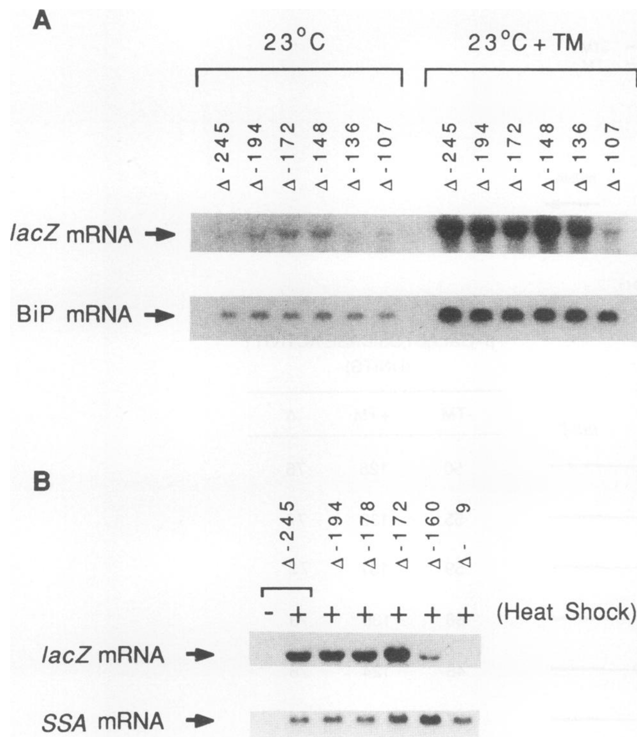


FIG. 6. Effects of 5′ deletions on *KAR2* promoter activity. Wild-type yeast cells were transformed with single-copy plasmids containing 5′-deleted versions of the *KAR2* promoter fused to the *lacZ* gene (diagrammed in Fig. 5). (A) Total RNAs (10 μg) isolated from cells grown at 23°C for 1 h in the absence or presence of tunicamycin (TM; 1 μg/ml) were separated by gel electrophoresis, transferred to a solid support, and hybridized with radiolabeled DNA probes specific for *lacZ* and *KAR2* (BiP) mRNAs. (B) Total RNAs (10 μg) isolated from control (23°C) or heat-shocked cells (37°C, 10 min) were separated by gel electrophoresis, transferred to a solid support, and hybridized with radiolabeled DNA probes specific for *lacZ* and *SSA1* as described in Materials and Methods.

−138) including the HSE and the GC-rich sequence (Fig. 7A), or (ii) a mutant (+HSE) promoter that retains the HSE (nucleotides −168 to −149) but has a deletion of 30 nucleotides (−142 to −113) that disrupts the GC-rich sequence and the tunicamycin-responsive element (Fig. 7A). In the latter cases, deletion of multiples of 10 bp (each corresponding to a helical turn of double-stranded DNA) should not cause rotational displacement of the upstream and downstream sequences relative to one another and therefore should minimize disruption of any protein binding sites that lie along one face of the helix. The abilities of the WT and mutant promoters to drive expression of the *lacZ* gene were then assayed by measuring the amount of *lacZ* mRNA (Fig. 7B) and β-galactosidase activity (Fig. 8A) in transformants cultured in the presence or absence of stress.

Cells grown at 23°C were incubated at 23°C or at 23°C for 1.5 h in the presence of tunicamycin (1 μg/ml) or were shifted to 37°C for 10 min before total RNAs were extracted and analyzed by Northern hybridization using DNA probes specific for *lacZ* and *KAR2* (BiP) (Fig. 7B). As seen previously, transcription of *lacZ* mRNA from the WT promoter construct was induced in response to heat shock and tunicamycin treatment in a pattern identical to that of transcription of BiP mRNA from the endogenous *KAR2* promoter. As expected, the pattern of induction of transcription of BiP mRNA from its endogenous promoter was unaltered in cells transformed with the +HSE and +UPR mutant constructs. However, the basal level of expression of *lacZ* mRNA from both the +HSE and +UPR promoters was decreased, presumably because the GC-rich element is deleted or disrupted in both constructs. Heat shock had no effect on transcription from the +UPR promoter but resulted in several-fold induction of transcription of *lacZ* mRNA from the +HSE promoter, confirming that the HSE maps between nucleotides −167 to −143 of the *KAR2* promoter. Significant levels of tunicamycin-mediated induction of *lacZ* mRNA were observed only from the +UPR promoter, confirming that the tunicamycin-responsive element is located in the promoter between nucleotides −137 and −113.

When the amount of β-galactosidase activity in these transformants was measured following a 3-h incubation at 23°C (Fig. 8A), the basal activities of the +HSE and +UPR promoters in unstressed cells were approximately 20 and 15%, respectively, of that of the WT promoter. Treatment of cells with tunicamycin (2 μg/ml) for 3 h at 23°C caused increased expression of β-galactosidase activity when the *lacZ* sequences were transcribed from either the WT or +UPR promoter. Expression of β-galactosidase activity from the +HSE promoter was not significantly altered following treatment of the cells with tunicamycin. These results are consistent with those obtained by analysis of mRNA levels (Fig. 7B), and together these experiments clearly show that the HSE and the tunicamycin-responsive element mapped in the *KAR2* promoter respond to the appropriate stress independently both of each other and of the GC-rich region.

The tunicamycin-responsive domain also responds to the accumulation of secretory precursors in the ER. Tunicamycin, which induces synthesis of BiP in both mammalian and yeast cells, specifically inhibits N-linked glycosylation (12, 61), thereby causing accumulation of unglycosylated and malfolded proteins in the ER (see, for example, references 18 and 25). In yeast cells, BiP is induced when *sec* mutants that accumulate precursors of secretory proteins in the ER are shifted from permissive to semi- or nonpermissive temperatures (44, 52) or when a malfolded protein constructed from the prepro sequence of yeast killer protein and mouse α-amylase is expressed in wild-type cells (63). We have therefore named the induction of the *KAR2* promoter by these diverse stimuli the unfolded protein response (44). To verify that the same regulatory element responds to the different types of stimulus, we used three *sec* mutants (*sec11*, *sec53*, and *sec62*) that affect different steps in the secretory pathway. *SEC11* encodes a peptidase that removes hydrophobic signal peptides from newly synthesized transmembrane and secretory proteins (3). At a nonpermissive temperature, *sec11* mutants accumulate in their ER core-glycosylated forms of secretory precursors that still retain their signal peptides. As discussed above, *sec53* cells are defective in phosphomannomutase (29) and accumulate full-length precursors of secretory proteins that are abnormally glycosylated and malfolded (1, 15). Finally, *SEC62* encodes a transmembrane protein that is a component of the translocation machinery (9). *sec62* mutants accumulate unglycosylated polypeptides that are associated with the cytosolic face of the ER membrane (53). In addition to these *sec* mutants, we used the *kar2-1* mutant (48) that displays a temperature-sensitive growth defect.

These mutants were transformed with single-copy (pSEYc102-based) plasmids carrying the WT, +HSE, and

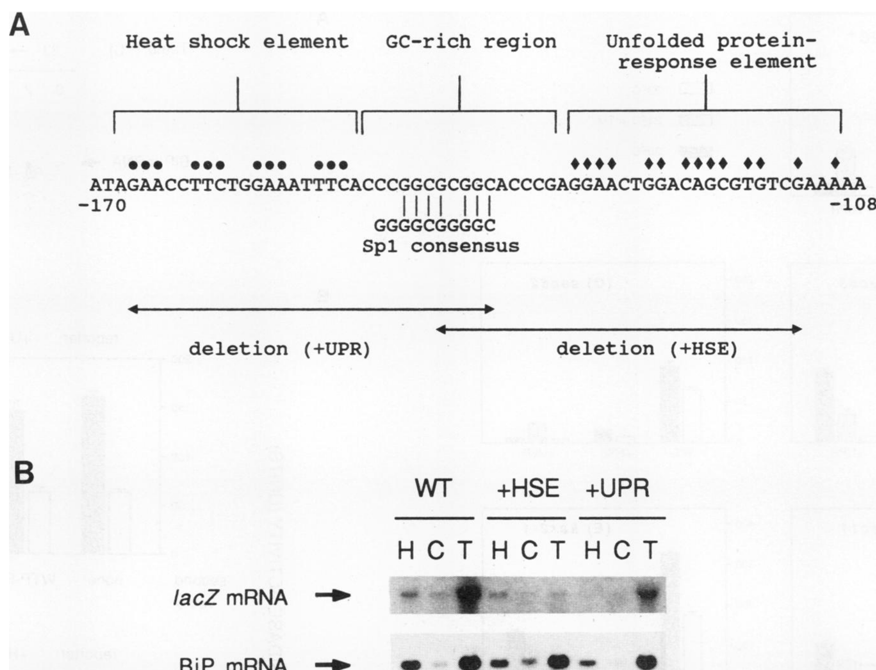


FIG. 7. Internal deletion analysis of the *KAR2* promoter. (A) Sequence of the *KAR2* promoter from nucleotides −170 to −108, including an HSE consensus sequence defined by Lis et al. (34) that contains four 5-bp modular units of nGAAn alternating with nTTCn (marked by filled circles) and the UPR element containing nucleotides (marked with diamonds) conserved in the promoters of yeast *KAR2* (BiP) and mammalian *grp78* (BiP) and *grp94* genes (6, 50). The locations of 30-bp deletions used to generate the +UPR and +HSE mutant promoters (see Materials and Methods) are indicated by double-headed arrows. (B) Northern blot. Cultures of wild-type yeast cells transformed with single-copy (pSEYc102-based) plasmids carrying the WT, +UPR, and +HSE constructs were grown at 23°C and then incubated at 23°C (C) or at 23°C for 1.5 h in the presence of 1 μg of tunicamycin per ml (T) or were shifted to 37°C for 10 min (H) before total RNAs were extracted and analyzed by Northern hybridization using DNA probes specific for *lacZ* and *KAR2* (BiP).

+UPR promoters described above and in Fig. 7A. The amount of β-galactosidase activity produced from each construct was assayed in cultures growing logarithmically at 23°C, and in parallel cultures that had been treated with tunicamycin (2 μg/ml) for 3 h, or shifted for 3 h to 30°C, a temperature that results in expression of the *sec* mutant phenotypes but causes little activation of the heat shock response (44). As expected, temperature upshift of the wild-type (*SEC*$^+$ *KAR2*$^+$) cells had no significant effect on transcription from the +UPR promoter (Fig. 8A). However, although tunicamycin-mediated induction of transcription from the +UPR promoter occurred in all of the mutant cells, β-galactosidase activity was induced after a temperature shift to 30°C in *sec53*, *sec11*, and *kar2-1* cells but not in *sec62* cells (Fig. 8B to E). This result is consistent with the first three mutants, but not *sec62*, displaying temperature-conditional defects that affect newly synthesized proteins in the ER lumen. By contrast, deletion of the tunicamycin-responsive element in the +HSE construct eliminated significant induction of β-galactosidase activity in all types of mutant cells after the temperature upshift. The small amount of induction observed for the +HSE construct in mutant cells following incubation at 30°C is almost certainly due to a minor heat shock response at this temperature, because the +UPR promoter, which lacks the HSE, shows no induction at all in *sec62* cells. Finally, although the levels of basal activity of all three constructs in *sec53* and *sec62* cells grown at 23°C were similar to those in wild-type cells, significantly increased levels of basal activity occurred in *sec11* and *kar2-1* cells containing the two constructs (WT and +UPR). This observation suggests that even at 23°C, *sec11* and *kar2-1* cells display defects that result in partial induction of the UPR response.

These results demonstrate that the UPR element, which lies between nucleotides −137 and −113, responds specifically both to treatment of cells with tunicamycin and to the presence of precursors of secretory proteins in the ER. In a separate study, we have shown that 22 nucleotides (−131 to −110) from this element are sufficient to confer responsiveness both to tunicamycin treatment and to the *sec53* defect on an unregulated (*CYC1*) promoter (37).

UPR element-mediated transcriptional activation of the *KAR2* promoter is down-regulated following expression of BiP protein in the ER. We then examined the effect of expression of wild-type BiP protein on the activities of the +HSE and +UPR promoters in *kar2-1* cells. Synthesis of BiP mRNA is significantly induced following a temperature upshift of *kar2-1* cells to 30°C (Fig. 9A). When a *lacZ* reporter plasmid containing either the +UPR or +HSE promoter was present in these cells, significant induction of β-galactosidase activity was observed only from the +UPR promoter (Fig. 9B; see also Fig. 8E). Very similar results were obtained when the cells carried a second plasmid (WT-PiB) containing the BiP coding sequences in reverse orientation behind the *KAR2* promoter. However, when the second plasmid in the *kar2-1* cells contained the BiP coding sequences in the correct orientation, both the basal activity and the degree of inducibility of the +UPR promoter on the *lacZ* reporter plasmid were decreased to the levels observed in wild-type (*SEC*$^+$ *KAR2*$^+$) cells (compare Fig. 8A and Fig. 9B, top

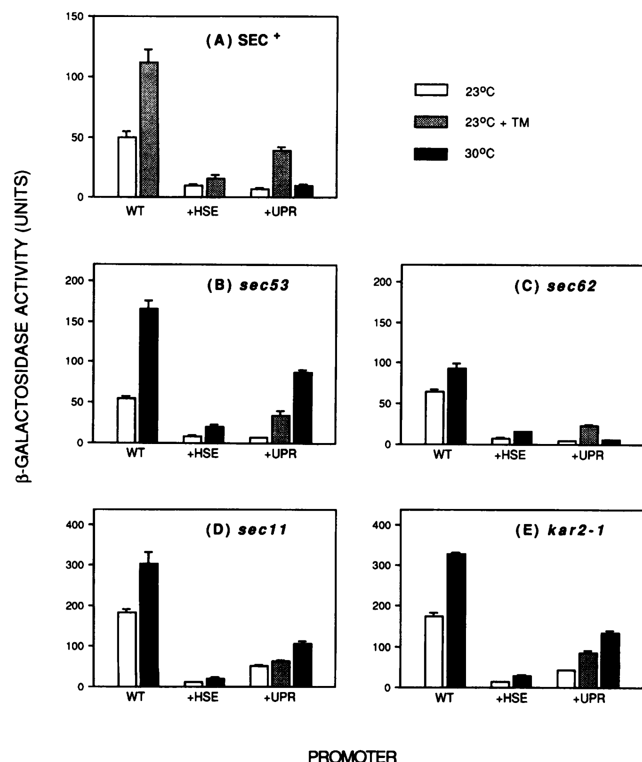


FIG. 8. Analysis of the unfolded protein response of the *KAR2* promoter. SEC$^+$ *KAR2*$^+$ yeast cells (strain SEY6210) and the *sec53*, *sec62*, *sec11*, and *kar2-1* mutants were transformed with a single-copy plasmid (pSEYc102) carrying versions of the *KAR2* promoter (WT, +HSE, and +UPR; see text) fused to the *lacZ* gene. Cultures grown at 23°C were incubated at 23°C (open boxes), at 23°C for 3 h in the presence of 2 μg of tunicamycin per ml (TM; dotted boxes), or at 30°C for a further 3 h (closed boxes) before cell extracts were prepared and assayed for β-galactosidase activity. Values are expressed as the mean ± SE (bar) of duplicate determinations of three independent transformants.

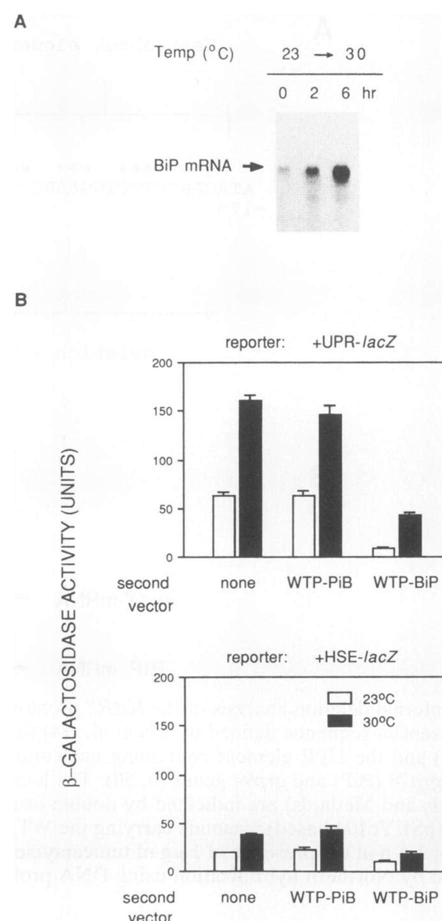


FIG. 9. Effect of BiP expression on the activity of the *KAR2* promoter in *kar2-1* cells. (A) *kar2-1* cells grown at 23°C were incubated at 30°C for the periods shown, and then total RNAs (10 μg) were isolated, separated by gel electrophoresis, transferred to a solid support, and hybridized with a radiolabeled DNA probe specific for *KAR2* (BiP) mRNA. (B) *kar2-1* cells were transformed with a *lacZ* reporter plasmid containing either the +UPR or +HSE promoter construct (see text). Transformants either were used directly (none) or were further transformed with an expression vector containing wild-type BiP coding sequences in either the correct orientation (WTP-BiP) or the reverse orientation (WTP-PiB) under the control of the wild-type *KAR2* promoter. Cells grown at 23°C were incubated at 23°C (open boxes) or at 30°C (closed boxes) for 3 h before cell extracts were prepared and assayed for β-galactosidase activity. Values are expressed as the mean ± SE (bar) of duplicate determinations of three independent transformants.

panel). Thus, expression of the wild-type BiP protein in the ER not only complements the temperature-sensitive growth defect and the karyogamy defect of *kar2-1* cells (44) but also suppresses the UPR element-mediated up-regulation of transcription of the *KAR2* gene in the mutant cells. Introduction of neither the WT-PiB nor the WT-BiP second vector had any significant effect on expression of β-galactosidase activity from the +HSE promoter at either 23 or 30°C (Fig. 9B, lower panel), confirming that the HSE is not involved in transcriptional activation of the *KAR2* gene in *kar2-1* cells.

Finally, we examined the effect of overexpression of BiP protein in the ER of yeast cells on the unfolded protein response of the *KAR2* promoter. First, we used the glucose-repressible *GAL10* promoter, which is 5 to 10 times more active than the wild-type *KAR2* promoter in galactose-containing media but totally inactive in glucose-containing media (42). As shown in Fig. 10A (left panel), there was no difference in the degree of tunicamycin-mediated induction of β-galactosidase activity expressed from the +UPR-*lacZ* construct in wild-type yeast cells grown in medium containing either glucose or galactose. The presence of a second vector, GALP-BiP, had no effect on the unfolded protein response when the cells were cultured in glucose medium (Fig. 10A, right panel). The immunoblot shown in Fig. 10C demonstrates that under these growth conditions, the amount of BiP protein produced by the cells is not changed from that in cells containing no second vector. However, following growth in media containing galactose, overexpression of BiP protein from the *GAL10* promoter (Fig. 10C) significantly suppressed the tunicamycin-mediated induction of β-galactosidase activity (Fig. 10A, right panel). The size of the overexpressed BiP protein is exactly the same as that of the BiP present in uninduced cells, demonstrating that the polypeptides have been efficiently translocated into the ER and undergone cleavage of their 42-residue signal peptides.

Because the observed suppression of the tunicamycin-mediated induction of β-galactosidase activity could be due, at least in part, to differences in growth rate and secretory activity between cells grown on glucose and galactose, we

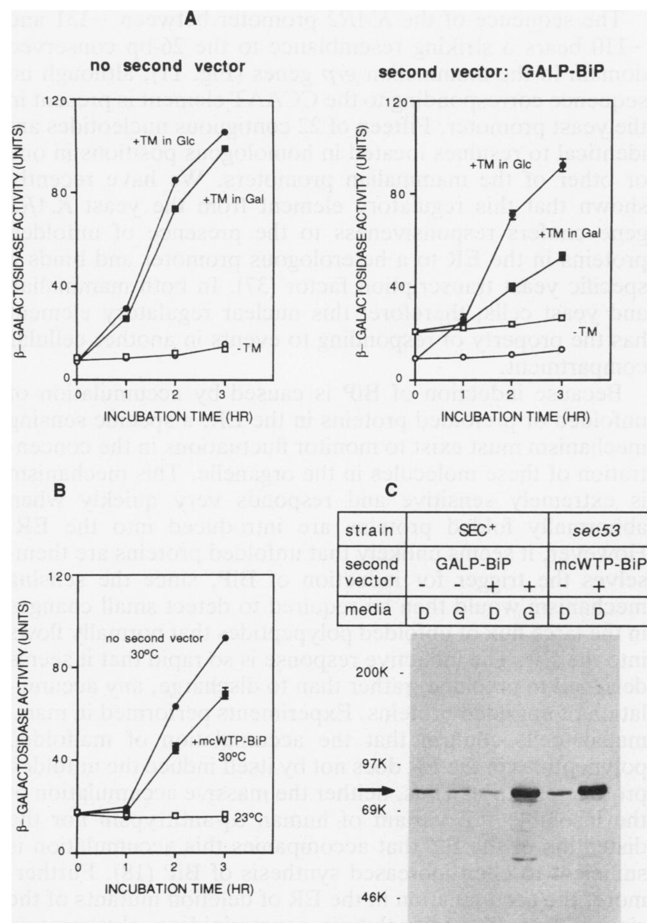


FIG. 10. Effect of overexpression of BiP protein on the unfolded protein response of the *KAR2* promoter. (A) *SEC*$^+$ wild-type yeast cells were transformed with the +UPR-*lacZ* reporter construct. Transformants were used directly (no second vector; left panel) or further transformed with the second vector GALP-BiP (right panel), which expresses BiP protein under the control of the *GAL10* promoter (see text and Materials and Methods). Identical transformants grown at 30°C in selection medium containing either 2% glucose (circles) or 2% galactose (squares) were further incubated at 30°C for 3 h in the presence (filled symbols) or absence (open symbols) of tunicamycin (TM; 2 μg/ml) before cell extracts were prepared and assayed for β-galactosidase activity. (B) *sec53* cells were transformed with the +UPR-*lacZ* reporter construct. Transformants were used directly (no second vector; circles) or further transformed with the second vector mcWTP-BiP, a 2 μm-based multicopy plasmid expressing BiP protein under the control of the wild-type *KAR2* promoter (squares). Transformants grown at 23°C were further incubated at 23°C (open symbols) or 30°C (closed symbols) for 3 h before cell extracts were prepared and assayed for β-galactosidase activity. Values are expressed as the mean ± SE (bar) of duplicate determinations of three independent transformants. (C) The amount of BiP protein in extracts of the yeast transformants described in panels A and B was measured by immunoblotting by the enhanced chemiluminescence Western blotting procedure as described in Materials and Methods. The arrow marks the position of migration of the mature (signal-cleaved) BiP polypeptide. D, dextrose (glucose); G, galactose.

also overexpressed the BiP protein in *sec53* cells under the control of the wild-type *KAR2* promoter, using a 2μm-based multicopy plasmid. In this system, transformation of *sec53* cells carrying the +UPR-*lacZ* construct with the second vector (mcWTP-BiP) had no effect on either the growth rate of transformants (results not shown) or their basal level of expression of β-galactosidase activity at 23°C (Fig. 10B). However, when these *sec53* transformants were cultured at 30°C to cause accumulation of abnormal secretory precursors (1, 15), the level of expression of BiP was greatly increased (Fig. 10C) and the unfolded protein response was reduced (Fig. 10B). Again, the great majority of the overexpressed protein was the same size as the mature protein, indicating its efficient translocation and signal cleavage in the ER lumen. These results indicate that the activity of UPR promoter element in the *KAR2* gene in the nucleus is modulated by the amount of wild-type BiP protein in the ER.

DISCUSSION

Four different stimuli induce synthesis of BiP in yeast cells: (i) treatment of cells with inhibitors of glycosylation, such as tunicamycin (44, 52); (ii) incubation at the nonpermissive temperature (30°C) of *sec* mutants that accumulate precursors of secretory proteins in the ER (44, 52); (iii) expression in wild-type cells of a malfolded protein constructed from the prepro sequence of yeast killer protein and mouse α-amylase (63); and (iv) exposure of cells to heat shock (37°C) (44, 52).

The first three stimuli cause an accumulation of partially processed secretory proteins in the early stages of the secretory pathway, suggesting that a proximal signal for induction of BiP in yeast cells, as in mammalian cells (30), might be the presence of unusually high concentrations of unfolded proteins in the ER. The fourth stimulus, heat shock, works by a different mechanism since induction of BiP by heat-shock is more rapid and shorter in duration than is induction by tunicamycin; induction by the two stimuli is additive; and the *KAR2* promoter contains regulatory sequences that respond independently to tunicamycin and to heat shock.

The most distal element that we have mapped in the *KAR2* promoter responds to heat shock and has a structure corresponding to the modular sequence shown by Lis et al. (34) to be the core element of functional HSEs in both yeast and mammalian cells. Typically, these elements contain several tandemly arranged repeats of the canonical sequence nGAAn and its complement nTTCn. A minimum of three modules (i.e., nGAAnnTTCnnGAAn or nTTCnnGAAnnTTCn) is required to form a functional HSE (34). The region of the *KAR2* promoter from nucleotides −168 to −149 contains four such modules, and a 30-bp deletion spanning these sequences ablates the heat shock response. Pelham (46) previously suggested that the consensus sequence CnnGAAnnTTCnnG, which contains only two Lis modules, defined a heat shock regulatory element. Three motifs nearly identical to the Pelham consensus sequence are contained in a 42-bp segment of the yeast *KAR2* promoter (nucleotides −188 to −147). One of these elements (nucleotides −188 to −175) lies outside the region required for induction and thus cannot act as a functional HSE of the yeast *KAR2* promoter. Interestingly, this element lies within the region (−194 to −172) that apparently includes a negative regulatory sequence (see below). This region contains no sequence similarity to either of the upstream repression sequences that negatively modulate transcription of *SSA1* (45) and *SSA4* (5). The remaining two Pelham elements overlap by four nucleotides such that the combined sequences contain within them the four Lis modules. When the two promoter-distal modules are removed by 5′ truncation, the *KAR2* promoter

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                                    Unfolded Protein-Response Element
                                    ┌───────────────────────┐
Yeast KAR2      -145  GGCGCGGCACCCGAGGAACTGGACA GCGTGTC GAAA  -110
                      :::           :: :  :: :: :: ::   :  :
Rat GRP78       -171  GGCCGCTTCGAATCGGCAGCGGCCA GCTTGGT GGCA  -136
                      ::::::::::::::::: ::::::: ::::::: :::
Human GRP78     -134  GGCCGCTTCGAATCGGCGGCGGCCA GCTTGGT GGCC   -99
                                ::::::  :  :::: :::: :  :::
Human GRP94     -193            AATCGGAAGGAGCCACGCTTCG  GGCA  -168
                                :::::      :::::::: ::   :::
Chicken GRP94   -202            AATCGACGCCGGCCACGCTCCGTCCGCA  -175
```

FIG. 11. Sequences conserved in mammalian and yeast BiP (*KAR2*) promoters. The sequence in the *S. cerevisiae KAR2* promoter that is important for the induction by unfolded proteins in the ER is compared with sequences in the promoters of the human and rat *grp78* (BiP) and human and chicken *grp94* genes. The 5′ flanking regions of the mammalian genes were isolated and characterized by Lee and coworkers (6, 7, 62).

loses its ability to respond to heat shock, confirming that the remaining two modular repeats are insufficient to form a functional HSE.

Deletion of the HSE from the *KAR2* promoter has little effect on the basal level of expression of the gene. By contrast, when the GC-rich region (nucleotides −148 to −133) is deleted, basal expression of the reporter gene decreases to a low level, although inducibility by tunicamycin is not seriously affected. Seven of ten contiguous nucleotides in the GC-rich region are identical to the consensus sequence for binding of the mammalian transcription factor Sp1 (28), which is known to be involved in transcription of a human stress-70 gene (36). Basal expression of the *KAR2* promoter is not governed solely by the GC-rich region, since removal of upstream sequences (−1254 to −245) causes a modest reduction in basal expression, and deletion of nucleotides −194 to −172 results in a modest but significant increase in expression, suggesting the presence in this region of a negative regulatory element. Our present interpretation of our data is that the GC-rich region has a powerful and perhaps dominant influence on the level of basal expression. Further testing of this hypothesis will become possible only when a yeast homolog of Sp1 or an unrelated transcription factor that binds this GC-rich sequence has been identified and characterized.

The region from −136 to −107 of the *KAR2* promoter contains a UPR element that responds to treatment with tunicamycin and to the disruption of the maturation and transport of secretory proteins in the ER. As discussed earlier, two proteins of the mammalian ER, BiP (GRP78) and GRP94, are induced by various forms of stress (32) whose common denominator is likely to be the expression of unfolded proteins in the ER (30). The regulatory sequences of the genes coding for these proteins have been studied extensively by Lee and colleagues (6, 50, 65). By comparing the sequences of the promoters of the genes encoding human, rat, and chicken GRP78 and GRP94, Chang et al. (6) identified a common domain of 26 bp that interacts with a specific DNA-binding protein(s) (50) and is required, at least in part, for the induction of transcription of both genes by calcium ionophore. In the rat *grp78* promoter, a 50-bp region which includes this conserved domain contains a regulatory element for induction by calcium ionophore, by glycosylation block, and by the presence of malfolded proteins in the ER (65). This regulatory element appears to have been duplicated further upstream in the promoter (65). The activity of this element, as well as the basal expression of the promoter, requires in addition a 10-bp region spanning nucleotides −99 to −90, which includes a CCAAT element that binds the transcription factor CTF/NF-I (65).

The sequence of the *KAR2* promoter between −131 and −110 bears a striking resemblance to the 26-bp conserved domain in the mammalian *grp* genes (Fig. 11), although no sequence corresponding to the CCAAT element is present in the yeast promoter. Fifteen of 22 contiguous nucleotides are identical to residues located in homologous positions in one or other of the mammalian promoters. We have recently shown that this regulatory element from the yeast *KAR2* gene confers responsiveness to the presence of unfolded proteins in the ER to a heterologous promoter and binds a specific yeast transcription factor (37). In both mammalian and yeast cells, therefore, this nuclear regulatory element has the property of responding to events in another cellular compartment.

Because induction of BiP is caused by accumulation of unfolded or prefolded proteins in the ER, a specific sensing mechanism must exist to monitor fluctuations in the concentration of these molecules in the organelle. This mechanism is extremely sensitive and responds very quickly when abnormally folded proteins are introduced into the ER. However, it seems unlikely that unfolded proteins are themselves the trigger for induction of BiP, since the sensing mechanism would then be required to detect small changes in the large flux of unfolded polypeptides that normally flows into the ER. The inductive response is so rapid that it seems designed to preclude, rather than to discharge, any accumulation of unfolded proteins. Experiments performed in mammalian cells confirm that the accumulation of malfolded polypeptides in the ER does not by itself induce the unfolded protein response. Thus, neither the massive accumulation of the insoluble PiZ variant of human α_1-antitrypsin nor the distention of the ER that accompanies this accumulation is sufficient to elicit increased synthesis of BiP (18). Furthermore, the accumulation in the ER of deletion mutants of the simian virus 5 hemagglutinin-neuraminidase glycoprotein that do not bind to BiP does not cause induction of the unfolded protein response (41). Finally, stimuli other than the presence of unfolded proteins in the ER may also lead to induction of BiP, since in the case of one *sec* mutant, *sec18* (13, 20), in which BiP is induced at the nonpermissive temperature, secretory proteins that accumulate in the ER appear to be properly folded (14).

It is therefore likely that the sensing mechanism detects changes either in the concentration of complexes formed between BiP and unfolded proteins or in the concentration of free BiP itself. Our data support the latter possibility. Thus, the activation of the unfolded protein response in *kar2-1* cells is abrogated following expression in the cells of wild-type BiP protein, and overexpression of BiP in the ER mitigates UPR element-mediated transcriptional activation of the *KAR2* promoter either in wild-type cells treated with tunicamycin or in *sec53* cells cultured at the nonpermissive temperature. In each of these circumstances, it is likely that the concentration of complexes would be increased, and yet the induction of synthesis of BiP is suppressed. Overexpression of mammalian BiP similarly mitigates the unfolded protein response in Chinese hamster ovary cells (11). These results suggest that the formation of complexes between unfolded proteins and functional BiP protein is not the signal for UPR induction. That the sensing mechanism monitors the concentration of free, functional BiP in the ER is further supported by studies on a yeast BiP mutant lacking its C-terminal ER retention signal (22). Although this mutant protein was secreted from yeast cells, its intracellular concentration was maintained at wild-type levels by a compensatory up-regulation of its rate of synthesis.

The question then arises as to how information about the free BiP concentration is transduced from the ER lumen to the nucleus. Genetic evidence (56a) suggests that BiP interacts with at least one resident ER protein that spans the lipid bilayer, i.e., the transmembrane protein encoded by the *SEC63* gene (54). An attractive possibility is that Sec63p or another transmembrane protein acts as a BiP receptor and transmits signals about changes in the concentration of free BiP across the membrane of the ER. On the cytosolic and/or nuclear side of the membrane, this receptor could affect, directly or indirectly, the transcription factor that specifically binds to the sequences in the *KAR2* promoter which respond to the presence of unfolded proteins in the ER. We are now testing this model by using genetic methods to select yeast mutants that are defective at various stages of the signalling pathway and by characterizing further the biochemical properties of the protein that binds to the regulatory elements of the *KAR2* promoter.

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