Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 5450–5454, June 1993
Cell Biology

The *FKB2* gene of *Saccharomyces cerevisiae*, encoding the immunosuppressant-binding protein FKBP-13, is regulated in response to accumulation of unfolded proteins in the endoplasmic reticulum

(FK506/rapamycin/peptidyl-prolyl cis–trans isomerase/chaperonin/heat shock)

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Communicated by Fred Sherman, March 10, 1993

ABSTRACT **The *FKB2* gene of *Saccharomyces cerevisiae* encodes a homolog of mammalian FKBP-13, an FK506/rapamycin-binding protein that localizes to the lumen of the endoplasmic reticulum (ER). We have found that *FKB2* mRNA levels increase in response to the accumulation of unfolded precursor proteins in the ER. *FKB2* mRNA levels are elevated in cells blocked in N-glycosylation—i.e., in wild-type cells treated with tunicamycin and in the *sec53-6* mutant grown at the nonpermissive temperature. Mutations that block other steps in secretion have no effect on *FKB2* mRNA levels, indicating that increases in *FKB2* mRNA are not the consequence of a general block in secretion. The increase in *FKB2* mRNA in response to unfolded proteins in the ER is mediated through a 21-bp unfolded-protein response (UPR) element located in the 5′ noncoding region of *FKB2*. UPR elements present in other ER chaperone genes, such as yeast *KAR2* (BiP), mammalian GRP78 (BiP), and GRP94, function in an analogous manner to that in *FKB2*. As with *KAR2*, *FKB2* mRNA levels are also elevated by heat shock. The similarities in the regulation of *FKB2* and other ER chaperone genes suggest that FKBP-13 may play a role in protein trafficking in the ER.**

Immunophilins are proteins that bind with high affinity to the immunosuppressants FK506, rapamycin, and cyclosporinA (CsA). The immunophilins consist of two structurally distinct families: the FK506/rapamycin-binding proteins (FKBPs) and the CsA-binding cyclophilins. The FKBPs and cyclophilins are conserved proteins whose members localize to different subcellular compartments. Although the cyclophilins and FKBPs possess peptidyl-prolyl cis–trans isomerase (PPIase) activity inhibitable by CsA and by FK506 or rapamycin, respectively, inhibition of PPIase activity is not sufficient to mediate drug action. Rather, an immunophilin complexed with drug is the active entity, blocking signal transduction pathways in a variety of cell types as diverse as human T cells and yeast (reviewed in refs. 1 and 2; subsequent reports in refs. 3 and 4).

The cellular functions of the immunophilins in the absence of drug have not been established. PPIases accelerate the refolding of proline-containing polypeptides *in vitro* (5–7), suggesting a role for the immunophilins in protein folding *in vivo*. Consistent with this notion is the finding that CsA inhibits the folding of the triple helix of type I collagen in chicken embryo fibroblasts (8). Another possibility is that the immunophilins function in a manner analogous to chaperones, binding to unfolded protein intermediates and facilitating correct protein–protein interactions and intracellular protein trafficking. Support for this idea comes from studies of the *Drosophila melanogaster* photoreceptor-specific cyclophilin homolog ninaA, which is required for transport of rhodopsin through the secretory pathway (9). Mutant alleles of *ninaA* define a region required for rhodopsin biogenesis which encompasses the peptidyl-prolyl substrate-binding site, indicating that this is the functionally relevant region of the molecule (10). The recent finding that a CsA-sensitive immunophilin can accelerate the rate of protein folding of carbonic anhydrase by PPIase-dependent and -independent mechanisms suggests that immunophilins may be multifunctional (11).

Other immunophilins are found as constituents of protein complexes. FKBP-52 (12), also called FKBP-59 (13), p56 (14), and hsp56 (15), associates with the 90-kDa heat shock protein (hsp90) in the inactive steroid receptor complex (16); FKBP-12 binds with high affinity to the Ca^{2+}-release channel in skeletal muscle (17); and s-cyclophilin colocalizes with the Ca^{2+}-storage protein calreticulin (18). These observations suggest a role for immunophilins in steroid receptor function and in calcium regulation. However, the functional significance of these protein–protein interactions has not been established.

Yeast FKBPs, like their mammalian counterparts, possess PPIase activity that is inhibited by FK506 and rapamycin (19–21). We recently reported the isolation of *FKB2*, a yeast gene encoding a homolog of the human FKBP-13 (22). Yeast FKBP-13 is 57% and 52% identical with human FKBP-13 and yeast cytosolic FKBP-12, respectively. Both yeast and human FKBP-13 are membrane-associated proteins, containing hydrophobic leader peptides that precede the amino terminus of the mature protein (21–23). Recently, mammalian FKBP-13 has been localized to the endoplasmic reticulum (ER) (S. Burakoff, personal communication). However, information regarding the function or regulation of mammalian FKBP-13 has been lacking.

In yeast, a deletion of *FKB2* has no obvious effect on cell growth (ref. 21; V.B., unpublished results), suggesting that FKBP-13 is dispensable or that another member of the FKBP family substitutes functionally for FKBP-13 in its absence. Since certain genes specifying cellular chaperones are regulated in response to stress (24–26), we considered that this might be the case for FKBP-13. We report here that *FKB2* is regulated by two forms of stress: heat shock and conditions that cause accumulation of unglycosylated precursor proteins in the ER. The regulation of *FKB2* by increased levels of precursors in the ER parallels that of other ER chaperone genes: yeast *KAR2* (BiP), required for entry of proteins into the ER (27), and mammalian GRP78 (BiP) and GRP94 (26).

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Abbreviations: ER, endoplasmic reticulum; UPR, unfolded-protein response; FKBP, FK506/rapamycin-binding protein; CsA, cyclosporin A; PPIase, peptidyl-prolyl cis–trans isomerase.

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Yeast *EUG1* also exhibits similar regulation and encodes an ER protein that is a functional homolog of protein disulfide-isomerase (28). The latter is multifunctional, participating in protein transit through the secretory pathway as well as catalyzing protein disulfide bond formation, isomerization, or reduction (reviewed in ref. 29). The induction of *FKB2* by conditions that cause the accumulation of precursor proteins in the ER suggests that FKBP-13, like the ER chaperones, interacts with nascent polypeptides in the ER.

MATERIALS AND METHODS

Yeast Strains and Growth Media. Normal *Saccharomyces cerevisiae* strains used for the expression analysis were F119 (*MATa ade2 his3-11,15 ura3-1 trp1-1 leu2-3,112 can1-100 KSS1*$^+$; original source, R. Rothstein, Columbia University, New York) and VB86-19B (*MATa ura3-52 his3-Δ200 leu2-3,112 lys2-Δ201*). The temperature-sensitive *sec* mutants used in this study were VB13-17B (*MATa sec53-6 ade2 ura3*), VB10-3B (*MATa sec18 ade2*), VB8-2C (*MATa sec7 ade2*), and F159 (*MATa sec1-1 PMR1/HA169 ade2 his3-Δ200 leu2-3,112 lys2-Δ201 ura3-52*; obtained from G. R. Fink, Massachusetts Institute of Technology). Strains were grown in YPD or minimal media containing 0.67% yeast nitrogen base without amino acids (Difco) supplemented with the required amino acids and 2% glucose (30).

Growth Conditions. Tunicamycin experiments were performed by growing cells to midexponential phase ($OD_{600} \approx 0.4$) in YPD at 30°C. Tunicamycin (10 μg/ml) was added 2.5 hr prior to harvesting. Temperature-sensitive *sec* mutants were grown to midexponential phase ($OD_{600} \approx 0.4$) in YPD at 24°C. The culture was split in half: one half was maintained at 24°C and the other half was pelleted, resuspended in prewarmed YPD at 37°C, and grown for 2 hr prior to harvesting for Northern blot analysis. Growth of *sec* mutants for assaying the unfolded-protein response (UPR) element was performed as described by using the semipermissive temperature of 30°C, which is optimal for measuring β-galactosidase activity in the *sec53-6* mutant (31). Heat shock was performed by growing cells to midexponential phase in YPD at 25°C. The cells were pelleted, suspended in prewarmed YPD at 39°C, and maintained in a shaking water bath. Aliquots of cells were taken at various times, harvested on ice, and immediately frozen in a dry ice/ethanol bath.

RNA Isolation and Northern Blot Analysis. Total yeast cellular RNA was prepared by a glass-bead miniprep procedure (32). Denatured RNA was resolved in a 1.2% agarose/6% formaldehyde gel and transferred to a nylon membrane (Micron Separations, Westboro, MA). Equal sample loading was visualized by staining the gel with ethidium bromide. The hybridizations were performed as described (33). The probes used in the hybridization analysis were prepared by random hexamer-primed [α-^{32}P]dCTP labeling (34). The probes for *FKB1* and *FKB2* were derived from cDNA clones 1-8-1 and Z13-2, respectively (V.B., unpublished results; ref. 22). A 560-bp *KAR2*-specific probe extending from positions 1991 to 2559 (24) was generated by the polymerase chain reaction (PCR) using oligonucleotides 5′-ACGCTTCTATCAAGGCCAAGG-3′ and 5′-CCCCAGTC-TCTATACTCTTCA-3′. The ≈1.6-kb *Eco*RI–BamHI *ACT1* fragment was derived from plasmid pRB147 (35).

Plasmid Constructions. Plasmid pSLF178K, a 2μ yeast vector containing the *CYC1–lacZ* fusion, was kindly provided by L. Guarente (Massachusetts Institute of Technology). This plasmid contains the start site of transcription for *CYC1* but lacks all *CYC1* upstream activation sequences, producing very low levels of expression of *lacZ* in yeast (36). A PCR fragment containing *FKB2* sequence extending from −92 to −251 relative to the start site of translation (see Fig. 3) was cloned into pSLF178K digested with *Kpn* I and *Xho* I, restriction enzymes whose recognition sites are located upstream of *CYC1*. The PCR fragment contained *FKB2* sequence flanked by *Kpn* I and *Xho* I sites and several nucleotides of random sequence lost upon digestion with *Kpn* I and *Xho* I. The sequences of the oligonucleotides used for PCR were 5′-GGGTTTGGGTACCCGCATATATAATG-CAC-3′ and 5′-GGGTTTCCGCTCGAGCGGCCTGTATT-TGAAGATGC-3′. Synthetic double-stranded oligonucleotides encoding *FKB2* and *KAR2* UPRs (see Fig. 3) were cloned into the *Kpn* I and *Xho* I sites of pSLF178K. The double-stranded oligonucleotides had 5′ recessed and 3′ protruding sequences complementary to the single-stranded sequences generated by *Kpn* I and *Xho* I.

Enzyme Assays. Assays of β-galactosidase activity in extracts of yeast cells were performed by a procedure in which units are defined as $(OD_{420} \times 10^3)/(OD_{600} \times tv)$ where t is incubation time in minutes and v is volume in milliliters (37).

RESULTS

Accumulation of Unfolded Proteins in the ER Increases the Levels of *FKB2* mRNA. Tunicamycin is a drug that inhibits core oligosaccharide addition to nascent polypeptides in the ER, blocking proper protein folding and transit through the secretory pathway (38). Treatment of cells with tunicamycin is one of several perturbations of protein glycosylation that induces transcription of ER chaperone genes such as yeast *KAR2* (24) and mammalian GRP78 and GRP94 (26), as well as *EUG1*, encoding a functional homolog of protein disulfide-isomerase (28). *FKB2* mRNA, like that of the ER chaperone genes, accumulates in cells treated with tunicamycin. The effect of tunicamycin on *FKB2* mRNA levels is comparable to its effect on *KAR2* mRNA levels. Tunicamycin treatment has no effect on the expression of *FKB1*, which encodes the cytosolic FK506/rapamycin-binding protein FKBP-12, or on the expression of the gene for actin (Fig. 1).

Induction of *FKB2* transcription by tunicamycin treatment could be due to a general block in secretion or to the specific accumulation of precursors in the ER. To distinguish between these two possibilities, we analyzed the effect of mutations which block specific steps of secretion on the levels of the *FKB2* transcript. *FKB2* mRNA is elevated in the *sec53-6* mutant grown at the nonpermissive temperature. Like tunicamycin, the *sec53-6* mutation, which abolishes phosphomannomutase activity, blocks core glycosylation,

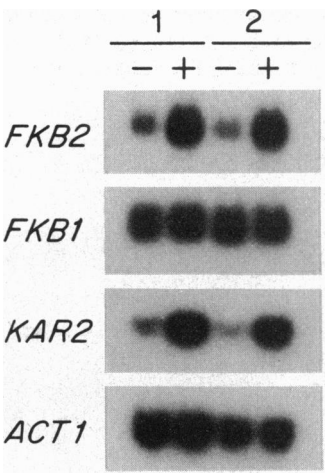

FIG. 1. *FKB2* mRNA levels are elevated in cells treated with tunicamycin. Shown are Northern blots containing total RNA samples (20 μg per lane) isolated from two different wild-type strains, VB86-19B (lanes 1) and F119 (lanes 2), untreated (−) or treated (+) with tunicamycin for 2.5 hr. Blots were hybridized with probes specific for the genes indicated at left.

causing the accumulation of unfolded precursors in the lumen of the ER (39, 40). Mutants blocked at other steps of secretion do not have elevated *FKB2* mRNA levels. These include *sec61-2*, *sec62-1*, and *sec63-2* mutants, which are blocked in entry of polypeptides into the ER (results not shown) (41–43), the *sec18* mutant, which is blocked in ER-to-Golgi transport (44), and the *sec7* mutant, which accumulates polypeptides in the Golgi apparatus (45). A modest increase in *FKB2* mRNA levels occurs in *sec1-1* cells grown at the nonpermissive temperature, which blocks exocytosis, causing the accumulation of secretory vesicles (46). *FKB1* mRNA levels, in contrast to those of *FKB2*, are not elevated in any of the *sec* mutants that were tested (Fig. 2). These results and those described below indicate that accumulation of unglycosylated precursors in the ER, and not a block in secretion, induces the *FKB2* gene.

***FKB2* Contains a UPR Element That Regulates Transcription in Response to the Accumulation of Unfolded Proteins in the ER.** A 22-bp UPR element present upstream of the transcription start site of *KAR2* mediates transcriptional induction in response to a block in core glycosylation (31). Sequences homologous to the *KAR2* UPR element are present in the promoters of mammalian GRP78 and GRP94 genes (47) and the yeast *EUG1* gene (28). We found a putative UPR element in *FKB2* at positions −101 to −121 relative to the start site of translation that shows significant homology to the other UPR elements (Fig. 3).

We tested the ability of the putative *FKB2* UPR element to activate transcription of the *lacZ* gene of *Escherichia coli* fused to the yeast *CYC1* promoter lacking its upstream activator sequences (50). When introduced into yeast cells, the parent *CYC1–lacZ* fusion displays no stress inducibility. Oligonucleotides containing the putative *FKB2* UPR (Fig. 3) inserted directly upstream of the *CYC1–lacZ* fusion increase β-galactosidase levels ≈13-fold in cells treated with tunicamycin compared with those that were untreated. A 160-bp DNA fragment (−92 to −251 from translation start) encompassing the putative *FKB2* UPR element increases β-galactosidase activity to the same extent as the 27- or 21-bp oligonucleotide, indicating that the latter is sufficient to induce transcription of a foreign promoter in response to stress (Table 1).

The *FKB2* UPR oligonucleotides or the 160-bp DNA fragment containing the UPR element (Fig. 3) fused to *CYC1–lacZ* also increases β-galactosidase levels ≈7-fold in *sec53-6* cells but are not active in *sec18* or *sec1-1* cells grown at the semipermissive temperature (Table 1). These results indicate that the *FKB2* UPR element acts specifically as a transcriptional activator in response to accumulation of unglycosylated, unfolded precursors in the ER.

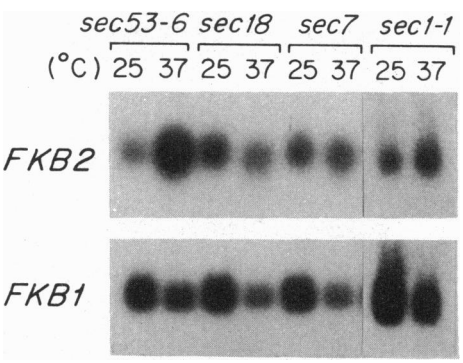

FIG. 2. *FKB2* mRNA levels are elevated in certain secretory-pathway mutants. Shown are Northern blots containing total RNA samples (20 μg per lane) isolated from various *sec* mutants, as indicated, incubated at permissive (25°C) or restrictive (37°C) temperatures. Blots were hybridized with probes specific for the genes indicated at left. Equal sample loading was visualized by staining the gel with ethidium bromide.

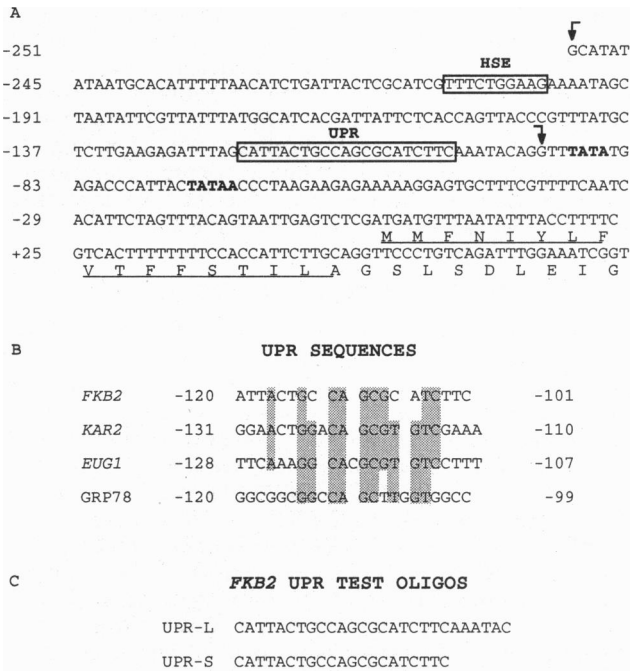

FIG. 3. (*A*) The sequence of the *FKB2* promoter region (22). Negative numbers refer to the nucleotide positions upstream of the translational start site at +1. The hydrophobic signal sequence (amino acids 1–17) is underlined. The TATA boxes are shown in bold. A putative heat shock element (HSE) and a UPR element are boxed. The general HSE consensus sequence is nTTCnnGAAn, consisting of two 5-bp inverted repeats (48). The yeast HSE consensus sequence is TTCTAGAA (49). Arrows bracket the 160-bp *FKB2* region (−92 to −251) cloned into pSLF178K. (*B*) Alignment of UPR sequences from *FKB2*, *KAR2* (31), *EUG1* (28), and human GRP78 (47). Each sequence is numbered relative to the translational start site. Shaded regions highlight identical nucleotides in the UPR elements. (*C*) Oligonucleotides containing the *FKB2* UPR element. The *FKB2* UPR sequences were cloned immediately upstream of the TATA box of *CYC1–lacZ* in pSFL178K (36) and were tested for function.

***FKB2* Is Regulated by Heat Shock.** Among the genes that encode chaperones are those, like *KAR2*, that are induced by several forms of cellular stress (24, 25, 51). Upstream of the UPR element in the promoter region of *FKB2* is a conserved element that has been shown to mediate transcriptional induction in response to heat shock (48). When wild-type cells are shifted to a temperature above that which is optimal for growth, *FKB2* mRNA levels decrease within the first 10 min but then steadily rise above non-stressed levels, reaching maximum levels at 50 min. *KAR2* mRNA levels also increase in response to heat shock, but without exhibiting the initial decline observed for *FKB2* mRNA levels. *FKB1* mRNA levels, on the other hand, do not increase, but progressively decline during the first 30 min of heat shock (Fig. 4).

DISCUSSION

Examination of steady-state mRNA levels indicates that *FKB2*, encoding FKBP-13, the ER member of the FKBP family (refs. 21–23; S. Burakoff, personal communication), is regulated in response to cellular stress. *FKB2* is expressed constitutively but is also induced by conditions that cause the accumulation of unglycosylated, unfolded proteins in the ER. *FKB2* mRNA levels are elevated by tunicamycin treatment or the *sec53-6* mutation, both of which block N-glycosylation and preclude the proper folding of precursors in the ER

Table 1. Activity of *FKB2* promoter elements in stressed versus unstressed cells

Strain	Promoter element	β-Galactosidase units		Induction
		Unstressed	Stressed	
		− TUNI	+ TUNI	
SEC+	None	4.5	2.7	0.6
	160 bp	4.2	80.2	19.1
	UPR-L	1.5	19.5	13.0
	UPR-S	1.1	13.7	12.5
		24°C	30°C	
sec53	None	8.4	7.4	0.9
	160 bp	10.0	44.5	4.5
	UPR-L	6.3	22.9	3.6
	UPR-S	4.2	31.3	7.5
sec18	None	3.6	4.0	1.1
	160 bp	6.0	9.0	1.5
	UPR-L	3.3	5.3	1.6
	UPR-S	5.8	6.5	1.1
sec1	None	1.6	2.3	1.4
	160 bp	2.3	3.5	1.5
	UPR-L	1.7	1.3	0.8
	UPR-S	1.2	1.6	1.3

β-Galactosidase activity was measured in triplicate in SEC+ (F119) and *sec* mutant cells transformed with pSLF178K (36) containing the indicated *FKB2* promoter elements. The 160-bp region and the UPR-L and UPR-S sequences are described in Fig. 3. SEC+ cells were grown in the absence (− TUNI) or presence (+ TUNI) of tunicamycin. The *sec* mutants were grown at the permissive (25°C) or semipermissive (30°C) temperature (see *Materials and Methods*). Induction refers to the ratio of β-galactosidase activity in stressed cells to that in unstressed cells.

necessary for their transit through the secretory pathway (38–40). We investigated whether the accumulation of *FKB2* mRNA occurs in response to a general block in secretion and found that *FKB2* mRNA levels are unaffected by other mutations that either block entry of proteins into the ER or affect later steps of secretion. These results, in light of the recent finding that mammalian FKBP-13 is localized to the lumen of the ER (S. Burakoff, personal communication), suggest that FKBP-13 participates in protein folding or trafficking in this subcellular compartment.

We have identified a UPR transcriptional regulatory element located ≈100 nucleotides 5′ of the putative translation start site in *FKB2*. The UPR element in *FKB2* shows homology to those in the ER chaperone genes, yeast *KAR2*/BiP and mammalian GRP78/BiP and GRP94, that are regulated in response to the accumulation of unfolded precursors in the ER (27, 31, 47). A UPR element is also found in the promoter region of the yeast *EUG1* gene (28). It is noteworthy that *EUG1* and *FKB2*, both of which encode ER proteins implicated in protein folding and trafficking, are near each other on chromosome IV (22), located ≈300 bp apart. However, the orientation of *EUG1* and *FKB2* is such that they are transcribed from the same DNA strand and, consequently, do not share promoter elements (J.A.P., unpublished results).

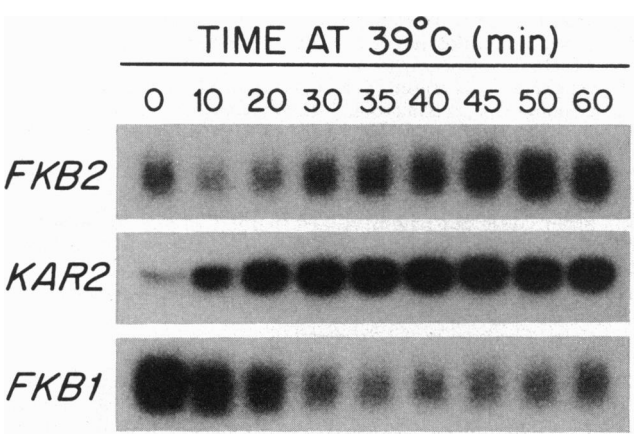

FIG. 4. *FKB2* mRNA levels are regulated by heat shock. Shown are Northern blots containing total RNA samples (15 μg per lane) isolated from the wild-type strain F119 grown for the indicated times at 39°C. Blots were hybridized with probes specific for the genes indicated at left. Equal sample loading was visualized by staining the gel with ethidium bromide.

The UPR element in *FKB2*, like that in *KAR2*, is sufficient to induce transcription in response to a block in N-glycosylation. When fused immediately upstream of the TATA box of a heterologous promoter, the *FKB2* UPR element mediates transcriptional induction in wild-type cells treated with tunicamycin or in *sec53-6* cells incubated at the semipermissive temperature. Transcriptional induction mediated by the *FKB2* UPR element occurs specifically in response to the accumulation of unglycosylated, unfolded precursors in the ER; no transcriptional induction occurs via the *FKB2* UPR element in response to earlier blocks in secretion that cause the accumulation of secretory precursors in the cytoplasm or later blocks that cause the accumulation of precursors that have undergone core glycosylation and folding in the ER. In this regard the specificity of the *FKB2* UPR element differs from that in *KAR2*, which mediates transcriptional induction in both the *sec53-6* and the *sec18* mutant (24, 25). In contrast to the *sec53-6* mutant, the *sec18* mutant is unaffected in N-glycosylation but rather is blocked in the transit of normally folded, glycosylated precursors from the ER to the Golgi apparatus (44).

The ER chaperone genes are coordinately regulated in response to the accumulation of unfolded precursors in the ER (26, 31, 47) and are implicated in protein folding and formation of multisubunit complexes destined for secretion. Recent studies have demonstrated physical association of KAR2/BiP, GRP78/BiP, and GRP94 with polypeptides in the ER. Mammalian GRP78/BiP and GRP94 associate with the heavy and light chains of immunoglobulin and are thought to maintain the immunoglobulin subunits in a state that enables them to fold and assemble into functional antibody molecules (52, 53). Yeast KAR2/BiP is part of the translocation machinery responsible for import of proteins into the ER and recently has been shown to bind to a modified form of a secretory protein (prepro-α-factor) trapped during translocation across the ER membrane (27). Transcriptional induction of *FKB2* by accumulation of precursors in the ER suggests FKBP-13, like the ER chaperones, may interact with nascent polypeptides upon their transport into the ER.

Many chaperone genes are regulated by a variety of stresses, including heat shock. Among the heat shock genes are those that are critical to the cell's ability to survive stress and, additionally, function as chaperones under normal growth conditions (51). *FKB2*, like *KAR2*, is regulated in response to heat shock. Although the 5′ noncoding region of *FKB2* contains a heat shock element consisting of two conserved 5-bp modules (48), neither the heat shock element alone nor the 160-bp fragment containing this element (Fig. 3) was sufficient to induce the expression of the *CYC1–lacZ* fusion in response to prolonged heat shock (J.A.P., unpublished results). No additional heat shock elements are present upstream of the one found in *FKB2*. Sequences flanking heat shock elements can affect both basal and heat-inducible expression (54). Therefore, further characterization of *FKB2* 5′ noncoding sequences is required to define their effect on the regulation of *FKB2* by heat shock.

The ninaA protein, the photoreceptor-specific cyclophilin in *D. melanogaster* that is required for transit of a specific rhodopsin isoform through the secretory pathway, localizes

to the ER and secretory vesicles (9, 55). By analogy with ninaA, FKBP-13 may function as an ER chaperone or, alternatively, facilitate protein folding by virtue of its PPIase activity, two possibilities which are not mutually exclusive. Unlike *KAR2*, *FKB2* is not an essential gene (ref. 21; V.B., unpublished results), nor does a deletion of *FKB2* affect growth rate (J.A.P., unpublished results). This suggests that FKBP-13 does not play a global role in protein trafficking but rather, like ninaA, may be required specifically for the transit of one or a few nonessential proteins. Alternatively, other proteins may be redundant with respect to FKBP-13 function, making FKBP-13 dispensable.

The immunosuppressants FK506 and CsA are thought to act by binding to their cytosolic receptors, FKBP-12 and cyclophilin, respectively, which then complex with and inhibit calcineurin, a Ca^{2+}-dependent phosphatase (56). Since these immunosuppressants, as well as rapamycin, bind with high affinity to other members of the immunophilin family (12, 21, 57), modulation of other cellular functions may contribute to their overall biological activity. In this regard it is interesting to consider whether inhibition of FKBP-13 could affect the processing and presentation of antigen, which is thought to occur in the lumen of the ER in antigen-presenting cells (58). Recently, CsA and another immunosuppressant, deoxyspergualin, have been shown to bind to hsp70 and the heat shock cognate protein hsc70, respectively (59, 60). Binding of immunosuppressants to chaperones and proteins, such as FKBP-13, that are implicated in protein trafficking suggests that immunosuppressants may exert their effects, in part, by interfering with protein folding mechanisms within the cell.

We thank Leonard Guarente for providing pSLF178K; Matt Harding, Patricia Nelson, and Debra Peattie for critical reading of the manuscript; and Stephen Burakoff for kindly communicating results prior to publication.

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