Conservation and Divergence of the Yeast and Mammalian Unfolded Protein Response

ACTIVATION OF SPECIFIC MAMMALIAN ENDOPLASMIC RETICULUM STRESS ELEMENT OF THE grp78/BiP PROMOTER BY YEAST Hac1

(Received for publication, May 3, 1999, and in revised form, July 20, 1999)

Dolly M. Fotić, Ajith Welihinda, Randal J. Kaufman, and Amy S. Lee

From the Department of Biochemistry and Molecular Biology and the University of Southern California/Norris Comprehensive Cancer Center, University of Southern California School of Medicine, Los Angeles, California 90089 and the Department of Biological Chemistry, and the Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor, Michigan 48109

Yeast Hac1 (yHac1), the transcription factor that binds and activates the unfolded protein response element of endoplasmic reticulum (ER)-chaperone gene promoters, only accumulates in stressed cells after an unconventional splicesome-free mRNA processing step and escape from translation block. In determining whether the novel regulatory mechanisms for yHac1 are conserved in mammalian cells, we discovered a unique unfolded protein response element-like sequence within the endoplasmic reticulum stress element 163, one of the three ER stress elements recently identified in the rat grp78 promoter. The unspliced form of yHac1 is stably expressed in nonstressed mammalian cells and is as active as the spliced form in stimulating the promoter activities of grp genes. Further, the yHac1 mRNA is not processed in response to ER stress in mammalian cells. We identified a CCAGC motif as the yHac1 binding site, which is contained within a YY1 binding site previously shown to be important for mammalian UPR. Dissection of the yHac1 and the YY1 binding sites uncovered specific contact points for an activator protein predicted to be the mammalian homolog of yHac1, the activity of which can be stimulated by YY1. A model of the conserved and unique features of the yeast and mammalian unfolded protein response transcription machinery is proposed.

The endoplasmic reticulum (ER) is an essential organelle for cell viability. In addition to serving as a major intracellular store for calcium and the production site for lipids and sterols, ER is the cellular organelle for the synthesis, assembly, and glycosylation of proteins that are destined for secretion or transport to the cell surface. Perturbations of ER result in stress signaling from the ER to the nucleus, leading to the activation of specific sets of genes (1, 2). Conserved from yeast to human is a mechanism referred to as the unfolded protein response (UPR) (3, 4). In Saccharomyces cerevisiae, several key components of the UPR have been identified through genetic analysis and biochemical characterizations. Thus, the yeast UPR is mediated by a novel signaling pathway that requires ER stress-inducible splicing of the primary transcript of Hac1, also referred to as Ern4p (5, 6). The open reading frame of the Hac1 gene encodes a protein of 230 amino acids (aa), whereas the active transcription factor is a 238-aa protein. In nonstressed yeast cells, the unspliced Hac1 mRNA is prevented from translation into the 230-aa protein by the presence of a translation attenuator in a 252-bp intron (6, 7). Upon induction of the UPR, unconventional splicing mediated by Ire1p results in the elimination of the intronic sequence, which contains the codons encoding the last 10 aa of the 230-aa Hac1 and part of the 3′-untranslated region. Although the last 10 aa of 230-aa Hac1 is deleted through this process, the newly spliced Hac1 mRNA contains an additional exon of 18 aa, resulting in the translation of the 238-aa Hac1. Finally, in yeast cells, 230-aa Hac1 and 238-aa Hac1 produced from exogenous vectors are stable and can bind UPRE; however, the transactivating activity of the 230-aa Hac1 is lower than that of the 238-aa Hac1 (6).

The recognition sequence for yHac1 is a 22-bp sequence referred to as the unfolded protein response element (UPRE), which is found in the ER stress-inducible promoter of yeast ER resident protein genes such as KAR2 and PDI1. The yeast UPR contains an imperfect palindromic spacer with a single nucleotide (CAGGTG). The steric configuration of the UPRE appears to be critical because insertion or deletion of a single nucleotide within the spacer region results in the complete loss of its ability to mediate the UPR (8). One of the best characterized ER stress-inducible genes is grp78, the mammalian homolog of KAR2 (1). Contrary to yeast, the rat grp78 promoter is functionally redundant and contains three ER stress elements termed ERSE (9, 10). The consensus ERSE derived from comparison of a variety of ER stress-inducible mammalian promoters consists of a tripartite structure CCAAT(N9)CCACG, with N9 being a strikingly GC-rich region of 9-bp. The ERSE physically and functionally interacts with multiple mammalian transcription factors including that of YY1, Y-box proteins, YB-1, dbpA, NF-Y/CBF, and a newly discovered ER stress-inducible complex termed ERSF (10–13). Although the various ERSEs share similar sequence motifs and can independently confer the mammalian ER stress response to heterologous promoters (10, 14, 15), there are subtle differences that are evolutionarily conserved and could have functional implications for their regulation. For example, ERSE-163 contained within the grp78 core (16) bears the sequence CCAGC instead of the consensus CCACG found in ERSE-98 contained...
within the C1 element, which is most proximal to the TATA element (10). The CCAGC sequence within ERSE-163 of the grp78 core is of particular significance because in vivo genomic footprinting revealed that it is the major region on the grp78 promoter where ER stress-induced factor occupancy changes are detected (16), correlating with its functional importance based on 5′ deletion analysis (17).

We have previously identified the multifunctional transcription factor YY1 as a major DNA-binding protein to ERSE-163 (12). The unconventional YY1 binding site encompasses the CCAGC motif and its flanking sequence. In co-transfection studies using NHI3T3 cells, YY1 had little effect on the basal expression of the grp78 promoter but specifically enhanced induction of the grp78 promoter in a DNA binding-dependent manner by a variety of ER stress conditions. Consistent with divergent transcriptional regulatory mechanisms for the different ERSEs, YY1 stimulates the grp78 core but the same effect was not observed for the C1 element containing ERSE-98 (10). Interestingly, YY1 has not been found in yeast, and the mammalian homolog of Hac1 has not yet been identified.

Based on the similarity between the mammalian and yeast UPR, we seek to determine whether the unique regulatory mechanism for Hac1 discovered in S. cerevisiae are conserved in mammalian cells. Our studies revealed several novel observations suggesting both conservation and divergence of the mammalian and yeast UPR. We discovered that among the three ERSEs of the rat grp78 promoter, only ERSE-163 contained within the grp78 core shares sequence identity with the partial palindromic sequence of the yeast UPRE. This is the only ERSE that can bind and can be transactivated by yHac1. We further showed that yHac1 mRNA is not processed in response to ER stress in mammalian cells. Both the unspliced and spliced form of yHac1 can be stably expressed in non-stressed mammalian cells and exhibit similar stimulatory activity toward the grp78 promoter. The grp94 promoter, which shares sequence identity with the 78core (17), is also strongly activated by yHac1. The binding site of yHac1 is contained within the YY1 binding site, and binding of yHac1 to ERSE-163 is strictly dependent on the sequence integrity of the CCAGC motif. Through base mutations that selectively destroy either the yHac1 or YY1 binding site, we defined critical contact points for an activator protein predicted to be the mammalian homolog of yHac1, the activity of which can be stimulated by YY1. Based on these observations, we propose a model of the conserved and unique features of the yeast and mammalian UPR transcription machinery.

MATERIALS AND METHODS

Plasmid Constructions—To construct the mammalian expression vector for 230-aa Hac1, Hac1 was released from the plasmid plasmid T74E5/CCAGC (gift of R. Buettner, University of Regensburg, Germany) to generate CMV-YY1 (gift of Y. Shi, Harvard Medical School) was used undigested.

Gel Mobility Shift Assays—For EMSAs with in vitro translated proteins, 2 μl of the TNT in vitro coupled transcription/translation reaction mixture were used in the binding reactions. In some reactions, 5 μl of cell lysate from transfected cells were used. Hac1 binding conditions were preincubated in 20 μl of the TNT translation reaction buffer and harvested in Nonidet P-40 lysis buffer. Cell extracts were analyzed by SDS-polyacrylamide gel electrophoresis and Western blotting.

Activation of the grp78 Promoter by Yeast Hac1

RESULTS

Unique Occurrence of a UPRE-like Sequence within the Conserved grp78 Core—The rat grp78 promoter contains three ERSEs (ERSE-98, ERSE-131, and ERSE-163) upstream of the TATA element (Fig. 1A). In slight variation to the consensus ERSE sequence of CCAGAT<TAG<CCAGC, ERSE-163 contains a CGAT motif separated by 9-bp from a CCAGC motif. Further, in contrast to other ERSEs, ERSE-163 contained within the grp78 core region spanning 170 to 135 exhibits two distinct features, suggesting its functional importance in the in vivo regulation of the endogenous grp78 promoter. First, in vivo genomic footprinting revealed that stress-inducible footprint changes occurred primarily within the GGCCAGCTTG se-
sequence. A, localization of the ERSEs in the rat grp78 promoter and comparison of the grp78 core sequence, the yeast UPRE, as well as the one nucleotide insertion mutant UPREm, that were used in EMSAs to investigate binding activity and specificity of yHac1. Boxed nucleotides in the 78core sequence represent the two conserved motifs separated by 9-bp regions. Open stars and arrows denote inducible changes detected by in vitro genomic footprinting (18). In the UPRE and UPREM sequences, the yHac1 binding site is boxed, with the single base addition within UPREm indicated by an italicized lowercase letter. Other lowercase letters represent linker sequences. Bold letters indicate the nucleotides identical between 78core and the UPRE. B, schematic drawing of the expression vector used for in vitro transcription/translation of Hac1 (230 aa), as well as for co-transfection experiments in NIH3T3 cells. The relative locations of the ATG translation initiation codon, the basic (b) and the leucine zipper (LLV) domains within the BamHI (B) and EcoRI (E) fragment are indicated. C, lysates from cells transfected with pCMX-Hac1 (230 aa) were tested by EMSA to detect UPRE-specific binding activity versus control cells, which were transfected with the empty vector. 5 μl of the cell lysate were preincubated in binding buffer containing no competitor (lanes 1 and 4), 50-fold molar excess of UPRE (lanes 2 and 5), or UPREM (lanes 3 and 6) as competitor, and binding reactions were further carried out with 100,000 cpm of labeled UPRE sample.

The binding of Hac1 to the UPRE is strictly dependent on the sequence integrity of the UPRE, because insertion of only 1 bp within the UPRE as in the case of UPREM (Fig. 1A) completely eliminates Hac1 binding. Scanning of the rat 78 promoter sequence reveals no other substantial match with the yeast UPRE, and no Hac1 binding was detectable with the other ERSEs (data not shown).

Functional Conservation of yHac1 as a Transcription Regulator of the Mammalian grp78 Promoter—With the identification of a UPRE-like sequence within the grp78 core, we next tested whether yHac1 can be stably expressed in mammalian cells and what effect, if any, it has on the grp78 promoter activity under stressed and nonstressed conditions. First, 230-aa Hac1 was subcloned into a mammalian expression vector (Fig. 1B) and transiently transfected into NIH3T3 cells. To test for the ability of the mammalian cells to stably express yHac1, cell lysates were prepared from cells either transfected with the empty vector or the Hac1 expression vector. The cell extracts were used in EMSAs with the UPRE as probe (Fig. 1C). In the control cells transfected with empty vector, no UPRE binding activity was detected. In cells transfected with the Hac1 expression vector, a strong UPRE binding complex was readily detected. The specificity of UPRE binding was confirmed by efficient competition with the wild type but not the mutated UPRE.

To test the effect of overexpression of 230-aa Hac1 on grp78 promoter activity, the expression vector for Hac1 was co-transfected into NIH3T3 cells with −154CAT, which retains all the ER stress-inducible properties of the rat grp78 promoter (14). As expected, in cells transfected with the empty vector, an 8-fold induction of the promoter activity was detected with the ER stress inducer Tg, which depletes the ER calcium store and tunicamycin, which blocks protein glycosylation (Fig. 2). In nonstressed cells, co-transfection of 230-aa Hac1 resulted in a 7-fold induction of the basal grp78 promoter activity. Upon treatment of the cells with Tg or tunicamycin, the overall promoter activity further increased to about 25-fold (Fig. 2). Similarly, the grp94 promoter, which shares sequence identity with the 78core (17), was also strongly induced by yHac1. These results indicate that yeast 230-aa Hac1, when overexpressed in mammalian cells, is highly functional as a transcription factor and acts as a potent stimulator of the grp promoter activities in both nonstressed and stressed cells.

Stable Expression of Unspliced yHac1 in Mammalian Cells—Because yHac1 can exist as a 230-aa protein if the translation is terminated at the first termination codon or as a 238-aa protein if the primary transcript has undergone splicing, we compared the potency of the various forms of yHac1 to enhance grp78 promoter activity. Prior to these experiments, it is of interest to determine whether transfection of the intact yHac1 gene into nonstressed mammalian cells would result in translation block as observed in S. cerevisiae or whether translation would proceed resulting in a 230-aa protein that could be stably expressed. To facilitate detection of the yHac1, the HA epitope was inserted into the amino end of the Hac1 coding sequence, which was subcloned into the mammalian expression vector, pED (Fig. 3A). Similarly, an HA-tagged Hac1 gene encoding the induced form of 238-aa Hac1 was also constructed. Upon tran-
sient transfection into COS cells, yHac1 protein expression level was determined by Western blotting with antibodies against the HA-epitope (Fig. 3B). The unusual electrophoretic mobility of the 230-aa Hac1 and 238-aa Hac1 was in agreement with that previously reported for Hac1 (6). As expected, 238-aa Hac1 was produced from the vector expressing the induced form of Hac1. However, with the expression vector containing the unspliced form of the Hac1 gene, predominantly 230-aa Hac1 was produced. These results revealed that in contrast to S. cerevisiae, the unspliced form of yHac1 can be translated and stably expressed in nonstressed mammalian cells.

To investigate further whether yHac1 mRNA is processed to produce the 238-aa Hac1 in response to ER stress in mammalian cells, COS-1 cells transiently transfected with the expression vectors for HAC1 wild type but not the mutant UPRE. Further, as reported for yHac1 (8), this binding activity was enhanced by the addition of 10 mM magnesium in the binding reaction (data not shown).

To map more precisely the critical DNA contact points for yHac1 within the 78core sequence, the ability of a set of mutants of the 78core (M1 through M4) with clustered 4-bp mutations around the UPRE-like site to bind the UPRE were included as positive controls. As expected, the strongest binding of yHac1 was observed with the UPRE probe. Among the 78core sequence, yHac1 bound with similar affinity to M3 and M4 as with the wild-type sequence. In contrast, binding of Hac1 to M2 was severely diminished. In M2, the CAGCTTG sequence motif at 3' half of the UPRE-like sequence may not be critical for yHac1 binding. For M4, which lies outside the boundary of the UPRE-like sequence, this mutation was without effect on yHac1 binding. Interestingly, for M1, yHac1 binding was slightly stronger. This could be due to the fact that the last mutated base in M1 changed a C residue to A, resulting in more similarity with the UPRE core sequence ACAGCTTG.
Activation of the grp78 Promoter by Yeast Hac1

of mutations to −152 to −141 (16). For YY1 binding to the 78core, M1 and M3 mutation severely diminished YY1 binding, whereas M2 retained weak binding affinity for YY1 (16). Lastly, in vitro translated YY1 while exhibiting strong binding to the 78core does not bind the UPRE (Fig. 6B).

**Hac1 Stimulation of Specific Mammalian ERSE**—If yHac1 stimulation is dependent on its binding to the grp78 promoter, the prediction is that yHac1 should be able to stimulate the promoter activity mediated by ERSE-163 contained within the grp78 core sequence but not the C1 element, which does not contain a UPRE-like sequence and does not bind yHac1 (Fig. 7A). Further, mutation of the UPRE-like sequence to eliminate specifically yHac1 binding within the 78core should diminish Hac1-mediated stimulation. Because the M2 mutation resulted in the loss of Hac1 binding and reduction in YY1 binding activity (Fig. 6), we created a new 78core mutant M5, which contains a single base mutation that changes the critical CAGC sequence to CATC. This mutation resulted in even stronger binding for YY1 (Fig. 7A), because the CATC motif corresponds to the consensus YY1 binding site (24). However, the ability to bind yHac1 was severely diminished for M5 as compared with the wild-type core (Fig. 7A). For 78C1, although it does not bind yHac1, YY1 binding can be detected and is mediated by the CCACG motif (11).

![Fig. 5. Binding of yHac1 to the grp78 core.](image)

**Fig. 5. Binding of yHac1 to the grp78 core.** 1.5 μl of in vitro translated yHac1 were used in EMSAs with labeled 78core as probe (lanes 1–3). The complex formed as shown in lane 1 was competed by a 10-fold molar excess of unlabeled UPRE (lane 2) or UPREmut (lane 3). In lane 4, labeled UPRE was used as probe in the absence of competitor.

![Fig. 6. Delimitation of the boundary of the yHac1 binding site within the grp78 core.](image)

**Fig. 6. Delimitation of the boundary of the yHac1 binding site within the grp78 core.** A, the EMSAs were carried out with 2 μl of in vitro translated yHac1 and 100,000 cpm of each of the oligomers indicated on top. B, sequences of the 78core and its mutants (M1 and M4) as compared with UPRE. Solid boxes highlight the UPRE or UPRE-like site. The broken boxes indicate the boundaries of the YY1 binding site previously defined using the same set of mutant oligomers (16). Italicized, bold lowercase letters indicate mutated sequences. The ability of each oligomer to bind yHac1 and YY1 is summarized. +, binding; ++, strong binding; +/−, partial binding; −, no binding.

![Fig. 7. Sequence-specific induction of ERSEs by yHac1.](image)

**Fig. 7. Sequence-specific induction of ERSEs by yHac1.** A, sequences of 78core, 78core with the M5 mutation, and 78C1 are shown. The ERSE sequences are bracketed. The partially conserved sequence motifs within the ERSEs are boxed. In the M5 mutant, a single point mutation converts the G residue of the 78core CCAGC to T. The ability of each of the oligomers to bind translated yHac1 and YY1 is summarized. B, NIH3T3 cells were co-transfected by the SuperFct method with 4 μg of promoter construct (78coreCAT, 78core(M5)CAT, or 78C1CAT), 2 μg of pMCX-Hac1(230aa), and empty vector to a final DNA concentration of 10 μg/μl. 1 μg of SV40 β-galactosidase was included in the assay to monitor the efficiency of transfection. The transfected cells were either untreated or treated with 300 nM Tg. The fold stimulation of the promoter activities with standard deviations are shown.

To test the promoter sequence requirements for yHac1 stimulation in vitro, CAT reporter genes 78coreCAT, 78core(M5)CAT, and 78C1CAT containing tandem copies of the synthetic oligomers shown in Fig. 7A linked to the minimal promoter of mouse mammary tumor virus were co-transfected with the Hac1 expression vector or empty vector into NIH3T3 cells. In nonstressed cells, overexpression of yHac1 was able to stimulate the 78core promoter activity by about 4-fold (Fig. 7B). For M5, there was minimal increase of 1.2-fold, and for 78C1, no increase was observed. In cells treated with Tg, yHac1 further stimulated the overall 78core promoter activity about 6-fold. For M5, Tg stress induction was lost, and in the presence of yHac1, only 1.5-fold increase was detected. In the case of 78C1, its promoter activity increased to about 5-fold in Tg-treated cells and was not affected by overexpression of yHac1. Thus, the stimulation of the grp78 promoter activity by yHac1 is promoter sequence-specific and strictly dependent on the sequence integrity of the CAGC sequence motif. Further, results from the 78core(M5) mutation shows that YY1 binding is not sufficient to confer stress inducibility, suggesting the involvement of an as yet unidentified mammalian factor with CAGC as its critical binding site.

**Optimal Hac1 Stimulation of the grp78 Promoter Requires an Intact YY1 Binding Site Adjacent to the UPRE-like Sequence**—To test whether yHac1 binding alone is sufficient for its stimulatory activity, mutation M6 was created within the context of the native grp78 promoter. In M6, the sequence from −144 to −140 containing the 3′ half of the UPRE-like sequence within ERSE-163 was mutated from TTGGT to ACGGA, whereas the critical CAGC sequence motif was preserved (Fig. 8A). Consistent with the boundaries previously defined for YY1 binding site (16), YY1 was not able to bind M6 in EMSA experiments, whereas yHac1 binding was fully retained (Fig. 8B). Thus, through M5 and M6 mutations of ERSE-163, we discovered that yHac1 binding to the UPRE-like sequence of the grp78 promoter is primarily mediated through the CAGC sequence, and YY1 binding requires the sequence immediately 3′ to the CAGC motif. Despite the ability of yHac1 to bind to the
promoter sequence bearing the M6 mutation, when the construct −154(M6)CAT was tested in co-transfection experiments, yHac1-mediated stimulation of the grp78 promoter was reduced to half of that observed with the wild-type promoter under both nonstressed and Tg stressed conditions (Fig. 8C). These results showed that the YY1 binding site is required for the optimal stimulation of the rat grp78 promoter by yHac1. Further, it is noted that in the absence of exogenously expressed yHac1, the stress-inducibility of −154(M6)CAT was similar to −154CAT. This is consistent with the functional redundancy of the grp78 promoter because of multiple ERSEs (25).

Enhancement of yHac1 Stimulation of the grp78 Core Sequence by YY1—The requirement of the YY1 binding site for optimal Hac1 stimulating activity on ERSE-163 suggests that YY1 may be a co-activator for yHac1. To test this, expression vectors for YY1 and yHac1 were co-transfected into NIH3T3 cells using either 78coreCAT or 78core(M5)CAT as reporter genes. Our results confirmed that yHac1 stimulates both basal and Tg-induced promoter activity of the 78core, whereas YY1 only exerts a stimulatory effect under Tg stressed conditions (Fig. 9A). When both proteins were overexpressed, the 78core promoter activity was enhanced over the level achieved by yHac1 alone. The stimulation of yHac1 activity by YY1, although only in the range of 1.5–2-fold, is strictly dependent on the sequence integrity of the yHac1 binding domain CAGCTG, which distinctively undergoes stress-inducible changes (16). ERSE-163 is also strongly trans-activated by overexpression of yHac1. Because other ERSEs such as ERSE-98, although unable to bind Hac1 are nonetheless strong mediators of the UPR (10), 14–15, one explanation is that the organization of the mammalian grp78 promoter has diverged from the yeast gene to ensure the induction of mammalian UPR by multiple pathways. Alternatively, the mammalian homolog of yHac1 may differ from yHac1 such that it is able to interact with all the ERSEs, which might be regulated through...

Discussion

The recent discovery of the tripartite structure of the mammalian ER stress response element ERSE provides important information on the regulatory components mediating the mammalian UPR (10). Thus, within the rat grp78 promoter are three ERSEs, namely ERSE-98, ERSE-131, and ERSE-163, with highly similar but distinct sequences that are evolutionarily conserved among promoter elements of ER chaperone genes. The fact that each ERSE is able to confer ER stress inducibility to a heterologous promoter (14) suggests that the grp78 promoter contains functionally redundant ERSEs. Our studies reveal several new findings leading to the proposal of a more complete model for mammalian stress induction mediated by the ERSE-163 (Fig. 10). Importantly, our model suggests that despite major differences, some key features of the mammalian UPR are conserved with the yeast UPR.

The yeast promoter contains two simple regulatory sequences, UPRE and heat shock element, that allow the yeast KAR2 gene to respond to the accumulation of misfolded proteins in the ER and in the cytoplasm. In analyzing transcription factors that interact with various ERSEs, we discovered that ERSE-163 is the only ERSE of the grp78 promoter that shares near sequence identity with the yeast UPRE. We provide here evidence that yHac1 selectively binds the ERSE-163, and the binding occurs within the sequence motif GGC-CAGCTG, which distinctively undergoes stress-inducible changes (16). ERSE-163 is also strongly trans-activated by overexpression of yHac1. Because other ERSEs such as ERSE-98, although unable to bind Hac1 are nonetheless strong mediators of the UPR (10, 14, 15), one explanation is that the organization of the mammalian grp78 promoter has diverged from the yeast gene to ensure the induction of mammalian UPR by multiple pathways. Alternatively, the mammalian homolog of yHac1 may differ from yHac1 such that it is able to interact with all the ERSEs, which might be regulated through...
one common mechanism. The resolution of this awaits the isolation of mammalian Hac1.

Unexpectedly, mutational analysis revealed that the core binding site required for yHac1 binding on the mammalian grp78 promoter is considerably less stringent than the yeast UPRE. In *S. cerevisiae*, Hac1 is the only trans-acting factor demonstrated to bind the UPRE so far, whereas the more complex mammalian ERSEs have the ability to bind multiple transcription factors, as the CCAAT or CCAAT-like motif binding NF-Y/CFB, and the 9-bp region required for the stress-inducible ERSF binding (10). The model predicts that a Hac1-like protein yet unidentified in mammalian cells binds to the UPRE-like sequence within ERSE-163 and, in cooperation with YY1 and other co-factors, activates grp78 stress induction.

\[ \text{YHac1} + \text{YY1} + \text{co-factors} \rightarrow \text{transcription} \]

The induction for the yeast KAR2 promoter as proposed by Welihinda et al. (31) (A) is compared with that proposed for the mammalian grp78 gene (B). Both promoters contain a UPRE or UPRE-like functional element that has the ability to bind Hac1, a bZIP protein. The Ada family of proteins serve as adapter proteins between Hac1 and Gen5 in *S. cerevisiae*, whereas YY1 as an interactive partner of p300 and its associating protein PCAF could serve as the bridge protein in mammalian cells. Both Gen5 and PCAF possess histone acetylase activity that could activate transcription through modification of chromatin structure. There are also unique features of each promoter system. The yeast promoter contains a heat shock element that is absent from the rat grp78 promoter, whereas the grp78 promoter contains two other functional ERSEs downstream of ERSE-163. In addition, the mammalian ERSE contains a tripartite structure, with the CCAAT or CCAAT-like motif binding NF-Y/CFB, and the 9-bp region required for stress-inducible ERSF binding (10). The model predicts that a Hac1-like protein yet unidentified in mammalian cells binds to the UPRE-like sequence within ERSE-163 and, in cooperation with YY1 and other co-factors, activates grp78 stress induction.
support, the M2 mutation of the grp78 core, which has mutated the CAGC sequence but contains intact the adjacent AC-rich sequence and the rest of the larger YY1 binding site, was able to compete partially with the wild-type grp78 core for YY1 binding when compared with other mutated sequences (16). Several properties of YY1 suggest that it could play a role similar to that of the co-activator complexes that act in concert with Hac1 and Ire1p in yeast. YY1 and p300/CBF are known to form a physical complex in vivo and in vitro. Binding assays further mapped the p300-interacting domain to the C-terminal half of YY1 (33). By recruiting p300/CFB and its associating factors such as PCAF with intrinsic histone acetylase activity (34), YY1 binding to ERSE-163 could lead to targeted histone acetylation, resulting in change of chromatin structure and activation of gene transcription (Fig. 10). Preliminary analysis suggests that histone acetylation is part of the regulatory machinery of grp expression in mammalian cells.2

Despite the strong prediction of the existence of a mammalian Hac1 based on the isolation of yHac1 in 1996, the mammalian homologs of yeast Ire1p have only been isolated recently. The identification of a mammalian Hac1 activator protein defined as a direct substrate of the Ire1p kinase and the ability to bind a UPRE-like sequence in mammalian chaperone promoters remains elusive. Because of the lack of a genetic screen in mammalian cells, the isolation of mammalian Hac1, if one exists, relies on precise definition of its binding site and transactivating properties on the mammalian ERSE. Here we identified the boundaries of a unique binding site for yHac1 within ERSE-163 and have created mutants that can dissect its binding from the overlapping YY1 binding site. This will greatly facilitate the isolation and characterization of a mammalian equivalent of yHac1, which will lead to a direct test of the model proposed for the ER stress induction of grp78 and other coordinately regulated ER-chaperone gene promoters in mammalian cells.

Acknowledgments—We thank Peter Baumeister, Xin Wang, and Trevor Phan for technical assistance and Wilfred Li for helpful discussions.

REFERENCES
1. Lee, A. S. (1987) Trends Biochem. Sci. 12, 20–23
2. Pahl, H. L., and Baeuerle, P. A. (1995) EMBO J. 14, 2580–2588
3. McMillan, D. R., Gething, M. J., and Sambrook, J. (1994) Curr. Opin. Biotechnol. 5, 540–545
4. Kaufman, R. J. (1999) Genes Dev. 13, 1211–1233
5. Cox, J. S., and Walter, P. (1996) Cell 87, 391–404
6. Kawahara, T., Yanagi, H., Yura, T., and Mori, K. (1997) Mol. Biol. Cell 8, 1845–1862
7. Chapman, R. E., and Walter, P. (1998) Curr. Biol. 7, 850–859
8. Mori, K., Kawahara, T., Yoshida, H., Yanagi, H., and Yura, T. (1996) Gene Cells 1, 803–817
9. Yoshida, H., Haze, K., Yanagi, H., Yura, T., and Mori, K. (1998) J. Biol. Chem. 273, 33741–33749
10. Roy, B., and Lee, A. S. (1999) Nucleic Acids Res. 27, 1437–1443
11. Roy, B., Li, W. W., and Lee, A. S. (1996) J. Biol. Chem. 271, 28995–29002
12. Li, W. W., Hsiung, Y., Zhou, Y., Roy, B., and Lee, A. S. (1997) Mol. Cell. Biol. 17, 54–60
13. Li, W. W., Hsiung, Y., Wang, V., Galvin, K., Zhou, Y., Shi, Y., and Lee, A. S. (1997) Mol. Cell. Biol. 17, 61–68
14. Li, W. W., Alexandre, S., Cao, X., and Lee, A. S. (1993) J. Biol. Chem. 268, 12003–12009
15. Zhou, Y., and Lee, A. S. (1998) J. Natl. Cancer Inst. 90, 381–388
16. Li, W. W., Sistonen, L., Morimoto, R. I., and Lee, A. S. (1994) Mol. Cell. Biol. 14, 5533–5546
17. Liu, E. S., and Lee, A. S. (1991) Nucleic Acids Res. 19, 5425–5431
18. Kaufman, R. J., Davies, M. H., Weisley, L. C., and McInruck, D. (1991) Nucleic Acids Res. 19, 4485–4490
19. Shi, Y., Seto, E., Chang, L. S., and Shenk, T. (1991) Cell 67, 377–388
20. Chang, S. C., Erwin, A., and Lee, A. S. (1989) Mol. Cell. Biol. 9, 5612–5623
21. Tirasophon, W., Weilhinda, A. A., and Kaufman, R. J. (1998) Genes Dev. 12, 1832–1844
22. Chen, C. A., and Okayama, H. (1988) BioTechniques 6, 632–638
23. Harlow, E., and Lane, D. (1989) Antibodies: A Laboratory Manual, pp. 471–506 Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
24. Yant, S. R., Zhu, W., Millinson, D., Slightom, J. L., Goodman, M., and Gumucio, D. L. (1995) Nucleic Acids Res. 23, 4353–4362
25. Wooden, S. K., Li, J. L., Navarro, D., Qdri, I., Pereira, L., and Lee, A. S. (1991) Mol. Cell. Biol. 11, 5791–5801
26. Lee, T., Bradley, M. E., and Walowitz, J. L. (1998) Nucleic Acids Res. 26, 3215–3220
27. Ramakrishnan, M., Tugizov, S., Pereira, L., and Lee, A. S. (1995) DNA Cell Biol. 14, 373–384
28. Marcus, N., and Green, M. (1997) DNA Cell Biol. 16, 1123–1131
29. Shi, Y., Lee, J.-S., and Galvin, K. M. (1997) Biochim. Biophys. Acta 1332, 45–56
30. Wang, X. Z., Harding, H. P., Zhang, Y., Jolicoeur, E. M., Kuroda, M., and Ron, D. (1998) EMBO J. 17, 5768–5771
31. Weihinda, A. A., Tirasophon, W., Green, S. R., and Kaufman, R. J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 377–382
32. Zhou, Q., Gedrich, R. W., and Engel, D. A. (1995) J. Virol. 69, 4323–4330
33. Lee, J.-S., Galvin, K. M., See, R. H., Ecker, R., Livingston, D., Moran, E., and Shi, Y. (1995) Genes Dev. 9, 1188–1198
34. Yang, X.-J., Ogryzko, V. V., Nishikawa, J.-I., Howard, B. H., and Nakatani, Y. (1996) Nature 382, 319–324

I. Christadoupoulos and A. S. Lee, unpublished data.