MOLECULAR AND CELLULAR BIOLOGY, Oct. 1988, p. 4579–4584
0270-7306/88/104579-06$02.00/0

Vol. 8, No. 10

Identification of Highly Conserved Regulatory Domains and Protein-Binding Sites in the Promoters of the Rat and Human Genes Encoding the Stress-Inducible 78-Kilodalton Glucose-Regulated Protein

ELPIDIO RESENDEZ, JR., SCOTT K. WOODEN, AND AMY S. LEE*

Department of Biochemistry and the Norris Cancer Research Institute, University of Southern California School of Medicine, Los Angeles, California 90033

Received 23 March 1988/Accepted 24 June 1988

The gene encoding GRP78 has been shown to be constitutively expressed in many cell types and is inducible by the calcium ionophore A23187. To understand the regulation of GRP78 transcription, we analyzed the components that control its basal-level expression. By transfecting deletions into cells, we have identified a 54-nucleotide *cis*-acting regulatory element important for high basal-level expression and a contiguous 50-nucleotide element for both basal-level expression and A23187 induction. Using DNase footprinting assays with both rat and human GRP78 promoters, we demonstrated that the protein factors present in the HeLa cell nuclear extracts bind to the regulatory regions identified by the deletion studies. This domain contains a palindromic sequence and is highly conserved among GRP genes in *Caenorhabditis elegans*, chicks, rats, and humans.

A set of stress-inducible proteins known as the glucose-regulated proteins (GRPs) are constitutively expressed in mammalian cells. When the cells are deprived of glucose or treated with reagents that inhibit protein glycosylation, perturb intracellular calcium stores, or denature proteins, the synthesis of the GRPs is rapidly increased (11). The major GRP in mammalian cells has a molecular mass of about 78,000 daltons and is localized within the endoplasmic reticulum (ER) (18, 25a). This protein, generally referred to as GRP78, shares about 60% sequence homology with the 70,000-dalton heat-shock protein (HSP70) and is thought to have a function analogous to that of HSP70 but within the ER (18). By immunoprecipitation and comparisons of peptide maps and amino-terminal sequences, GRP78 was found to be identical to the immunoglobulin-binding protein referred to as BiP (1, 4, 15). In lymphoid and fibroblast cells, GRP78-BiP has been shown to bind to immature immunoglobulin and aberrant proteins (1, 18). Recently, the sequence of the 3,215-dalton steroidogenesis activator polypeptide isolated from a rat cell tumor has been reported (17). Strikingly, this 30-residue peptide, which has the ability to facilitate cholesterol side-chain cleavage to pregenolone, is identical, except for two residues, with the last 30 amino acids of the GRP78 carboxyl terminus as predicted from the DNA sequence of the rat GRP78 gene (15, 23).

The gene encoding GRP78 has recently been isolated from a human genomic library (22a). Both the rat and the human GRP78 genes are split into eight exons and contain a signal sequence which targets GRP78 into the ER (22a, 25a). The expression of the GRP genes is regulated at the transcriptional level (9, 10, 13, 19). Thus, when the cells are treated with potent inducers such as the calcium ionophore A23187, β-mercaptoethanol, or tunicamycin, the transcription of the GRP78 gene is increased 10- to 25-fold within 5 h (3, 9, 19). The GRP78 promoter is highly active, and a 291-nucleotide (nt) fragment from the promoter region functions as an enhancer when fused to other cellular promoters (12). This region of the GRP78 promoter is G+C rich and contains several CCAAT sequences and extensive arrays of tandem and inverted repeat motifs characteristic of other cellular and viral enhancers (6). In addition, this 291-nt fragment, when placed upstream of a heterologous gene, can confer inducibility by calcium ionophore (12).

GRP78 transcript levels in HeLa cells. To facilitate the search for cellular factors which interact with the DNA regulatory domain, we utilized the HeLa cell system, which can be grown in suspension to provide high yields of cellular extracts. We have shown previously that GRP78 transcripts can be detected in a variety of mammalian cell lines (20). Since the focus of this study was on the utilization of the human HeLa S3 cell line as a source of functional protein extracts, we first investigated the expression of the endogenous GRP78 gene in these cells. GRP78 mRNA was detectable in noninduced HeLa cells grown in suspension cultures (Fig. 1). When the cells were further treated with 0.5 or 2 μM of the calcium ionophore A23187 or were grown continuously in the culture medium without a medium change, the endogenous GRP78 transcript levels were further increased (Fig. 1). These results demonstrate that the GRP78 gene is constitutively expressed in HeLa cells to provide a basal level of GRP78 transcripts. Furthermore, A23187 is capable of eliciting a strong stress response in the HeLa cells. However, A23187 is relatively toxic to HeLa S3 cells compared with fibroblast cell lines from hamster, rat and mouse. The optimum conditions for the induction of the GRP78 gene in HeLa S3 cells are 2 μM A23187 and an incubation period not longer than 6 h. Higher concentrations of A23187 or longer incubation periods or both result in substantial cell death (unpublished results).

Transcriptional activities of rat GRP78 promoter-deletion mutants in human cells. It has been shown previously that a 291-nt SmaI-StuI fragment upstream of the TATA element of the rat GRP78 promoter is important for both high basal level and A23187 induction of the reporter gene after transfection

* Corresponding author.

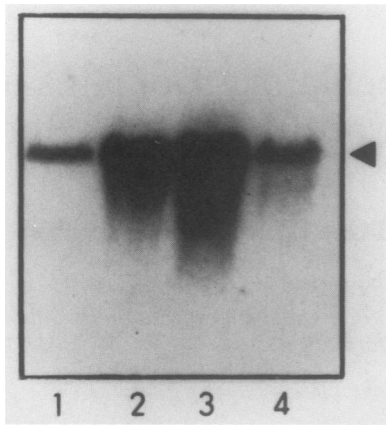

FIG. 1. Enhanced expression of GRP78 mRNA levels in HeLa cells. Cytoplasmic RNA was extracted from HeLa cells after incubation under the following conditions: control (lane 1), 0.5 μM A12387 (lane 2), 2 μM A23187 (lane 3), and medium starvation (lane 4). For control and calcium ionophore (A23187) treatment, cells were placed in fresh medium 8 h before cytoplasmic RNA extraction; for calcium ionophore treatment, cells were incubated for 5 h with the ionophore before RNA extraction; and for the medium starvation condition, the cells were kept in the original medium and incubated for an additional 24 h before RNA extraction. The cytoplasmic RNA samples (5 μg) were electrophoresed on a formaldehyde denaturing gel, trans-blotted onto a nitrocellulose filter, and hybridized with radiolabeled hamster GRP78 cDNA plasmid p3C5 (23a) as described previously (10, 20). The arrow indicates the position of the 2.7-kilobase GRP78 transcript.

into hamster fibroblast K12 cells (2, 12). The sequence of this region and the proximal promoter sequences are shown in Fig. 2A. To delineate further the functional domains within this region, a series of 5′-deletion mutants were constructed and their deletion endpoints were determined by DNA sequencing. To test the functionality of these deleted 5′-flanking sequences, they were fused upstream to the bacterial chloramphenicol acetyltransferase (CAT) transcriptional unit (Fig. 2B). These 5′-deletion plasmids were transfected into HeLa, HepG2, and K12 cells. The cells were transfected with 3 or 5 μg of cesium chloride gradient-purified plasmid DNA with 5 or 7 μg of high-molecular-weight carrier HeLa DNA. The transfection conditions and the assay of CAT activity were performed as described previously (16, 19, 24). The promoter strengths of the deletion mutants were determined by the CAT enzymatic activities under noninduced and A23187-induced conditions (Table 1). In these transient transfection experiments, we failed to detect CAT activities in the HeLa cells transfected with the CAT plasmids, probably due to the low transfection efficiency of the HeLa cells used. However, our results with the human HepG2 and hamster K12 cells consistently showed that between deletions −208 and −154, a fivefold reduction in the basal level of expression was observed, suggesting that this 54-nt domain contains important *cis*-acting regulatory elements for high basal-level expression. When the promoter is deleted to −130, the basal level was further reduced and the 2.5- to 8-fold induction by A23187 observed in pGRP78 (−154)CAT was reduced by one-half. In the deletion mutants pGRP78(−104)CAT and pGRP78(−85)CAT, only minimal activities were detected. While these activities were higher than the promoterless plasmid pSV0CAT, the basal level was further reduced and A23187-induced expression was eliminated. This implies that the 50-nt domain between deletions −154 and −104 contains important DNA elements for both basal level and A23187 induction.

TABLE 1. Relative CAT activities[a]

Deletion endpoint	HepG2		K12	
	Basal level	Level with A23187 (increase [fold])	Basal level	Level with A12387 (increase [fold])
−208	100	150 (1.5)	100	500 (5.0)
−154	20	50 (2.5)	20	160 (8.0)
−130	3	4 (1.3)	6	20 (3.5)
−104	2	2 (1.0)	2	3 (1.5)
−85	1.5	1.5 (1.0)	2	3 (1.5)
pSV0CAT	0.5	0.5 (1.0)		

[a] Level of CAT activity of pGRP78(−208)CAT under noninduced conditions was set as 100. Results represent averages of three sets of transfections for each cell line.

The HeLa extract contains protein factors that bind to the rat GRP78 regulatory domain. Using an in vitro transcription assay, we observed that the nuclear extracts from HeLa cells were able to transcribe the rat GRP78 promoter in a template- and extract-dependent manner. In addition, by gel mobility shift experiments we detected multiple complexes formed between HeLa nuclear extract and the regulatory region of the GRP78 promoter defined above (unpublished

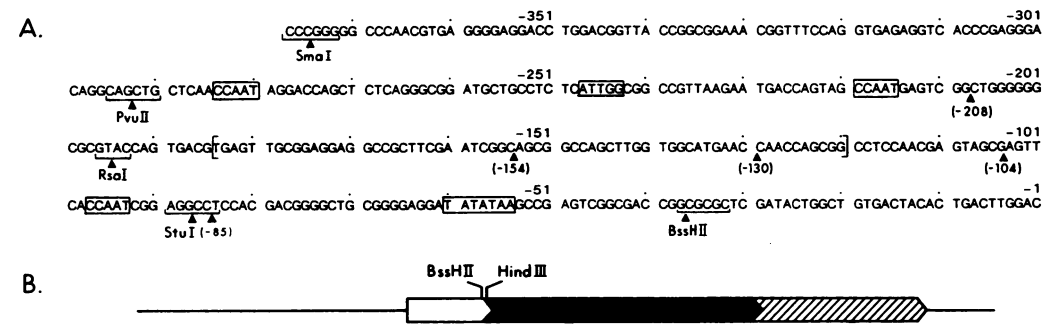

FIG. 2. Promoter deletions and activities of CAT constructs. (A) The sequence of the rat GRP78 promoter. Bases are numbered in reference to the major mRNA cap site of GRP78 (12). Several relevant restriction sites are noted. The deletion mutants were generated by BAL 31 digestion of pI10 (19). The deletion endpoints for each of the deletion plasmids used for the transfection are indicated. The CCAAT and TATA regions are boxed. The region protected from DNase I digestion described below is bracketed. (B) The organization of the deletion mutant plasmids. The GRP78-deleted promoter with an uniform 3′ deletion endpoint at the *Bss*HII site is fused to the *Hin*dIII site of pSV0CAT (19). Other regions of the plasmid include: the CAT gene (▬), the SV40 polyadenylation site (▨), and sequences from pBR322 (——).

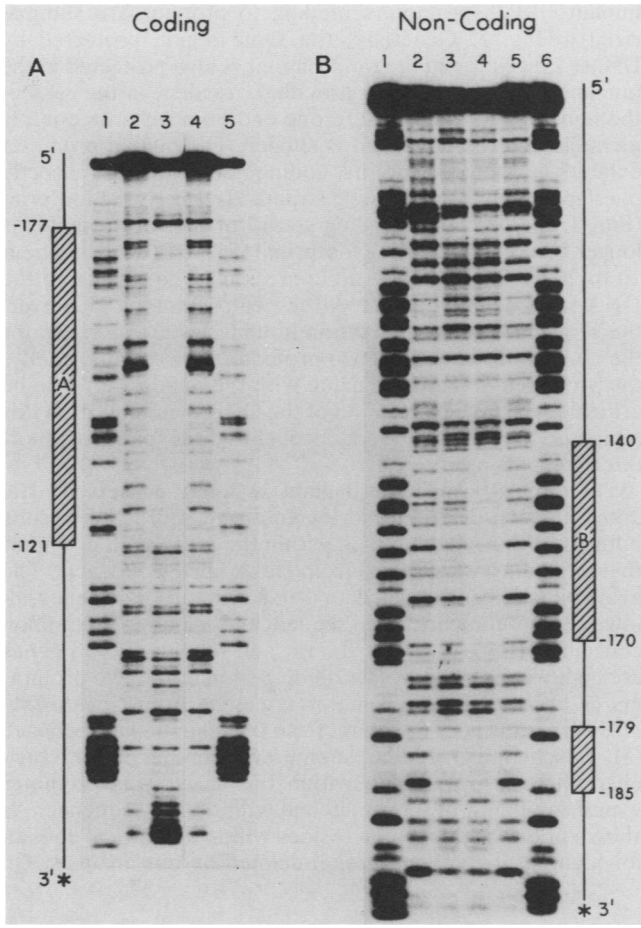

FIG. 3. Footprint analysis of the rat GRP78 promoter. A 159-nt EcoRI-HindIII fragment (spanning −195 to −88) was end labeled, cut with *Asp*718 to obtain the labeled coding strand or with *Sal*I to obtain the labeled noncoding strand, and gel purified. The HeLa nuclear protein extract was prepared as described previously (21). (A) Coding strand footprint. Lanes: 2 and 4, DNA probe incubated without protein extract; 3, DNase-treated probe with 44 μg of HeLa nuclear protein extract; 1 and 5, Maxam and Gilbert G sequencing reaction (14) of the labeled strand. (B) Noncoding strand footprint. Lanes: 2 and 5, DNase-treated probe without protein extract; 3 and 4, 44 and 66 μg, respectively, of HeLa extracts; 1 and 6, Maxam and Gilbert G sequencing reactions. Diagrams of the coding and noncoding regions are shown next to the gels. The footprint regions A and B are boxed. The numbers denote nucleotides correspond to the locations of the footprints relative to the transcription start site (+1) of the rat GRP78 gene.

results). To define the binding sites of these factors within the rat GRP78 promoter, the subfragment spanning −195 to −88 was end labeled, mixed with HeLa nuclear extracts, and subjected to DNase I digestion. Binding reaction mixtures (20 μl) contained 10 mM Tris-hydrochloride (pH 7.5), 50 mM NaCl, 10 mM β-mercaptoethanol, 1 mM EDTA, 4% glycerol, 2 mM $MgCl_2$, 1 μg poly(dI-dC), and 0.4 to 1 ng of end-labeled DNA. After 20 min of incubation at room temperature, 20 μl of 5 mM $CaCl_2$–1 mM EDTA was added; freshly prepared DNase I was added, and the mixture was incubated for 60 s at room temperature. To terminate the nuclease reaction, 50 μl of buffer (200 mM NaCl, 20 mM EDTA, 1% sodium dodecyl sulfate, 250 μg of tRNA per ml) was added. Samples were deproteinized by phenol-chloroform extraction, and the DNA was ethanol precipitated, suspended in formamide-dye mix, and electrophoresed on a denaturing 6% polyacrylamide–8M urea sequencing gel. The analysis was performed for both the coding and the noncoding strands of the DNA. The results revealed that a region spanning positions −121 to −177 (Fig. 3, A box) was protected on the coding strand. In the case of the noncoding strand, two regions spanning positions −140 to −170 (Fig. 3, B box) and −179 to −185 of the noncoding strand were

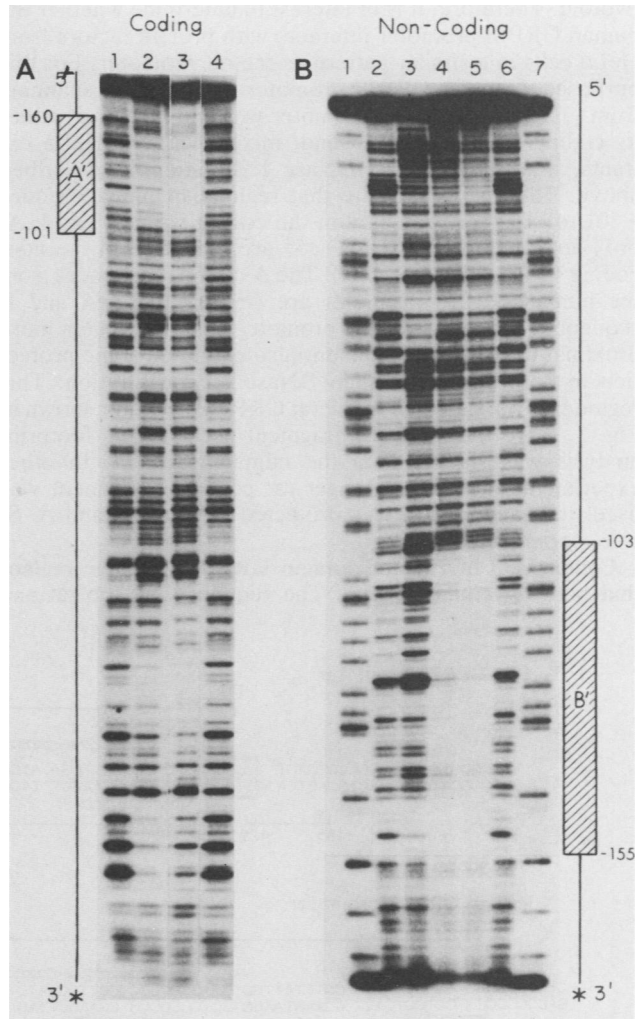

FIG. 4. Footprint analysis of the human GRP78 promoter. pUCH223 (−170, +53), which contains the 223-nt PvuII-NruI fragment from the human GRP78 promoter (22a) subcloned into the *Sma*I site of pUC8 (with the *Pvu*II site proximal to the *Eco*RI site of the pUC8 polylinker sequence), was linearized with *Hin*dIII, end labeled with T4 DNA polymerase, and cut with *Eco*RI to obtain the labeled coding strand. The labeled noncoding strand was obtained by labeling the *Eco*RI-*Hin*dIII insert of pUCH223 and recutting with *Sal*I. (A) Coding strand footprint. Lanes: 1 and 4, DNase-treated probe without nuclear extract; 2 and 3, DNase-treated probe with 44 and 66 μg, respectively, of HeLa nuclear protein. (B) Noncoding strand footprint. Lanes: 2 and 6, DNase-treated probe without nuclear extract; 3, 4, and 5, DNase-treated probe with 22, 44, and 66 μg, respectively, of HeLa nuclear protein; 1 and 7, Maxam and Gilbert G sequencing reaction of the labeled strand. Diagrams of the coding and noncoding regions are shown next to the gels. The boxes represent the footprint regions (A′ and B′). The numbers denote nucleotides and correspond to the locations of the footprints relative to the transcription start site (+1) of the rat GRP78 gene.

protected from DNase I digestion. These domains reside within the functional domains (−104 to −208) defined by in vivo transfection experiments described above.

Binding of protein factors to the human GRP78 promoter. We have previously shown that the rat and the human GRP78 promoter sequences are highly conserved (22a). The 170 nt proximal to the TATA sequence in the human GRP78 promoter have been identified as important for basal-level expression and inducibility by A23187 (22a). Sequences further upstream enhance the basal-level expression by twofold. Therefore, it is of interest to determine whether the human GRP78 promoter interacts with protein factors from HeLa cells in a similar pattern as the rat promoter. For this purpose, a human GRP78 promoter subfragment spanning from −170 to +53 of the promoter was end labeled at either its coding or noncoding strand, mixed with the HeLa extracts, and subjected to DNase I digestion as described above. The results indicate that regions spanning residues −101 to −160 are protected in the coding strand (Fig. 4, A′ box) and residues −103 to −155 are protected in the noncoding strand (Fig. 4, B′ box). The A′ and B′ sequences from the human GRP78 promoter are similar to the A and B sequences of the rat GRP78 promoter. Another region more proximal to the TATA element also exhibited some protection in both strands at high DNase I concentration. This region was not detected in the rat GRP 78 promoter shown in Fig. 3, since the rat DNA fragment used for the footprint analysis was shorter than the human fragment. In other experiments in which a longer rat promoter fragment was used, the same region was protected (T. Nakaki and A. S. Lee, unpublished results).

Comparison of rat and human GRP78 promoter regions that bind to cellular factors. The sequences of the rat and human GRP78 promoters binding to proteins are summarized in Fig. 5. Generally, the same region protected by DNase I digestion in the rat promoter is also protected in the human promoter. This is particularly evident in the case of the noncoding strand, where one end point of protection is identical and the other end is similar. The human promoter exhibits a footprint on its coding strand which directly overlaps that of its noncoding strand. However, the footprint (Fig. 4, A box) of the coding strand of the rat promoter is longer than the noncoding footprint (Fig. 4, B Box) by about 10 to 20 nt. These subtle differences may be related to the DNA sequence divergence of the two promoters. However, the sequences at the protection boundaries are identical for the coding strands of the two promoters. Most importantly, the protected domains coincide with the region shown to be critical for in vivo expression of the GRP78 gene. Within this region, a palindromic sequence and three pairs of short direct repeats are present.

The GRP78 regulatory domain is highly conserved. The above in vivo deletion and the footprint protection results both point to a small domain within the rat GRP78 promoter that interacts with proteins from HeLa nuclear extracts. The availability of sequence data from several GRP genes allowed us to compare their sequences for possible common consensus (Fig. 5). While the rat and human GRP78 genes are highly conserved over a 200-nt region of the two promoters (22a), the GRP78 promoter sequence from *Caenorhabditis elegans* is very divergent from the mammalian sequence (M. Heschl and D. Baillie, submitted for publication). However, there is one region within the *C. elegans* promoter which has 19 out of 23 nt matched with the rat sequence. As shown in Fig. 5, this region resides within the critical domain for high basal-level expression defined by our analysis. On

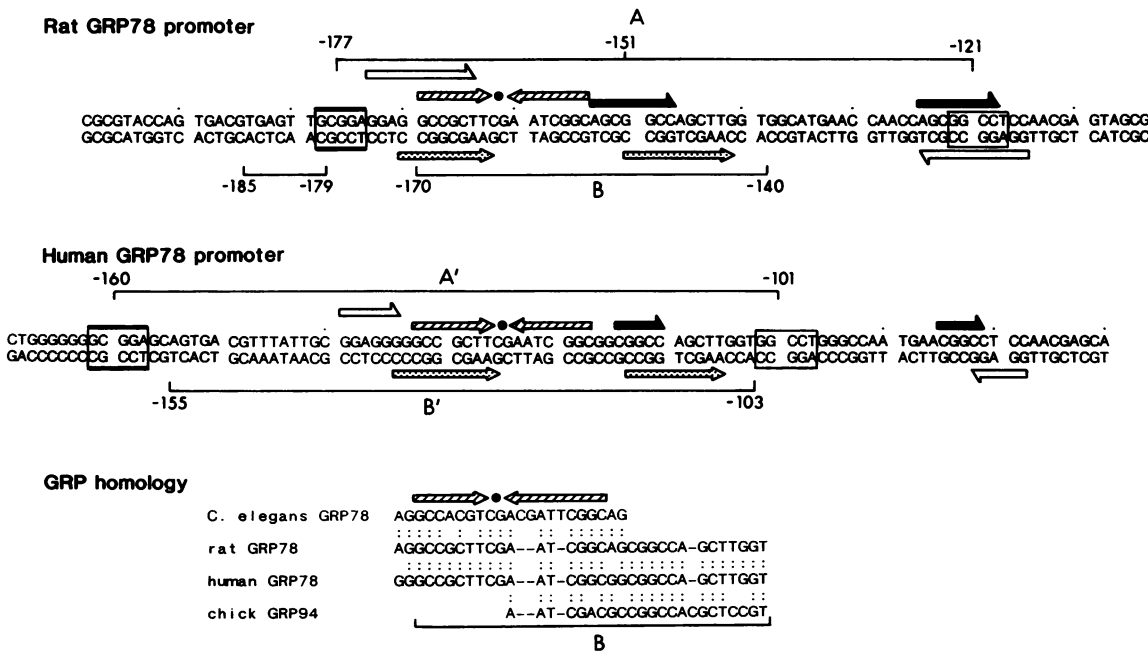

FIG. 5. Footprint sequence of the rat and human DNA and its conservation. The sequences that footprint on the coding strand of rat (A) and human (A′) GRP78 gene and on the noncoding strand of rat (B) and human (B′) GRP78 gene are bracketed. The palindromic sequence is indicated by a pair of hatched arrows, with the center of symmetry highlighted by a black dot. Other pairs of direct repeats are indicated by the other types of arrows. The homologous sequences between the rat and human promoter at the coding strand footprint boundaries are boxed. The highly conserved domain of the rat GRP78 promoter with the 5′-flanking regions of GRP genes of the other species is also shown. The location of the palindromic sequence and the footprint B domain shown above for the rat GRP78 promoter are indicated. Identical nucleotides are indicated by the vertical dots.

the other hand, GRP94, which is expressed at a lower basal level than GRP78 but is inducible by A23187, has 18 out of 23 nt matched with the rat and human GRP78 promoters within the region important for A23187 induction (22a). These two domains are also the same domain (Fig. 3, B box) detected by our footprint analysis. The strict conservation of these short domains within such phylogenetically diverse species as worms, chicks, rats, and humans strongly implies that they have a functional significance in the regulation of GRP gene expression.

The GRP78 gene system represents a unique model for the study of the regulation of genes encoding ER-localized proteins. Most interestingly, GRP78 transcriptional activity appears to correlate directly with the amount of ER activity or ER damage in a variety of cell types, bringing up the question of how the state of another organelle, such as the ER, can regulate a gene in the nucleus. Clearly, intermediate molecules must exist and traverse the membranes to communicate the signal to the GRP gene system. Since the ER membranes are associated with the perinuclear membranes, traffic between the two organelles can be envisioned.

By 5'-deletion analysis, we show here that a 100-nt sequence within this region is critical for both high basal and A23187-induced expression. This same DNA segment is highly conserved among the GRP genes and is protected by cellular factors in DNase protection assays. If the footprinted domain represents the core for the binding activities of cellular factors that regulate GRP78 gene expression, this region may represent binding sites for a novel class of transcriptional factors that have not yet been described. By sequence analysis, it is devoid of recognition sites for characterized transcriptional factors such as Sp1, CTF, AP1, AP2, and TFIID (5). Since this region contains a pair of inverted repeats which basically results in the same sequence independent of orientation, it may explain the orientation-independent characteristics of the DNA enhancer element contained within the GRP78 promoter.

Although GRP78 shares partial sequence homology with members of the 70,000-dalton heat shock protein family and may share the properties of binding to abnormal proteins under stressful conditions, the regulation of GRP78 expression is distinct from that of HSP70 in many ways. First, the transcriptional activation of the GRP78 gene is sensitive to the protein synthesis inhibitor cycloheximide (8, 20), whereas the induction of the heat-inducible HSP70 gene does not require de novo protein synthesis. In fact, it has been demonstrated that the heat shock regulatory factor can bind to the heat shock element in the presence of cycloheximide (26). In addition, the most potent inducers of GRP78 expression, such as calcium ionophores and β-mercaptoethanol, do not affect HSP70 expression (7, 9, 25). Therefore, whatever is the mechanism of induction of GRP78, it is likely to be different from that of the HSP70 gene and probably involves multiple steps (7, 11, 25). The identification of the *trans*-acting factors which regulate the expression of the GRP78 gene holds the key to uncovering the intermediate steps whereby a signal from the ER can be transmitted to the nucleus to enhance GRP78 transcription.

We thank Mark Heschl and David Baillie for communication of the *C. elegans* promoter sequence before publication and Walter Miller for alerting us to the similarity between SAP and GRP78.

E.R., Jr. was supported in part by a predoctoral fellowship (GM 9805-MC) from the National Institutes of Health, and S.W. was supported by NIH training grant T32 CA09569-01A1. This investigation was supported by Public Health Service Grant R37 CA 27607 awarded by the National Cancer Institute, Department of Human and Health Services. A. S. Lee is a recipient of a Faculty Research Award from the American Cancer Society.

The GenBank accession numbers are J03377 for the rat sequence and M19645 for the human sequence.

LITERATURE CITED

1. **Bole, D., L. Hendershot, and J. F. Kearney.** 1986. Post-translational association of immunoglobulin heavy chain binding protein with nascent heavy chains in non-secreting and secreting hybridomas. J. Cell Biol. **102**:1558–1566.
2. **Chang, S. C., S. K. Wooden, T. Nakaki, Y. K. Kim, A.-Y. Lin, L. Kung, J. W. Attenello, and A. S. Lee.** 1987. Rat gene encoding the 78 kDa glucose-regulated protein GRP78: its regulatory sequences and the effect of protein glycosylation on its expression. Proc. Natl. Acad. Sci. USA **84**:680–684.
3. **Drummond, I. S., A. S. Lee, E. Resendez, Jr., and R. A. Steinhardt.** 1987. Depletion of intracellular calcium stores by calcium ionophores A23187 induces the gene for the glucose-regulated proteins in hamster fibroblasts. J. Biol. Chem. **262**: 12801–12805.
4. **Hendershot, L., J. Ting, and A. Lee.** 1988. Identity of the immunoglobulin heavy-chain-binding protein with the glucose-regulated protein and the role of posttranscriptional modifications in its binding function. Mol. Cell. Biol. **8**:4250–4256.
5. **Jones, N. C., P. W. J. Rigby, and E. B. Ziff.** 1988. Trans-acting protein factors and the regulation of eukaryotic transcription: lessons from studies on DNA tumor viruses. Genes Dev. **2**:267–281.
6. **Khoury, G., and P. Gruss.** 1983. Enhancer elements. Cell **33**: 313–314.
7. **Kim, K. S., and A. S. Lee.** 1986. The effects of extracellular Ca^{++} and temperature on the induction of the heat shock and glucose-regulated protein in hamster fibroblast. Biochem. Biophys. Res. Comm. **140**:881–887.
8. **Kim, Y. K., K. S. Kim, and A. S. Lee.** 1987. Regulation of the glucose-regulated protein genes by β-mercaptoethanol requires de novo protein synthesis and correlates with inhibition of protein glycosylation. J. Cell Physiol. **133**:553–559.
9. **Kim, Y. K., and A. S. Lee.** 1987. Transcriptional activation of the glucose-regulated protein genes and their heterologous fusion by β-mercaptoethanol. Mol. Cell. Biol. **7**:2974–2976.
10. **Lee, A. S., A. M. Delegeane, V. Baker, and P. C. Chow.** 1983. Transcriptional regulation of two genes specifically induced by glucose starvation in a hamster mutant fibroblast cell line. J. Biol. Chem. **258**:597–603.
11. **Lee, A. S.** 1987. Coordinated regulation of a set of genes of glucose and calcium-ionophore in mammalian cells. Trends Biochem. Sci. **12**:20–23.
12. **Lin, A. Y., S. C. Chang, and A. S. Lee.** 1986. A calcium ionophore-inducible cellular promoter is highly active and has enhancer-like properties. Mol. Cell. Biol. **6**:1235–1243.
13. **Lin, A. Y., and A. S. Lee.** 1984. Induction of two genes by glucose starvation in hamster fibroblasts. Proc. Natl. Acad. Sci. USA **81**:988–992.
14. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
15. **Munro, S., and H. R. B. Pelham.** 1986. An hsp-70-like protein in the ER: identity with the 78 kd glucose-regulated protein and immunoglobulin heavy chain binding protein. Cell **46**:291–300.
16. **Parker, B. A., and G. R. Stark.** 1979. Regulation of simian virus 40 transcription: sensitive analysis of the RNA species present early in infections by virus or viral DNA. J. Virol. **31**:360–369.
17. **Pedersen, R. C., and A. C. Brownie.** 1987. Steroidogenesis-activator for polypeptide isolated from a rat Leydig cell tumor. Science **236**:188–190.
18. **Pelham, H. R. B.** 1986. Speculations on the functions of the major heat shock and glucose-regulated proteins. Cell **46**:959–961.
19. **Resendez, E., Jr., J. W. Attenello, A. Grafsky, C. S. Chang, and A. S. Lee.** 1985. Calcium ionophore A23187 induces expression of glucose-regulated genes and their heterologous fusion genes. Mol. Cell. Biol. **5**:1212–1219.

20. **Resendez, E., Jr., J. Ting, K. S. Kim, S. K. Wooden, and A. S. Lee.** 1986. Calcium ionophore A23187 as a regulator of gene expression in mammalian cells. J. Cell. Biol. **103**:2145–2152.
21. **Shapiro, D. J., P. A. Sharp, W. W. Wahli, and M. J. Keller.** 1988. A high efficiency HeLa cell nuclear transcription extract. DNA **7**:47–55.
22. **Sorger, P. K., and H. R. B. Pelham.** 1987. Cloning and expression of a gene encoding hsc73, the major hsp70-like protein in unstressed rat cells. EMBO J. **6**:993–998.
22a. **Ting, J., and A. S. Lee.** 1988. Human gene encoding the 78,000 dalton glucose-regulated protein and its pseudogene: structure, conservation, and regulation. DNA **7**:275–286.
23. **Ting, J., S. K. Wooden, R. Kritz, K. Kelleher, R. J. Kaufman, and A. S. Lee.** 1987. The nucleotide sequence encoding the hamster 78-dDa glucose-regulated protein (GRP78) and its conservation between the hamster and rat. Gene **55**:147–152.
24. **Wang, M.-L., and A. S. Lee.** 1983. Polymerization of vector DNA after transfection into hamster fibroblast cells. Biochem. Biophys. Res. Commun. **110**:593–601.
25. **Watowich, S. S., and R. I. Morimoto.** 1988. Complex regulation of heat shock- and glucose-responsive genes in human cells. Mol. Cell. Biol. **8**:393–405.
25a. **Wooden, S. K., R. P. Kapur, and A. S. Lee.** 1988. The organization of the rat GRP78 gene and A12387 induced expression of fusion gene products targeted intracellularly. Exp. Cell Res. **178**:84–92.
26. **Zimarino, V., and C. Wu.** 1987. Induction of sequence-specific binding of *Drosophila* heat shock activator protein without protein synthesis. Nature (London) **327**:727–730.