Cloning of a functional Burkitt's lymphoma polypeptide-binding protein/78 kDa glucose-regulated protein (BiP/GRP78) gene promoter by the polymerase chain reaction, and its interaction with inducible cellular factors

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The promoter of the human gene encoding the stress-responsive protein polypeptide-binding protein/78 kDa glucose-regulated protein (BiP/GRP78) was isolated from Burkitt's lymphoma cells by PCR. This promoter DNA segment (termed BiP670) or one of its 5' deletion derivatives was fused to the bacterial chloramphenicol acetyltransferase gene and introduced into HeLa cells for transient expression. BiP670 retained transcriptional activity at both the basal and Ca^{2+} ionophore A23187-inducible levels. However, there was no significant increase in promoter activity following a 5 h induction with 7 μM -A23187, and less than 5-fold induction at 15 h. In contrast, the steady-state mRNA level was induced by 18-fold at 5 h. The *in vivo* transactivation assays with BiP670 5' deletion derivatives indicate that the putative A23187-inducible element is located within a 70 bp DNA segment (i.e. spanning –39 to –107 bp upstream of the transcriptional initiation site). Using an *in vitro* gel mobility shift assay, A23187-inducible nuclear factors were identified from HeLa cell extracts. DNA-binding competition experiments also suggest that the 70 bp DNA segment contains a potential sequence motif for the binding of the A23187-inducible nuclear factors.

INTRODUCTION

Polypeptide binding-protein/78 kDa glucose-regulated protein (BiP/GRP78) is conserved in mammalian and lower eukaryotic cells. There is accumulated evidence indicating that a major physiological function of BiP/GRP78 is to assist in the assembly and transport of cellular proteins (reviewed in [1,2]). Recent studies have implied that BiP/GRP78 may also be involved in the control of cell growth [3]. In response to certain stresses, including pH shift, hindrance of Ca^{2+} metabolism and, in some cases, glucose starvation, the levels of the steady-state mRNA and encoded protein of BiP/GRP78 gene are transiently elevated in cells (reviewed in [4]). Using inhibitors of cellular metabolism, Morimoto and colleagues [5] have demonstrated that although BiP/GRP78 can respond promptly to stresses, the regulation of the gene is complicated because there is no single mechanism that can be applied to explain the observed phenomenon. These results indicate that the regulation of the expression of structurally conserved BiP/GRP78 may be dispersive. However, little is known about the molecular basis of its regulation in cells. In order to gain a better understanding of the regulation of expression of this stress-responsive protein, the control of BiP/GRP78 transcription at the promoter level was investigated in the present study.

The induction of steady-state BiP/GRP78 mRNA levels has been extensively demonstrated by Lee's group and others in rodent cells [4]. The regulatory mechanism of human BiP/GRP78 gene expression is mostly implied from studies of the rat BiP/GRP78 gene. Although the human BiP/GRP78 gene has been cloned from embryonic liver cells that contain sufficient 5' promoter sequence for both basal and inducible transcription [6], direct evidence for the molecular basis of its induction is lacking.

In the present study, we isolated a functional BiP/GRP78 promoter from human Burkitt's lymphoma Namalwa cells by the PCR. This promoter DNA segment (BiP670) and its 5' deletion mutants were either fused to the bacterial chloramphenicol acetyltransferase (CAT) gene for transient expression in cells, or used in an *in vitro* gel mobility shift assay with cellular proteins. The questions that we investigated were: (a) whether promoter strength affects the steady-state mRNA level of the BiP/GRP78 gene, (b) whether a sequence motif of the promoter mediating induction by Ca^{2+} ionophore A23187 is present, (c) whether abundant, inducible trans-acting factors are present in stressed cells, and (d) what is their binding target on the promoter. The A23187-inducible nuclear factors were identified in HeLa cells that interact with the putative sequence motif of the BiP/GRP78 promoter.

MATERIALS AND METHODS

Chemicals and standard methods

All of the chemicals used in this study were purchased from Sigma, St. Louis, MO, U.S.A., unless otherwise specified. Genomic DNA preparation, gel electrophoresis, isolation and sub-cloning of plasmid DNA fragments, and DNA sequencing were carried out by standard methods [7].

Cell lines and culture conditions

The cell cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air according to the supplier's specifications (American Type Culture Collection). HeLa S3 and Namalwa Burkitt's lymphoma cells (a gift from Dr. Y.-S. Chang, Chang Gung Medical College) were maintained in Dulbecco's modified Eagle's medium and RPMI1640 medium (GIBCO

Abbreviations used: BiP/GRP78, polypeptide-binding protein/78 kDa glucose-regulated protein; AINF, A23187-inducible nuclear factor; AIE, A23187-inducible element; CAT; chloramphenicol acetyltransferase.

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Laboratories, Gaithersburg, MD, U.S.A.) respectively. The medium was supplemented with 10% (v/v) fetal bovine serum (JRH Biosciences, Lenexa, KS, U.S.A.), 100 µg of streptomycin/ml and 100 units of penicillin/ml. For some cultures, the cells were treated with Ca^{2+} -ionophore A23187 (7 µM) for the indicated time period.

PCR cloning and plasmid construction

PCR was carried out with Namalwa genomic DNA in a DNA thermal cycler (MJ Scientific) according to the method described by Saiki *et al.* [8]. A 50 pmol sample of oligonucleotide primers (see below) and 2 µg of DNA template were used in a final volume of 50 µl, containing 1 unit of *Taq* DNA polymerase (Stratagene Cloning System, La Jolla, CA, U.S.A.). Each cycle of PCR included 1 min of denaturation at 95 °C, 1 min of annealing at 55 °C and 2 min of extension at 72 °C.

Oligonucleotides were synthesized using an Applied Biosystems DNA synthesizer (model 380B); primer yield and quality were tested by u.v. spectroscopy and gel electrophoresis. The primers 5'-CCCGGGGTCACTCCTGC-3' (sense strand) and 5'-TCCTTCTTGTCCTCCTCCT-3' (antisense strand) were designed according to the published sequence of the human fetal liver BiP/GRP78 gene [6]. The primer pairs correspond respectively to -371 and +294 bp relative to the transcriptional initiation site. PCR products were separated on 2% agarose gels and stained with ethidium bromide. The expected 667 bp DNA bands (designated BiP670) were isolated from the gel by a NA45 DEAE membrane (Schleicher & Schuell), eluted and cloned into a pBS(+) plasmid (Stratagene Cloning System) at a *Sma*I site to generate pBSBiP670. 5' deletion mutants of BiP670 (i.e. BiP500, BiP300, BiP237, BiP168 and BiP126) were generated by restriction digestion of BiP670 with *Sma*I and a second restriction enzyme cutting the upstream region (see Fig. 2). DNA sequences were directly determined on this plasmic using a commercial sequencing kit (United States Biochemicals). BiP670 or its 5' deletion mutants were subcloned into the mammalian expression vector pGCAT [9]; a gift from Dr. Olivier Brison, Institut Gustave Roussy, France) using the restriction enzymes *Kpn*I and *Bam*HI, to form a recombinant pGBiPCAT. The pGBiPCAT constructs were used for transient expression assay in cells.

Isolation of total RNA and RNA blot analysis

Total RNA preparation and blotting were performed by standard methods [7]. A 20 µg portion of RNA from each sample was separated on a 1% agarose gel containing 6.7% formaldehyde. The integrity and relative amounts of RNA were analysed by ethidium bromide staining. The RNA was then transferred to a Hybond N membrane (Amersham Corp., Arlington Heights, IL, U.S.A.), and cross-linked to the membrane by a u.v. cross-linker (Stratalinker; Stratagene Cloning System) according to the supplier's instructions. Hybridization was carried out in 1 M-NaCl/1% SDS at 65 °C, using $\sim 5 \times 10^5$ c.p.m. of probe per ml. The probe was prepared by labelling the DNA with [α - ^{32}P]dCTP (3000 Ci/mmol; Amersham) using the hexamer method [10], to specific radioactivities of $\sim 10^8$ c.p.m./µg of DNA. The DNA fragment of the probe was isolated from plasmid containing a near full-length transcript of mouse BiP/GRP78 CDNA (a gift from Dr. Joe Sambrook, University of Texas Southwestern Medical Center, Dallas, TX, U.S.A.).

Electroporation and CAT assay

pGBiPCAT carrying BiP670 or its 5' deletion mutants was introduced into cells by electroporation (Bio-Rad electroporator) as previously described [11]. A 1000 µF capacitor was charged

to 250 V and then discharged through a 1 ml cell suspension containing 3×10^6 cells and 20 µg of plasmid DNA. Under these transfection conditions there were no significant morphological changes to the cells; cell membranes remained intact as tested by dye exclusion. After 72 h, cell extracts were prepared for CAT activity analysis [12]. The reaction was incubated at 37 °C for 1 h, followed by development on a silica t.l.c. plate (Merck, Darmstadt, Germany). After autoradiography, the regions corresponding to the substrate chloramphenicol and the acetylated products were scraped off the t.l.c. plate and their radioactivity was counted to determine CAT activity. Activity was calculated as the percentage of [^{14}C]chloramphenicol converted into acetylated derivatives.

Cell extracts and gel mobility shift assay

Nuclear and cytosolic extracts were prepared according to Dignam *et al.* [13]. The protein concentration was measured by the Bradford method [14] and visualized by SDS/PAGE. The gel mobility shift assay was performed in buffer containing 12% glycerol; 12 mM-Hepes (pH 7.9); 100 mM-KCl, 5 mM-MgCl₂, 4 mM-Tris/HCl; 1 mM-EDTA; 1 mM-dithiothreitol and 300 µg of BSA/ml as previously described [15]. Briefly, the DNA probe (0.3 ng) was incubated with specified cell extracts in 15 µl at 30 °C for 30 min. The reaction mixtures were then subjected to 4% polyacrylamide gel electrophoresis under low ionic strength (6.7 mM-Tris/HCl, pH 7.9, 3.3 mM-sodium acetate and 1 mM-EDTA) at 20 °C and 15 mA constant current. The resolved gels were dried and exposed to Kodak XAR-5 X-ray film at -70 °C with an intensifying screen. The intensity of the shifted DNA bands was determined by scanning the X-ray film through a scanning densitometer (Hoefer GS 300). DNA probes used for the gel mobility shift assay were prepared as follows. The DNA fragments BiP300, BiP237, BiP168 and BiP126 were isolated from plasmids pBSBiP300, pBSBiP237, pBSBiP168 and pBSBiP126 respectively by restriction with *Hind*III and *Eco*R1. The DNA fragments were dephosphorylated with calf intestine phosphatase, labelled with [γ - ^{32}P]ATP and kinase (3×10^4 c.p.m./ng of DNA), and then purified in spin columns using standard methods [7].

RESULTS

Cloning of human BiP/GRP78 promoter by PCR amplification

A 667 bp genomic DNA sequence was isolated from Burkitt's lymphoma cells using PCR amplification. The primer pair (see the Materials and methods section for details) is located at -371 bp upstream and +294 bp downstream of the transcription initiation site, according to the reported human liver BiP/GRP78 gene sequence [6]. In contrast to the liver sequence [6], there is an additional G within the 5' untranslated region of the cloned BiP/GRP78 gene fragment (base 524; EMBL Data Library accession no. X59969). The significance of this sequence variation is not clear. This PCR-cloned lymphoma BiP/GRP78 promoter segment is designated BiP670.

The cloned BiP/GRP78 promoter sequence retains basal and inducible activity

To test whether the cloned DNA fragment was functional, the BiP670 promoter sequence was subcloned into the mammalian expression vector pGCAT [9], which carries the bacterial CAT gene as a reporter. CAT mRNA was transcribed from the inserted upstream BiP670 sequence. A 20 µg portion of the BiP/CAT recombinant plasmid was introduced into HeLa cells by electroporation, and processed for transient analysis. Cell extracts were isolated, and CAT activity was assayed *in vitro*. A typical result is shown in Fig. 1(a). A 10 µg portion of the

Fig. 1. Transient CAT assay of BiP670 promoter activity and the steady-state mRNA level in HeLa cells

(a) A 20 µg portion of pGBiPCAT carrying BiP670 was transfected into cells for transient expression, and 10 µg of protein extract was used for the CAT assay. Lane 1, mock transfection; lane 2, without A23187; lane 3, A23187 (7 µM), 5 h; lane 4, A23187, 15 h. CM, chloramphenicol; 1Ac and 3Ac, acetylated chloramphenicol. (b) Northern blot hybridization of steady-state HeLa mRNA. A 20 µg portion of total RNA was used for each treatment. Lane 1, mock treatment; lane 2, A23187, 4 h; lane 3, A23187, 6 h; lane 4, A23187, 20 h. BiP/GRP78 mRNA is indicated by the arrowhead.

transfected cell extracts gave significant CAT activity (lane 2, indicated with 1Ac and 3Ac). A 5 h induction of transfected cells with 7 µM-A23187 did not result in increased CAT activity (lane 3), whereas a 3-fold induction of CAT activity was detected following 15 h of induction (lane 4). Lane 1 shows mock-transfected cells, in which only substrate was seen (indicated with CM). The results indicate that the cloned sequence retains transcriptional activity at both basal and induced levels in cells.

The steady-state level of BiP/GRP78 mRNA was also analysed in HeLa cells following A23187 treatment. A 20 µg portion of total RNA was assayed. Fig. 1(b), lane 1, shows the level of BiP/GRP78 mRNA in untreated cells. The mRNA was significantly induced following a 3 h treatment of cells with A23187 (lane 2); an even greater induction was observed after 6 h of induction (lane 3). However, the BiP/GRP78 mRNA level was dramatically decreased following 20 h of induction (lane 4). The treated living cells were checked by dye exclusion, suggesting that the low level of BiP/GRP78 message at 20 h of induction was not because of cytolysis. The data may suggest that the cloned BiP670 contains only part of the regulatory sequence for BiP/GRP78 expression.

Determination of the A23187-inducible element (AIE) of the cloned BiP/GRP78 promoter sequence by 5' deletion/CAT assay in HeLa cells

The transactivation of the cloned BiP/GRP78 promoter was tested in HeLa cells. A 20 µg portion of pGBiPCAT plasmid carrying BiP670 or one of its derivatives (BiP500, BiP300, BiP237, BiP168 or BiP126) (Fig. 2) was electroporated into HeLa cells and transient expression was measured. The upper part of Fig. 2 shows some functional elements (as stated in the Figure legend) and restriction enzyme sites used to generate deletion mutants.

Fig. 2. Schematic presentation of the 5' deletion mutants of BiP670

Restriction enzyme sites used to generate deletion mutants are indicated. Symbols: closed box, TATATAA; open box, CCAAT; shaded box, putative Sp1-binding sequence. BiP500 is the 5'-end 500 bp of BiP670. The line length representing each mutant is shown, each linked in the same orientation to the CAT gene, with the corresponding names indicates to the right.

Fig. 3. Transient CAT assay of BiP promoter deletion mutants

Numbers represent the corresponding BiP constructs shown in Fig. 2. A 20 g portion of pGBiPCAT carrying individual BiP promoter mutants was transfected into cells for transient expression without (-) or with (+) A23187 treatment, and 2 µg protein extracts were used for the CAT assay. Open and closed arrowheads indicate chloramphenicol and acetylated chrloamphenicol respectively.

The putative *cis*-acting elements are indicated with boxes: a closed box is TATATAA, five open boxes are for CCAAT, and two shadowed boxes are the Sp1-binding sequence (see also legend to Fig. 2). Typical CAT t.l.c. patterns are shown in Fig. 3. Cells were mock-treated or treated with 7 µM-A23187 for 15 h, and CAT activity was determined 72 h later. Numbers above lanes represent the pGBiPCAT carrying the corresponding BiP/GRP78 promoter 5' deletion mutants (for example, 500 indicates BiP500). Chloramphenicol substrate and acetylated forms are indicated with open and closed arrowheads, respectively. The basal activity was relatively low; in contrast, the CAT activity was induced by 7–8-fold for the BiP500 and BiP300 constructs. For BiP237, the induced CAT activity appeared much lower; however, the basal activity was also low. Quantification of the radioactivity from the t.l.c. plates (see the Materials and methods section for details) of three independent experiments is shown in Table 1. BiP500, BiP300 and BiP237 gave respectively 7.0-, 8.2- and 8.0-fold induction of transactivation activity. Although the CAT activity of BiP237 was less than 15 % of that of BiP500 in induced cells, which was probably due to the decreased CAT activity (15 %) in non-induced cells, BiP237 retained a near-complete inducibility. Interestingly, BiP670 gave slightly less inducibility than BiP500. The reason for this is not clear; however, it should be noted that the differential expression of these promoter constructs in cells is not the result of variations in the amount of plasmid DNA. There is about 15–20 % variation

Table 1. Relative CAT activities of the BiP/GRP78 promoter constructs

CAT activities (means $\pm$ s.d.) were determined from three experiments, and expressed relative to that of the BiP670 CAT construct under non-induced conditions, which was set at 100%. Fold induction is the ratio of induced to basal levels.

Construct	CAT activity (%)		Fold induction
	-A23187	+A23187	
BiP670	100 $\pm$ 20	550 $\pm$ 60	5.5
BiP500	100 $\pm$ 25	700 $\pm$ 52	7.0
BiP300	55 $\pm$ 10	450 $\pm$ 90	8.2
BiP237	15 $\pm$ 2	90 $\pm$ 20	6.0
BiP168	5 $\pm$ 1	5 $\pm$ 0.5	1.0
BiP126	2 $\pm$ 0.2	2 $\pm$ 0.1	1.0

Fig. 4. *In vitro* gel mobility shift assay

HeLa cells were treated with 7 μ M A23187 for 0, 5 or 15 h. A 4 μ g portion of partially purified nuclear extract (lanes 2–4) or cytosolic extract (lanes 5–7) was used for binding with 0.3 ng of BiP300 probe (a) or BiP237 probe (b). Lane 1 contains probe alone. Arrowheads denote bound probe; f, free probe.

Fig. 5. Competition of *in vitro* DNA-binding assay

Portions of 8 μ g of nuclear extract were used for binding with 0.3 ng of BiP300 probe. Competitors BiP300 (lanes 3–5), BiP237 (lanes 6–8), BiP168 (lanes 9–11) or BiP126 (lanes 12–14) were included in the binding reactions at a 1-, 10- or 100-fold molar excess, as indicated. Control-lanes: lane 1, probe alone; lane 2, without competitor. Arrowheads denote bound probe; f, free probe.

Table 2. *In vitro* inhibition of AINF binding to BiP300 (expt. A) and BiP237 (expt. B)

The relative levels of binding activity (means $\pm$ s.d.) were determined by scanning densitometry of the X-ray films from three experiments. Competitors were 100-fold molar excesses of the probe indicated. B, bound probe; F, free probe.

Expt.	Competitor	B/(B + F) (%)	Binding (%)
A	Control	74.0 $\pm$ 10	100
	BiP300	6.0 $\pm$ 0.5	8.1
	BiP237	10.6 $\pm$ 1.5	14.3
	BiP168	47.2 $\pm$ 4.0	63.8
	BiP126	51.8 $\pm$ 8.0	70.6
B	Control	50.4 $\pm$ 5.0	100
	BiP300	4.1 $\pm$ 0.7	8.1
	BiP237	10.9 $\pm$ 1.5	21.6
	BiP168	36.8 $\pm$ 5.0	72.9
	BiP126	43.5 $\pm$ 6.4	86.2

of differential expression. These results suggest that the DNA segment between BiP237 and BiP168 (i.e. between the *AluI* and *MnII* sites) contains an AIE.

Identification by an *in vitro* gel mobility shift assay of A23187-inducible nuclear factors (AINF) that interact with the BiP/GRP78 AIE

A 4 μ g portion of partially purified nuclear or cytosolic extract from HeLa cells which had been mock-treated (Fig. 4, lanes 2 and 5) or treated with A23187 (Fig. 4, lanes 3, 4, 6 and 7) was assayed for AINF *in vitro*. Two AINFs were identified (indicated with arrowheads in Fig. 4). The binding of HeLa extract to BiP300 increased with the period of A23187 treatment. For example, a 15 h treatment resulted in 2.5-fold enhancement of binding (Fig. 4a, compare lane 4 with lane 2). In contrast, only a trace amount, if any, of binding activity was detected in the cytosolic extract from cells given the same treatments (Fig. 4a, lanes 5–7), indicating that the AINF is not present in the cytosolic fraction. The same binding pattern, but with slightly less binding activity, was seen when the BiP237 probe was used. As shown in Fig. 4(b), AINF increased by 1.5-fold after 15 h of A23187 treatment (lanes 2–4), whereas no significant binding activity was detected in cytosolic extracts (lanes 5–7). These results suggest that the sequence motif itself between the *PvuII* and *AluI* sites (i.e. the sequence present in BiP300 but not in BiP237) (see Fig. 2) is not important for AINF binding.

The specificity of the identified AINF was tested by competition analysis; typical results are shown in Fig. 5. Unlabelled BiP300 (lanes 3–5), BiP237 (lanes 6–8), BiP168 (lanes 9–11) or BiP126 (lanes 12–14) at the indicated molar excess (1-, 10-, or 100-fold) was included in the binding reaction. More than 60% and 90% of the AINF was displaced by 10- and 100-fold excesses respectively of BiP300 competitor (compare lanes 4 and 5 with lane 2, indicated with arrowhead). A similar competition pattern was seen when BiP237 was used as competitor. In contrast, there was less than 20–30% competition observed when an increasing molar excess of BiP168 (lanes 9–11) or BiP126 (lanes 12–14) was used. The results indicate that the detected AINF is probably specific for BiP300 and BiP237. The results also suggest that the region that differs between BiP237 and BiP168 (i.e. between the *AluI* and *MnII* sites; see Fig. 2) contains the sequence motif for AINF binding. The same competition pattern was seen when the BiP237 probe was used with the same competitors (see Table 2). In this study two AINFs were detected, as for the BiP300 probe. Quantification of the competition assays from three experiments

in the CAT assay because of differences in transfection of the plasmid and the *in vitro* CAT assay. Therefore, the detected differences in CAT activity in these tested cell lines is the result

is shown in Table 2. BiP300 competed more effectively than did BiP237 (resulting in 8.1% and 14.3% binding respectively) against BiP300 binding (Table 2, expt. A). Similarly, 8.1% and 21.6% binding was observed with BiP300 and BiP237 as competitors against BiP237 binding (expt. B). In any case, binding of both AINFs was displaced by BiP300 and BiP237. However, there is no evidence so far to indicate whether the two factors are independent or are just conformational isoforms of the same factor.

DISCUSSION

In this study we have cloned a functional human BiP/GRP78 promoter sequence from Burkitt's lymphoma cells (Namalwa) by PCR amplification. The DNA segment retains an additional G in the 5'-end untranslated region, compared with the previously reported human liver sequence [6]. This was confirmed by the restriction mapping of four clones from independently isolated PCR products (results not shown). Transient expression of BiP670 in cells indicates that it retains basal and inducible activity. Induction of transfected cells with 7 μ M-A23187 for 15 h significantly increased CAT activity. However, induction with the same agent for 5 h, which is sufficient to elicit a near-optimum accumulation of steady-state mRNA, did not cause transcriptional induction of the gene (Fig. 1). These results suggest that the cloned BiP670 probably represents only part of the control machinery for transcriptional regulation. There may be a flanking enhancer-like sequence which assists the transcription of BiP670. However, one should also consider post-transcriptional regulation when analysing the data comparing the time course of the cloned promoter activity following induction and the endogenous steady-state BiP/GRP78 mRNA levels. Since different RNAs are involved, one would expect turnover differences to be at least as influential as transcriptional differences. Transactivation of BiP670 and its deletion mutants in HeLa cells revealed that the sequence motif present in BiP300 but not in BiP237 (i.e. between the *Pvu*II and *Alu*I sites shown in Fig. 2) is important for basal expression. An additional motif in BiP500, but not in BiP300, must exist, as indicated by a 50% decrease in BiP300 basal activity. Sequences in BiP237 but not in BiP168 (i.e. between *Alu*I and *Mn*II) contain elements essential for both basal and inducible activity. Using a relatively rough deletion assay, Lee and colleagues [16] have previously suggested that the human BiP/GRP78 promoter contains an A23187-inducible region within 170 nucleotides upstream from the transcriptional initiation site (corresponding to BiP237).

We have also identified two AINFs in HeLa cells. These cellular factors interact with the AIE-containing sequence motif present in BiP237 but not in BiP168. More than 80% of the AINF binding to the AIE was competed by a 100-fold molar excess of BiP237 or a longer BiP promoter sequence, but competition was not as effective with BiP168 or any shorter sequence, supporting the conclusion from *in vivo* CAT assays that sequences present in BiP237 but not in BiP168 (i.e. between residues -107 and -39) contain the binding element for these AINFs. These results suggest that the AINFs may be important for the transcriptional

activation of the BiP/GRP78 gene. Since there are two CCAAT boxes located in the AIE-containing domain, the identified AINF may be the CCAAT-binding nuclear factor. However, a 100-fold CTF/NF1-binding sequence did not significantly affect AINF binding to BiP300 (C. C.-K. Chao, unpublished work), suggesting that CTF/NF1 is not itself sufficient for inducibility. Studies of the rat BiP/GRP78 gene promoter using *in vitro* DNA binding have suggested that a CG/CAAT sequence and a GC-rich sequence motif are important for basal and induced expression of the gene [16]. These motifs are conserved and correspond to the human promoter spanning residues -135 to -83. The human GC-rich motif overlaps with the 5' part of the putative AIE-containing motif proposed in our study. It is possible that this motif is a critical region for the binding of the AINF. More recently, Wooden *et al.* [17] have shown a proximal region containing a CCAAT motif in the rat BiP/GRP78 gene promoter as being an essential mediator for gene induction by A23187 and other stresses. The corresponding human proximal CCAAT motif is within the proposed AIE-containing motif, but is further downstream than the GC-rich motif. Therefore our results, together with those of others, suggest that the proposed AIE-containing motif and the putative AINF, along with CTF/NF1, are important in mediating the transactivation of the human BiP/GRP78 gene.

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REFERENCES

1. Pelham, H. R. (1986) *Cell* **46**, 959-961
2. Rothman, J. E. (1989) *Cell* **59**, 591-601
3. Li, X. & Lee, A. S. (1991) *Mol. Cell. Biol.* **11**, 3446-3453
4. Lee, A. S. (1987) *Trends Biochem. Sci.* **12**, 20-23
5. Watowich, S. S. & Morimoto, R. I. (1988) *Mol. Cell. Biol.* **8**, 393-405
6. Ting, J. & Lee, A. S. (1988) *DNA* **7**, 275-286
7. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor
8. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487-491
9. Frebourg, T. & Brison, O. (1988) *Gene* **65**, 315-318
10. Fernberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6-13
11. Chao, C. C.-K., Huang, S.-L., Huang, H. & Lin-Chao, S. (1991) *Mol. Cell. Biol.* **11**, 2075-2080
12. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2**, 1044-1051
13. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11**, 1475-1489
14. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248-254
15. Hannighausen, L. & Lubon, H. (1987) *Methods Enzymol.* **152**, 721-735
16. Liu, E. S. & Lee, A. S. (1991) *Nucleic Acids Res.* **19**, 5425-5431
17. Wooden, S. K., Li, L.-J., Navarro, D., Qadri, I., Pereira, L. & Lee, A. S. (1991) *Mol. Cell. Biol.* **11**, 5612-5623