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COORDINATED INDUCTION OF TWO UNRELATED GLUCOSE-REGULATED PROTEIN GENES BY A CALCIUM IONOPHORE: HUMAN BiP/GRP78 AND GAPDH

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SUMMARY: The induction of human BiP/GRP78 and GAPDH protein genes by the calcium ionophore A23187 was determined. Steady-state levels of mRNA for both the glucose starvation-responsive BiP/GRP78 gene and the glucose-responsive GAPDH gene were dramatically induced in a variety of human cells. There is a homologous palindromatic sequence GCCGTTAACGGC in the active promoter region of the two genes that is known to be required for the induction of mammalian BiP/GRP78 by A23187. The evidence confirms in general the function of this element in the regulation of calcium-associated gene activity.

Mammalian BiP/GRP78 is a stress-inducible protein. It was first identified as protein when cells were depleted of glucose [1, 2]. Subsequently, it was found that a variety of reagents which disrupt intracellular calcium stores or block protein glycosylation also enhance the BiP/GRP78 synthesis (reviewed in [3]). This 78-kD protein is present as a soluble protein in the lumen of the rough endoplasmic reticulum (ER; Bole et al. (4)), and has been identified as the immunoglobulin heavy-chain-binding protein [5] by Munro and Pelham [6]. Substantial lines of evidence imply that BiP/GRP78 is a catalyst of protein folding and is crucial for the maturation of mammalian cell membrane-associated and secreted proteins (reviewed in [7, 8]).

The glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.2.12) (GAPDH) is a tetramer composed of identical 37-kD MW subunits [9, 10]. It catalyzes the conversion of glyceraldehyde 3-phosphate to 1,3-diphosphoglycerate [9-11].

Abbreviations: GRP78, glucose-regulated protein; BiP, immunoglobulin binding protein; gapdh, glyceraldehyde-3-phosphate dehydrogenase.

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This reaction is regulated by several of the glycolytic metabolites and also by the coupling of the enzyme with the cell membrane [12, 13].

Since cell metabolism demands for BiP/GRP78 and GAPDH proteins, the corresponding genes are expected to be constitutively active or readily inducible. The response to the diverse signals of genes for the BiP/GRP78 [3] and GAPDH [14-17] in the cell suggests that a converged signal for gene transcription probably exists. In support of this hypothesis, we found that the BiP/GRP78 and GAPDH mRNA are coordinately induced by a calcium ionophore in a variety of human cells. In addition, through DNA sequence comparison of two gene promoters, we found that a palindromatic sequence required for BiP/GRP78 induction by ionophore A23187 also exists in the GAPDH active promoter region.

METHODS AND MATERIALS

Cell Lines and Culture Conditions. Monolayers of human WI-38 VA13 cells, and cancer cell lines CaSki, C-33A, HeLa, suspension cultures of CA46, EB-1, HL-60, HeLa-S_3, and monkey COS-1 cells were maintained according to the supplier's specifications (ATCC).

Induction Conditions. The cells were maintained as described above until they reach approximately 90% confluency. Seven uM ionophore A23187 (Sigma) was then added to the culture medium and left for 4 h or as otherwise indicated and stopped immediately. Additional type of inductions were: heat shock (45°C, 1 h) and labeled 4 h later; 254-nm UV (10 J/m^2) and labeled 4 h later; cisplatin (3 ug/ml, 4 h) and labeled immediately. For protein labeling, see below.

Labeling and Detection of Inducible Proteins. After the induction treatment, HeLa cells were labeled with [^{35}S]methionine (1163 Ci/mmol, NEN Research Products, DuPont) (100 uCi per 10^6 cells in medium lacking methionine) for 1 h, then washed with ice-cold phosphate-buffered saline. Cell protein extracts were then prepared as described by Dignam et al. [18]. Protein concentrations were quantitated by Bradford method [19]. Equal amount of proteins from each induction treatment were separated in a 4-10% gradient SDS-polyacrylamide gel (SDS-PAGE) after heating at 70°C for 10 mins. in gel sample buffer (20 mM Tris-HCl, pH 6.8 containing 10% glycerol, 1% SDS, 0.1% DTT, and 0.2% bromophenol blue), followed by gel drying and autoradiography.

Isolation of Cytoplasmic RNA and RNA Blot Analysis. RNA preparation and blotting were performed by standard methods [20]. Total cytoplasmic RNA was isolated as described by Chirgwin et al. [21]. Twenty ug of RNA from each sample were separated on 1% agarose gel containing 6.7% formaldehyde. The integrity and relative amounts of RNA were analyzed by ethium bromide staining. The RNA were then transfered to Hybond N membrane (Amersham

Corp.), and cross-linked by exposure to UV light (Stratalinker, Stratagene). Hybridization was carried out in 1 M NaCl-1% SDS at 65°C, using ~ 5 x 10^5 cpm of probe per ml. The probe was prepared by the hexamer method [22] to specific activities of ~10^8 cpm/ug of DNA. The DNA fragments of the probe were from a cDNA plasmid encoding mouse GRP78/BiP [23] or human GAPDH [24].

RESULTS AND DISCUSSION

Induction of BiP/GRP78 by Ionophore A23187. HeLa cells were treated with various inducing agents and cells were then labeled to visualize the induction of specific proteins. Cellular proteins were partially fractionated into nuclear and cytosolic extracts. A 50 ug extract was run in a 4%-10% gradient SDS-PAGE. As shown in Fig. 1a, a protein band corresponding to Bip/GRP78 was clearly detected in the ionophore A23187-treated cytosolic extract (CE) (indicated with an arrowhead, see lane A in the panel CE). Since the rough ER is associated with the nuclear membrane in the cell, it is not surprising to have some ER fractions attached to the nuclear extract (NE) during partial fractionation. This may explain the cross contamination of a small proportions of the 78-kD molecules in the NE. In contrast, heat shock, UV and cisplatin (Pt) were incapable of inducing the

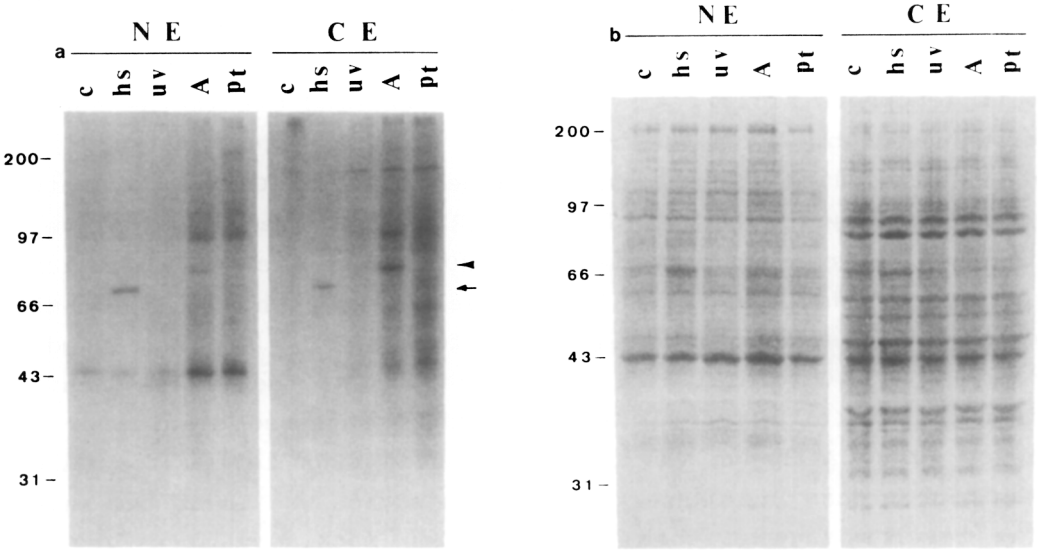

Figure 1. Effects of various treatments on the BiP/GRP78 synthesis. (1a) Autoradiogram of [^{35}S]methionine-labeled proteins synthesized by HeLa cells untreated (c), treated with ionophore A23187 (A), heat shock (hs), UV, or cisplatin (Pt). (1b) The same gel as stained with Coomassie blue. Position corresponding to BiP/GRP78 and hsp70 is indicated with an arrowhead and an arrow, respectively, at the right. Molecular masses (kD) are indicated at the left.

BiP/GRP78. The results are consistent with the notion that BiP/GRP78 is located in the rough ER [7, 8]. On the other hand, a protein band representing hsp70 protein was specifically induced by heat shock (indicated with an arrow, see lane hs in the panel CE). And an even stronger hsp70 signal was also detected in the NE. This was unexpected because hsp70 proteins have no targeting signal for the destination to the nucleus and therefore should be present exclusively in the cytosol [25]. In addition, hsp70, although it has been found transiently bound to newly synthesized but incompletely assembled oligomeric enzymes [7, 8], has not yet been detected in the nucleus. However, it is unlikely due to the sample preparation because the major portion of the cytosolic BiP/GRP78 was present in the CE. A possible explanation for this is that hsp70 proteins may also function as binding proteins in the nucleus. The regulation of hsp70 and BiP/GRP78 is complex [26]. It needs further studies to confirm this hypothesis. Nevertheless, it is certain that BiP/GRP78 is effectively induced by ionophore A23187 in HeLa cells and other tested cell lines (data not shown) in our assay conditions. Fig. 1b shows the coomassie blue staining pattern of the same gel before processing for autoradiography.

Induction of BiP/GRP78 and GAPDH mRNA by Ionophore A23187. To determine the optimal induction period of BiP/GRP78 and GAPDH, mRNA of both proteins were measured in HeLa cells after treatment with ionophore A23187. All assays were carried out in the normal growth medium. As shown in Fig. 2, the constitutive levels of GAPDH (indicated with grpdh) is higher than BiP/GRP78 (indicated with grp) (see lane c). The BiP/GRP78 mRNA was progressively induced and reached a maximum of ~20-fold 6 h after induction, and quickly decline to the basal level after 24 h. GAPDH, on the other hand, was induced at least 10-fold after 2 h and reached a maximum of ~30-fold after 4 h, then decline to the level of early induction (i.e., 10-fold) and thereafter remained stable for at least 24 h. Although the overall induced mRNA levels for GAPDH was higher than BiP/GRP78, the fold induction was about the same. The results suggest that a degradation machinery specific for BiP/GRP78 was activated by prolonged exposure of the cells to ionophore A23187 whereas GAPDH was stable under this growth conditions (see Fig. 2, also see [27, 28]).

Conserved Induction of BiP/GRP78 and GAPDH mRNA in Human Cells. To study further whether the induction of BiP/GRP78 and GAPDH is

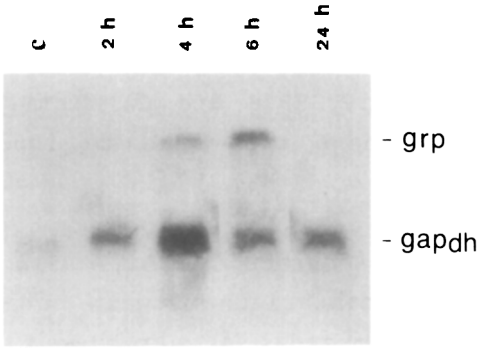

Figure 2. Northern blot analysis of RNA from ionophore A23187-treated HeLa cells. Total cytoplasmic RNA was isolated from untreated cells (lane c) or cells treated with 7 uM A23187 for 2, 4, 6, or 24 h. The RNA was electrophoresed, blotted to a membrane and hybridized as described in the Materials and Methods. grp, BiP/GRP78 mRNA; gapdh, GAPDH mRNA.

cell specific or conserved, nine cell lines were assayed 5 h after treatment (comparable to the maximum induction for both mRNA). The levels of GAPDH mRNA were effectively induced and are less variable between cell lines (Fig. 3a and 3b). In contrast, the levels of induced BiP/GRP78 varies slightly between suspension cultures (see Fig. 3a), and even greater between monolayer cells (see Fig. 3b). The exact reason for this is not clear. However, it may be partly due to the instability of the BiP/GRP78 mRNA relative to GAPDH mRNA (see Fig. 2). Alternatively, the variation of the level of BiP/GRP78 may be associated with the culture condition, because the levels of BiP/GRP78 mRNA between monolayer and suspension cultured HeLa cells vary slightly (compare HeLa-S and HeLa in Fig. 3a). In addition, the distribution of cells in the cell cycle phase should also be considered because metabolism (e.g., glucose) in G_0 cells is very different from actively "cycling" cells [29]. It is interesting to note that although the constitutive GAPDH mRNA

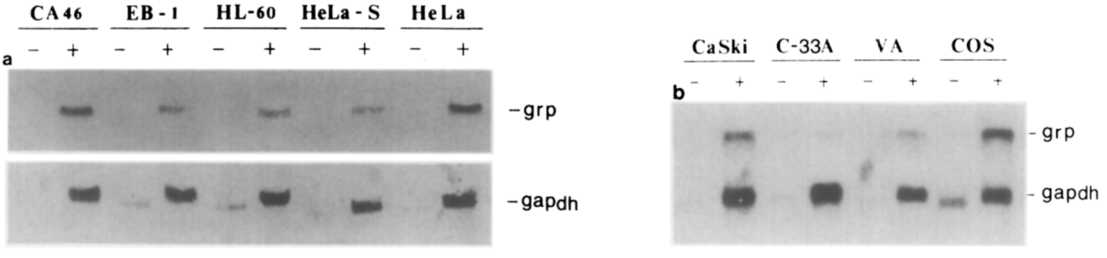

Figure 3. Induction of BiP/GRP78 and GAPDH mRNA levels by maximum ionophore A23187 treatment. (3a) mRNA levels from suspension cultured cells except HeLa are indicated above the panel. (3b) mRNA levels from monolayer cells. Symbols: -, untreated; +, treated.

level in monkey COS cells is ~10-fold higher than in human cells, the induced mRNA level is about the same. In conclusion, although the levels of induced BiP/GRP78 and GAPDH mRNA may vary, the effective response of these two genes to ionophore A23187 is conserved in human cells.

A Common Palindromatic Sequence in BiP/GRP78 and GAPDH Gene Promoters. A sequence comparison between human BiP/GRP78 [30] and GAPDH [31] gene promoters was carried out through computer analysis (PC/Gene, IntelliGenetics). We found that a palindromatic sequence GCCGTTAACGGC is present in both promoter regions (Fig. 4). It has been shown that this element is crucial for the induction of human BiP/GRP78 mRNA by a variety of agents, including ionophore A23187 [32, 33; our unpublished data]. There is no reason not to believe that this element is also required in GAPDH induction by ionophore A23187.

In this study, a putative regulatory element for calcium ionophore A23187 induction was identified in human GAPDH gene promoter. This element has been found conserved in mammalian BiP/GRP78 gene [32]. The similarity of fold induction of BiP/GRP78 and GAPDH mRNA observed in this study suggests that the induction by ionophore A23187 is likely to be dependent upon this common element. Therefore, the findings of GAPDH induction by ionophore A23187 may provide an additional model to study mammalian gene regulation. However, the steady-state levels of mRNA for BiP/GRP78 following induction is significantly lower than GAPDH, suggesting that mRNA stability is a crucial determinant for the expression of BiP/GRP78. In contrast, mRNA stability for GAPDH plays a lesser role in its expression. Works aiming to clarify these uncertainties are ongoing.

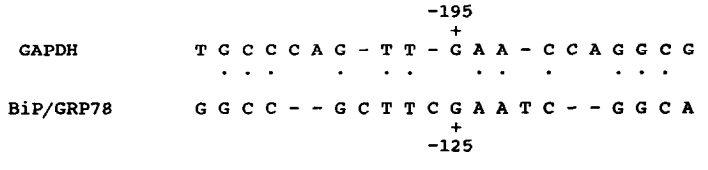

Figure 4. Common sequences among the human BiP/GRP78 and GAPDH promoters. Sequences shared between the promoters of genes for BiP/GRP78 [23] and GAPDH [24] are shown by dots. The location of each sequence is numbered on the basis of its own transcriptional initiation site.

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REFERENCES

1. Shiu, R.P.C., Pouyssegur, J. and Pastan, I. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 3840-3844.
2. Lee, A.S. (1981) J. Cell Physiol. 106, 119-125.
3. Lee, A.S. (1987) Trends. Biochem. Sci. 12, 20-23.
4. Bole, D.G., Hendershot, L.M. and Kearney, J.. (1986) J. Cell Biol. 102, 1558-1566.
5. Hass, I.G. and Wabl, M. (1983) Nature 306, 387-389.
6. Munro, S. and Pelham, H.R.B. (1986) Cell 46, 291-300.
7. Rothman, J.E. (1989) Cell 59, 591-601.
8. Pelham, H.R.B. (1989) EMBO J. 8, 3171-3176.
9. Fox, J.B. and Dandliker, W.B. (1956) J. Biol. Chem. 218, 53-57.
10. Harris, I. and Perham, R.N. (1963) Biochem. J. 89, 60P
11. Dandliker, W.B. and Fox, J.B. (1955) J. Biol. Chem. 214, 275-283.
12. Wang, C. and Alapovic, P. (1980) Arch. Biochem. Biophys. 205, 136-145.
13. Tsai, I.-H., Murthy, S.N.P. and Steck, T.L. (1982) J. Biol. Chem. 257, 1438-1442.
14. Holland, M.J. and Holland, J.P. (1979) J. Biol. Chem. 254, 5466-5474.
15. Aithal, H.N., Walsh-Reitz, M.M. and Toback, F.G. (1983) Proc. Natl. Acad. Sci. U.S.A. 50, 2941-2945.
16. Alexander, M., Curtis, G., Avruch, J. and Goodman, H. (1985) J. Biol. Chem. 260, 11978-11985.
17. Alexander, M., Lomanto, M., Nasrin, N. and Ramaika, C. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 5092-5096.
18. Dignam, J.D., Lebovitz, R.M. and Roeder, R.G. (1983) Nucleic Acids Res. 11, 1475-1489.
19. Bradford, M.M. (1976) Anal. Biochem. 72, 248-254.
20. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning, Cold Spring Harbor Laboratory, New York.
21. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) J. Biochem. 18, 5294-5299.
22. Fernberg, A.P. and Vogelstein, B. (1983) Anal. Biochem. 132, 6-13.
23. Kozutsumi, Y., Normington, K., Press, E., Slaughter, C., Sambrook, J. and Gething M.-J. (1989) J. Cell Sci. in press.
24. Tso, J.Y., Sun, X.-H., Kao, T., Reece, K.S. and Wu, R. (1985) Nucleic Acids Res. 13, 2485-2502.
25. Lindquist, S. and Craig, E.A. (1988) Annu. Rev. Genet. 22, 631-677.
26. Watowich, S.S. and Morimoto, R.I. (1988) Mol. Cell. Biol. 8, 393-405.
27. Ciccarelli, C., Philipson, L. and Sorrentino, V. (1990) Mol. Cell. Biol. 10, 1525-1529.
28. Manfioletti, G., Ruaro, M.E., Sal, G.D., Philipson, L. and Schneider (1990) Mol. Cell. Biol. 10, 2924-2930.
29. Denhardt, D.T., Edwards, D.R. and Parfett, C.L.J. (1986) Biochim. Biophys. Acta 865, 83-125.

30. Ting, J. and Lee, A.S. (1988) DNA 7, 275-286.
31. Ercolani, L., Florence, B., Denaro, M. and Alexander, M. (1988) J. Biol. Chem. 263, 15335-15341.
32. Resendez, E., Jr., Wooden, S.K. and Lee, A.S. (1988) Mol. Cell. Biol. 8, 4579-4584.
33. Chang, S.C., Erwin, A.E. and Lee, A.S. (1989) Mol. Cell. Biol. 9, 2153-2162.