Brefeldin A as a Regulator of grp78 Gene Expression in Mammalian Cells

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We report here that brefeldin A (BFA), which specifically blocks protein transport from the endoplasmic reticulum to the Golgi apparatus and causes resorption of Golgi membrane to the endoplasmic reticulum, specifically induced the endoplasmic reticulum-resident protein GRP78. Treatment of a human hepatoma cell line Alex-PC with BFA at a concentration of 5 μg/ml increased the grp78 transcript level by 12-fold. Analyses of the transcriptional rate of grp78 and the transcription with grp78 promoter suggested that this cell line utilized a posttranscriptional mechanism to increase the expression of grp78 in response to BFA. The induction process was partially dependent on de novo protein synthesis. Interestingly, in a hamster lung fibroblast cell line, K12, the induction of grp78 by BFA could be mediated by a transcriptional control mechanism. We further demonstrated that in K12 cells the region of the grp78 promoter responsive to BFA was within a 40-base pair region between −169 and −130, containing the conserved grp core and a 10-base pair region between −99 to −80 that contained a proximal CCAAT element. A model of how BFA regulates grp78 expression at both the transcriptional and posttranscriptional level is presented.

The glucose-regulated proteins (GRPs), along with the heat shock proteins, belong to a superfamily of stress-inducible proteins that are overproduced when cells respond to adverse physiological conditions (Hightower, 1991). The GRPs were first identified as proteins whose syntheses were greatly enhanced when cells were grown in medium depleted of glucose (Shiu et al., 1977). The most prominent member of the GRP family is a 78,000 molecular weight species known as GRP78 or BiP (Ting et al., 1987; Hendershot et al., 1988). GRP78 is constitutively expressed and localized in the lumen of the endoplasmic reticulum (ER). Other potent inducers for GRPs were identified, such as calcium ionophore A23187, which perturbs intracellular calcium ion concentration, tunicamycin, which prevents N-linked glycosylation, and GRP78 have been identified, such as calcium ionophore A23187, which perturbs intracellular calcium ion concentration, tunicamycin, which prevents N-linked glycosylation, and the accumulation of malfolded proteins in the ER (Lee, 1987; Chang et al., 1987; Kozutsumi et al., 1988; Watowitch and Morimoto, 1988; Wooden et al., 1991).

Recently, a chemical known as brefeldin A (BFA) has been isolated from the fungus Eupenicillium brefeldianum. This chemical appears to inhibit protein transport from the ER to the Golgi apparatus and causes resorption of Golgi membrane into the ER (Misumi et al., 1986; Fujiwara et al., 1988). These effects suggest the possibility of BFA as a novel inducer for the GRPs, since it introduces physiological stress to the ER by disrupting the normal protein efflux from the ER to the Golgi, and as a result, non-ER-resident proteins could be accumulating in the lumen of the ER (Wang et al., 1991). GRP78 has been identified as one of the molecular chaperone proteins that have the ability to associate with polypeptides (Gething et al., 1986; Hendershot et al., 1988; Rothman, 1989). Previously, we and others have postulated that accumulation of non-ER-resident proteins may serve as a stimulus for GRP78 induction, as more GRP78 may be required to associate with the proteins blocked in the ER (Lee, 1987; Kozutsumi et al., 1988; Nakaki et al., 1989; Wooden et al., 1991).

In the present study, we tested whether BFA induces grp78 and if so, by what mechanism. The systems that we chose to study were a transformed human hepatoma cell line Alex-PC, and its parental cell line Alexander. The Alex-PC cells contain the genome of hepatitis B virus (HBV) driven by a Rous sarcoma virus promoter stably integrated into the human Alexander hepatoma cell line (PLC/PRF/5) (Yeh and Ou, 1991). The difference between Alexander and Alex-PC is the predominant expression of the HBV precore protein in the latter. Previously, we have established that BFA at 5 μg/ml blocks the transport of HBV precore protein from the ER to the Golgi (Wang et al., 1991); therefore, this cell line has been proven to be responsive to BFA. For comparison, we also studied the effect of BFA in a hamster fibroblast cell line K12 (Lee, 1981).

Here, we observed unexpectedly that whereas the induction of grp78 by most inducers in a variety of cell lines is controlled at the transcriptional level (Lee, 1987; Resendez et al., 1988; Wooden et al., 1991), BFA is able to regulate the expression of grp78 at both transcriptional and posttranscriptional levels, dependent on the cell lines. The regions of the rat grp78 promoter where BFA exerts its effect were mapped.

MATERIALS AND METHODS

Cells and Culture Conditions—The human hepatoma cell line Alex-PC was established from the Alexander hepatoma cell line (PLC/PRF/5) (Yeh and Ou, 1991). The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum at 36 °C. The K12 cell line was described elsewhere (Lee, 1981) and maintained in DMEM supplemented with 10% cadet calf serum at 35 °C.
Plasmid DNA—The hamster cDNA plasmids p3C5 and p4A3, encoding hamster GRP78 and GRP94 respectively, have been described previously (Lee et al., 1983; Ting et al., 1987). pJ is a cDNA clone coding for mouse α-skeletal actin (Artishevsky et al., 1984).

The grp78/CAT fusion plasmids used are (−1290)CAT, (−656)CAT, (−169)CAT, and (−130)CAT. These are the grp78 promoters containing 5′−3′ endpoints at 1290, 456, 169, and 130 bp, respectively, upstream of the transcriptional start site and an identical 3′−endpoint at −38 fused to the chloramphenicol acetyltransferase (CAT) gene (Resendez et al., 1985). LS90 is a linker−and 130 bp, respectively, upstream of the transcriptional start site and 10 bp substitution at −99 to −90. A129/90 is identical with (−456)CAT, with the exception of a 10-bp substitution at −99 to −90. Δ129/90 is identical with (−456)CAT, with the exception that it has a 40-bp internal deletion from −129 to −90. The construction of all the plasmids has been previously described (Wooden et al., 1991).

Transfection and CAT Assays—Alexander, Alex-PC, and K12 cells were seeded on 10-cm dishes at about 30% confluency. Sixteen to 24 h after seeding, cells were transfected with 5−10 μg of test plasmids using the calcium phosphate precipitation method, as described previously (Lee et al., 1986). After 4 h of incubation with the DNA precipitate, the cells were shocked with 15% glycerol. The cells were harvested 4 h after transfection. Sixteen h prior to harvesting, the cells were all changed to fresh medium. For the treated cells, either 5 μg/ml of BFA or 7 μM of A23187 was added. The preparation of protein extracts and the CAT assays were performed as described previously (Resendez et al., 1985). Ten to 50 μg of protein extract was used in each assay in a 1-h incubation reaction at 37°C. The CAT activities were quantitated by an AMBIS Radioanalytic Imaging System (AMBIS Systems, Inc., San Diego, CA).

Preparation of Cytoplasmic RNA—Extraction of cytoplasmic RNA has been described previously (Lee et al., 1983). Briefly, confluent cell cultures from 10-cm diameter dishes were washed once with cold phosphate-saline buffer. Cells were pelleted and resuspended in 0.6 ml of isosoy buffer (0.14 M NaCl, 10 mM Tris-HCl, pH 8.4, 1.5 mM MgCl2). Cells were lysed by the addition of 70 μl of 5% Nonidet P-40. The supernatant was recovered and mixed with 0.5 ml of NETS buffer (0.1 M NaCl, 10 mM Tris-HCl, pH 8.4, 1 mM EDTA, 1% SDS). The mixture was extracted twice with phenol/chloroform/isooamyl alcohol (25:24:1) and once with chloroform. The RNA samples in aqueous phase were precipitated with 0.1 volume of 3 M sodium acetate (pH 5.4) and 2 volumes of 95% ethanol. The samples were spun down, dried, and resuspended in 40 μl of 10 mM Tris-HCl, pH 8.0, and 1 mM EDTA and stored at −80°C. Generally, 50−100 μg of RNA were obtained per 10-cm diameter dish by this method.

RNA Blot Hybridization—Ten to 15 μg of cytoplasmic RNA from each sample were applied onto a 1% denaturing formaldehyde-formamide agarose gel, subjected to electrophoresis for 3−4 h at 90 V at 4°C, and transferred onto nitrocellulose filters (Lee et al., 1983). The hybridization procedures have been described previously (Lee et al., 1983). Generally, 2.5 × 106 cpm of [32P]dCTP-labeled probes were used in each hybridization reaction. The specific activities of the labeled DNA were about 0.5−1 × 106 cpm/μg. All hybridization probes were labeled by the hexamer method (Feinberg and Vogelstein, 1983).

Protein Labeling and SDS-Polyacrylamide Electrophoresis—Subconfluent cells (80−90% confluency) were grown in DMEM as described before. The cells were changed into the methionine-free medium supplemented with 5% of dialyzed serum and 20 μCi/ml of [35S]methionine (1000 Ci/mmol), treated either with 5 μg/ml BFA, or 7 μM A23187, and incubated for 16 h at 35°C. At the end of labeling, cells were harvested and spun down by centrifugation. The cell pellets were then resuspended in 100 μl of a lysis buffer containing 9.5% urea, 2% Nonidet P-40, 5% β-mercaptoethanol, and subjected to three cycles of freezing and thawing. The cell debris was removed by centrifugation and the supernatant stored at −20°C. Equal amounts of counts/min (2 × 104 cpm) for each sample were loaded onto a 8.5% SDS-polyacrylamide gel. The gels were dried and exposed to Kodak X-Omat AR films at −70°C with intensifying screen.

In Vitro Transcription Measurements—Alex-PC cells were grown to 80−90% confluency in 15-cm diameter culture dishes. Two dishes were used for each treatment condition. After a change of fresh medium, either no drug, 7 μM of A23187, or 5 μg/ml of BFA was added. Sixteen h after addition of the drugs, the cells were resuspended in 100 μl of a lysis buffer containing 9.5% urea, 2% Nonidet P-40, 5% β-mercaptoethanol, and subjected to three cycles of freezing and thawing. The cell debris was removed by centrifugation and the supernatant stored at −20°C. Equal amounts of counts/min (2 × 104 cpm) for each sample were loaded onto a 8.5% SDS-polyacrylamide gel. The gels were dried and exposed to Kodak X-Omat AR films at −70°C with intensifying screen.

RESULTS

Treatment of BFA Increases the mRNA Level of grp78 in Alex-PC Cells—BFA has been shown to inhibit protein transport from the ER. To determine whether BFA can specifically enhance the expression of the two stress-inducible ER-resident glucose-regulated proteins, GRP78 and GRP94, we treated the Alex-PC cells with 5 μg/ml of BFA and measured the steady state mRNA levels of grp78 and grp94 by Northern blot analysis. As shown in Fig. 1A, upon addition of BFA, the level of grp78 mRNA increased gradually. By 6 h of treatment, a saturated level of 12-fold induction was observed. In contrast, the steady-state level of grp94 and actin transcripts were only minimally affected by BFA. Thus, BFA appears to induce predominantly the grp78 mRNA in these cells.

BFA Induction of grp78 mRNA Level Requires de Novo Protein Synthesis—To assess whether new protein synthesis is required to mediate the induction, Alex-PC cells were pretreated with cycloheximide for 2 h to block new protein synthesis prior to the addition of BFA. For comparison, the cells were also treated with A23187, a potent transcriptional activator for grp78. The cells were harvested after 6 h of

![Fig. 1. A. RNA blot analysis of grp78, grp94, and actin transcripts in BFA-treated cells. Cytoplasmic RNA were extracted from Alex-PC cells treated with 5 μg/ml BFA at the times indicated, blotted, and hybridized with hexamer-labeled p3C5 and p4A3 and pJ. The autoradiogram shown was quantitated by an LKB ultrascan XL densitometer. The relative transcript levels of grp78 (•) were normalized against that of actin and plotted against the time of BFA treatment. B. Effect of cycloheximide on the BFA induction of grp78. Cytoplasmic RNA were prepared from Alex-PC cells grown to 80% confluency in DMEM + 5% fetal calf serum and subjected to the following conditions prior to extraction. Lane 1, 5 μg/ml BFA was added for 6 h; lane 2, 7 μM A23187 was added for 6 h; lane 3, 0.1 mM cycloheximide (CHX) was added for 8 h; lane 4, 1 mM CHX was added for 2 h prior to the addition of 5 μg/ml BFA in DMEM for further incubation for 6 h; lane 5, 0.1 mM CHX was added for 2 h prior to the addition of 7 μM A23187 for further incubation for 6 h; lane 6, untreated control cells.](image-url)
incubation, and cytoplasmic RNA was extracted. The amount of grp78 was determined by Northern blot analysis. As shown in Fig. 1B, cycloheximide treatment reduced the grp78 transcript level in both A23187- and BFA-treated cells by about 50%, as revealed by densitometry, suggesting that new protein synthesis was partially required to mediate the BFA induction response. The result for A23187 is consistent with our previous observation that de novo protein synthesis is required for the transcriptional activation of grp78 by A23187 (Resendez et al., 1986). In contrast, the mRNA level of a stress-invariant transcript, p3A10 (Lin and Lee, 1984), was neither induced by BFA nor affected by cycloheximide treatments. Treatment of Alex-PC cells with cycloheximide, in the absence of BFA or A23187, had a minimal effect on the grp78 mRNA levels.

The Accumulation of grp78 Transcripts Results in an Increase in grp78 Protein—To investigate whether the elevation of grp78 mRNA level leads to an increase in GRP78 protein, we labeled Alexander and Alex-PC cells in vivo with [35S]methionine for 16 h. Lanes 1 and 4, untreated cells; lanes 2 and 5, cells treated with 5 µg/ml BFA for 16 h before harvest; lanes 3 and 6, cells treated with 7 µM A23187 for 16 h before harvest. The locations of GRP78 and protein X are indicated by arrows.

456 bp upstream of the transcription start site of the rat grp78 gene promoter (Chang et al., 1987). To test whether BFA also regulates grp78 through a similar mechanism, we transfected grp78/CAT fusion constructs into Alex-PC cells and measured their relative CAT activities under induced and noninduced conditions.

To our surprise, the same concentration of BFA that induced a 12-fold increase in grp78 mRNA levels failed to transactivate the grp78 promoter contained within (−456)CAT (Fig. 3A). Since this plasmid contains all the important regulatory elements of grp78 promoter for other stress inductions, BFA apparently utilizes a different mechanism to induce the grp78 mRNA levels. Similarly, two other grp78/CAT fusion constructs, Δ129/90 and LS90 were also not inducible by BFA (Fig. 3A). These two constructs contain deleted or mutated sequences shown to be important for the induction of grp78 by other stress conditions (Wooden et al., 1991).

To test the possibility that BFA may act through the grp78 promoter at a region further upstream of −456, we transfected into Alex-PC cells (−1290)CAT that contains the grp78 promoter up to 1290 bp. The relative CAT activities from this study are shown in Fig. 3B. As in the case of the −456 deletion, the grp78 promoter contained within this fusion gene was not affected by BFA treatment. In the case of A23187 treatment, we detected about a 2-fold increase for the −1290 and −456 CAT plasmids. These results, taken together, suggested that in Alex-PC cells BFA cannot transactivate the grp78 promoter in transient transfection assays.

BFA Does Not Increase the Rate of grp78 Transcription in Alex-PC Cells—To determine whether the endogenous grp78 gene in the Alex-PC cells is affected by BFA at the transcriptional level, we measured the transcriptional rates of grp78 in the Alex-PC cells treated with 5 µg/ml of BFA. For compari-

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*E. S. Liu and A. S. Lee, unpublished results.

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**Fig. 2. Analysis of in vivo labeled protein by SDS-polyacrylamide electrophoresis.** Alexander (Alex) cells (lanes 1–3) and Alex-PC cells (lanes 4–6) were labeled with 20 µCi/ml of [35S]methionine for 16 h. Lanes 1 and 4, untreated cells; lanes 2 and 5, cells treated with 5 µg/ml BFA for 16 h before harvest; lanes 3 and 6, cells treated with 7 µM A23187 for 16 h before harvest.

**Fig. 3. Promoter activities of grp78/CAT fusion constructs in Alex-PC cells.** A, Alex-PC cells were transiently transfected with 10 µg each of the grp78/CAT fusion constructs (−456, Δ129/90, and LS90) as indicated at the top. Protein extracts from control and BFA-treated (5 µg/ml for 16 h) cells were assayed for CAT activities. The positions of chloramphenicol (CM) and its acetylated forms (3Ac and 1Ac) are indicated. B, the same cells were transiently transfected with either (−1290)CAT or (−456)CAT. Protein extracts from control, BFA-, or A23187-treated cells were assayed for CAT activities.
son, we also measured the transcription rates of grp94 and actin by both BFA and A23187; the latter has been previously determined to increase the transcriptional rate of the grp genes in a variety of cell lines (Resendez et al., 1985; Day and Lee 1989). Nuclei were obtained from cells treated with BFA or A23187 and in vitro labeled with [\(\alpha^{32}\)]PUTP. Labeled nuclear RNA were hybridized with grp78, grp94, and actin DNA bound to nitrocellulose filters. The autoradiograms as shown in Fig. 4A were quantitated by densitometry. Although A23187 increased the transcriptional rates of grp78 and grp94 genes by about 4.0- and 2.7-fold, respectively (Fig. 4B), BFA did not increase the transcriptional rate of these two genes. These results were in agreement with the lack of induction at the promoter level (Fig. 3) and further supported the implication that BFA causes accumulation of grp78 transcripts by a posttranscriptional mechanism.

**Cell Line-specific Regulation of grp78 by BFA**—To determine whether the observed posttranscriptional regulation of grp78 by BFA is a general property of the drug in mammalian cells, we tested the BFA response in two other cell lines. One is the parental Alexander cell line (PLC/PRF/5), and the other is the hamster lung fibroblast cell line K12. Since the Alex-PC cell line contains the complete HBV genome and express only the HBV envelope protein. To test whether BFA can transactivate the grp78 promoter in these two cell lines, we transfected the grp78/CAT fusion constructs into these cells and measured the CAT activities. We found that the grp78 promoter in the Alexander cell line, as in the case of Alex-PC cells (Fig. 5A), was nonresponsive to BFA but responsive to A23187. Although detectable, the fold induction by A23187 in these cells is lower than that in other cell lines (Resendez et al., 1988). These results suggested to us that in this human hepatoma cell line, irrespective of the presence or absence of a complete HBV genome, the regulation of grp78 at the transcriptional level is subdued and the increase in grp78 mRNA by BFA occurs primarily through posttranscriptional regulatory events. However, in the hamster fibroblast K12 cell line that we have extensively characterized and in which we demonstrated that the grp78 gene can be regulated at the transcriptional level by A23187 and a variety of other stress conditions, we observed that BFA and A23187 were able to induce the grp78 promoter by about 4- and 5-fold, respectively (Fig. 5B). At the steady state mRNA level, BFA induced grp78 transcript level by about 20-fold in K12 cells. These results implied that both transcriptional and posttranscriptional mechanisms could be utilized by BFA to regulate the expression of grp78 in mammalian cells. Depending on the cell types, one mechanism can be preferred over the other. Nonetheless, an overall increase in grp78 mRNA level is achieved.

**Localization of the BFA-responsive Element in the grp78 Promoter**—To delineate further where the signal of BFA stress acts on the grp78 promoter in K12 cells, we transfected into K12 cells several grp78/CAT fusion constructs and compared the CAT activities from control and BFA-treated cells. The plasmid (–456)CAT, as previously demonstrated to be inducible by BFA, was used as the wild type control. Other test constructs included the (–169)CAT, (–130)CAT, and LS90 (Fig. 6A). These test constructs were used because we have shown in previous studies that, in the rat grp78 promoter, the region between –169 and –130 contains a grp core regulatory element responsible for stress induction (Resendez et
K12 cells under normal and BFA-induced conditions.

and the site for transcriptional initiation regulatory elements, a conserved domain among endpoints for the 5' promoter deletion mutants are indicated. The indicated of (-456)CAT, (-169)CAT, (-130)CAT, or LS90. Protein extracts were prepared from untreated by BFA to about 4-fold. Deletion to -169 showed similar for CAT activities as described in the legend for Fig. 3.

al., (CORE), a proximal CCAAT motif at -95 motif responsible for mediating the regulatory functions of nonetheless, grp78 promoter containing C1 alone is not sufficient for grp78 expression. The CAT assay results are shown in Fig. 6A. As expected, the wild type construct was induced by BFA to about 4-fold. Deletion to -169 showed similar fold induction, suggesting that the region between -456 and -170 was not needed for BFA induction. Further 5' deletion down to -130 dropped the BFA inducibility by 50%, suggesting that the conserved element between -169 and -130 was partially responsible for BFA induction. The linker-scanning mutation LS90 between -99 and -90, which maintains basal level expression of the grp78 promoter, completely blocked the induction by BFA, demonstrating the C1 region was essential for mediating the induction response by BFA. Thus, the same region required for stress induction of grp78 was utilized for BFA induction in K12 cells.

DISCUSSION

GRP78 is an integral component of the ER function postulated to facilitate the proper assembly and transport of membrane-bound and secretory proteins. GRP78 has been shown to bind transiently to nascent polypeptides and stably to malfolded and underglycosylated proteins (Lee, 1987; Hendershot et al., 1988; Kozutsumi et al., 1988). Our combined results of RNA and protein analysis showed that in response to stress caused by BFA, GRP78 and a 50,000-dalton protein are specifically induced in mammalian cells. This provides the first example that BFA, a potent drug now commonly used to block ER to Golgi transport, can act as a specific regulator of gene expression. One plausible explanation on how overproduction of GRP78 can relieve BFA stress is by binding to the proteins that are blocked from transport. Perhaps the binding of GRP78 to these proteins within the ER can prevent their degradation by proteinases while their transit is blocked in the ER. Once the cell is relieved from stress, these proteins can quickly dissociate themselves from GRP78, refold into proper conformation and are ready for immediate transport to the Golgi apparatus.

Our results suggested that BFA, which causes disruption to the ER functions, can specifically regulate the expression of grp78 by two different mechanisms: a transcriptional regulation and a posttranscriptional control (Fig. 7). In the human Alexander hepatoma cells, we observed that the accumulation of grp78 transcripts by BFA was not due to transcriptional control, strongly suggesting that a posttranscriptional regulation event occurred. By transfection with grp78/CAT fusion constructs into K12 cells, we could detect induced expression of the exogenous reporter gene, implying that BFA can regulate the grp78 promoter in this cell line. Why would the expression of grp78 be regulated at multiple and different levels in different cell lines? Possibly, the functions of grp78 are so vital to cell survival (Li and Lee, 1991) that regulation at multiple steps would ensure its induction when cells are subjected to severe ER stress. If the first step at the transcriptional level is limited, as in the case with the human Alexander hepatoma cell line, regulation at the posttranscriptional event could compensate for the inability to induce grp78 transcriptionally. With these dual controls, in both cell lines, the levels of grp78 transcript and protein are rapidly elevated by BFA.

The BFA response in Alex-PC cells was reduced to half by pretreatment with cycloheximide. It is possible that cycloheximide blocks the synthesis of a labile protein that specifically regulates grp78 transcript levels. Alternatively, cycloheximide reduces the overall protein synthesis, since it blocks polypeptide elongation in the ribosomes. With reduced protein trafficking through the ER, the signal for increased GRP synthesis may also be reduced correspondingly. A combination of these pleiotropic effects caused by cycloheximide may contribute to the overall reduction in the BFA response. There are several interesting features in the untranslated regions (UTRs) of the grp78 transcripts that may serve a role in the posttranscriptional regulatory event. In contrast to most genes, the 5'- and 3'-UTRs of the grp78 gene are highly conserved among human, rat, and hamster (Ting et al., 1987). The 5'-UTR is relatively long and shares about 70% sequence identity among these species. The conserved region contains an imperfect invert-repeat sequence that is capable of forming

Fig. 7. A model outlining how BFA, a reagent that disrupts ER to Golgi protein transport, enhances the expression of GRP78, a binding protein in the ER, through transcriptional and/or posttranscriptional mechanisms.
stable dyad structure. The sequence identity of the 3'-UTR among rat and hamster is even more striking. Although the size of the 3'-UTR differs in each species, close to 95% sequence homology was observed throughout the UTRs (Ting et al., 1987). The functional significance of the conserved 5'- and 3'-UTRs is not clear. However, it is tempting to speculate that the UTRs may confer preferential stability of the mRNA in the ER during translation. Recently, it has been shown that the grp78 message is unique in that it can be translated by an internal ribosome-binding mechanism at a time when the general cap-dependent translation is blocked (Macejak and Sarnow, 1991).

Although it has been assumed that the induction of grp78 in the ER is a consequence of sequestering grp78 into complexes with other polypeptides that serve as part of the signaling mechanism of grp78 induction at the transcriptional level (Lee et al., 1987; Kozutsumi et al., 1988; Hightower, 1991), little is known about the mechanisms involved. Consistent with this hypothesis, underglycosylated proteins resulted from glycosylation block, malformed proteins, and abnormal proteins have all been shown directly to complex stably with GRP78 and induce grp78 transcription. In addition to the grp core sequence important for grp78 expression (Resendez et al., 1988; Li and Lee, 1991; Liu and Lee, 1991), we have recently located a 10-base pair region containing a proximal CCAAT motif between -99 to -90 of the grp78 promoter to be crucial for mediating the responses by BFA, a drug specific for blocking ER to Golgi transport, can also act as a positive regulator of the grp78 expression and that the stress signal is mediated through the same 10-bp region. Since the signal conferred by BFA acts through the same region in the promoter, it is likely to follow the same signal transduction pathway as the malformed proteins blocked in ER.

Our work established that BFA can be a regulator of gene expression at the transcriptional and posttranscriptional level. Since our analysis has only focused on grp78, it would be interesting to identify other specific genes, including the gene encoding the protein X, that are regulated by this drug. Other candidate genes may also exist but are nondetectable at the protein level. Since BFA blocks protein transport, the genes regulated under BFA control may encode proteins that are important for protein trafficking and their stabilization.

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REFERENCES