*Acta Physiologica Hungarica, Volume 83 (4), pp. 333–342 (1995)*

# Changes of the 78 kDa glucose-regulated protein (grp78) in livers of diabetic rats#

Ildikó **Szántó**, P. **Gergely**, Z. **Marcsek***, T. **Bányász****, J. **Somogyi**, P. **Csermely**

Institute of Medical Chemistry I, Semmelweis University of Medicine, Budapest; * Department of Cell Biology, Joint Research Organization of the Hungarian Academy of Sciences, Budapest, and ** Institute of Physiology, University Medical School of Debrecen, Hungary

Received March 29, 1995
Accepted May 7, 1995

The 78 kDa glucose-regulated protein (grp78) is an abundant member of the 70 kDa molecular chaperone family in the lumen of the endoplasmic reticulum participating in the quality control of secretory proteins. In the present paper we have analysed the synthesis and level of grp78 in livers of control, streptozotocin-diabetic, and the spontaneously diabetic Zucker rats. The level of grp78 mRNA significantly decreased in streptozotocin-diabetic rats. The effect was reversed by insulin treatment. In case of Zucker rats we did not detect any significant change in grp78 mRNA, grp78 protein level showed opposite changes being essentially unchanged in streptozotocin-diabetes and significantly reduced in Zucker rats. Autoradiograms of Ca-dependent phosphorylation of postmitochondrial supernatants of control and streptozotocin-diabetic livers indicated no significant changes in the 70 kDa region. Decrease in the availability of grp78 may participate in the attenuation of hepatic protein secretion in diabetes.

**Keywords:** diabetes, glucose regulated proteins, chaperones, heat shock proteins, insulin, protein phosphorylation

# This paper is dedicated to the memory of Professor Tibor Kovács (1929–1994), who actively participated in the initiation of this project, and whose talent and spirit helped a lot to those who were privileged to know him.

Correspondence should be addressed to
Péter **Csermely**
Institute of Medical Chemistry, Semmelweis University of Medicine
H–1444 Budapest, P.O. Box 260, Hungary
Telephone/Fax: 361-266-6550
email: csermely@puskin.sote.hu

0231-424X / 95 / $5.00

The 78 kDa glucose-regulated protein (grp78, BiP)* is an abundant member of the 70 kDa molecular chaperone family in the lumen of the endoplasmic reticulum. grp78 synthesis is induced after either glucose starvation or treatment with calcium ionophores. The protein was shown to bind the immunoglobulin heavy chain and its possible involvement in the quality control of secretory proteins was also suggested [13–16, 19, 25, 44].

Diminished extracellular glucose level induces the synthesis of numerous glucose-regulated proteins such as grp78, grp94 and GLUT–1 [13, 19, 44, 45]. Parfett et al. [27] reported a transient increase of grp78 mRNA in NOD mice. GLUT–1 mRNA as well as GLUT–1 protein levels were also reported to increase in diabetic animal models [39, 40]. In spite of the intimate link between changes in extracellular glucose level and the regulation of the synthesis of glucose regulated proteins, our knowledge of their function in diabetes is rather limited. As an initial attempt to characterize the effect of diabetes on molecular chaperones we analyzed the mRNA, protein levels of grp78, as well as its autophosphorylation in the liver of control and diabetic rats. Our results suggest that a decreased availability of grp78 may participate in the impaired protein secretion of diabetic livers.

## Materials and Methods

### *Chemicals*

The chemicals used for polyacrylamide gel electrophoresis were from Bio-Rad (Richmond, CA). Streptozotocin was purchased from Boehringer Mannheim (Germany). Anti-KSEKDEL and anti-CTGEEDTSEKDEL [38] antibodies (recognizing the endoplasmic reticulum retention signal present in grp78) were obtained from Stressgen (Victoria, B.C., Canada) and from Affinity BioReagents (Neshanic Station, NJ), respectively. The grp78 (clone p3C5) and β-actin (clone RβA–1) cDNA probes were generous gifts of drs. Amy S. Lee [20] and Laurence H. Kedes (both from the Univ. of Southern California, Los Angeles), respectively. Chicken blood DNA, SalI was a GIBCO BRL (Berlin, Germany) product. The Multiprime random oligonucleotide priming system, ECL immunodetection kit, [alpha-$^{32}$P]-dCTP (110 TBq/mmol) and [gamma-$^{32}$P]-ATP (185 TBq/mmol) were from Amersham Life Science (Braunschweig, Germany). All the other chemicals used were from Sigma Chemicals Co. (St. Louis, MO).

### *Animals*

Eighteen weeks old (200–220 g) male Sprague–Dawley rats (LATI, Gödöllő, Hungary) were treated with 50 mg/kg i.p. streptozotocin under ether anaesthesia. A group of six diabetic rats was sacrificed 2 weeks, another 4 weeks after streptozotocin treatment, respectively. Six animals received daily insulin injections of an individual dose to normalize their blood glucose level for two weeks prior sacrification. The weight and blood glucose levels of the animals were monitored and showed characteristic changes of

* The abbreviations used are: dnaK, *Escherichia Coli* 70 kDa heat shock protein homologue; GLUT–1, glucose transporter; grp78, 78 kDa glucose-regulated protein (BiP); Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulphate.

diabetic and "reversed" state, respectively [43]. Twelve weeks old spontaneously diabetic male Zucker (fa/fa) and lean (fa/0) rats (IFFA-CREDO, l'Albresle, France) were also examined.

*Measurement of grp78 mRNA levels*

The recombinant pBR322 plasmid (clone p3C5) containing the grp78 specific 2.5 kbase long insert [20] was opened by SalI digestion for 60 minutes at 37 °C. The opened plasmid was labelled with [alpha-$^{32}$P]-dCTP by the random oligonucleotide priming system (Multiprime) of Amersham overnight at room temperature. The radiolabelled probe was separated on a Sephadex G–50 column. Total cellular RNA was isolated from rat livers by the method of Chirgwin et al. [7]. RNA was quantitated by measuring its absorbance at 260 nm. 20 $\mu$g of RNA was subjected to electrophoresis on a 1.2% agarose-formaldehyde gel and the gel was blotted onto Amersham Hybond-N nylon filters by capillary transfer. RNA blots were stabilized by heating in vacuum at 80 °C for 2 hours and by UV light transillumination for 15 min. The blots were prehybridized at 42 °C for 60 min in a mixture containing 5 × SSC (0.75 M NaCl, 75 mM sodium-citrate, pH 7.0), 5 × Denhardt solution (1 mg/ml each of bovine serum albumin, Ficoll 400 and poly-vinyl-pirrolidone), 50% deionized formamide and 1% SDS. After the addition of approximately $10^6$ cpm of alpha-$^{32}$P-dCTP labelled grp78 specific cDNA probe and 200 $\mu$l/ml chicken blood DNA, hybridization was performed at 42 °C for 16 h. The filters were washed for 2 × 10 min at room temperature with 2 × SSC and 0.1% SDS, and for 2 × 5 min at 65 °C with 0.2 × SSC and 0.1% SDS. mRNA levels were quantitated by autoradiography and densitometry on an LKB-laserdensitometer. Results were normalized to the amount of $\beta$-actin mRNA. Full length rat $\beta$-actin cDNA probe was excised by BglI digestion from clone pR$\beta$A-1 and was used for hybridization utilizing a procedure identical to that of grp78.

*Quantitation of grp78 and Ca-dependent phosphorylation*

Livers of control and diabetic rats were homogenized in two volumes of a buffer containing 20 mM Hepes, pH 7.4, 30 mM KCl, 2 mM PMSF, 100 $\mu$g/ml aprotinin, 2 mM dithio-threitol and 1 mM EDTA. Postmitochondrial supernatants were obtained by sequential centrifugation of the samples at 2,000 × g, 10 min, 4 °C and 15,000 × g, 20 min, 4 °C, respectively. Protein concentration was determined by the Bradford method [3] using bovine gamma-globuline as a standard. 200 $\mu$g of proteins were subjected to SDS-PAGE [18] and transferred to nitrocellulose filters [42]. grp78 levels were analysed by probing the Western-blots with anti-grp 78 antibodies [38] recognizing the endoplasmic reticulum retention signal of grp78. Immunocomplexes with peroxidase-conjugated anti-rabbit antibodies were quantitated using the ECL immunodetection kit and by densitometry with an LKB laser-densitometer.

In phosphorylation assays 200 $\mu$g protein of hepatic postmitochondrial supernatant was incubated in 50 mM Hepes buffer, pH 7.4, at 37 °C for 20 minutes in the presence of 5 mM $CaCl_2$ and 400 kBq of 0.1 mM [gamma-$^{32}$P]-ATP. Reaction was stopped with boiling for 5 minutes in Laemmli sample buffer and samples were subjected to SDS-PAGE [18] and autoradiography.

*Statistics*

Data represent means ± SDs. Levels of significance were determined using the Student's *t*-test.

## Results

### *Changes of grp78 mRNA in diabetic rats*

Rat hepatic grp78 mRNA levels were significantly decreased both 2 weeks and 4 weeks after streptozotocin injection (to 47 and 38% of control level, respectively). Normalization of blood glucose level by 2 weeks insulin treatment completely reversed the effects observed. In contrast to the results in streptozotocin-diabetes, the spontaneously diabetic Zucker (fa/fa) rats did not show any significant decrease of hepatic grp78 mRNA (Fig. 1).

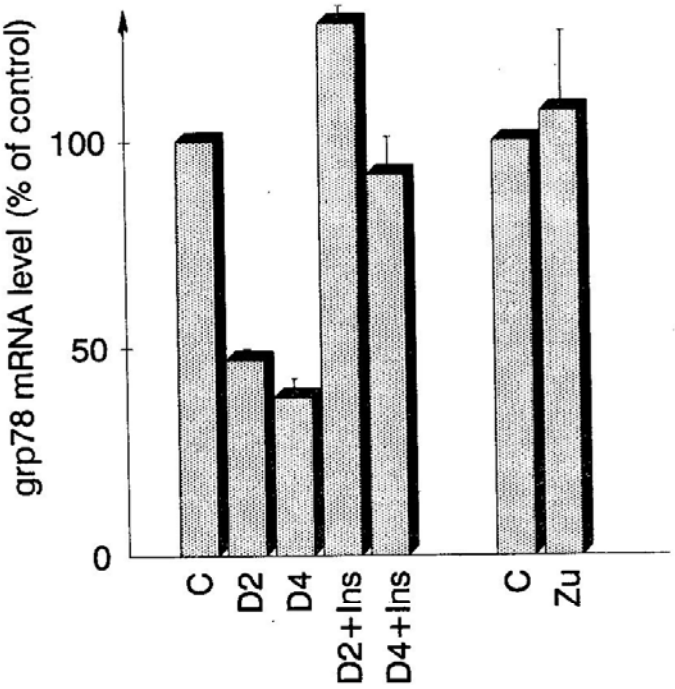


Fig. 1. Changes of grp78 mRNA in diabetic rats Hepatic grp78 mRNA levels were determined as described in Materials and Methods. Experimental groups: C, control; D2, two weeks of streptozotocin-diabetes; D4, four weeks of streptozotocin-diabetes; D2+Ins, two weeks of streptozotocin-diabetes + 2 weeks of insulin treatment; D4+Ins, four weeks of streptozotocin-diabetes + 2 weeks of insulin treatment; Zu, spontaneously diabetic Zucker (fa/fa) rats. Data are means ± SDs of the densitometric analysis of three autoradiograms of mRNA-s from two animals per each group compared to their respective controls

### *Changes of grp78 level in diabetic rats*

Levels of hepatic immunodetectable grp78 were essentially unchanged in streptozotocin-diabetic rats and significantly ($p < 0.025$) diminished in spontaneously diabetic Zucker (fa/fa) rats (Fig. 2). In some samples from livers of Zucker rats more than one anti-KSEKDEL immunopositive lanes could be detected in the 70–80 kDa region, which may reflect an increased posttranslational modification (limited

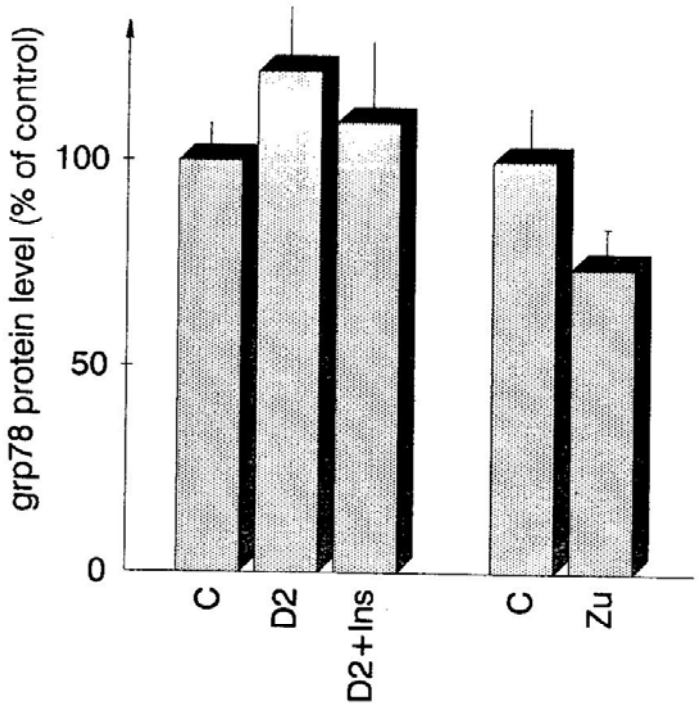


Fig. 2. Changes of grp78 level in diabetic rats grp78 levels were determined by subjecting 200 μg protein or rat liver postmitochondrial supernatant from control and diabetic animals to SDS-PAGE and Western-blot analysis as described in Materials and Methods. Western blots were probed with an anti-KSEKDEL antibody recognizing grp78. Experimental groups: C, control; D2, two weeks of streptozotocin-diabetes; D2+Ins, two weeks of streptozotocin-diabetes + 2 weeks of insulin treatment; Zu, spontaneously diabetic Zucker (fa/fa) rats. Data are means ± SDs of the densitometric analysis of three Western blots from five animals per each group

proteolysis?) of grp78 in diabetes. Similar grp78 levels could be detected using two antipeptide antibodies (anti-KSEKDEL and anti-CTGEEDTSEKDEL) against the C-terminus of grp78 (data not shown). In Zucker diabetic rats a 57 kDa anti-KSEKDEL immunopositive lane (detecting possibly protein-disulphide isomerase) was also diminished compared to the respective controls (to 31 ± 18% $p < 0.005$). The observed decrease noes not reflect an overall attenuation of protein levels of all molecular chaperones, since the levels of immunodetectable 27, 70 and 90 kDa heat shock proteins (hsp27, hsc70 and hsp90) did not change significantly in diabetic liver (data not shown).

### *Ca-dependent phosphorylation of postmitochondrial supernatant in control and diabetic rats*

Ca-ATP-dependent phosphorylation is a sensitive tool to discriminate between the general (Mg-ATP-dependent) phosphorylation of proteins and the Ca-ATP-dependent (auto)phosphorylation of various molecular chaperones including hsp90 [10], grp94 [11] and grp78 [22]. Phosphorylation of postmitochondrial supernatants

from livers of control, streptozotocin-diabetic and insulin-treated diabetic rats in the presence of Ca-ATP did not reveal any major differences in the 70–80 kDa region (data not shown) which indicates no major changes in the autophosphorylation rate of hepatic grp78 in streptozotocin-diabetes.

## Discussion

Exposure of cells to glucose starvation and calcium ionophores stimulates the synthesis of a specific set of proteins including grp78 [19]. However, the situation seems to be more complex, since dietary glucose restriction leads to a decrease in the mRNA of hepatic grp78 [36] and Parfett et al. [27] reported a transient increase of grp78 mRNA in spontaneously diabetic NOD mice. Our results show a significant decrease in grp78 mRNA in rat livers after streptozotocin treatment which was reversible after insulin treatment. Two and four week streptozotocin-diabetic rats may better correspond to the overt diabetic NOD mice, where the increase in hepatic grp78 mRNA has been already levelled off [27]. As a further example for the complex regulation of grp-genes in various models of diabetes, in case of Zucker (fa/fa) rats we found no significant change in grp78 mRNA compared to control rats. Heat shock is known to increase the stability of grp78 mRNA [32]. Different changes of grp78 mRNA stability may also contribute to the differences of grp78 mRNA levels in various models of diabetes.

Synthesis of grp78 mRNA is known to be mediated by an ER-resident protein kinase [9, 26]. The fact that protein kinase inhibitors were reported to prevent, and the phosphorprotein phosphatase inhibitor ocadaic acid promoted the induction of glucose regulated proteins [29, 30] also indicates the involvement of protein phosphorylation in the process. Protein kinases, and phosphatases such as the insulin receptor tyrosine kinase itself, are differently affected in various models of diabetes [17] which may indicate the involvement of a phosphorylation-dependent signalling step in changes of grp78 induction in diabetes.

grp94 mRNA was also decreased in livers of streptozotocin-diabetic rats.* The similar regulation of grp78 and grp94 mRNA in diabetes may reflect the high similarity in their promoter region [19, 23, 37].

grp78 protein levels showed opposite changes in spontaneously diabetic Zucker (fa/fa) and streptozotocin-diabetic rats than the corresponding mRNA levels. Decrease of grp78 in spontaneously diabetic Zucker (fa/fa) rats cannot be explained by a transcriptional defect, since the corresponding mRNA level was unchanged. Both increase [4] and decrease [35] of hepatic translation rates of various proteins have been reported in diabetes. Thus the observed changes may result from the impaired translation of the protein but may also reflect a shortening of the extraordinarily long

*P. Csermely, P. Gergely, I. Szántó, Z. Marcsek, T. Bányász and J. Somogyi, manuscript in preparation.

half-life (> 48 hours [14]) of grp78 in diabetes. Similar findings were reported in the case of hepatic 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase where the diabetic enzyme level has been decreased parallel with an unchanged mRNA level [8]. An increased susceptibility for proteolytic degradation may reflect a defect in the structure of the protein and may account for the decrease of grp78 protein levels in spontaneously diabetic Zucker (fa/fa) rats all the more, since grp78 mRNA uses both cap-dependent scanning and internal ribosome binding mechanisms for translation [24, 31] which makes a translational defect in this case less likely. On the contrary, the extraordinarily stable translational mechanism of grp78 (together with the possible lack of accelerated proteolysis) may account for the preservation of grp78 protein levels in spite of reduced mRNA in streptozotocin-induced diabetes.

An increase of malfolded proteins in the lumen of endoplasmic reticulum (which is supposed to occur in diabetes) should provoke an increase in the synthesis and level of glucose regulated proteins. However, grp synthesis - and glycosylation - may itself be influenced by diabetes, worsening the adaptation of hepatocytes to the presumably increased demand of the "quality control" of secretory proteins.

grp78 is known to phosphorylate itself [22]. The autophosphorylation of grp78 plays a role in the dissociation of grp78 from other proteins [5, 12, 41] in the analogy of similar effects on hsp60 [33] and hsp90 [21].* On the other hand, heat-induced phosphorylation of the hsp70 homologue, dnaK is reported to increase its binding to polypeptides [34]. Phosphorylation may impair the activity of grp78 [37]. In spite of these intimate links between the phosphorylation and activity of grp78, we did not observe any change in Ca-dependent (auto)phosphorylation in the 70 kDa region. This might reflect a true lack of detectable grp78 phosphorylation but fluctuations in overall grp78 levels may also obscure some minor changes in the phosphorylation of the protein in diabetes.

We have no direct information on the possible alterations of hepatic protein secretion in diabetes. Though the secretion of numerous proteins, such as albumin [28] and the VLDL constituent apolipoproteins B and E [35] are markedly decreased, these effects are largely attributed to defects of mRNA synthesis and translation, respectively. Glycoproteins seem to be less sensitive to diabetes-induced attenuation of protein secretion [2]. Studies on pancreatic protein secretion of streptozotocin-induced diabetic rats indicate an inhibition of amylase secretion and a higher amount of immunodetectable pancreatic lipase along the secretory pathway [1, 6].

These findings may indicate an increased retention of abnormal proteins by the endoplasmic reticulum. Reduced availability of grp78 may contribute to impaired functioning of the "quality control" mechanisms of the endoplasmic reticulum resulting in the accumulation of abnormal proteins in the luminal compartimentum.

* M.S.Z. Kellermayer and P. Csermely, BBRC, **211**, 166-174 (1995).

## Acknowledgements

We would like to express our thanks to drs Amy S. Lee and Laurence H. Kedes (both from the Univ. of Southern California, Los Angeles), for the cDNA-s of grp78 and β-actin, respectively. The authors are thankful to Katalin Mihály (Institute Biochemistry I, Semmelweis Univ. Medical School) for her excellent technical assistance and for dr. Agnes Turi (Institute Biochemistry I, Semmelweis University of Medicine) and Zoltán Bori for their advice and help in working with grp cDNA-s. We would like to thank to Prof. Jürgen Eckel (Diabetes Forschungsinstitut, Düsseldorf, Germany) for the livers of control and Zucker rats. This work was supported in part by scientific grants from the Hungarian Academy of Sciences (OTKA-T5534, T12962, T17720), from the Hungarian Ministry of Social Welfare (ETT-681/93, 746/93), from COST Human Exchange and Mobility programs ERBCIPDCT 930188/940604 and from the AB-Aegon Ins. Co. P.C. is an International research Scholar of the Howard Hughes Medical Institute (# 75195-541701).

## REFERENCES

1. Bendayan, M. and Levy, E.: Immunocytochemical and biochemical evaluation of pancreatic lipase in acinar cells of control and streptozotocin-induced diabetic rats. Pancreas, **3**, 269–273 (1988).
2. Berry, E. M., Ziv, E. and Bar On, H.: Protein and glycoprotein synthesis and secretion by the diabetic liver. Diabetologia, **19**, 535–540 (1980).
3. Bradford, M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. Anal. Biochem., **72**, 248–254 (1976).
4. Brady, P. S. and Brady, L. J.: Hepatic carnitine palmitoyltransferase turnover and translation rates in fed, starved, streptozotocin-diabetic and diethylhexyl phthalate-treated rats. Biochem. J., **246**, 641–649 (1987).
5. Carlino, A., Toledo, H., Skaleris, D., DeLisio, R., Weissbach, H. and Brot, N.: Interactions of liver grp78 and *Escherichia coli* recombinant grp78 with ATP: multiple species and disaggregation. Proc. Natl. Acad. Sci. USA, **89**, 2081–2085 (1992).
6. Chey, W. Y., Shay, H. and Shuman, C. R.: External pancreatic secretion in diabetes mellitus. Ann. of Internal. Med., **59**, 812–821 (1963).
7. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. and Rutter, W. J.: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry, **18**, 5294–5299 (1979).
8. Colosia, A. D., Marker, A. J., Lange, A. J., el Maghrabi, M. R., Granner, D. K., Tauler, A., Pilkis, J. and Pilkis, S. J.: Induction of rat liver 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase mRNA by refeeding and insulin. J. Biol. Chem., **263**, 18669–18677 (1988).
9. Cox, J. S., Shamu, C. E. and Walter, P.: Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. Cell, **73**, 1197–1206 (1993).
10. Csermely, P. and Kahn, C. R.: The 90 kDa heat shock protein (hsp-90) possesses an ATP-binding site and autophosphorylating activity. J. Biol. Chem., **266**, 4943–4950 (1991).
11. Csermely, P., Miyata, Y., Schnaider, T. and Yahara, I.: Autophosphorylation of grp94 (endoplasmin). J. Biol. Chem., **270**, 6381–6388 (1995).
12. Dorner, A. J., Wasley, L. C. and Kaufman, R. J.: Protein dissociation from grp78 and secretion are blocked by depletion of cellular ATP levels. Proc. Natl. Acad. Sci. USA, **87**, 7429–7432 (1990).
13. Gething, M. J. and Shambrook, J.: Protein folding in the cell. Nature, **355**, 33–45 (1992).
14. Haas, I. G.: BiP (grp78), an essential hsp70 resident protein in the endoplasmic rehculum. Experientia, **50**, 1012–1020 (1994).

15. Hammond, C. and Helenius, A.: Folding of VSV G protein: sequential interaction with BiP and calnexin. Science, **266**, 456–458 (1994).
16. Hendrick, J. P. and Hartl, F-U.: Molecular chaperone functions of heat shock proteins. Ann. Rev. Biochem., **62**, 349–384 (1993).
17. Kahn, C. R., White, M. F., Shoelson, S. E., Backer, J. M., Araki, E., Cheatham, B., Siddle, K., Sun, X., Wilden, P. A., Yamada, K., Csermely, P., Folli, F., Goldstein, B. J., Huertas, P., Rothenberg, P. L. and Saad, M. J. A.: The insulin receptor and its substrate: molecular determinants of early events in insulin action. Recent Prog. in Horm. Res., **48**, 291–339 (1993).
18. Laemmli, U. K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, **227**, 680–685 (1970).
19. Lee, A. S.: Coordinated regulation of a set of genes by glucose and calcium ionophores in mammalian cells. Trends in Biochem. Sci., **12**, 20–23 (1987).
20. Lee, A. S., Delegeane, A. and Scharff, D.: Highly conserved glucose-regulated protein in hamster and chicken cells: preliminary characterization of its cDNA clone. Proc. Natl. Acad. Sci. USA, **78**, 4922–4925 (1981).
21. Legagneux, V., Morange, M. and Bensaude, O.: Heat shock increases turnover of 90 kDa heat shock protein phosphate groups in HeLa cells. FEBS Letters, **291**, 359–362 (1991).
22. Leustek, T., Toledo, H., Brot, N. and Weissbach, H.: Calcium-dependent autophosphorylation of the glucose-regulated protein, grp78. Arch. Biochem. Biophys., **289**, 256–261 (1991).
23. Liu, E. S. and Lee, A. S.: Common sets of nuclear factors bind to the conserved promoter sequence motif of two coordinately regulated ER protein genes, grp78 and grp94. Nucl. Acids Res., **19**, 5425–5431 (1991).
24. Macejak, D. G. and Sarnow, P.: Internal initiation of translation mediated by the 5'leader of a cellular mRNA. Nature, **353**, 90–94 (1991).
25. Melnick, J., Aviel, S. and Argon, Y.: The endoplasmic reticulum stress protein grp94, in addition to BiP, associates with unassembled immunoglobulin chains. J. Biol. Chem., **267**, 21303–21306 (1992).
26. Mori, K., Ma, W., Gething, M. J. and Shambrook, J.: A transmembrane protein with a cdc2+/CDC28-related kinase activity is required for signalling from the ER to the nucleus. Cell, **74**, 743–756 (1993).
27. Parfett, C. L. J., Brudzynski, C. and Stiller, C.: Enhanced accumulation of mRNA for 78-kilodalton glucose-regulated protein (grp78) in tissues of nonobese diabetic mice. Biochem. Cell Biol., **68**, 1428–1432 (1990).
28. Peavy, D. E., Taylor, J. M. and Jefferson, L. S.: Correlation of albumin production rates and albumin mRNA levels in livers of normal, diabetic, and insulin-treated diabetic rats. Proc. Natl. Acad. Sci. USA, **75**, 5879–5883 (1978).
29. Price, B. D., Mannheim, R. L. A. and Calderwood, S. K.: Brefeldin A, thapsigargin and AlF4- stimulate the accumulation of GRP78 mRNA in a cycloheximide dependent manner, whilst induction by hypoxia is independent of protein synthesis. J. Cell Physiol., **152**, 545–552 (1992).
30. Resendez, E. J., Ting, J., Kim, K. S., Wooden, S. K. and Lee, A. S.: Calcium ionophore A23187 as a regulator of gene expression in mammalian cells. J. Cell Biol., **103**, 2145–2152 (1986).
31. Sarnow, P.: Translation of glucose-regulated protein 78/immunoglobulin heavy-chain binding protein mRNA is increased in poliovirus-infected cells at a time when cap-dependent translation of cellular mRNAs is inhibited. Proc. Natl. Acad. Sci. USA, **86**, 5795–5799 (1989).
32. Schiaffonati, L., Tacchini, L. and Pappalardo, C.: Heat shock response in the liver: expression and regulation of the HSP70 gene family and early response genes after *in vivo* hyperthermia. Hepatology, **20**, 975–983 (1994).
33. Sherman, M. Y. and Goldberg, A. L.: Heat shock in *Escherichia coli* alters the protein-binding properties of the chaperonin groEL by inducing its phosphorylation. Nature, **357**, 167–169 (1992).

34. Sherman, M. Y. and Goldberg, A. L.: Heat shock of *Escherichia coli* increases binding of dnaK (the hsp70 homolog) to polypeptides by promoting its phosphorylation. Proc. Natl. Acad. Sci. USA, **90**, 8648–8652 (1993).
35. Sparks, J. D., Zolfaghari, R., Sparks, C. E., Smith, H. C. and Fisher, E. A.: Impaired hepatic apolipoprotein B and E translation in streptozotocin diabetic rats. J. Clin. Invest., **89**, 1418–1430 (1992).
36. Spindler, S. R., Crew, M. D., Mote, P. L., Grizzle, J. M. and Walford, R. L.: Dietary energy restriction in mice reduces hepatic expression of glucose-regulated protein 78 (BiP) and grp94 mRNA. J. Nutr., **120**, 1412–1417 (1990).
37. Sugawara, S., Takeda, K., Lee, A. and Dennert, G.: Suppression of stress protein grp78 induction in tumor B/C10ME eliminates resistance to cell mediated cytotoxicity. Cancer Res., **53**, 6001–6005 (1993).
38. Takemoto, H., Yoshimori, T., Yamamoto, A., Miyata, Y., Yahara, I., Inoue, K. and Tashiro, Y.: Heavy chain binding protein (BiP/grp78) and endoplasmin are exported from the endoplasmic reticulum in rat exocrine pancreatic cells, similar to protein disulfide-isomerase. Arch. Biochem. Biophys., **296**, 129–136 (1992).
39. Tal, M., Schneider, D. L., Thorens, B. and Lodish, H. F.: Restricted expression of the erythroid/brain glucose transporter isoforms to perivenous hepatocytes in rats. Modulation by glucose. J. Clin. Invest., **86**, 982–992 (1990).
40. Tal., M., Kahn, B. and Lodish, H. F.: Expression of the low Km GLUT-1 glucose transporter is turned on in perivenous hepatocytes of insulin-deficient diabetic rats. Endocrinology, **129**, 1933–1941 (1991).
41. Toledo, H., Carlino, A., Vidal, V., Redfield, B., Nettleton, M. Y., Kochan, J. P., Brot, N. and Weissbach, H.: Dissociation of glucose-regulated protein grp78 and grp78-IgE Fc complexes by ATP. Proc. Natl. Acad. Sci. USA, **90**, 2505–2508 (1993).
42. Towbin, H., Staehelin, T. and Gordon, J.: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**, 4350–4354 (1979).
43. Vér, Á., Csermely, P., Bányász, T., Kovács, T. and Somogyi, J.: Alterations in the properties and isoform ratio of brain $Na^+/K^+$ATPase in streptozotocin diabetic rats. Biochem. Biophys. Acta, **1237**, 143-150 (1995).
44. Welch, W. J.: Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. Physiological Reviews, **72**, 1063–1081 (1992).
45. Wertheimer, E., Sasson, S., Cerasi, E. and Ben-Neriah, Y.: The ubiquitous glucose transporter GLUT-1 belongs to the glucose-regulated protein family of stress-inducible proteins. Proc. Natl. Acad. Sci. USA, **88**, 2525–2529 (1991).