FEBS Letters 403 (1997) 100–104 FEBS 18178

Dexamethasone differently modulates TNF-α- and IL-1β-induced transcription of the hepatic Mn-superoxide dismutase gene

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Received 28 November 1996

Abstract The effects of cytokines, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and the synthetic glucocorticoid dexamethasone on the gene expression of antioxidant enzymes have been investigated in rat hepatocytes in primary culture. First, we observed that the hepatocyte culture process induced a strong but transient induction of manganese superoxide dismutase (Mn-SOD) gene expression, whereas copper-zinc superoxide dismutase, glutathione peroxidase and catalase genes were down-regulated. IL-1β and TNF-α both stimulated specifically Mn-SOD gene expression in a time-dependent manner. TNF-α rapidly induced Mn-SOD gene expression while IL-1β was a strong but slow inducer of this gene. Both cytokines acted at the transcriptional level as shown by nuclear run on assays. Dexamethasone prevented the TNF-α- but not the IL-1β-induced up-regulation of Mn-SOD gene transcription by a mechanism likely to involve the glucocorticoid receptor. Moreover this glucocorticoid did not suppress the TNF-α-induced increase of NF-kB binding activity. These results suggest that IL-1β and TNF-α regulate Mn-SOD gene transcription by different pathways.

Key words: Cytokine; Superoxide dismutase; Glucocorticoid; Hepatocyte; Transcription

1. Introduction

Reactive oxygen species which are generated in the liver during oxidative stress conditions, such as metabolism of toxic compounds and inflammation, have been suggested to produce tissue injury notably by initiating lipid peroxidation. Tissue damage occurs when the production of reactive oxygen species exceeds the capacity for free radical detoxication. In order to protect cells from these damaging effects, the organism possesses antioxidant systems, including enzymatic ones. The main enzymes are superoxide dismutases (SOD) which convert superoxide anion to hydrogen peroxide, catalase (CAT) which breaks down hydrogen peroxide, likewise glutathione peroxidase (GPx) which in addition reduces lipid peroxides to their hydroxylated derivatives. In eukaryotic cells, there are two forms of SOD enzymes, the manganese SOD (Mn-SOD) found in the mitochondrial matrix, and the copper-zinc SOD (CuZn-SOD) which is mainly cytoplasmic [1].

During inflammation, hepatocytes are subjected to a reprogramming of the pattern of gene expression [2]. The factors responsible for this hepatic response to inflammation have been identified and include at least the cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), as well as glucocorticoids. The latter compounds act through different pathways in order to reduce inflammation and cellular damage [3]. Several studies have reported an induction of Mn-SOD by TNF-α [4–7] and IL-1β [5,8] in different cell types. IL-1β was found to induce Mn-SOD gene expression in rat hepatocytes in primary culture and in human hepatoma cells [9,10]. However, the effectiveness of TNF-α seems to be cell-type specific, since it was unable to increase Mn-SOD expression in hepatocytes in primary culture [10,11]. Surprisingly, administration of TNF-α to rats increased Mn-SOD gene expression and activity in liver and prevented lipid peroxidation caused by carbon tetrachloride [12]. These results further put into question a possible role for TNF-α in the induction of Mn-SOD in hepatocytes. The molecular basis for the changes in gene expression mediated by TNF-α and IL-1β is still unclear; however, it has been reported that intracellular reactive oxygen species could be involved in their mechanism of action [13]. The activation of the nuclear transcription factors NF-kB and AP1 has been demonstrated to mediate their effects [14].

In the present study, we examined the effects of TNF-α, IL-1β and dexamethasone on the expression of Mn-SOD, CuZn-SOD, CAT and GPx genes in rat hepatocytes in primary culture. We provide evidence for the induction of Mn-SOD gene transcription by IL-1β and TNF-α through different pathways and we show, for the first time, that dexamethasone suppresses the TNF-α-induced Mn-SOD gene transcription.

2. Materials and methods

2.1. Recombinant cytokines and chemicals

Human recombinant IL-1β and TNF-α were purchased from Genzyme (Cambridge, UK). RU 486 was a gift from Roussel-Uclaf (Romainville, France).

2.2. Cell isolation and culture

Hepatocytes were isolated by perfusion of the liver with a collagenase solution as previously described [15]. Cell viability was estimated by trypan blue exclusion and was found to range between 85 and 90%. Cells were maintained at 37°C in a humidified atmosphere of air/CO_2 (95:5). Four hours after cell seeding, a serum-free medium consisting of 75% minimum essential medium and 25% Medium 199 supplemented with 10 μg bovine insulin/ml, 0.1% bovine serum albumin

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Abbreviations: TNF, tumor necrosis factor; IL, interleukin; Mn-SOD, manganese superoxide dismutase; CuZn-SOD, copper-zinc superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase; NF-kB, nuclear factor kappa B

PII S0014-5793(97)00033-1

and 100 nM dexamethasone was added to the cultures. The medium was changed every day. In some experiments medium lacking dexamethasone was added 24 h prior to cell harvesting. The cytokines were used at 100 units/ml for IL-1β and 50 units/ml for TNF-α.

2.3. RNA blot analysis

After extraction by the method of Chomczynski and Sacchi [16], total RNA (10 μg) was electrophoresed on a 1% agarose-formaldehyde gel and transferred onto nylon membranes as described previously [17]. Membranes were hybridized overnight at 65°C according to Church and Gilbert [18] with DNA probes labeled with [α^{32}P]dCTP by random priming. The final wash was carried out in 0.2×SSC (20×SSC is 3 M NaCl and 0.3 M Na citrate, pH 7.0) and 0.1% SDS at 65°C except for catalase (55°C). Specific cDNA probes were rat MnSOD (pSP65-RMS), rat CuZn-SOD (pUC13-RCS) and rat GPx (LK440-cGP) kindly provided by Dr. Y. Ho (Detroit, MI, USA). The cDNA for human catalase (AdCL) was obtained from Dr. P. Lemarchand (Paris, France). An oligonucleotide specific for the 18S ribosomal RNA was used as a control [19]. Relative mRNA amounts were determined by densitometric analysis and were corrected for differences in RNA loading by comparison with the signal obtained for 18S rRNA.

2.4. Nuclear run on assay

Nuclear run on experiments were done as described previously [17] with some modifications for the preparation of rat hepatocyte nuclei. About 10^8 hepatocytes were used for each point. Cells were scraped in phosphate buffered saline and pelleted at 500×*g* for 5 min. The pellet was resuspended in a hypotonic buffer for 10 min as described by Shapiro [20], and NP40 0.1% was added for 5 min. Cells were then disrupted in a Dounce homogenizer by 4 strokes using a B pestle and nuclei were pelleted at 400×*g* for 5 min. Nuclei were then layered on a 2 M sucrose cushion and centrifuged at 40 000 rpm for 1 h using a SW-41 rotor at 4°C. Specific probes used were β-actin, albumin, Mn-SOD, CuZn-SOD and GPx. The background hybridization signal was determined using plasmid DNA.

2.5. Nuclear protein extracts and gel retardation assay

Nuclear extracts from cultured hepatocytes were prepared by the method of Cereghini et al. [21], except that they were not dialyzed. The binding reactions were carried out as previously described using 1 μg poly(dI-dC) [22]. The NF-kB binding activity was determined using a double stranded oligonucleotides: 5′-GACAGAGGGGACTTTCCGAGAGG-3′ [23]. 5 μg of protein was added to the reaction mixture and incubated for 10 min on ice. The DNA-protein complexes were resolved on a 6% acrylamide gel in 0.5×TBE (45 mM Tris-borate, 1.25 mM EDTA) at 22 mA for 2 h. The gel was then fixed, dried and subjected to autoradiography.

3. Results

RNA blot hybridization was used to measure the relative levels of the mRNAs encoding antioxidant enzymes as a mean for studying the effect of culturing hepatocytes on their expression. CuZn-SOD, GPx and especially CAT mRNAs were down-regulated whereas Mn-SOD mRNA was transiently up-regulated (Fig. 1). Indeed, a marked increase of the level of Mn-SOD mRNA was noted during the first 24 h of culture. The basal level of Mn-SOD mRNA, corresponding to that found in freshly isolated cells, was recovered after 48 h of culture in a medium containing 100 nM dexamethasone. Therefore hepatocytes were cultured for 48 h before addition of TNF-α or IL-1β.

These cytokines induced a stimulation of Mn-SOD gene expression while they had no significant effect on the expression of CuZn-SOD and GPx genes (Fig. 2). The predicted TNF-α induction of Mn-SOD gene expression was observed only in the absence of dexamethasone, demonstrating that this compound blocked the TNF-α-induced up-regulation of Mn-SOD gene expression. This effect was abolished by the synthetic antiglucocorticoid RU 486, suggesting the involvement of the glucocorticoid receptor in the inhibitory effect of dexamethasone (Fig. 2A). In contrast, dexamethasone did not affect the IL-1β-induced Mn-SOD up-regulation.

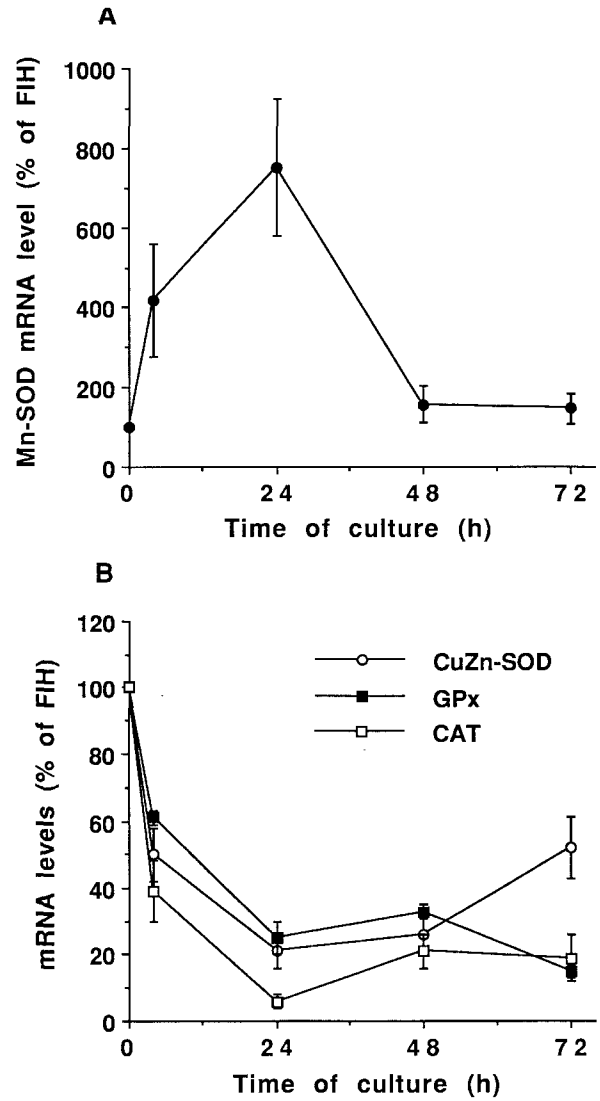

Fig. 1. Time course of Mn-SOD, CuZn-SOD, GPx and catalase gene expression in rat hepatocytes in primary culture. Total RNA was prepared from rat hepatocytes at the indicated time of culture. RNA blot analysis was carried out using rat Mn-SOD cDNA, rat CuZn-SOD cDNA, rat GPx cDNA, human CAT cDNA and an oligonucleotide specific for the 18S rRNA as probes. A: Time course of Mn-SOD gene expression. B: Time course of CuZn-SOD, GPx and CAT gene expression. The mRNA signal was quantitated by densitometry and normalized for differences in RNA loading using the 18S rRNA signal. Data are expressed as the percentage of expression in freshly isolated hepatocytes (FIH). Each value is the mean ± S.E.M. of results from four separate experiments carried out in duplicate.

Thus the kinetics of Mn-SOD gene induction by TNF-α and IL-1β were determined in the absence of dexamethasone (Fig. 3). TNF-α induced a rapid 3–4-fold increase in Mn-SOD gene expression with a maximum induction after 8 h of treatment. In contrast, IL-1β induced a slower but stronger increase in Mn-SOD expression, about 6-fold after 24 h and 10-fold 48 h after cytokine addition.

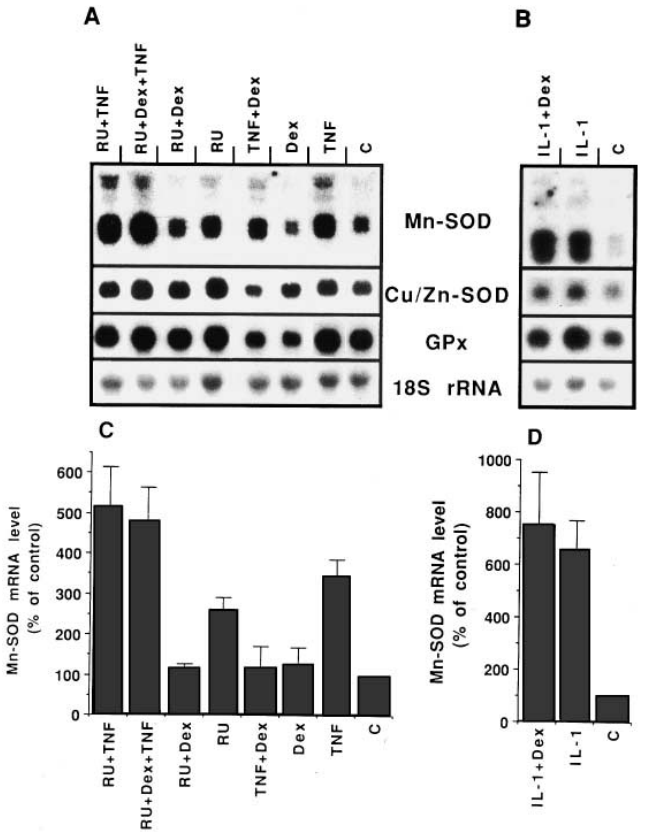

Fig. 2. RNA blot analysis of Mn-SOD, CuZn-SOD and GPx mRNAs in rat hepatocytes under various conditions. Total RNA was prepared from rat hepatocytes following incubation with 50 U/ml TNF-α, 100 nM dexamethasone (Dex), 1 μM RU 486 (A, C) and 100 U/ml IL-1β, 100 nM Dex (B, D). C, control untreated hepatocytes. RNA blot analysis was performed using rat Mn-SOD cDNA (top panel), rat CuZn-SOD cDNA (second panel), rat GPx cDNA (third panel) and an oligonucleotide specific for the 18S rRNA (bottom panel) as probe. A and B are representative of four independent experiments. C and D show results obtained from densitometric quantification of Mn-SOD autoradiograms. Each value is the mean ± S.E.M. of results from four separate experiments carried out in duplicate.

The mechanisms involved in the regulation of the Mn-SOD gene by these cytokines was investigated using nuclear run on experiments. A 2 h exposure of hepatocytes to TNF-α induced a specific 2-fold increase of Mn-SOD gene transcription (Fig. 4A). Moreover, the TNF-α-induced gene transcription was blocked by dexamethasone. The glucocorticoid had no effect on the basal rate of Mn-SOD transcription, while inducing albumin gene transcription. IL-1β induced a 2-fold increase of Mn-SOD gene transcription after 12 h of treatment (Fig. 4B). As expected, albumin gene transcription was reduced with IL-1β treatment. Actin gene transcription, included as a control, was not affected by the treatments.

Since the NF-kB transcription factor is a good candidate for the control of Mn-SOD gene transcription [24], the DNA binding activity of this factor was estimated by gel retardation with nuclear extracts from rat hepatocytes treated for 30 min with or without TNF-α and dexamethasone (Fig. 5). TNF-α was found to increase the specific DNA binding activity of NF-kB. However, dexamethasone did not modify the DNA binding activity resulting from TNF-α stimulation.

4. Discussion

Mn-SOD plays an important role in the cellular defense against reactive oxygen species. In this study, we demonstrated that in rat hepatocyte primary cultures, Mn-SOD gene expression was highly sensitive to cytokines as well as to the culture process. Moreover, among all the antioxidant enzymes studied, Mn-SOD was the only one being regulated by IL-1β and TNF-α.

Induction of Mn-SOD gene expression by these cytokines has been shown in several cell lines [4–11]. However, this is the first demonstration that TNF-α induces Mn-SOD gene expression in rat hepatocytes in primary culture. Although TNF-α-induction of Mn-SOD gene expression has been described in many cell types, there is less information concerning the mechanisms involved. We demonstrate in this study that TNF-α acts at the transcriptional level. Since TNF-α is a known inducer of NF-kB binding activity, it was suggested that the cytokine could act on Mn-SOD gene transcription through NF-kB activation [24]. In this study, TNF-α stimulates the NF-kB binding activity within 30 min (Fig. 5). This result is consistent with a rapid activation of Mn-SOD gene transcription by TNF-α.

We provide evidence that dexamethasone inhibits the TNF-α-induced Mn-SOD gene transcription. Thus, these results might explain the lack of effect of TNF-α reported by Kayanoki et al. [11] since these investigators maintained their hepatocytes in the presence of 100 nM dexamethasone. In addition, this glucocorticoid has been shown to repress basal Mn-SOD expression in rat intestinal epithelial cells [25]. In rat hepatocytes, dexamethasone has no effect on the basal level of Mn-SOD gene expression, suggesting a tissue or cellular specificity of glucocorticoid action. Glucocorticoid hormones mediate their effects by binding to the intracellular glucocorticoid receptor, which then binds to the regulatory elements in the 5′ flanking region of target genes [26]. Protein-protein interaction between the glucocorticoid receptor and the tran-

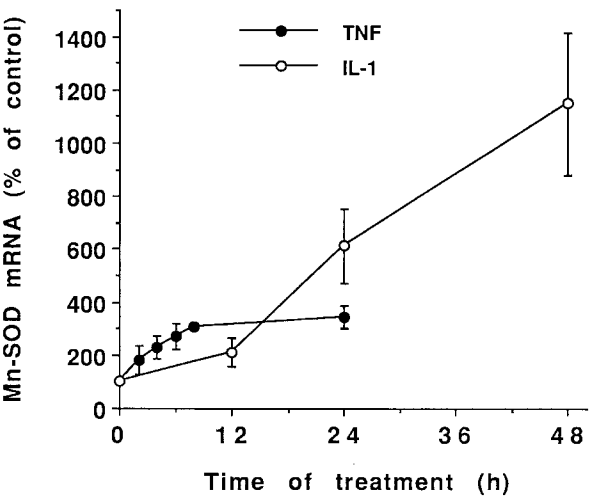

Fig. 3. Time course of induction of Mn-SOD mRNA by TNF-α and IL-1β in rat hepatocytes. At the indicated time points, RNA was isolated and analyzed using radiolabeled Mn-SOD cDNA. The mRNA signal was quantitated by densitometry and normalized for differences in RNA loading using the 18S rRNA probe. Data are expressed as the percentage of control untreated hepatocytes. Each value is the mean ± S.E.M. of results from four independent experiments carried out in duplicate.

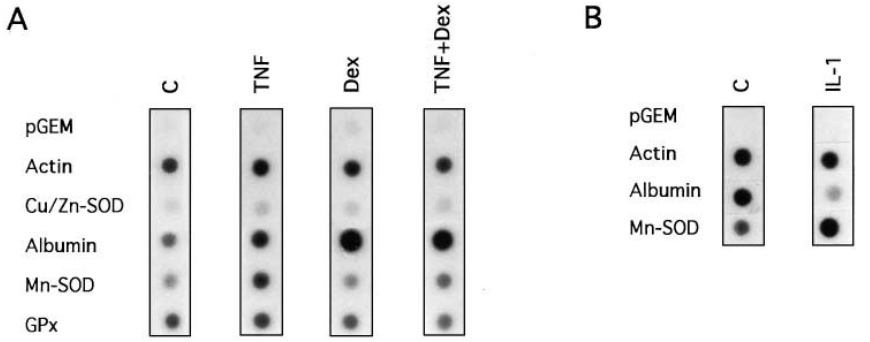

Fig. 4. Mn-SOD, CuZn-SOD, GPx, albumin and β-actin gene transcription in rat hepatocytes under various conditions. Nuclei were prepared following 2 h treatment with either TNF-α (50 U/ml) or dexamethasone (Dex) (100 nM), alone or in combination (A), or 12 h treatment with 100 U/ml IL-1β (B). A representative autoradiogram of labeled transcripts representing Mn-SOD, CuZn-SOD, GPx, β-actin and albumin gene transcription is shown. Plasmid DNA is used as a background control.

scription factor NF-kB has been suggested to mediate a negative cross-talk between dexamethasone and TNF-α-induced gene transcription [27]. In this study, we demonstrate the involvement of the glucocorticoid receptor in the dexamethasone effect but we provide no evidence for such negative cross-talk between the receptor and NF-kB. Thus dexamethasone could block the TNF-α-induced Mn-SOD gene transcription at a different step.

IL-1β is a strong inducer of Mn-SOD gene transcription in rat hepatocyte primary cultures; however, its effect is delayed as compared to that of TNF-α. Thus, it could be suggested, as it has been demonstrated for the induction of the α-1 acid glycoprotein, that a short-lived protein could be involved in the IL-1β effect [28]. In rat hepatocytes, the IL-1β-induced Mn-SOD gene transcription was not inhibited by dexamethasone, suggesting that IL-1β and TNF-α induced Mn-SOD gene transcription through different pathways. However, they both stimulated NF-kB activation (data not shown). Therefore it could be postulated that the two cytokines activated the transcription factor through different routes. Finally, it appears clear that TNF-α and IL-1β induced Mn-SOD gene transcription through different pathways, the former being highly sensitive to dexamethasone inhibition.

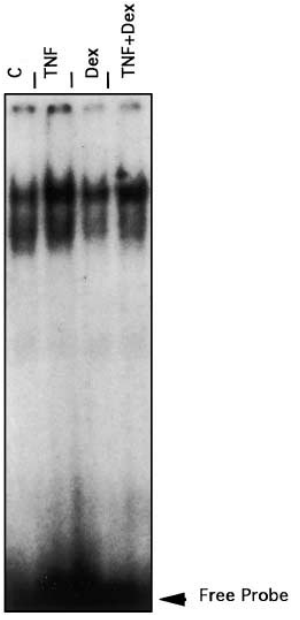

Fig. 5. Influence of TNF-α and dexamethasone on the protein binding activity for the NF-kB site. Nuclear protein extracts were prepared from rat hepatocytes following a 30 min incubation with either TNF-α or dexamethasone (Dex) alone or in combination. NF-kB-protein complexes were resolved on acrylamide gels. C, control untreated hepatocytes. This result is representative of three separate experiments.

Acknowledgements: We gratefully acknowledge Dr. Y. Ho (Detroit, MI, USA) for kindly providing us with the GPx, Mn and CuZn-SOD plasmids. We wish to thank Dr. Patricia Lemarchand (Paris, France) for the gift of CAT plasmid. We would like to thank Drs. Laurent Corcos and Odile Sergent for helpful discussion. This research was supported by INSERM, the Association pour la Recherche sur le Cancer and la Ligue contre le Cancer, comité d'Ille et Vilaine. K.M. is a recipient of a fellowship from the Ministère de la Recherche et de l'Espace.

References

[1] Marklund, S.L. (1984) Biochem. J. 222, 649–655.
[2] Kushner, I. (1988) Methods Enzymol. 163, 373–383.
[3] Dewaal, R.M.W. (1994) Mol. Biol. Rep. 19, 81–88.
[4] Wong, G.H.W. and Goeddel, D.V. (1988) Science 242, 941–944.
[5] Visner, G.A., Dougall, W.C., Wilson, J.M., Burr, I.A. and Nick, H.S. (1990) J. Biol. Chem. 265, 2856–2864.
[6] Warner, B.B., Burhans, M.S., Clark, J.C. and Wispe, J.R. (1991) Am. J. Physiol. 260, L296–301.
[7] Pang, X.P., Ross, N.S., Park, M., Juillard, G.J.F., Stanley, T.M. and Hershman, J.M. (1992) J. Biol. Chem. 267, 12826–12830.
[8] Bigdeli, N., Niemann, A., Sandler, S. and Eizirik, D.L. (1994) Biochem. Biophys. Res. Commun. 203, 1542–1547.
[9] Ono, M., Kohda, H., Kawaguchi, T., Ohhira, M., Sekiya, C., Namiki, M., Takeyasu, A. and Taniguchi, N. (1992) Biochem. Biophys. Res. Commun. 182, 1100–1107.
[10] Kayanoki, Y., Fujii, J., Susuki, K., Kawata, S., Matsuzawa, Y. and Taniguchi, N. (1994) J. Biol. Chem. 269, 15488–15492.
[11] Dougall, W.C. and Nick, H.S. (1991) Endocrinology 129, 2376–2384.
[12] Sato, M., Sasaki, M. and Hojo, H. (1995) Arch. Biochem. Biophys. 316, 738–744.
[13] Feng, L., Xia, Y., Garcia, G.E., Hwang, D. and Wilson, C.B. (1995) J. Clin. Invest. 95, 1669–1675.
[14] Baeuerle, P.A. (1991) Biochim. Biophys. Acta 1072, 63–80.
[15] Guguen, C., Guillouzo, A., Boisnard, M., Le Cam, A. and Bourel, M. (1975) Biol. Gastroenterol. 8, 223–231.
[16] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[17] Antras-Ferry, J., Robin, P., Robin, D. and Forest, C. (1995) Eur. J. Biochem. 234, 390–396.
[18] Church, G.M. and Gilbert, W. (1984) Proc. Natl. Acad. Sci. USA 81, 1991–1995.

[19] Chan, Y.L., Gutell, R., Noller, H.F. and Wool, I.G. (1984) J. Biol. Chem. 259, 224–230.
[20] Shapiro, D.J., Sharp, P.A., Wahli, W.W. and Keller, M. (1988) DNA 7, 47–55.
[21] Cereghini, S., Blumenfeld, M. and Yaniv, M. (1988) Genes Dev. 2, 957–974.
[22] Langouët, S., Corcos, L., Abdel-Razzak, Z., Loyer, P., Ketterer, B. and Guillouzo, A. (1995) Biochem. Biophys. Res. Commun. 216, 793–800.
[23] Edbrooke, M.E., Burt, D.W., Cheshire, J.K. and Woo, P. (1989) Mol. Cell. Biol. 9, 1908–1916.
[24] Das, K.C., Lewis-Molock, Y. and White, C.W. (1995) Mol. Cell. Biochem. 148, 45–57.
[25] Valentine, J.F. and Nick, H.S. (1994) Gastroenterology 107, 1662–1670.
[26] Beato, M. (1989) Cell 56, 335–344.
[27] Caldenhoven, E., Liden, J., Wissink, S., Van de Stolpe, A., Raaijmakers, J., Koenderman, L., Okret, S., Gustafsson, J.A. and Van der Saag, P.T. (1995) Mol. Endocrinol. 9, 401–412.
[28] Geiger, T. Andus, T., Klapproth, J., Northoff, H. and Heinrich, P.C. (1988) J. Biol. Chem. 263, 7141–7146.