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Interleukin-1β-induced cytosolic phospholipase A_2 activity and protein synthesis is blocked by dexamethasone in rat mesangial cells

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Interleukin-1β induces gene expression and secretion of the secretory phospholipase A_2 ($sPLA_2$) and prostaglandin E_2 (PGE_2) release from rat mesangial cells. We have previousy shown that prolonged treatment of rat mesangial cells with interleukin-1β (IL-1β) also enhances the cytosolic phospholipase A_2 ($cPLA_2$) activity. This effect of IL-1β on the $cPLA_2$ activity is inhibited by actinomycin D and cycloheximide, indicating that both transcription and translation are involved. Here, we describe that IL-1β increases mRNA levels and protein synthesis of $cPLA_2$ itself. In parallel with the effect of dexamethasone on the $sPLA_2$, this glucocorticoid inhibits the IL-1β-enhanced $cPLA_2$ activity as a result of the suppression of IL-1β-induced $cPLA_2$ gene expression. This report suggests that the pro-inflammatory action of interleukin-1β may, in part, be mediated by its effects on $cPLA_2$ activity.

Phospholipase A_2; Interleukin-1β; Dexamethasone; Rat mesangial cell

1. INTRODUCTION

Interleukin-1β (IL-1β) is a potent pro-inflammatory cytokine produced mainly by stimulated macrophages and monocytes [1]. IL-1β shows a wide variety of biological activities. It plays an important role in the mediation of inflammatory responses. Stimulation of target cells with IL-1β enhances the production of inflammatory mediators, in particular prostaglandins, leukotrienes and platelet-activating factor. It is now well established that activation of PLA_2 is the key controlling event in the synthesis of these inflammatory mediators. PLA_2s are a diverse family of enzymes which catalyze the hydrolysis of the 2-acyl ester bond of phospholipids. The action of PLA_2 is of particular interest for the release of arachidonic acid, which serves as a the rate-limiting step for the biosynthesis of the eicosanoids [2].

Mammalian cells contain several PLA_2 enzymes, including the 14 kDa secretory PLA_2 ($sPLA_2$) and the recently described cytosolic PLA_2 ($cPLA_2$) [3,4]. The role of the $sPLA_2$ in inflammation has recently been reviewed by Vadas and Pruzanski [5]. The $cPLA_2$ is found in a variety of cell types and selectively cleaves arachidonic acid from phospholipids [6]. In several cell lines it has been shown that $cPLA_2$ is coupled to hormone-induced arachidonic acid release [7,8]. However, despite recent progress it is presently still unclear which PLA_2s are involved in the release of arachidonic acid during inflammatory diseases.

Glomerular mesangial cells are one of the target cells for potent inflammatory cytokines. Both $sPLA_2$ and $cPLA_2$ have been described in rat mesangial cells [7,9]. We and others have recently shown that IL-1β and tumor necrosis factor (TNF) enhanced the synthesis and secretion of the $sPLA_2$ and PGE_2, which was accompanied by increased mRNA levels coding this PLA_2 in rat mesangial cells [9–12]. Such observations have also been made in other cell types [13–16]. In addition, we have recently shown that prolonged treatment of rat mesangial cells with IL-1β and transforming growth factor-β2 enhanced the $cPLA_2$ activity [17]. Whether the increased $cPLA_2$ activity was associated with a de novo synthesis rather than an activation by enzyme modification was not established. IL-1 was also shown to induce the accumulation of $cPLA_2$ and the release of PGE_2 in human fibroblasts [18] and rheumatoid synovial fibroblasts [19]. However, in contrast to rat mesangial cells, the $sPLA_2$ was not affected in fibroblasts. Furthermore, TNF induced $cPLA_2$ activity in human epithelial carcinoma cells [20], HeLa cells[21] and a human endothelial cell line [22].

It has been shown that the cytokine-induced $cPLA_2$ activity can be inhibited by glucocorticoids [18,20]. We have shown previously that the cytokine-induced expression of $sPLA_2$ in rat mesangial cells was prevented by dexamethasone [23]. However, the effect of dexamethasone on the $cPLA_2$ activity has not yet been elucidated in rat mesangial cells. In the present study, we

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Abbreviations: Il-1β, interleukin-1β; $cPLA_2$, cytosolic phospholipase A_2; $sPLA_2$, secretory phospholipase A_2; TNF, tumor necrosis factor; PGE_2, prostaglandin E_2.

show that IL-1β increased $cPLA_2$ mRNA levels and $cPLA_2$ mass in rat mesangial cells, resulting in enhanced $cPLA_2$ activity. This IL-1β-induced de novo synthesis of the $cPLA_2$is completely suppressed by dexamethasone.

2. MATERIALS AND METHODS

2.1. *Materials*

Recombinant IL-1β was prepared by the Biotechnology Department of Ciba-Geigy Ltd., Basel, Switzerland. 1-Stearoyl-2-[1-^{14}C]arachidonoylphosphatidylcholine, deoxycytidine 5'-[α-^{32}P]triphosphate ([α-^{32}P]dCTP, 3,000 Ci/mmol) and [^{35}S]methionine and -cysteine were obtained from Amersham International, UK. The random primer labeling kit was from Boehringer-Mannheim, Germany. The PGE_2 assay kit and the nylon membranes (GeneScreen) were purchased from New England Nuclear, Boston, USA. Rabbit anti-$cPLA_2$ antibody was kindly donated by Dr. J. Clark, Genetics Institute, Boston, USA. A cDNA probe for mouse $cPLA_2$ comprising residues 529–2,390 was provided by Dr. R.M. Kramer, Lilly Research Laboratories, Indianapolis, USA.

2.2. *Cell culture and incubation*

Mesangial cells were cultivated and characterized as described previously [24] with slight modifications. The cells were grown in RPMI 1640 supplemented with 14% fetal calf serum, penicillin (10 IU/ml), streptomycin (10 μg/ml) and insulin (0.13 IU/ml). The cells were maintained in an incubator at 37°C in an atmosphere of 92.5% air and 7.5% CO_2. Cells were passaged using split ratios of 4–5 at 3–5 day intervals. For the experiments only cells up to passage 30 were used. Confluent mesangial cells cultured in 25 cm^2 tissue culture flasks were washed once with PBS and incubated with 5 ml of RPMI 1640 containing 0.1 mg/ml of fatty acid-free bovine serum albumin with or without IL-1β for 24 h. After the indicated time periods, the supernatant was removed and stored at −40°C until further analysis for PGE_2 formation.

2.3. *Preparation of cytosolic fractions*

After incubation and withdrawal of the supernatant, the cells were washed once with PBS and scraped with a plastic policeman into 0.5 ml of homogenization buffer: 50 mM HEPES, pH 7.4, 0.25 M sucrose, 1 mM EDTA, 1 mM EGTA, 50 μM sodium fluoride, 10 μM leupeptin, 0.15 mM phenylmethanesulfonyl fluoride. The cells were homogenized with 10 strokes using a Potter-Elvehjem motor-driven Teflon pestle at 600 rpm. The homogenate obtained was then centrifuged at 200,000 × g for 30 min in a Beckman TL-100 Ultracentrifuge with a TLA 100.3 rotor. The resulting supernatant was designated the cytosolic fraction.

2.4. *Assays*

The $cPLA_2$ enzymatic activity was measured with 2.5 μM 1-Stearoyl-2-[1-^{14}C]arachidonoylphosphatidylcholine as substrate in 0.2 ml of 0.1 M Tris-HCl, pH 8.5, containing 1 mM $CaCl_2$ in excess of EGTA and EDTA and 5 mM DTT to inactivate the $sPLA_2$. After incubation for 5 min at 37°C the reaction was stopped and the released [1-^{14}C]arachidonate was extracted by a modified Dole extraction procedure [25].

Protein concentrations were determined with a Bio-Rad protein assay solution with bovine serum albumin as standard.

PGE_2 was determined by a radioimmunoassay.

2.5. *Immunoprecipitation*

For immunoprecipitation of $cPLA_2$, mesangial cells were harvested and lysed in RIPA-buffer: 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1 mM PMSF, 1% sodium deoxycholate and 0.1% Triton X-100. The lysates were clarified by centrifugation at 14,000 × g for 10 min. After a pre-clearance step with pre-immune serum, the $cPLA_2$ was isolated from the lysates by immunoprecipitation using 3 μl of rabbit anti-$cPLA_2$ antibody. After incubation at 4°C overnight, the antigen–antibody complex was bound to 6.5 mg protein A-Sepharose. The immunoprecipitates were washed once with the RIPA buffer and twice with 20 mM Tris-HCl, pH 7.4, 0.5 M NaCl, 1% Triton X-100 and twice with 20 mM Tris-HCl pH 7.4, 0.15M NaCl, 1% Triton X-100. The samples were boiled for 3 min in 30 μl Laemmli's sample buffer and applied to SDS-PAGE (10% gel).

2.6. *Northern blot analysis*

Total mesangial cell RNA was extracted using the guanidinium thiocyanate/phenol/chloroform method [26]. RNA (20 μg) was separated on 0.8% agarose gels containing 6.6% formaldehyde and transferred to GeneScreen membranes. The filters were baked at 80°C for 2 h and prehybridized at 42°C in 50% (v/v))formamide, 10% (w/v) dextrane sulphate, 1% SDS (w/v), 1 M NaCl and 100 μg/ml denaturated herring sperm DNA. The filters were hybridized at 42°C overnight with a cDNA probe coding for mouse $cPLA_2$, comprising residues 529–2,390, and labeled with [α-^{32}P]dCTP according to the random primer labeling technique described by Feinberg and Vogelstein [27]. After washing, the signal was detected by autoradiography.

3. RESULTS AND DISCUSSION

As illustrated in Fig. 1, prolonged treatment of rat mesangial cells with IL-1β enhanced the $cPLA_2$ activity 3-fold. The $cPLA_2$ activities were measured in the presence of DTT to inactivate the $sPLA_2$ completely [17]. The stimulation of the $cPLA_2$ by IL-1β was completely blocked by actinomycin D and cycloheximide, demonstrating that synthesis of RNA and new protein is involved in IL-1β-enhanced $cPLA_2$ activities. The effect of IL-1β on the $cPLA_2$ is accompanied by the effect of IL-1β on PGE_2 synthesis. IL-1β treatment of mesangial cells resulted in a 100-fold increase in the formation of PGE_2. These results are in agreement with previously observed data [10,17]. Similar results were also reported for IL-1-stimulated fibroblasts [18,19]. However, in contrast to what was shown in fibroblasts, IL-1β also induced the synthesis and secretion of $sPLA_2$ in rat mesangial cells [9]. Thus, both PLA_2s could be involved in the IL-1β-induced release of arachidonate for the observed PGE_2 formation. The relative contributions of both IL-1β-induced $sPLA_2$ and $cPLA_2$ activities in the release of arachidonate for the enhanced synthesis of PGE_2 in rat mesangal cells remains to be established. Recently, we provided evidence that an enhanced $cPLA_2$ activity is involved in transforming growth factor-β2-induced PGE_2 synthesis in rat mesangial cells [29].

Glucocorticosteroids are widely used anti-inflammatory drugs. The anti-inflammatory activity has been attributed to their ability to inhibit prostaglandin synthesis. The contribution of $sPLA_2$ to PGE_2 formation is supported by the dose-dependent inhibition of IL-1β-induced $sPLA_2$ and PGE_2 synthesis by dexamethasone in rat mesangial cells [23]. However, the effect of dexamethasone on the $cPLA_2$ activity has not been elucidated in rat mesangial cells so far. Therefore, we started to determine the effect of dexamethasone on the IL-1β-enhanced $cPLA_2$ activity. As shown in Fig. 2, the IL-1β-

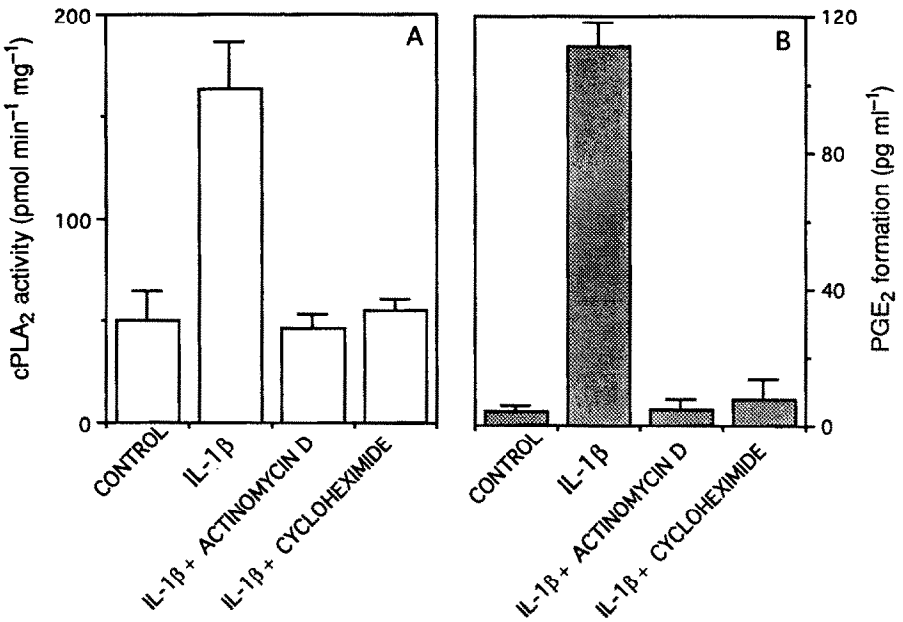

Fig. 1. Effect of actinomycin D and cycloheximide on IL-1β-enhanced $cPLA_2$ activity and PGE_2 release from rat mesangial cells. Rat mesangial cells were incubated with actinomycin D (10 μM) or cycloheximide (10 μM) alone or together with IL-1β (1 nM) for 24 h. Thereafter, 200 μl of the culture supernatants were used for PGE_2 measurements. Cytosolic fractions of the cells were prepared and measurements of the $cPLA_2$ activities were done as described in section 2. Results are means ± S.E.M. from triplicate experiments.

induced $cPLA_2$ activity was dose-dependently inhibited by dexamethasone with a complete inhibition at 100 nM.

In order to determine whether the IL-1β-enhanced $cPLA_2$ activity was directly due to increased $cPLA_2$ mass levels or indirectly to the synthesis of proteins involved in the $cPLA_2$ activation, an immunoblot experiment using $cPLA_2$ antibodies was performed. With immunoprecipitation experiments using polyclonal antibodies raised against a mouse $cPLA_2$, 89% of the $cPLA_2$ activity could be precipitated, indicating that the antibodies are cross-reactive with the rat $cPLA_2$. However, we could not detect $cPLA_2$ protein with Western blot analysis, neither in control cells nor in stimulated cells (data not shown). This was probably due to the low amount of the $cPLA_2$ in rat mesangial cells (compare also Fig. 4A and B). To study the biosynthesis of the $cPLA_2$, rat mesangial cells were stimulated with or without IL-1β. In the presence of [^{35}S]methionine and [^{35}S]cysteine. The detection of the $cPLA_2$ was done by an immunoprecipitation with a $cPLA_2$ antibody, followed by SDS-PAGE and autoradiography (Fig. 3). Under these experimental conditions, treatment of rat mesangial cells with IL-1β for 24 h was associated with approximately a 2-fold increase in $cPLA_2$ protein, as estimated by densitometrical scanning. The increased $cPLA_2$ protein levels correlate with the increased $cPLA_2$ activity, indicating that the increased $cPLA_2$ activity upon treatment of mesangial cells with IL-1β is due to increased synthesis of the $cPLA_2$. Similar results have been noted for IL-1α-treated human fibroblasts [18], macrophage colony stimulating factor-treated human monocytes [30] and TNF-treated HeLa cells [21].

Next we determined the effect of dexamethasone on the induced synthesis of the $cPLA_2$. As shown in Fig. 3, the IL-1β induction of $cPLA_2$ protein was prevented by treatment of the mesangial cells with dexamethasone. Together with the above mentioned effect of dexamethasone on the $sPLA_2$, these observations imply that the inhibition of the IL-1β-induced PGE_2 synthesis by dexamethasone in rat mesangial cells is caused by direct inhibition of PLA_2 protein expression.

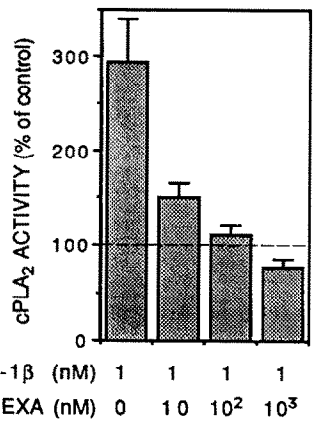

Fig. 2. Dose-dependent inhibition by dexamethasone of the $cPLA_2$ activity upon stimulation of mesangial cells. Rat mesangial cells were treated with IL-1β (1 nM) and with dexamethasone at various concentrations and 24 h. The $cPLA_2$ activities were determined in the cytosolic fractions as described in section 2.

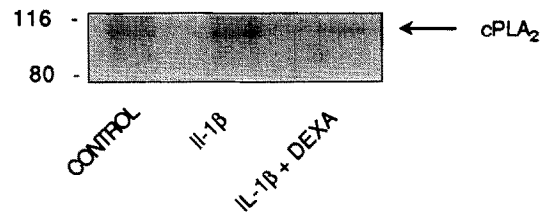

Fig. 3. Effect of IL-1β and dexamethasone on the $cPLA_2$ protein levels in rat mesangial cells. Rat mesangial cells were labeled with [^{35}S]methionine and [^{35}S]cysteine for 24 h in the presence of IL-1β (1 nM) or IL-1β (1 nM) plus dexamethasone (100 nM) as indicated. After immunoprecipitation and SDS-PAGE, the $cPLA_2$ was detected by autoradiography as described.

The inhibition of the IL-1β-induced PGE_2 synthesis by dexamethasone is probably not due to induced synthesis of PLA_2 inhibitory proteins such as annexins. In addition, we have recently shown that annexins I–VI are not induced by dexamethasone in rat mesangial cells (Vervoordeldonk, M. et. al, manuscript submitted). These data indicate that dexamethasone interferes with arachidonate release by repressing the IL-1β-induced expression of the $sPLA_2$ and $cPLA_2$ rather than by inducing the synthesis of PLA_2 inhibitory proteins.

The effect of IL-1β on the expression of the $cPLA_2$ was also tested by Northern blot analysis using a $cPLA_2$ probe. As shown in Fig. 4A, low levels of mRNA are present in growing rat mesangial cells. We included mouse 3T3 fibroblast mRNA as a positive control. The amount of $cPLA_2$ activity in 3T3 fibroblasts is approxomately 40-fold higher compared to rat mesangial cells (Fig. 4B), and this is accompanied by high mRNA

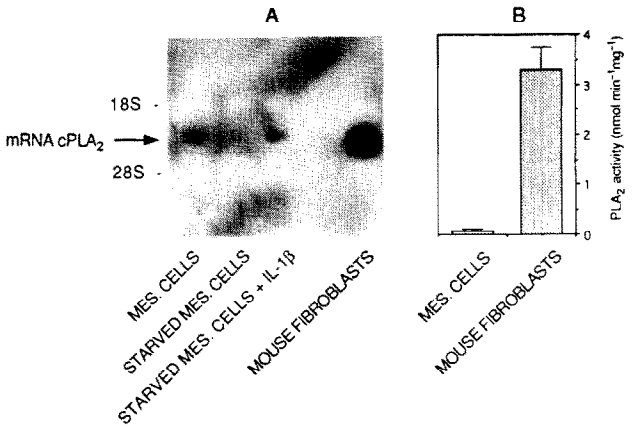

Fig. 4. (A) Effect of IL-1β and dexamethasone on the $cPLA_2$ mRNA levels in rat mesangial cells. Total RNA (20 μg) of growing rat mesangial cells, serum-starved rat mesangial cells, serum-starved rat mesangial cells treated with IL-1β and serum-starved rat mesangial cells treated with both IL-1β and dexamethasone were analyzed by Northern blotting as described in section 2. As a positive contol we included RNA from 3T3 mouse fibroblasts. (B) The $cPLA_2$ activities of growing rat mesangial cells and 3T3 fibroblasts. Cytosolic fractions of these cells were prepared and measurements of the $cPLA_2$ activities were done as described in section 2. Results are means ± SE from triplicate experiments.

levels (Fig. 4A). In serum-starved rat mesangial cells, no mRNA coding for $cPLA_2$ could be detected. Treatment of such cells with IL-1β was associated with induced $cPLA_2$ mRNA levels. The IL-1β-induced expression of the $cPLA_2$ gene occurred in paralleled with an increase in $cPLA_2$ protein levels (Fig. 3)

In summary, our studies provide evidence that the pro-inflammatory mediator, IL-1β, induces gene expression of the $sPLA_2$ and the $cPLA_2$ and the formation of PGE_2 in rat mesangial cells. The relative contribution of these PLA_2s for PGE_2 formation remains to be studied in order to establish the physiological role of these PLA_2s in the progression of renal inflammatory diseases.

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REFERENCES

[1] Oppenheim, J.J., Kovacs, E.J., Matsushima, K. and Durum, S.K. (1986) Immunol. Today 7, 44–45.
[2] Van den Bosch, H. (1980) Biochim. Biophys. Acta 604, 191–246.
[3] Clark, J.D., Milona, N. and Knopf, J.L. (1990) Proc. Natl. Acad. Sci. USA 87, 7708–7712.
[4] Kramer, R.M., Roberts, E.F., Manetta, J. and Putnam, J.E. (1991) J. Biol. Chem. 266, 5268–5272.
[5] Pruzanski, W. and Vadas, P. (1991) Immunol. Today 12, 143–146.
[6] Clark, J.D., Lin, L-L., Kriz, R.W., Ramesha, C.S., Sultzman, L.A., Lin, A.Y., Milona, N. and Knopf, J.L. (1991) Cell 65, 1043–1051.
[7] Gronich, J.H., Bonventre, J.V. and Nemenoff, R.A. (1988) J. Biol. Chem. 263, 16645–16651.
[8] Lin, L.-L., Lin, A.Y. and Knopf, J.L. (1992) Proc. Natl. Acad. Sci. USA 89, 6147–6151.
[9] Schalkwijk, C., Pfeilschifter, J., Märki, F. and van den Bosch, H. (1991) Biochem. Biophys. Res. Commun. 174, 268–275.
[10] Pfeilschifter, J., Pignat, W., Vosbeck, K. and Märki, F. (1989) Biochem. Biophys. Res. Commun. 159, 385–394.
[11] Nakazato, Y., Simonson, M.S., Herman, W.H., Konieczkowski, M. and Sedor, J.R. (1991) J. Biol. Chem. 266, 14119–14127.
[12] Mühl, H., Geiger, T., Pignat, W., Märki, F., van den Bosch, H., Vosbeck, K. and Pfeilschifter, J. (1991) FEBS Lett. 291, 249–252.
[13] Nakano, T., Ohara, O., Teraoka, H. and Arita, H. (1990) FEBS Lett. 261, 171–174.
[14] Kerr, J.S., Stevens, T.M., Davis, G.L., McLaughlin, J.A. and Harris, R.R. (1989) Biochem. Biophys. Res. Commun. 165, 1079–1084.
[15] Lyons-Giordano, B., Davis, G.L., Gailbraith, W., Pratta, N.A. and Arner, E.C. (1989) Biochem. Biophys. Res. Commun. 164, 488–495.
[16] Crowl, R.M., Stoller, T.J., Conroy, R.R. and Stouer, C.R. (1991) J. Biol. Chem. 266, 2647–2651.
[17] Schalkwijk, C., de Vet, E., Pfeilschifter, J. and van den Bosch, H. (1992) Eur. J. Biochem 210, 169–176.
[18] Lin, L.-L., Lin,A.Y. and DeWitt, D.L. (1992) J. Biol. Chem. 267, 23451–23454.
[19] Hulkower, K.I., Hope, W.C., Chen, T., Anderson, C.M., Coffey, J.W. and Morgan, D.W. (1992) Biochem. Biophys. Res. Commun. 184, 712–718.
[20] Goppelt-Struebe, M. and Rehfeldt, W. (1992) Biochim. Biophys. Acta 1127, 163–167.

[21] Hoeck, W.G., Ramesha, C.S., Chang, D.J., Fan N. and Heller, R (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 4475–4479.
[22] Murakami, M., Kudo, I. and Inoue, K. (1993) J. Biol. Chem. 267, 839–844.
[23] Schalkwijk, C., Vervoordeldonk, M., Pfeilschifter, J., Märki, F. and van den Bosch, H. (1991) Biochem. Biophys. Res. Commun. 180, 46–52.
[24] Pfeilschifter, J., Kurtz, A. and Bauer, C. (1984) Biochem. J. 223, 855–859.
[25] Van den Bosch, H., Aarsman, A.J. and van Deenen, L.L.M. (1974) Biochim. Biophys. Acta 348, 197–207.
[26] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[27] Feinberg, A.P. and Vogelstein, B. (1983) Anal. Biochem. 132, 6–13.
[28] Pfeilschifter, J., Pignat, W., Vosbeck, K. and Märki, F. (1989) Biochem. Soc. Trans. 17, 916–917.
[29] Schalkwijk, C.G., Pfeilschifter, J., Märki, F. and van den Bosch, H. (1992) *J. Biol. Chem. 267*, 8846–8851.
[30] Nakamura, T., Lin, L.-L., Kharbanda, S., Knopf, J. and Kufe, D. (1992) EMBO J. 11, 4917–4922.