Volume 296, number 2, 179–183 FEBS 10588 January 1992

The precursor form of the rat liver non-specific lipid-transfer protein, expressed in *Escherichia coli*, has lipid transfer activity

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Received 6 November 1991

The cDNA encoding the precursor form of non-specific lipid-transfer protein (pre-nsL-TP) from rat liver was cloned into the expression vector pET3d. The resulting plasmid was transformed to the *Escherichia coli* strain BL21(DE3). After induction of the bacteria with isopropyl-β-D-thiogalactopyranoside (IPTG) pre-nsL-TP was purified from the bacterial lysate by anion exchange chromatography followed by gelfiltration. From 1 l of culture, 6–7 mg of pre-nsL-TP was obtained, equal to approximately 7% of the cytoplasmic protein. By use of a fluorescence lipid transfer assay, pre-nsL-TP was found to have lipid transfer activity identical to mature nsL-TP.

Non-specific lipid-transfer protein; Sterol carrier protein-2; Lipid transfer; Bacterial expression; Rat liver

1. INTRODUCTION

The non-specific lipid-transfer protein (nsL-TP) catalyzes in vitro the transfer of phospholipids, glycolipids and cholesterol [1,2]. Due to this transfer activity it stimulates various aspects of cholesterol metabolism [3–6]. The protein is also known as sterol carrier protein-2 (SCP-2).

The amino acid sequence of nsL-TP (123 residues, mol. wt. 13 kDa) is very conserved among mammalian species. Bovine, rat, human and mouse nsL-TP are more than 90% identical [7–11]. A peroxisomal nsL-TP from the yeast *Candida tropicalis* (13.8 kDa) has 33% sequence identity with rat nsL-TP [12]. nsL-TP from plant sources show no sequence similarity with the mammalian proteins [13–15].

Rat liver nsL-TP contains the C-terminal peroxisomal targeting signal Ala-Lys-Leu [16]. However, immuno-electronmicroscopic labeling studies [17–19] as well as subcellular fractionation studies [20,21] have indicated that nsL-TP is not strictly peroxisomal. On the other hand, a specific relationship between nsL-TP and peroxisomes is supported by the observations that nsL-TP is absent from Chinese hamster ovary (CHO) cells deficient in peroxisomes, and from Zellweger liver [20,22]. In these CHO cells, the mRNAs encoding nsL-TP are still present [23]. Rat liver nsL-TP is synthesized as a 15 kDa precursor (pre-nsL-TP) on cytoplasmic free polyribosomes [24]. It was concluded from studies on somatic cell fusion with Zellweger fibroblasts that the processing of pre-nsL-TP (presequence of 20 amino acids) depends on the presence of peroxisomes [25].

In this study we have made use of an expression system based on T7 RNA polymerase, which elongates RNA-chains about 5-times faster than *Escherichia coli* RNA polymerase. The translation vector pET3d derives from the multicopy plasmid pBR322 and carries a T7 promoter. The T7 RNA polymerase gene, which is integrated in BL21(DE3) lysogen used as a host strain in the expression system, is inducable by IPTG. After induction, T7 RNA polymerase transcribes the target DNA in the plasmid. Translation of the target mRNA gives directly the desired protein [26]. Here we describe the over-expression of pre-nsL-TP in *E. coli* and its purification from the bacterial lysate. The lipid transfer activity of pre-nsL-TP is determined.

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2. MATERIALS AND METHODS

2.1. *Materials*

E. coli strain BL21(DE3) and the vector pET3d (formerly pET8c) [26] were obtained from Dr. J.H. Veerkamp (Department of Biochemistry, University of Nijmegen, The Netherlands). *E. coli* strain PC2495 was obtained from the Phabagen collection, Utrecht, The Netherlands. The primers used were synthesized on a Biosearch 8600 DNA synthesizer. dNTPs were from Boehringer (Mannheim, Germany). Taq polymerase and PCR buffer were obtained from Promega (Madison, WI). The Prep-a-Gene DNA purification kit was from Bio-Rad (Brussels, Belgium). The T7 sequencing kit, isopropyl-β-D-thiogalactopyranoside (IPTG), and Sephadex G-50 were from Pharmacia (Uppsala, Sweden). [α-^{35}S]dATP (1000 Ci/mmol) was obtained from The Radiochemical Centre (Amersham, UK). Nitrocellulose was from Schleicher & Schuell (Dassel, Germany). DEAE-cellulose (DE 52) was obtained from Whatman Biosystems Ltd. (Maidstone, UK). Egg yolk phosphatidylcholine (PC) and phosphatidic acid (PA) were obtained from Sigma (St. Louis, MO). Trinitrophenyl-phosphatidylethanolamine (TNP-PE) was prepared from egg-yolk PE and trinitrobenzenesulfonic acid [27]. 1-Palmitoyl-2-[pyrenylhexanoyl]-*sn*-glycero-3-

phosphocholine (Pyr(6)PC) was a kind gift of Dr. P.J. Somerharju (University of Helsinki, Finland). Rat liver nsL-TP and an affinity-purified polyclonal anti-nsL-TP antibody were isolated as described before [28,29].

2.2. *Cloning of the cDNA sequence encoding rat liver pre-nsL-TP in pET3d*

A cDNA clone (1851 bp) encoding a 58-kDa protein that contains the complete pre-nsL-TP sequence [23] (see discussion) was used in a polymerase chain reaction (PCR) [30] experiment to give a 505-bp DNA fragment encoding pre-nsL-TP. Two oligonucleotides were synthesized and used in the PCR to create the appropriate restriction sites in this cDNA. With oligonucleotide 1, 5'-ACC.CTC.TAC.ACC.ATG.GGT.TTT.CCC.GAA-3', an *Nco*I site was created around the startcodon, and with oligonucleotide 2, 5'-CGT.GGA.TTT.CTA.CGG.ATC.CAC.ACA.TCT.C-3', a *Bam*HI site was made 43–48 bp downstream of the stopcodon. The PCR reaction mixture contained: 300–1500 ng DNA, 1 μM of each oligonucleotide, 200 μM of each dNTP, 2 U Taq polymerase, and 1/10 volume of 10× reaction buffer (10×: 500 mM KCl, 100 mM Tris-HCl, pH 9.0 at 25 °C, 15 mM $MgCl_2$, 0.1% gelatin (w/v) and 1% Triton X-100). The final volume was 100 μl. The mixture was overlayered with 100 μl water-saturated paraffin. The PCR was carried out in a thermocycler (Techne Programmable Dri-Block PHC-1, New Brunswick Scientific Benelux B.V., The Netherlands) as follows: 7 min at 95°C, then 5 cycles for 1 min at 95°C, 2 min at 55°C, 1.5 min at 72°C, then 25 cycles for 1 min at 95°C, 2 min at 65°C, 1.5 min at 72°C, and finally 10 min at 72°C to ensure complete synthesis. Standard techniques were used for cloning [31]. Because the 505-bp DNA fragment formed contained in addition to the newly created *Nco*I site an internal *Nco*I site, cloning required partial digestion with *Nco*I. Partial digestion yielded, on a 1.5% TAE-agarose gel, 3 distinct bands of about 500, 300 and 200 bp. The 500-bp band, containing non-digested DNA (505 bp) and the DNA digested at the 5' *Nco*I site (494 bp), was isolated from the gel by the freeze-sweep method [32]. This DNA was digested with *Bam*HI and ligated in pET3d, which was previously digested with *Bam*HI and *Nco*I and also purified from the gel. The resulting plasmid was transformed to *E. coli* strain PC2495. In order to check the nucleotide sequence of the insert, double-strand sequencing was performed according to instructions supplied with the T7 sequencing kit on plasmid DNA that was isolated according to the boiling method [31] and extensively purified by using Prep-a-Gene purification matrix.

2.3. *Expression of pre-nsL-TP in* E. coli

In order to overexpress pre-nsL-TP in *E. coli*, pET3d containing pre-nsL-TP cDNA was transformed to *E. coli* strain BL21(DE3). A 10 ml culture, grown overnight in LB containing 50 μg/ml ampicillin, was centrifuged for 10 min at 2000 × *g*. The pellet was resuspended in 3 ml of LB and used to inoculate 1 l of LB containing 50 μg/ml ampicillin which was prewarmed to 37°C prior to inoculation. Bacteria were grown at 37°C while shaking. At an A_{600} of 0.5 the culture was induced with 0.5 mM IPTG and grown for an additional 3 h.

2.4. *Purification of pre-nsL-TP*

All manipulations were performed at 4°C. Bacteria were harvested by centrifugation at 5000 × *g* for 10 min in a Sorvall RC2-B centrifuge. The pellet was washed once with buffer A (50 mM Tris-HCl, pH 8.0, 2 mM EDTA, 10 mM β-mercaptoethanol) and then resuspended in 25 ml buffer A. The resuspended cells were sonicated in portions of 5 ml for 4 × 30 s at 50 W output with the broad tip of a Sonifier B12 (Branson Sonic Power Co., Connecticut). The suspension was spun at 30,000 × *g* for 30 min in an ultracentrifuge (Beckman Spinco L2 65B). The supernatant was applied to a DEAE-cellulose column (12 × 2.5 cm) equilibrated with buffer A. The column was rinsed with buffer A at a flow rate of 30 ml/h (fractions of 10 ml). Pre-nsL-TP eluted in the run-through. The fractions containing pre-nsL-TP were pooled, put into a dialysis bag and concentrated to 8 ml by using solid polyethyleneglycol 2000. Aliquots of 4 ml were applied to a Sephadex G-50 column (110 × 1 cm) and the proteins were eluted with buffer B (50 mM Tris-HCl, pH 7.0, 2 mM EDTA, 10 mM β-mercaptoethanol) at a flow rate of 4 ml/h (fractions of 1 ml). Purification of pre-nsL-TP was followed by SDS-PAGE. Immunoblotting was carried out on gradient gels (7.5–25% acrylamide) [20]. Protein concentration was determined by a microtiter-plate version of the Lowry method [33].

2.5. *Fluorescence lipid transfer assay*

Transfer activity of pre-nsL-TP was determined by measuring the transfer of Pyr(6)PC from quenched donor vesicles to unquenched acceptor vesicles as described in [34].

3. RESULTS

The DNA fragment encoding pre-nsL-TP was obtained by PCR. As shown in Fig. 1, the PCR product was void of contaminating fragments. After digestion with *Nco*I and *Bam*HI, the ensuing DNA fragment was ligated in pET3d and transformed to *E. coli* strains PC2495 and BL21(DE3). Double-stranded sequencing of the plasmid DNA isolated from PC2495 demonstrated that the nucleotide sequence of the insert was identical to that of pre-nsL-TP. After induction with IPTG, pre-nsL-TP was effectively expressed in *E. coli* BL21(DE3) as shown by submitting total cell protein to SDS-PAGE (Fig. 2, lanes 1 and 2). It was observed that bacteria containing the plasmid grew somewhat slower than untransformed cells. Pre-nsL-TP was very prominently present in the bacterial lysate (Fig. 2, lane 3). The protein was purified from the lysate by anion exchange chromatography on DEAE-cellulose and gelfiltration on Sephadex G-50. At pH 8.0, most of the bacterial proteins bind to the DEAE-cellulose column, while pre-nsL-TP and minor amounts of contaminating proteins appear in the run-through (Fig. 2, lane 4). After gelfiltration, the protein was pure (Fig. 2, lane 5). In agreement with the presequence of 20 amino acid residues, pre-nsL-TP has a distinctly higher molecular weight than nsL-TP (i.e. an estimated mol. wt. of 16 and 14 kDa, respectively; see Fig.2, lanes 5 and 6). From 1 l of culture 6–7 mg pre-nsL-TP was obtained. It was

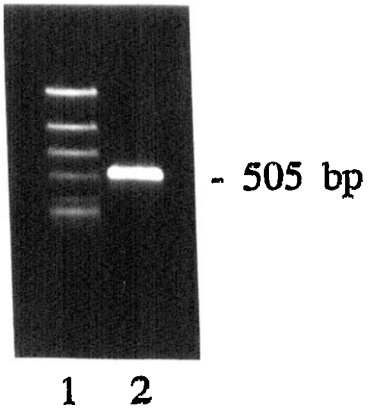

Fig. 1. Analysis of the PCR product encoding pre-nsL-TP on a 1.5% agarose gel. (Lane 1) φx-174-RF/*Hin*cII (marker, Pharmacia: 1057, 770, 612, 495, 392, 340, 295 bp); (lane 2) 1/25 of the PCR product (4 μl).

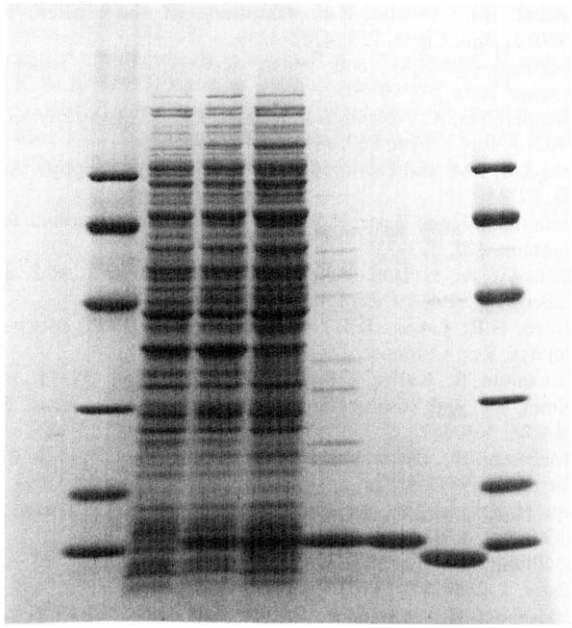

Fig. 2. The purification of rat liver pre-nsL-TP from *E. coli* as shown by SDS-PAGE. The gel was stained with Coomassie brilliant blue. (Lane 1) BL21(DE3) culture before induction with IPTG (0.5 ml); (lane 2) BL21(DE3) culture 3 h after induction with IPTG (0.25 ml); (lane 3) bacterial lysate (90 μg protein); (lane 4) DEAE column eluate (13 μg protein); (lane 5) purified rat liver pre-nsL-TP after G50 gel filtration (10 μg protein); (lane 6) pure rat liver nsL-TP (10 μg protein); (lane M) mol. wt. markers (Bio-Rad: 97.4, 66.2, 45.0, 31.0, 21.5 and 14.4 kDa).

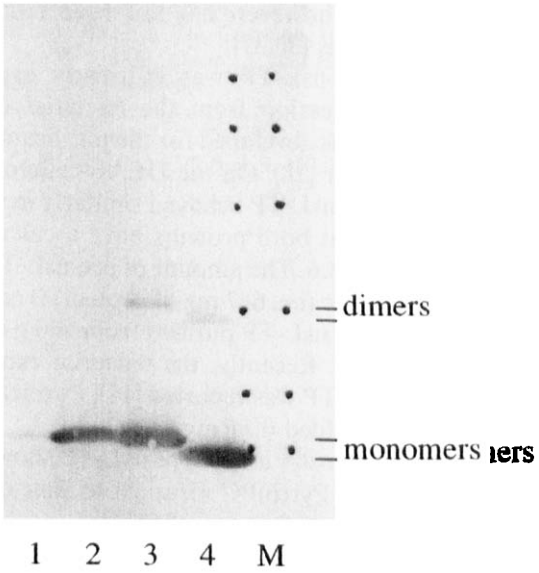

Fig. 3. Immunoblot analysis of rat liver pre-nsL-TP expressed in *E. coli*. (Lane 1) BL21(DE3) culture before induction with IPTG (0.5 ml); (lane 2) BL21 (DE3) culture 3 h after induction (0.25 ml); (lane 3) pure rat liver pre-nsL-TP (10 μg); (lane 4) pure rat liver nsL-TP (10 μg); (lane M) mol. wt. markers, as in Fig. 2.

estimated that after 3 h of induction approximately 7% of the cytoplasmic protein is pre-nsL-TP. Western blot analysis of the bacterial culture before and after induction showed that induction gave rise to one very prominent immunoreactive band representing pre-nsL-TP (Fig. 3, lanes 1 and 2). Immunoblotting of pure pre-nsL-TP and nsL-TP yielded 1 major band (Fig. 3, lanes 3 and 4). In addition, 1 minor band was observed at mol. wt. 32 and 29 kDa, respectively. This band most likely represents the dimers of pre-nsL-TP and nsL-TP, in agreement with previous observations [6,8,28].

In a continuous fluorescence transfer assay, pre-nsL-TP was found to have lipid transfer activity (Fig. 4). The activity per mg protein as estimated from the slope of the progress curve, was identical to that of nsL-TP (data not shown). Also in lipid monolayer experiments (see [35]), pre-nsL-TP behaved identical to nsL-TP (Dr. R.A. Demel, personal communication).

4. DISCUSSION

Pre-nsL-TP has an N-terminal extension of 20 amino acid residues, i.e. Met-Gly-Phe-Pro-Glu^{5}-Ala-Ala-Ser-Ser-Phe^{10}-Arg-Thr-His-Gln-Ile^{15}-Ser-Ala-Ala-Pro-Thr^{20}. Based on cDNA analysis, it was found that the complete amino acid sequence of pre-nsL-TP (15 kDa) is present at the C-terminal end of a 58-kDa protein [23,36,37]. However, pre-nsL-TP and the 58-kDa protein are synthesized from separate mRNA's. In this study, the cDNA encoding the 58-kDa protein was used in a PCR experiment to amplify the pre-nsL-TP encoding sequence. In a previous study [38], we have reported that an incomplete cDNA clone encoding pre-nsL-TP, coded for Ser instead of Phe at position 10 of the pre-

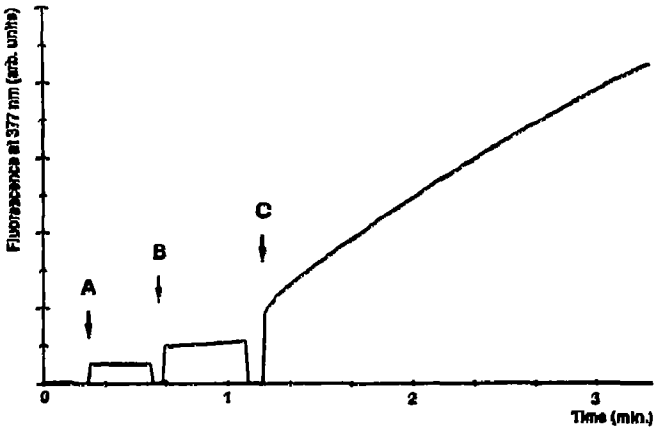

Fig. 4. Transfer of Pyr(6)PC from donor to acceptor vesicles by rat liver pre-nsL-TP as measured in a continuous fluorescence transfer assay. (Arrow A) Addition of 100 nmol acceptor vesicles consisting of PC and PA (95:5 mol%); (arrow B) addition of 2 nmol donor vesicles consisting of Pyr(6)PC and TNP-PE (90:10 mol%); (arrow C) addition of pre-nsL-TP (5 μg). Measurements were done at 25°C in a total volume of 2 ml. The assay buffer consisted of 20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA. The pyrene fluorescence was recorded at excitation 346 nm (bandpass 2.5 nm) and emission 377 nm (bandpass 10 nm).

sequence. This, however, has not been confirmed by other laboratories [36,37].

Rat liver pre-nsL-TP was efficiently expressed in *E.coli*. Its purification from the bacterial lysate was based on methods developed for the purification of nsL-TP from rat liver [28]. On the DEAE-cellulose column pre-nsL-TP and nsL-TP behaved similarly in agreement with the fact that both proteins have a calculated isoelectric point of 8.6. The amount of pre-nsL-TP purified from 1 l of culture (i.e. 6–7 mg of protein) is comparable to the amount of nsL-TP purified from 500 g of rat liver (50–60 rats) [28]. Recently, the bacterial expression of mouse liver nsL-TP was reported [11]. Purification from 1 l of culture yielded 0.11 mg of nsL-TP.

In the lipid transfer assay, pre-nsL-TP shows transfer activity towards Pyr(6)PC identical to that of nsL-TP. This indicates that cleavage of the pre-sequence is not necessary to activate the enzyme. In the absence of peroxisomes pre-nsL-TP is rapidly degraded [25]. This strongly suggests that processing of pre-nsL-TP is required to obtain a stable protein.

It was noted that the sequence Ala-Ala-Pro present in the pre-nsL-TP presequence at position −4 to −2 from the processing site and the Ser residue present at position +1 are also present in rat liver peroxisomal 3-oxoacyl-CoA thiolase, another peroxisomal protein which is synthesized as a precursor [39,40]. This sequence identity around the cleavage site could mean that the same protease(s) is involved in the processing of pre-nsL-TP and the thiolase [37]. It is not yet clear whether proteolytic processing of pre-nsL-TP and thiolase is functionally coupled to import into peroxisomes. It was suggested that the presequence of nsL-TP could be a mitochondrial targeting signal [11,41]. In vitro import studies should be carried out to establish whether indeed the nsL-TP presequence is able to target the protein to mitochondria.

The expression of pre-nsL-TP in *E. coli* offers the possibility to obtain a sufficient amount of protein for crystallization and 3D structure determination. nsL-TP has a low affinity lipid binding site [34]. The location of this binding site in the protein is not yet known. Site-directed mutagenesis can yield information on the nature of the lipid binding side.

Acknowledgements: The authors wish to thank J. Westerman for providing the rat liver nsL-TP, and Dr. R. Peeters for helpful discussions on the expression system. This research was carried out under the auspices of the Netherlands Foundation for Chemical Research (SON) and with financial aid from the Netherlands Organization for Scientific Research (NWO).

REFERENCES

[1] Bloj, B. and Zilversmit, D.B. (1977) J. Biol. Chem. 252, 1613–1619.
[2] Crain, R.C. and Zilversmit, D.B. (1980) Biochemistry 19, 1433–1439.
[3] Noland, B.J., Arebalo, R.E., Hansbury, E. and Scallen, T.J. (1980) J. Biol. Chem. 255, 4282–4289.
[4] Poorthuis, B.J.H.M. and Wirtz, K.W.A. (1982) Biochim. Biophys. Acta 710, 99–105.
[5] Chanderbhan, R., Noland, B.J., Scallen, T.J. and Vahouny, G.V. (1982) J. Biol. Chem. 257, 8928–8934.
[6] Trzaskos, J.M. and Gaylor, J.L. (1983) Biochim. Biophys. Acta 751, 52–65.
[7] Westerman, J. and Wirtz, K.W.A. (1985) Biochem. Biophys. Res. Commun. 127, 333–338.
[8] Pastuszyn, A., Noland, B.J., Bazan, J.F., Fletterick, R.J. and Scallen, T.J. (1987) J. Biol. Chem. 262, 13219–13227.
[9] Morris, H.R., Larsen, B.S. and Billheimer, J.T. (1988) Biochem. Biophys. Res. Commun. 154, 476–482.
[10] Yamamoto, R., Kallen, C.B., Babalola, G.O., Rennert, H., Billheimer, J.T. and Strauss III, J.F. (1991) Proc. Natl. Acad. Sci. USA 88, 463–467.
[11] Moncecchi, D., Pastuszyn, A. and Scallen, T.J. (1991) J. Biol. Chem. 266, 9885–9892.
[12] Tan, H., Okazaki, K., Kubota, I., Kamiryo, T. and Utiyama, H. (1990) Eur. J. Biochem. 190, 107–112.
[13] Bouillon, P., Drischel, C., Vergnolle, C., Duranton, H. and Kader, J.-C. (1987) Eur. J. Biochem. 166, 387–391.
[14] Takishima, K., Watanabe, S., Yamada, M., Suga, T. and Mamiya, G. (1988) Eur. J. Biochem. 177, 241–249.
[15] Bernhard, W.R. and Somerville, C.R. (1989) Arch. Biochem. Biophys. 269, 695–697.
[16] Gould, S.J., Keller, G.-A. Hosken, N., Wilkinson, J. and Subramani, S. (1989) J. Cell Biol. 108, 1657–4110.
[17] Van der Krift, T.P., Leunissen, J., Teerlink, T., van Heusden, G.P.H., Verkleij, A.J. and Wirtz, K.W.A. (1985) Biochim. Biophys. Acta 812, 387-392.
[18] Tsuneoka, M., Yamamoto, A., Fujiki, Y. and Tashiro, Y. (1988) J. Biochem. (Tokyo) 104, 560–564.
[19] Keller, G.A., Scallen, T.J., Clarke, D., Maher, P.A., Krisans, S.K. and Singer, S.J. (1989) J. Cell Biol. 108, 1353–1361.
[20] van Heusden, G.P.H., Bos, K., Raetz, C.R.H. and Wirtz, K.W.A. (1990) J. Biol. Chem. 265, 4105–4110.
[21] van Heusden, G.P.H., Bos, K. and Wirtz, K.W.A. (1990) Biochim. Biophys. Acta 1046, 315–321.
[22] van Amerongen, A., Helms, J.B., van der Krift, T.P., Schutgens, R.B.H. and Wirtz, K.W.A. (1987) Biochim. Biophys. Acta 919, 149–155.
[23] Ossendorp, B.C., van Heusden, G.P.H., de Beer, A.L.J., Bos, K., Schouten, G.L. and Wirtz, K.W.A. (1991) Eur. J. Biochem. 201, 233–239.
[24] Fujika, Y., Tsuneoka, M. and Tashiro, Y. (1989) J. Biochem. (Tokyo) 106, 1126–1131.
[25] Suzuki, Y., Yamaguchi, S., Orii, T., Tsuneoka, M. and Tashiro, T. (1990) Cell Struct. Funct. 15, 301–308.
[26] Studier, F.W., Rosenberg, A.H., Dunn, J.J. and Dubendorff, J.W. (1990) Methods Enzymol. 185, 60–89.
[27] van Duijn, G., Dekker, J., Leunissen-Bijvelt, J., Verkleij, A.J. and de Kruijff, B. (1985) Biochemistry 24, 7640–7650.
[28] Poorthuis, B.J.H.M. and Wirtz, K.W.A. (1983) Methods Enzymol. 98, 592–596.
[29] Teerlink, T., van der Krift, T.P., van Heusden, G.P.H. and Wirtz, K.W.A. (1984) Biochim. Biophys. Acta 793, 251–259.
[30] Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. (1988) Science 239, 487–491.
[31] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) in: Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[32] Tautz, D. and Renz, M. (1983) Analyt. Biochem. 132, 14–19.
[33] van Amerongen, A., van Noort, M., van Beckhoven, J.R.C.M., Rommerts, F.F.G., Orly, J. and Wirtz, K.W.A. (1989) Biochim. Biophys. Acta 1001, 243–248.

[34] Gadella, Jr., T.W.J. and Wirtz, K.W.A. (1991) Biochim. Biophys. Acta 1070, 237–245.
[35] van Amerongen, A., Demel, R.A., Westerman, J. and Wirtz, K.W.A. (1989) Biochim. Biophys. Acta 1004, 36–43.
[36] Seedorf, U. and Assmann, G. (1991) J. Biol. Chem. 266, 630–636.
[37] Mori, T., Tsukamoto, T., Mori, H., Tashiro, Y. and Fujiki, Y. (1991) Proc. Natl. Acad. Sci. USA 88, 4338–4342.
[38] Ossendorp, B.C., van Heusden, G.P.H. and Wirtz, K.W.A. (1990) Biochem. Biophys. Res. Commun. 168, 631–636.
[39] Hijikata, M., Wen, J.-K., Osumi, T. and Hashimoto, T. (1990) J. Biol. Chem. 265, 4600–4606.
[40] Bodnar, A.G. and Rachubinski, R.A. (1990) Gene 91, 193–199.
[41] Billheimer, J.T., Strehl, L.L., Davis, G.L., Strauss III, J.F. and Davis, L.G. (1990) DNA Cell Biol. 9, 159–165.