Biol. Chem. Hoppe-Seyler
Vol. 369, pp. 585–593, July 1988

Expression of the Human Serum Apolipoprotein AI and AII Genes in *Xenopus laevis* Oocytes

Lipid-Associated Secretion of Gene Products

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(Received 22 April 1988)

Summary: The two major apolipoproteins of plasma high-density lipoproteins (HDL) are apolipoprotein AI (apo AI) and AII (apo AII).

The apo AI and the correctly oriented apo CIII genes separated by 2.6 kb were obtained by fusion of two human λ-genomic clones. The apo AII gene was isolated as a 3 kb clone. These apolipoprotein genes have been injected independently and together into *Xenopus laevis* oocytes and their expression studied. Both apolipoprotein genes were transcribed and translated into their preproforms and processed in *Xenopus laevis* oocytes to their proforms. They were secreted into the medium associated with newly synthesized phospholipids and neutral lipids as particles floating in the high-density lipoprotein range between 1.12 and 1.21 g/ml.

Secreted apo AI is associated mainly with newly synthesized phosphatidylethanolamine and little triglyceride, apo AII with phosphatidylethanolamine, lysophosphatidylethanolamine and neutral lipids. Simultaneous injection of the apo AI and apo AII genes led to the secretion of both apoproteins which separated into two bands during CsCl-density gradient centrifugation. The heavier particles were associated with proapo AI and AII, phosphatidylethanolamine (> 90%) and traces of lysophosphatidylethanolamine as lipid components. Proapo AII was immunoprecipitated from the less dense fraction and found to be mainly associated with lysophosphatidylethanolamine. Radiolabelled newly synthesized apolipoproteins in secreted particles were characterized by immunoprecipitation after delipidation of the secreted lipoprotein particles. The oocyte-system proved very suitable for studies of the expression of serum apolipoprotein genes, the assembly of the apolipoproteins with specific lipids to lipoprotein particles and their secretion.

Expression der menschlichen Serum-Apolipoproteingene AI und AII in Xenopus-laevis-*Oozyten. Lipid-assoziierte Sekretion der Genprodukte*

Zusammenfassung: Die beiden Hauptproteine der High-Density-Lipoproteine des menschlichen Serums sind Apolipoprotein AI (Apo AI) und AII (Apo AII).

Das Apo-AI-Gen zusammen mit dem in der richtigen Orientierung (3′-3′) befindlichen, durch 2.6 kb getrennten Apo-CIII-Gen wurden durch Fusion zweier menschlicher genomischer Klone erhalten. Das Apo-AII-Gen wurde als 3 kb großer Klon isoliert. Diese beiden Gene wurden unabhängig und gemeinsam zur Expression in *Xenopus-laevis*-Oozyten injiziert. Beide Apolipoproteingene wurden in Oozyten transkribiert, zu den Präproformen translatiert, zu den Proformen prozessiert und dann in das Medium sezerniert. Mit Phospholipiden und neutralen Lipiden assoziert flottierten sie als Lipoproteinkomplexe im HDL-Dichtebereich 1,12–1,21 g/ml.

Abbreviations:
HDL, High-density lipoproteins; apo, apolipoprotein; PC, phosphatidylcholine; PE, phosphatidylethanolamine; lyso-PE; lysophosphatidylethanolamine; NL, neutral lipids.

Das sezernierte Proapo-AI ist hauptsächlich mit neu synthetisiertem Phosphatidylethanolamin und wenig neutralen Lipiden assoziert, Proapo-AII mit Phosphatidylethanolamin, Lysophosphatidylethanolamin und Neutrallipiden. Gleichzeitige Injektion beider Apolipoprotein-Gene führte zur Sekretion von Proapo-AI- und Proapo-AII-haltigen Partikeln, die durch CsCl-Gradienten-Ultrazentrifugation in zwei Banden aufgetrennt wurden. Die schwereren Partikel enthielten Proapo-AI und -AII, das mit Phosphatidylethanolamin (90%) und Spuren von Lysophosphatidylethanolamin assoziiert war. Proapo-AII wurde durch Immunpräzipitation aus der leichteren Fraktion isoliert. Dort liegt es mit neusynthetisiertem Lysophosphatidylethanolamin gebunden vor. Die radioaktiv markierten, neusynthetisierten Apolipoproteine in den Lipoproteinpartikeln wurden nach Delipidierung durch Immunpräzipitation charakterisiert. *Xenopus-laevis*-Oozyten erwiesen sich als ein für Expressionsstudien, die Assoziation mit Lipiden und die anschließende Sekretion der menschlichen Serumlipoproteine äußerst geeignetes System.

Key words: Human genomic clones of apo AI and AII, oocyte expression; protein processing; protein secretion; lipid association.

Plasma high-density lipoproteins (HDL) represent a population of spherical particles about 100 Å in diameter. The two main apolipoproteins, AI and AII, are assembled with phospholipids and cholesterol on the exterior surface and cholesterol esters and triacylglycerols as hydrophobic core. HDL subspecies undergo a different loading with cholesterol-esters as a consequence of its ability to take up cholesterol from peripheral tissue for transport to the liver where it is transformed to bile acids or secreted to the biliary system unchanged. Apo AI functions as activator of the lecithin cholesterol acyltransferase (LCAT) in this centripetal cholesterol transport. Serum lipoprotein lipase activation has been suggested as a function of apo AII in addition to its lipid-binding properties. Apo AI is synthesized in the liver and intestine at apparently the same rate[1–3]. Plasma mature apo AI is a 243-amino-acid-residues polypeptide. The in vitro primary translation product of liver and intestinal apo AI-specific poly(A)$^+$ mRNA of rat and human liver is a 267 amino-acid residue polypeptide, 18 of which resemble a prepeptide sequence, which is cotranslationally cleaved to proapo AI extended by the six amino-acid residues long N-terminal prosequence. The proform of apo AI is intracellularly stable and cleaved by a specific plasma protease only after secretion[1,2,19]. The mature apo AII is a dimer of two identical 77-residue long peptides which are linked by a disulfide bridge between the two Cys^6[4]. Apo AII synthesis so far has only been demonstrated in liver. Similarly to apo AI the primary translation product of apo AII carries a 23-amino-acid preprosequence at the N-terminus of the 77-residue long mature polypeptide[5–7], 18 N-terminal residues of which are cleaved by a signal peptidase cotranslationally. Part of the remaining pentapeptide prosequence is cleaved intracellularly, the rest in the plasma compartment[5–7]. Posttranslationally Cys^6 of the mature monomeric form dimerizes to a disulfide bond.

The integrity of the HDL particle structure is preserved by the surface location of apo AI and AII predominantly as tandem repeats of amphipatic α-helices of 22 amino-acid residues each.

Whether apo AI and AII or both carry recognition sites for a putative HDL receptor remains to be elucidated. Whereas the liver and intestinal origin and the processing of apo AI and AII is firmly established, little is known about the assembly and structure of the primary secretion particle, whether these apolipoproteins are secreted solely as polypeptides or associated with preexisting or newly synthesized cellular phospholipids and cholesterol thus forming a precursor particle of HDL. Furthermore the regulation of apo AI and AII synthesis is of utmost importance for cholesterol homeostasis.

In this study the *Xenopus laevis* oocyte system has been established as a suitable system for apo AI and AII gene expression and secretion process. In addition the assembly and secretion of apo AI and AII as lipoprotein-like particles has been studied.

Materials and Methods

Isolation and characterization of genomic apo AI and AII clones

300 000 clones of a human λ-genomic library[8] were screened with two [^{32}P]-labelled oligonucleotides (38^{mer} and 48^{mer}) probing the 5′ and 3′ region of the coding exons IV of the apo AI gene[9] and a 52^{mer} and 54^{mer} probing exon III and IV of the apo AII gene[10], respectively. Two identical apo AI clones were isolated as 10 kb *Eco*RI fragments, the 2.2 kb *Pst*I fragment of

which was subcloned into the plasmid pUC19 for double-strand nucleotide sequence analysis using the two universal M13 primers and an apo AI-specific 20^{mer} oligonucleotide[11]. The nucleotide sequence and restriction map of the apo AI gene in the *Pst*I fragment agreed completely with the previously published data[9]. The apo AII gene was isolated from the apo AII clone on a 3 kb *Hpa*II fragment, cloned into the compatible *Acc*I site of the polylinker of pUC19 and characterized by restriction analysis and double-strand sequence analysis. Oligodeoxynucleotides were synthesized by a DNA synthesizer, Model 380 A, Applied Biosystems.

They were 5′-end labelled with [γ-^{32}P]ATP and T_4-polynucleotide kinase[12] and used for filter and blot hybridization analysis as described before[13].

Total RNA from lysed proteinase K-treated oocytes was obtained as described before[14]. RNA was fractionated by electrophoresis in 1% agarose gels containing formaldehyde in 40mM Mops (4-morpholinopropanesulfonic acid) pH 7.0 at 2 V/cm[15]. RNA was transferred to a gene screen membrane by capillary blotting for Northern blot analysis according to the manufacturer's prescription.

Southern blot analysis of restriction fragments separated by agarose gel electrophoresis on gene screen filters and their hybridization with labelled oligonucleotides followed the prescription of the manufacturer.

Protein analyses

Lipoproteins secreted into the incubation medium were separated by density gradient centrifugation: 22% (w/w) CsCl, Beckman SW41, 38 000 rpm, 72 h. Each gradient was fractionated into 1-m*l* fractions and their density and the distribution of radioactivity determined. After desalting by Sephadex G-25 apo AI and AII were immunoprecipitated from the oocyte medium with rabbit anti human apo AI and AII IgG and separated by electrophoresis in polyacrylamide gradient gels (15–20%) with human apo AI and AII/2 as standards[1].

Lipid analyses

For lipid analyses the gradient fractions were extracted with chloroform-methanol[16], the combined organic phases washed with water, dried over Na_2SO_4 and concentrated under vacuum for thin-layer chromatography. Phospholipids were separated in the solvent system chloroform/methanol/water (65:25:4) and neutral lipids in ether/hexane/acetic acid (30:70:1). Labelled lipids were detected by radioscanning and/or autoradiography. Autoradiograms were analysed by densitometry, Densitometer Ultroscan XL, LKB.

Preparation of oocytes and microinjection

Limited removal of ovary tissue from *Xenopus laevis* and preparation of oocytes for injection by enzymatic

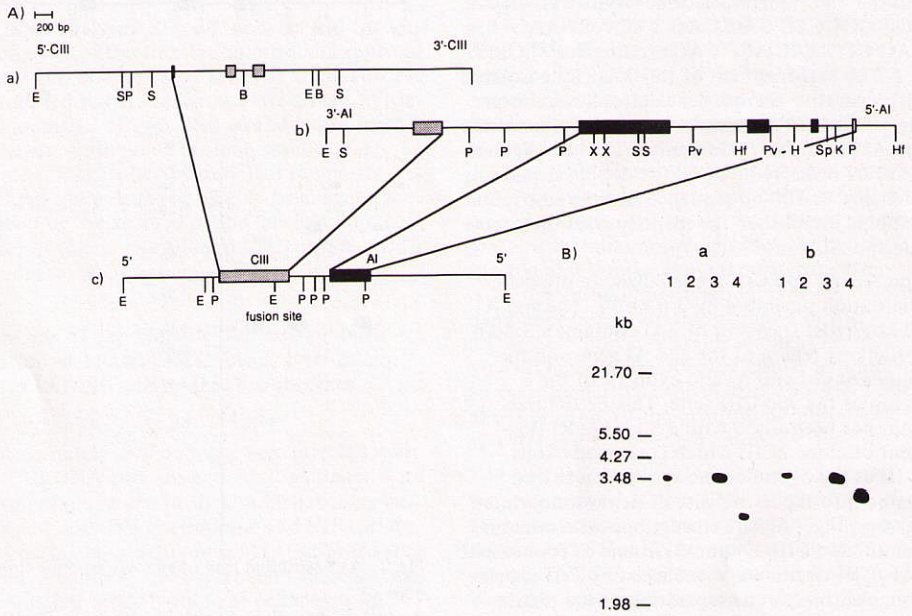

Fig. 1. A) Restriction maps of: a) λAI-clone, b) pCIII-clone, c) correctly fused apo AI-CIII gene.
E, *Eco*RI; B, *Bam*HI; Hf, *Hinf*I; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; S, *Sac*I; Sp, *Sph*I; X, *Xho*II.

B) Southern blot hybridization analysis of fusion λAI and λCIII *Eco*RI fragments restricted with *Pst*I.
a) 1–4, clones; b) 1–4, fusion products.
Hybridization with oligonucleotides: a) CIII-29, probing the exon IV of the apo CIII gene on the λAI clone (10 kb *Eco*RI fragment); b) CIII-36, probing exon III of the apo CIII gene contributed by pCIII (3 kb) to the fused λAI-CIII clone. The *Pst*I fragment of clones 1 and 3 hybridizes with both oligonucleotides proving the expected 3′-3′-fusion.

"stripping" with collagenase was essentially carried out as described before[17]. Micropipettes were produced with the vertical pipette puller, Kopff. The micromanipulator (Fa. Leitz, Wetzlar) with a hand-operated, motor-driven micrometer syringe was used. In general about 50 nl of DNA solution (appr. 10 ng DNA) were injected per nucleus. For RNA isolation appr. 25 to 40 oocytes were injected. Oocytes were incubated at room temperature with Barth's medium supplemented with 10 µg/ml penicillin, 10 µg/ml streptomycin and 5 kU/ml Trasylol. RNA was extracted 24 h after DNA injection.

Radioactive precursors, [^{35}S]methionine and [^{35}S]-cysteine and sodium [1-^{14}C]acetate, were added in Barth's solution 24 h after the DNA injection (300 µl/25 oocytes) and incubated for 10–12 h for translation in an Eppendorf tube.

Results

Isolation and characterization of genomic apo AI/CIII clones

Two identical genomic apo AI-specific clones were isolated by screening a human genomic library[8] with the 38^{mer} oligonucleotide (AGG GCC TGA GGG CAG GAG ATG AGC AAG GAT CTG GAG GA) coding Asp^{105}-Asp^{117} and the 48^{mer} oligonucleotide (AGC TTC CTG AGC GCT CTC GAG GAG TAC ACT AAG AAG CTC AAC ACC CAG) coding Ser^{229}-Gln^{243}. A 2.2 kb *Pst*I-fragment of this λAI clone isolated by preparative agarose gel electrophoresis from the 10 kb *Eco*RI genomic fragment harbored the apo AI gene. The *Pst*I fragment has been ligated into *Pst*I restricted pUC19 for double-strand sequencing. A 500-bp sequence and the restriction mapping established the identity with the genomic apo AI clone[9].

Apo AI and apo CIII are localized in opposite orientation separated by 2.6 kb[18]. The apo AI 10-kb *Eco*RI fragment of λAI contains a 3.5-kb 5′-flanking region of the apo AI gene and the four exons of apo AI and exon IV of the 3′ region of the apo CIII gene. This *Eco*RI fragment has been fused with a 3 kb *Eco*RI fragment of clone λCIII which contained exons I–III at the 5′ end of the apo CIII gene and ligated into the *Eco*RI site of dephosphorylated λgt wes, Fig. 1A. The correct opposite orientation (3′ apo CIII-3′ apo AI) in one of four fused λAI–CIII clones was established by *Pst*I restriction, electrophoretic separation of the restriction fragments in agarose gels and Southern blot hybridization with specific synthetic oligonucleotides (Fig. 1B). A CIII 29^{mer} homologous to a coding region of exon IV of the apo CIII sequence within the λAI clone (3′ end) and oligonucleotide CIII 36^{mer} probing the 5′ coding sequence of the apo CIII gene were used. Only a correct fusion of both *Eco*RI fragments, which will release the 3.6-kb *Pst*I fragment, hybridizes with both oligonucleotides.

Apo AII-genomic clone

Plaque hybridization of the same human λ-genomic library yielded one clone λAII which after plaque purification hybridized with oligonucleotides complementary to the four exons of the apo AII gene[10]. A 3-kb *Hpa*II fragment was isolated from λAII and cloned into the compatible *Acc*I site of the multicloning site of pUC19 yielding clone pAII. The restriction map and Southern blot hybridization analysis is given in Fig. 2.

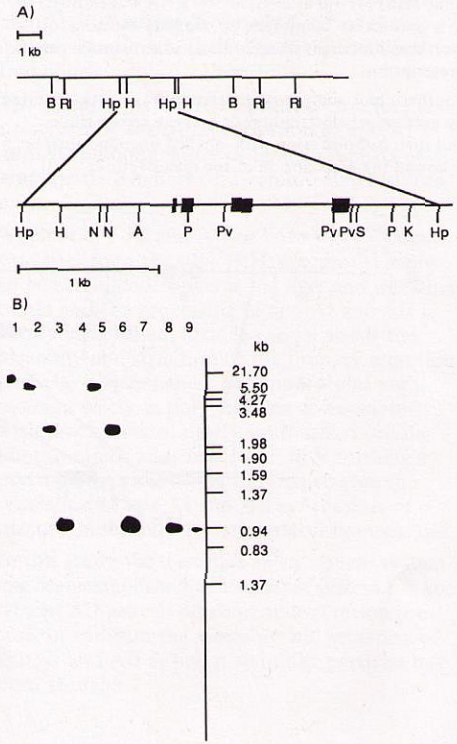

Fig. 2. A) Restriction map of apo AII genomic clone λAII.

A, *Ava*I; B, *Bam*HI; E, *Eco*RI; Hp, *Hpa*II; H, *Hin*dIII; K, *Kpn*I; N, *Nco*I; P, *Pst*I; Pv, *Pvu*II; S, *Sac*I.

B) Southern blot hybridization analysis of restriction fragments of λAII with apo AII homologous oligonucleotide AII-52.

Marker λ/*Hin*dIII/*Eco*RI at the right. 1, *Bam*HI; 2, *Eco*RI; 3, *Hin*dIII; 4, *Pst*I; 5, *Eco*RI/*Bam*HI; 6, *Bam*HI/*Hin*dIII; 7, *Bam*HI/*Pst*I; 8, *Eco*RI/*Pst*I; 9, *Hin*dIII/*Pst*I.

Further confirmation of the identity of the pAII clone was obtained by partial double-strand sequencing. The sequence data reveal the promoter region between − 70 to − 80 upstream with the CAAT consensus sequence 40 bases upstream from the TATA box and an inverted repeat between these two positions. The 5'- and 3'-flanking regions are each approximately 800 bp long.

Construction of a 5'-deleted apo AII clone (pAII ΔNco*)*

A deletion of 750 bp at the 5'-untranslated region of pAII was introduced by a combined restriction with *Nco*I and *Hin*dIII, filled in with Klenow fragment of DNA polymerase I and then blunt end ligated thus leaving only 250 bp upstream from the TATA box.

Apo AI and AII gene expression in Xenopus laevis *oocytes*

Oocyte production in *Xenopus laevis* was stimulated with gonadotropin and oocytes isolated as described under Materials and Methods.

For RNA analyses 20 to 30 oocytes and for the analysis of the translation products approximately 100 oocytes were injected and incubated in modified Barth's saline in Petri dishes, [^{35}S]-methionine (2 μCi/oocyte) was added to trace the newly synthesized protein, sodium [^{3}H]-acetate (20 μCi/oocyte) to detect the newly synthesized lipids in the double-labelling experiments. For the analysis of the labelled lipid classes of the oocyte sodium [^{14}C]acetate (2 μCi per oocyte) was used.

Three sets of experiments were carried out: injection of 1) λAI-CIII DNA, 2) pAII Δ*Nco* and 3) λAI-CIII and pAII Δ*Nco* together.

a) Expression of the apo AI gene

Approximately 100 oocytes were injected with λAI-CIII DNA and subsequently incubated with [^{35}S]methionine and in the double-labelling experiment with [^{35}S]methionine and [^{3}H]acetate. Oocytes injected with phosphate buffered saline served as control. The supernatant medium was fractionated by CsCl-gradient centrifugation (22%)[19]. An opalescent band at densities between 1.12 g/m*l* and 1.21 g/m*l* showed peak radioactivity. This was absent in the medium of the oocyte of the control experiment. ^{3}H-labelled lipids and ^{35}S-labelled proteins comigrated. The infranatant fraction contained only ^{35}S-labelled material.

Fractions with densities 1.12 g/m*l* to 1.21 g/m*l* were combined, desalted, delipidated and apo AI immunoprecipitated with anti-human apo AI IgG and analysed by gradient $NaDodSO_4$ polyacrylamide gel electrophoresis (15–20%) and autoradiography. The position of the labelled apo AI band is at a slightly higher molecular mass than that of the mature apo AI standard (Fig. 3a) identical with the position of proapo AI as shown in previous experiments[1,5].

b) Expression of the apo AII gene

To ascertain the correct transcription of the apo AII gene which is flanked by procaryotic sequences, λAII and pAII Δ*Nco* DNAs were injected and the RNA analysed by Northern blot hybridization analysis. The λAII and pAII-DNA yielded transcripts exceeding in size the human apo AII-specific mRNA, which was however identical with the pAII Δ*Nco* DNA. Therefore only the pAII Δ*Nco* DNA was used for further expression and analysis of the translation product.

Analogous to the apo AI experiment the oocytes injected with pAII Δ*Nco* DNA secreted radioactive protein which appeared in an opalescent band upon density gradient centrifugation at densities 1.12 g/m*l* to 1.21 g/m*l*. Again in the control experiment this band was missing. Also the double-labelling experiments showed a result comparable with the λAI experiment indicating the comigration of ^{3}H-labelled lipids and ^{35}S-labelled apo AII. Apo AII was isolated from the mixture of newly synthesized secreted proteins by immunoprecipitation with monospecific anti human apo AII antiserum after reductive carboxymethylation. One radioactive band appeared in autoradiography of the $NaDodSO_4$ polyacrylamide gradient gel (15–20%). The band was located clearly at a molecular mass higher than the mature apo AII/2 but at the position of the proform of monomeric apo AII as demonstrated before[5,6] Fig. 3b.

λAI-CIII DNA and pAII Δ*Nco* were injected into the oocyte nucleus simultaneously. Both genes were correctly transcribed into the apo AI and apo AII mRNA among which the apo AI-specific RNA was the abundant RNA in Northern blot hybridization analysis (not shown). For protein and lipid labelling the oocytes were incubated in [^{35}S]methionine and sodium [^{3}H]acetate-containing medium. Density gradient ultracentrifugation of the medium revealed the congruent distribution of the radiolabelled apolipoprotein and lipid in lipoprotein particles banding at density 1.12 to 1.21 g/m*l*.

These fractions were divided for immunoprecipitation with rabbit anti human apo AI and anti apo AII followed by gradient $NaDodSO_4$ polyacrylamide gel electrophoresis (15–20%) and autoradiographic analysis. Fig. 3c indicates that apo AI and AII were secreted and banded together as lipoprotein particles upon density gradient ultracentrifugation. The two apoproteins were secreted as proforms clearly different in electrophoretic mobility from their mature forms used as marker proteins. These experiments do not allow to decide, whether the two apolipoproteins are associated in one particle.

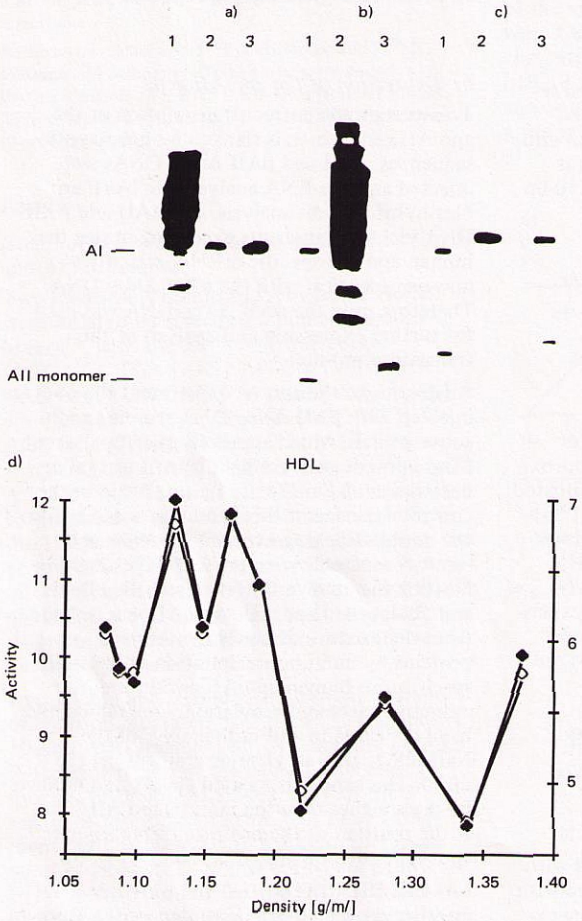

Fig. 3. a) Apo AI expression: Synthesis and secretion of proapo AI.
100 oocytes were injected with λAI-CIII DNA and incubated in supplemented medium. Proteins immunoprecipitated with anti apo AI antiserum from the medium were separated by $NaDodSO_4$ gradient (15–20%) polyacrylamide gel electrophoresis. Position of mature apo AI is indicated at the left of the fluorogram. $NaDodSO_4$ gradient polyacrylamide gel electrophoresis (15–20%) of
1) unfractionated, non-immunoprecipitated proteins in medium;
2) proapo AI immunoprecipitated from fractions with density 1.12 g/ml;
3) proapo AI immunoprecipitated from fraction with density 1.19 g/ml.
b) Apo AII expression: synthesis and secretion of proapo AII.
Xenopus laevis oocytes were injected with pAII Δ*Nco* DNA. The medium was fractionated by CsCl gradient centrifugation. The fractions with density 1.12–1.21 g/ml were delipidated and proapo AII isolated by immunoprecipitation, separated by 15–20% $NaDodSO_4$ gradient polyacrylamide gel electrophoresis and fluorographed.
1) Marker apo AI (22 kDa) + apo AII/2 (9 kDa);
2) labelled proteins in medium of untreated oocytes (control);
3) protein immunoprecipitated with anti human AII antiserum in fraction (1.10–1.16 g/ml).
c) Simultaneous expression of apo AI and apo AII genes: synthesis and secretion of proapo AI and proapo AII.
λAI-CIII and pAII Δ*Nco* DNAs were injected and the apolipoproteins isolated from the lipoprotein fraction of the density gradient fraction 1.12–1.21 g/ml after desalting by immunoprecipitation, $NaDodSO_4$ polyacrylamide gel electrophoresis (15–20%) and fluorography.
1) Marker proteins mature apo AI and AII/2.
2) proapo AI immunoprecipitated with anti human apo AI antiserum;
3) proapo AII immunoprecipitated with anti human apo AI and AII antisera.
d) Distribution of ^{35}S-labelled apo AI and AII and ^{3}H-labelled lipids in density gradient fractions of medium of *Xenopus laevis* oocytes injected with λAI-CIII and pAII Δ*Nco*, demonstrating cosegregation of apoproteins and lipids.
Empty symbols: ^{35}S; filled symbols: ^{3}H.

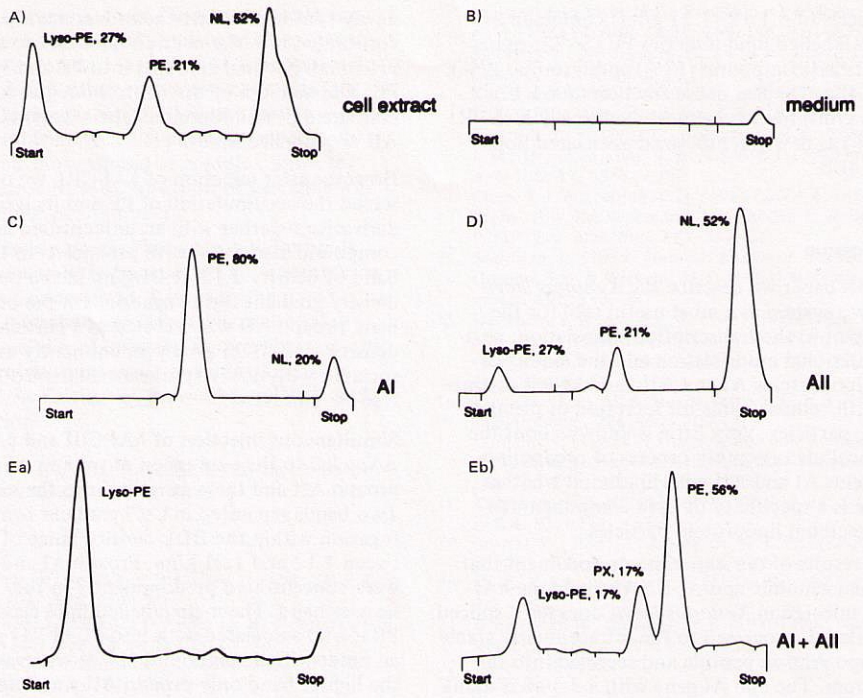

Fig. 4. Radio thin-layer chromatography of total lipids in the fractions of density range 1.12–1.16 and 1.19–1.21 g/ml of the medium of oocytes incubated in sodium [^{14}C]acetate- and [^{35}S]methionine-supplemented, lipid-free medium.

Lipid extract of A) control oocytes; B) total medium of control oocytes (injected with phosphate-buffered saline. Lipid extracts of medium of oocytes injected with C) λAI-CIII DNA; D) pAII Δ*Nco* DNA and E) λAI-CIII and pAII Δ*Nco* simultaneously; Ea) of lighter fraction; Eb) of heavier fraction (see text).

Apolipoproteins AI and AII are secreted as complexes with newly synthesized lipids

The lipid classes associated with newly synthesized and secreted apo AI and AII were analysed in similar experiments. λAI-CIII and pAII Δ*Nco* were injected into oocytes, incubated in Barth's medium supplemented with [^{35}S]methionine and sodium [^{14}C]acetate. The lipoprotein particles were isolated as described in the previous experiments.

Lipid extracts[16] of density gradient fractions of the two density ranges 1.12–1.16 and 1.19–1.21 g/m*l* were analysed separately by thin layer chromatography for neutral and phospholipids.

In the control experiments 100 untreated oocytes were incubated for 16 h with 50 μCi sodium [^{14}C]acetate. Total lipids of the oocytes and the medium were analysed for neutral and phospholipids by radio thin-layer chromatography. No labelled lipids were present in the medium of the control experiment (Fig. 4B).

The most abundant labelled lipids of the total lipid extract of the oocyte cell were lyso-PE (27%), PE (21%) and NL (52%) as documented in the densitometric tracing of the autoradiogram (Fig. 4A).

Oocytes expressing the apo AI gene secreted apo AI selectively associated with newly synthesized PE (80%) and NL (20%), Fig. 4C.

The injection of the pAII Δ*Nco* gene led to the secretion of lipoprotein particles (d = 1.12–1.21 g/m*l*) with apo AII complexed with lyso-PE (27%), PE (21%) and NL (52%), Fig. 4D.

The simultaneous expression of the apo AI and AII genes in *Xenopus laevis* oocytes induced the secretion of apolipoprotein particles with the proapo AI and AII, banding in the two density ranges 1.12–1.16 and 1.19–1.21 g/m*l*, proapo AI predominantly banding in the heavier and proapo AII in the lighter fraction. The lipids of the lipoprotein particles in the two density ranges were analysed separately. The heavier

particles (d = 1.19–1.21 g/m*l*) contained as main labelled lipid moieties PE (56%), an unidentified compound (17%) and lyso-PE (17%), Fig. 4Ea. The less dense fraction (d = 1.12–1.16 g/m*l*) revealed almost exclusively lyso-PE (92%) as newly synthesized associated lipid, Fig. 4Eb.

Discussion

In this paper we describe the *Xenopus laevis* oocyte system as a most useful tool for the study of a) the transcription, translation, post-translational modification and the export of apolipoproteins AI and AII and b) their assembly with cellular lipids for secretion of primary HDL particles. Very little is known about the intracellular assembly process of proapolipoproteins AI and AII with lipids and whether there is a specificity of lipid components in the secreted lipoprotein particles.

The results of our experiments document that human genomic apo AI DNA as in clone λAI-CIII injected in *Xenopus laevis* oocytes is spliced, translated, processed to the intracellularly stable proapo AI polypeptide and secreted into the medium. The apo AI gene with a 3.5-kb 5′-flanking region is expressed under its own upstream-regulatory sequences. We have not yet further studied the apo CIII expression in the oocyte system. Similarly apo AII was expressed to the proapo AII form and secreted as such. The secreted apolipoproteins isolated by immunoprecipitation had the exact electrophoretic mobility of the proforms identified earlier by radiosequencing[1,5,6,19]. The oocyte secretes the newly synthesized apolipoproteins AI and AII as proforms into the medium associated with oocyte-specific lipids. The *Xenopus* oocyte therefore expresses and secretes the foreign apolipoproteins identical to the Hep G2 cell, which normally secretes these HDL apolipoproteins.

The main phospholipids of oocytes amount to 13.5% and the neutral lipids to 86.5% of total lipids consisting of cholesterolesters (10%), cholesterol (20%) and triacylglycerols (56.5%). Cardiolipin amounts to 4%, PE to 4.6%, PC to 4.4%. Also traces of lyso-PE are present. We carried out labelling experiments of oocyte lipids with sodium [^{14}C]acetate during the incubation period following the injection of the apo AI and AII-specific DNAs in order to label the lipids newly synthesized during transcription and translation and identify the lipid classes secreted in association with the respective apolipoproteins. The radioactivity of newly synthesized fatty acids was mainly incorporated into the neutral lipids and to approximately equal amounts into PE and lyso-PE. The medium of the control oocytes neither contained [^{35}S]methionine-labelled apo AI and AII nor labelled lipids.

However after injection of λAI-CIII, we observed the accumulation of PE and its lyso-derivative together with an unidentified labelled compound associated with proapo AI in the band of density 1.12–1.21 g/m*l* in the CsCl density gradient centrifugation. On the other hand proapo AII was secreted as a particle of density 1.12–1.21 g/m*l* predominantly associated with newly synthesized labelled PE, lyso-PE and NL.

Simultaneous injection of λAI-CIII and pAII Δ*Nco* led to the expression of proapo AI and proapo AII and their secretion into the medium. Two bands separated in CsCl gradient centrifugation within the HDL density range of between 1.12 and 1.21 g/m*l*. Proapo AI and AII were concentrated predominantly in the heavier band. The main labelled lipid class was PE (56%) associated with lyso-PE (17%) and an unidentified compound (17%) whereas in the lighter band only proapo AII was detectable by immunoprecipitation but unlike in the heavier band almost exclusively lyso-PE was the complexed radiolabelled lipid with trace amounts of PE.

These results do not prove that two kinds of particles are assembled since the dissociation particularly of apo AII on gradient centrifugation has been observed. However two observations are remarkable: the secretion of proapo AI led to a preferential binding of newly synthesized PE whereas proapo AII bound preferentially neutral lipids associated with PE and lyso-PE. The assembly of the secreted proapo AI and AII and lipids is not a statistical process if one compares the lipids of the particles with those of the whole oocyte. This is particularly obvious for lyso-PE and PE. The resting oocyte contains small amounts of lyso-PE. The expression of foreign apolipoprotein genes apparently leads to the induction of the de novo synthesis of special phospholipids. The occurrence of lyso-PE as a component of the secreted particles raises the question whether this phospholipid with detergent properties is involved in a permeabilization of the oocyte plasma membrane for the extrusion of the lipoprotein particles, an event normally not occurring at this membrane. It is suggestive to assume an activation of a cellular phospholipase rather than assuming that lyso-PE resem-

bles an intermediate of PE de novo synthesis. This novel observation draws our attention to the expression and secretion of apolipoprotein AI and AII in other eucaryotic cells with different lipid patterns. It raises the question whether different cell types react specifically with their biosynthesis of complex lipids to the pressure of the expression of foreign apolipoprotein gene expression.

The assembly process between cellular lipids and intracellularly transported apolipoproteins is not yet understood. However, the oocyte system described here bears the potential of a very useful tool in serum lipoprotein research. Furthermore our results give additional support of the fidelity of transcription[20–24] and translation[25].

This work was generously supported by the *Deutsche Forschungsgemeinschaft*, SFB 74.

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