Involvement of the Ca^{2+} -dependent phosphorylation of a 20 kDa protein in the proliferative effect of high-density lipoproteins (subclass 3) on the adenocarcinoma cell line A549

Khalid A. TAZI, Monique BONNAFOUS, Gilles FAVRE, Georges SOULA and Francis LE GAILLARD*

Laboratoire de Ciblage en Thérapeutique (JE-DRED 175), Equipe de Biologie de la Cellule Tumorale, Faculté des Sciences Pharmaceutiques et Centre Claudius Regaud; 20–24, rue du Pont Saint Pierre, 31 052 Toulouse-Cedex, France

Previous studies from our laboratory demonstrated that high-density lipoproteins (subclass 3; HDL3) bind to sites specific for apolipoprotein AI on the human adenocarcinoma cell line A549 and that HDL3 binding promotes a mitogenic effect [Favre, Tazi, Le Gaillard, Bennis, Hachem and Soula (1993) *J. Lipid Res.* **34**, 1093–1106]. In the present study, we have examined the cell proteins that showed modified phosphorylation after binding of HDL3 to specific sites, and the roles of Ca^{2+} and protein kinase C. Native HDL3 (but not tetranitromethane-modified HDL3) and Ca^{2+} ionophore A23187 strongly enhanced the phosphorylation of a 20 kDa protein ($\times 3.6$) and, to a lower extent, the phosphorylation of 24 and 28 kDa proteins ($\times 2.2$ and 2.6 respectively). The two effectors were equally able to stimulate cell growth. Down-regulation of protein kinase C by a 24 h in-

cubation of cells with phorbol myristate acetate prevented the effects of HDL3 on the phosphorylation of 24 and 28 kDa proteins. However, the extent of phosphorylation of the 20 kDa protein was not affected. In contrast, activation of protein kinase C by a short incubation with phorbol myristate acetate resulted in inhibition of proliferation and an increase in 24 and 28 kDa (but not 20 kDa) protein phosphorylation. These results suggest that HDL3 putative receptors exert their proliferative effect on A549 cells through activation of a Ca^{2+} -dependent protein kinase. This kinase activity is not modulated by phorbol ester and thus may be a calmodulin kinase or an isoenzyme of protein kinase C that is independent of phorbol ester. It allows a subsequent 20 kDa protein to be phosphorylated.

INTRODUCTION

A major function attributed to high-density lipoproteins (HDLs) is the transport of excess cholesterol from peripheral tissues to the liver [1]. This can explain the inverse correlation between risk of coronary heart disease and plasma levels of HDL cholesterol [2]. In addition, HDLs have been reported to be mitogenic for several cell types [3–5].

The translocation of cholesterol from intracellular compartments to the plasma membrane requires the specific interaction of HDL apolipoproteins with high-affinity cell-surface receptors [6], but cholesterol efflux from endothelial cells can also be promoted by non-specific binding sites [7]. We have previously shown that the stimulation of proliferation of the human adenocarcinoma cell line A549 by HDL3 requires that lipoprotein particles bind to membrane protein sites specific for apolipoprotein AI; HDL3 binding to these sites promotes both cell proliferation and DNA biosynthesis [3].

For most cell types, this stimulation occurs in the absence of significant ligand internalization [8]. Thus there should be other ways for the HDL particles to induce their intracellular effects. HDLs may act through cell-surface receptors to activate an intracellular second-messenger pathway such as the cyclic AMP [9] and the phosphoinositide pathways [10]. No evidence of any initial intracellular event occurring on HDL binding to the cells has been demonstrated, and the nature of a possible second messenger involved in HDL action remains to be defined.

In the current study, we investigated the cellular signalling pathway by which HDLs are able to promote DNA synthesis in A549 cells. For this purpose, we looked at whether HDLs could

modulate protein phosphorylation. This mechanism plays an important role in the early biochemical events that occur during the mitogenic action of many growth factors [11] and tumour promoters [12].

MATERIALS AND METHODS

Materials

RPMI 1640 medium, foetal calf serum (FCS) and PBS were obtained from Seromed. Culture flasks (25 cm² and 75 cm²) and multiwell plates were obtained from Nunc. All the chemicals and solvents used were of analytical grade. 4 β -Phorbol 12-myristate 13-acetate (PMA) and Ca^{2+} ionophore A23187 were purchased from Sigma.

Cell culture

A549 cells were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). This epithelial-like cell line was initiated through explant culture of lung carcinoma tissue from a 58-year-old caucasian male. A549 cells were routinely grown in RPMI 1640 medium containing 3.5 mg/ml glucose and 2 mg/ml NaHCO_3 supplemented with 10% (v/v) FCS.

Cell-growth measurements

The cells were seeded at an initial density of 25000/ml in six-well multidishes and allowed to grow in RPMI 1640 supplemented

Abbreviations used: HDL3, high-density lipoproteins (subclass 3); PKC, protein kinase C; PMA, 4 β -phorbol 12-myristate 13-acetate; TNM-HDL3, tetranitromethane-modified HDL3; FCS, foetal calf serum; apoE, apolipoprotein E.

* To whom correspondence should be addressed.

with 10 % FCS in the absence (control) or presence of increasing concentrations of effectors. At indicated times, duplicate dishes were treated with trypsin and the cells counted using a haematocytometer.

Preparation of apolipoprotein E (apoE)-depleted HDL3

HDL3 (density 1.125–1.210 g/ml) were isolated from fresh human plasma by differential ultracentrifugal flotation [13]. They were then purified by chromatography on heparin-Sepharose (Pharmacia) to remove apoE-containing fractions [14]. ApoE-depleted HDL3 were dialysed against 10 mM Tris/HCl buffer, pH 7.4, containing 150 mM NaCl and 0.01 % disodium EDTA. HDL3 purity was assessed by SDS/PAGE [15]. Protein concentration was determined by the Bio-Rad protein assay reagent with BSA as standard.

Protein phosphorylation

At day 0, cells were seeded at 25000/well and allowed to grow in RPMI 1640 supplemented with 10 % FCS. At day 4, they were preincubated in RPMI 1640 alone for 24 h in order to remove lipoproteins and growth factors in FCS (this preincubation time does not allow cell synchronization). At day 5, cells were washed twice in Dulbecco's modified Eagle's medium, pH 7.4, containing 2 mg/ml BSA, and incubated for 3 h at 37 °C in 1 ml of the same medium containing 50 μ Ci of [32 P]phosphoric acid. Stimuli were then added for a further 30 min. Cells were washed twice with PBS buffer, and proteins were dissolved in 150 μ l of electrophoresis sample buffer containing 5 % (v/v) 2-mercaptoethanol. Samples were then boiled for 5 min. Proteins were separated by SDS/PAGE as described by Laemmli [15], fixed in cold trichloroacetic acid (12.5 %, v/v) and stained with Coomassie Blue. The gels were then dried and exposed to hyperfilm-MP (Amersham) for 24–72 h. The autoradiographs were scanned with an ultrascan 2202 laser densitometer (LKB). Incorporation of 32 P into a specific protein was evaluated by measuring the peak area above background on the densitometric tracings and dividing this value by the sum of the areas of other peaks not affected by the test agents. These values are given as arbitrary units. Results are expressed as percentages of the control value (100 %).

RESULTS

Effects of HDL3 binding on protein phosphorylation in A549 cells

A549 cells were labelled with $H_3^{32}PO_4$ for 3 h in Dulbecco's modified medium, and increasing concentrations of HDL3 (10–200 μ g of protein/ml) were then added over 30 min. The pattern of protein phosphorylation was analysed by SDS/PAGE and autoradiography. A typical pattern of phosphorylated proteins is shown in Figure 1. HDL3 markedly enhanced the phosphorylation of a protein band with an apparent molecular mass of 20 kDa and, to a lesser extent, the phosphorylation of 24 and 28 kDa protein bands. Densitometric scanning of the autoradiograph (Figure 1b) reveals that maximal 32 P incorporation into the 20, 24 and 28 kDa proteins was reached at a concentration of 20 μ g of protein HDL3/ml. This concentration has also previously been shown to be able to induce maximal stimulation of [H^3]thymidine incorporation into DNA and showed saturation of specific binding sites in these cells [3]. HDL3 (20 μ g of protein/ml) induced 3.7-, 2.1- and 2.3-fold increases in 32 P incorporation into the 20, 24 and 28 kDa proteins respectively in this experiment. Mean values $\pm$ S.D. of percentage phosphorylation obtained for quadruplicate experiments are shown in Table 1 (all these values are significantly higher than control; $P < 0.05$).

Figure 1 Effects of increasing HDL3 concentrations on protein phosphorylation in A549 cells

32 P incorporation was carried out as described in the Materials and methods section. A549 cells were incubated for 30 min in the absence [control (C)] or presence of increasing concentrations of HDL3 (from 10 to 200 μ g of protein/ml). Cell proteins were then analysed by SDS/PAGE (12 % gel) and autoradiography. (a) Autoradiograph of one representative experiment. Molecular mass was evaluated by the use of standard protein markers. The band present at the bottom of the gel co-migrated with the tracking dye and represents 32 P-labelled low-molecular-mass peptides. (b) Densitometric scan of the autoradiograph. 32 P incorporation into the 20 kDa protein (p20), 24 kDa protein (p24) and 28 kDa protein (p28) is expressed as percentage of the control value (100 %).

The effect of various incubation times when using a saturating dose of HDL3 over specific binding sites (20 μ g of protein/ml) was also studied. The increase in 32 P labelling (into the 20 kDa, 24 kDa and 28 kDa proteins) can be detected after a 5 min incubation and reaches a maximum after 30 min of treatment (results not shown).

As the maximum stimulation was obtained with an HDL3 concentration that allows saturation of specific binding sites, the question arises of whether these sites are implicated in signal transduction. In order to investigate this, HDL3 were modified with TNM, making them unable to bind to specific sites [16]. Moreover, such modified HDL3 have been shown to inhibit A549 cell growth [3]. When 20 μ g/ml TNM-modified HDL3 were substituted for native HDL3 under the same experimental conditions as in Figure 1, a stimulation of protein phosphorylation was not observed although a control with 20 μ g/ml native HDL3 had the same positive effect (Table 1). The stimulating effect of HDL3 on protein phosphorylation is therefore likely to be dependent on specific binding of HDL3 to

Table 1 Effects of HDL3 [native or modified with tetranitromethane (TNM), before or after prolonged treatment with PMA], 0.1 μ M PMA or 10 μ M Ca^{2+} ionophore A23187 on protein phosphorylation in A549 cells

Down-regulation of protein kinase C (PKC) was performed by preincubating A549 cells for 24 h in the presence of 0.1 μ M PMA; cells were then repeatedly washed in RPMI 1640 medium. ^{32}P incorporation was carried out as described in the Materials and methods section. Cells were incubated for 30 min in the absence (control) or presence of stimuli. Cell proteins were analysed by SDS/PAGE and autoradiography. ^{32}P incorporation into the 20 kDa protein (p20), 24 kDa protein (p24) and 28 kDa protein (p28) was expressed as a percentage of the control value (100%). Results are means $\pm$ S.D. of quadruplicate experiments for HDL3, PMA and Ca^{2+} ionophore or triplicate experiments for TNM-HDL3 and HDL3 after prolonged treatment with PMA.

Stimulus	Phosphorylation (%)		
	p 20	p 24	p 28
HDL3			
20 $\mu\text{g}/\text{ml}$	365 $\pm$ 17	216 $\pm$ 27	257 $\pm$ 35
200 $\mu\text{g}/\text{ml}$	353 $\pm$ 21	156 $\pm$ 26	172 $\pm$ 24
TNM-HDL3			
20 $\mu\text{g}/\text{ml}$	105 $\pm$ 21	106 $\pm$ 28	98 $\pm$ 16
200 $\mu\text{g}/\text{ml}$	115 $\pm$ 20	122 $\pm$ 26	164 $\pm$ 26
HDL3 after prolonged treatment with PMA			
20 $\mu\text{g}/\text{ml}$	310 $\pm$ 36	97 $\pm$ 23	100 $\pm$ 23
200 $\mu\text{g}/\text{ml}$	352 $\pm$ 28	109 $\pm$ 29	115 $\pm$ 28
PMA	114 $\pm$ 10	235 $\pm$ 38	250 $\pm$ 21
Ca^{2+} ionophore	360 $\pm$ 15	240 $\pm$ 20	265 $\pm$ 12

putative receptors. However, a high concentration of TNM-HDL3 (200 $\mu\text{g}/\text{ml}$) showed a small stimulation of phosphorylation of p24 and p28 that was not significantly different from that obtained with the same concentration of native HDL3 (122 compared with 156% and 164 compared with 172% respectively; $P > 0.05$) but a significant stimulation is only observed for p28 phosphorylation ($P < 0.05$). These results imply that, in contrast with p20 phosphorylation, p28 phosphorylation is dependent on both specific and non-specific binding of HDL3.

Phosphorylation of 20, 24 and 28 kDa proteins in the presence of PMA

As HDL3 have already been shown to stimulate PKC [6], we investigated the effect of PMA, a potent activator of PKC in A549 cells [17], on protein phosphorylation. As reported by others [18], exposure of A549 cells to PMA resulted in inhibition of cell proliferation. Under our conditions, incubation of A549 cells in the presence of increasing concentrations of PMA induces growth arrest. PMA does not induce an immediate toxicity in A549 cells because the number of cells after exposure to PMA did not decrease.

Like HDL3, PMA enhanced the degree of phosphorylation of 24 and 28 kDa proteins. However, in contrast with HDL3, PMA was unable to induce phosphorylation of the 20 kDa protein (Figure 2). Densitometric scanning of the autoradiograph revealed that PMA induced 1.1-, 1.8- and 2.2-fold increases in ^{32}P incorporation into the 20, 24 and 28 kDa proteins respectively. The values obtained with HDL3 were 3.8-, 1.7- and 2.1-fold respectively in this experiment (Figure 2b). The mean values $\pm$ S.D. of percentages obtained for quadruplicate experiments are shown in Table 1.

Figure 2 Effects of HDL3 and PMA on protein phosphorylation in A549 cells

^{32}P incorporation was carried out as described in the Materials and methods section. A549 cells were incubated for 30 min in the absence [control (C)] or presence of HDL3 (20 μg of protein/ml) or 0.1 μM PMA. Cell proteins were analysed by SDS/PAGE (12% acrylamide) and autoradiography; molecular mass was evaluated by the use of standard protein markers. (a) Autoradiograph. The band present at the bottom of the gel co-migrated with the tracking dye and represents ^{32}P -labelled low-molecular-mass peptides. (b) Densitometric scan of the autoradiograph. ^{32}P incorporation into the 20 kDa protein (p20), 24 kDa protein (p24) and 28 kDa protein (p28) is expressed as a percentage of the control value (100%). ■, p20; ▒, p24; □, p28.

Effects of HDL3 binding on protein phosphorylation after down-regulation of PKC

As a long-term exposure of A549 cells to phorbol esters leads to down-regulation of cellular PKC [17], it can be questioned whether the stimulation of the phosphorylation of 20, 24 and 28 kDa proteins by HDL3 is maintained in these conditions and what is the extent of this phosphorylation. To answer these questions, cells were pretreated for 24 h with 0.1 μM PMA, extensively washed, and incubated for 30 min in the presence of HDL3. Table 1 shows that down-regulation of PKC abolishes the effect of HDL3 on ^{32}P incorporation into the 24 and 28 kDa proteins but not into the 20 kDa protein. These results suggest that the 24 and 28 kDa proteins, in contrast with the 20 kDa protein, are physiological substrates for the PKC isoenzymes, the activity of which is down-regulated by PMA.

Figure 3 Time course of phosphorylation induced by a saturating dose ($10 \mu\text{M}$) of Ca^{2+} ionophore A23187

^{32}P incorporation was carried out as described in the Materials and methods section. A549 cells were incubated in the absence of Ca^{2+} ionophore A23187 [control (C)] or in the presence of $10 \mu\text{M}$ A23187 over 15–300 min. Cell proteins were analysed by SDS/PAGE (12% acrylamide) and autoradiography. (a) Autoradiograph; molecular mass was evaluated by the use of standard protein markers. The band present at the bottom of the gel co-migrated with the tracking dye and represents ^{32}P -labelled low-molecular-mass peptides. (b) Densitometric scan of the autoradiograph. ^{32}P incorporation into the 20 kDa protein (p20), 24 kDa protein (p24) and 28 kDa protein (p28) is expressed as a percentage of the control value (100%).

Effects of the Ca^{2+} ionophore A23187 on protein phosphorylation and cell proliferation

It is not clear whether stimulation of the phosphorylation of the 20 kDa protein is mediated by an activator that is able to stimulate all Ca^{2+} -dependent protein kinases. In an attempt to obtain evidence of the involvement of a Ca^{2+} -dependent protein kinase, the effect of the Ca^{2+} ionophore A23187 was examined. Like HDL3, this ionophore enhanced the phosphorylation of 20, 24 and 28 kDa proteins in a time- and dose-dependent manner. Figure 3 illustrates the effects of various incubation times on phosphorylation when a saturating concentration ($10 \mu\text{M}$) of A23187 was used. An increase in ^{32}P labelling was already observed after 5 min incubation and reached a maximum after 30 min of treatment. Densitometric scanning of the autoradiograph revealed that Ca^{2+} ionophore A23187 ($10 \mu\text{M}$) induced 3.7-, 2.3- and 2.6-fold increases in ^{32}P incorporation into the 20, 24 and 28 kDa proteins respectively for a 30 min incubation time in this experiment. The mean values $\pm$ S.D. of percentages obtained for quadruplicate experiments are shown in Table 1; they are not significantly different from those obtained with $20 \mu\text{g/ml}$ HDL3.

Figure 4 Effects of increasing concentrations of Ca^{2+} ionophore A23187 on the growth of A549 cells

At day 0, cells were seeded at 25 000/well and allowed to grow in RPMI 1640 supplemented with 10% FCS in the absence (control) or presence of increasing concentrations of Ca^{2+} ionophore A23187. After 6 days, cells from duplicate dishes were treated with trypsin and counted with an haematocytometer. Data are mean values $\pm$ S.D. of four measurements collected from one selected experiment.

We also investigated the effects of A23187 on cell proliferation; Figure 4 shows the effects of increasing concentrations of Ca^{2+} ionophore on cell proliferation. In contrast with PMA, A23187 induced a 2-fold stimulation in cell number, a maximum being reached at $10 \mu\text{M}$. At higher concentrations, cell proliferation was inhibited. This last effect might be explained by the toxicity of A23187 at high concentration. As cell death increases with Ca^{2+} concentration, high Ca^{2+} concentration may cause a Ca^{2+} overload which eventually leads to cell disorganization [19,20] and plasma-membrane disruption [19].

These results demonstrate that Ca^{2+} ionophore A23187 mimics the effects of HDL3 on both cell proliferation and protein phosphorylation.

DISCUSSION

The rate of cholesterol efflux as well as cell proliferation have been shown to be dependent on the extracellular concentration of HDL [4,16]. The precise mechanism of HDL action remains unknown, but a 110 kDa HDL protein receptor located on the surface of the cells has been fairly well characterized [21], and HDL binding to this receptor promotes stimulation of A549 cell proliferation [3]. In the present study, we attempted to elucidate the cellular signalling pathway used by HDL3 and we chose to determine whether protein kinase activity and subsequent protein phosphorylation are involved in the mitogenic effect of HDL3 in A549 cells.

The results show that HDL3 stimulated the phosphorylation of 20, 24 and 28 kDa proteins in A549 cells. This effect can be detected within 5 min of incubation and constitutes the earliest intracellular response to HDL3 reported so far in A549 cells. The stimulation of 20, 24 and 28 kDa protein phosphorylation required binding of HDL to specific sites, as TNM-HDL3, which do not bind to HDL3 specific sites, had no effect on protein phosphorylation although a low effect on the 28 kDa protein was observed at high concentration.

It has previously been shown that HDLs promote membrane-associated PKC activity which, in turn, phosphorylates a 27 kDa

protein involved in cell proliferation in vascular endothelial cells [22] and an 80 kDa protein involved in lactogen release in human placental cells [23]. HDL3 did not appear to stimulate the phosphorylation of 27 or 80 kDa proteins in A549 cells, although we cannot exclude the possibility that the 27 and 28 kDa proteins are the same. The fact that HDLs stimulate phosphorylation of 20, 24 and 28 kDa proteins in A549 cells but phosphorylation of a 27 kDa protein in vascular endothelial cells and an 80 kDa protein in placental cells suggests that HDLs have different effects on protein phosphorylation in different tissues. These differences in protein phosphorylation may account for the differences in the subsequent biological responses induced by stimulation of PKC activity. Activation of PKC is thought to be responsible for PMA-dependent growth inhibition of the adenocarcinoma cell line A549 [18]. Our results reinforce the hypothesis that PKC is involved in this biological response and strongly suggest that PMA-activated isoenzymes of PKC play a negative modulatory role in A549 cell proliferation. PMA treatment predominantly enhances incorporation of ^{32}P into 24 and 28 kDa proteins. These results suggest that PMA may inhibit growth of A549 cells via activation of PKC and subsequent protein phosphorylation.

In contrast with PMA, Ca^{2+} ionophore A23187, an activator of PKC and calcium protein kinase, was able to mimic the effects of HDL3 on both cell proliferation and protein phosphorylation. The differences observed between the effects of PMA and A23187 (on both cell proliferation and protein phosphorylation) suggest that the 20 kDa protein is a physiological substrate for a Ca^{2+} -dependent kinase in contrast with phorbol-activated isoenzymes of PKC [24]. Binding of HDL3 to their receptor on the A549-cell surface may induce the mobilization of intracellular Ca^{2+} and thus stimulation of the kinase. As the lipid composition of membranes is linked to the regulation of membrane-bound enzymes, transporters and ion channels [25], the direct interaction of HDL3 with the plasma membrane could induce changes in membrane lipid domains and trigger different metabolic events, such as Ca^{2+} transport, membrane phospholipase activity and then second-messenger signalling pathways. These events could be responsible for a wide range of cellular responses triggered by HDL, such as enhancement of intracellular Ca^{2+} [26], production of diacylglycerol in adipocyte cells [10] and stimulation of PKC activity [6,20].

Our data demonstrate the stimulation of phosphorylation of 20, 24 and 28 kDa proteins by the HDL3-specific binding sites is mediated by Ca^{2+} -dependent kinases. However, the mitogenic effect of these sites on A549 cells is related to the 20 kDa protein only. Phosphorylation of the 24 and 28 kDa proteins seems to be mediated by a phorbol-activated PKC. In contrast, phosphorylation of the 20 kDa protein seems to be mediated by a calmodulin kinase or an isoenzyme of PKC the activity of which is not modulated by phorbol ester [24]. In A549 cells, only the ζ isoenzyme of PKC is not down-regulated by PMA [27]. As this isoenzyme is Ca^{2+} -independent, it is unlikely that the 20 kDa protein is phosphorylated by PKC.

Work is in progress in our laboratory aimed at understanding the intracellular mechanism of the HDL3 mitogenic effect in A549 cells, especially by Ca^{2+} homeostasis and calmodulin-dependent kinase activity. The question also arises of whether this mechanism is related to the well-known HDL3 involvement in cell cholesterol homeostasis. It is not clear whether this mitogenic activity of HDL might have physiological or patho-

logical consequences; a high level of plasma HDL has been shown to correlate with an increased risk of breast cancer [28]. Total HDL concentration in extravascular fluids and in blood plasma is likely to be 10–100 times higher [29] than the concentration of HDL3 required to saturate specific sites on A549 cells. The other major density subclass of HDLs, HDL2, is known to behave as an antagonist, as it binds to the same cell sites as HDL3 but it unable to promote cholesterol efflux [30]. The relative proportions of HDL subclasses in the extracellular fluid should therefore be considered *in vivo*.

This work was supported by grants from the Groupe de Recherche du Centre Claudius Regaud (Toulouse, France) and from the Fédération Nationale des Centres de Lutte Contre le Cancer (France). We thank Mrs. Anne Lerne and Mr. Yvan Lescure for technical assistance, and Dr. John Woodley and Dr. Sophie Sixou for critical reading of the manuscript.

REFERENCES

- 1 Glomset, J. A. (1968) *J. Lipid Res.* **9**, 155–167
- 2 Boyd, N. F. and McGuire, V. (1990) *J. Natl. Cancer Inst.* **82**, 460–468
- 3 Favre, G., Tazi, K. A., Le Gaillard, F., Bennis, F., Hachem, H. and Soula, G. (1993) *J. Lipid Res.* **34**, 1093–1106
- 4 Gospodarowicz, D., Massoglia, S. and Cheng, D. K. (1986) *J. Cell. Physiol.* **127**, 121–136
- 5 Favre, G., Blanc, E., Tournier, J. F. and Soula, G. (1989) *Biochim. Biophys. Acta* **1013**, 118–124
- 6 Mendez, A. J., Oram, J. F. and Bierman, E. L. (1991) *J. Biol. Chem.* **266**, 10104–10111
- 7 Kilsdonk, E. P. C., Vangest, T., Dorsman, A. N. R. D. and van Tol, A. (1992) *Atherosclerosis* **97**, 131–142
- 8 Oram, J. F., Johnson, C. J. and Brown, T. A. (1987) *J. Biol. Chem.* **262**, 2405–2410
- 9 Wu, Y. Q., Jørgensen, E. V. and Handwerger, S. (1988) *Endocrinology* **123**, 1879–1884
- 10 Theret, N., Delbart, C., Aguilé, G., Fruchart, J. C., Vassaux, G. and Ailhaud, G. (1990) *Biochem. Biophys. Res. Commun.* **173**, 1361–1368
- 11 Kaur, P., Welch, W. J. and Saklatvala, J. (1989) *FEBS Lett.* **258**, 269–273
- 12 Regazzi, R., Eppenberger, U. and Fabro, D. (1988) *Biochim. Biophys. Acta* **152**, 62–68
- 13 Redgrave, T. G., Roberts, D. C. K. and West, C. E. (1975) *Anal. Biochem.* **65**, 42–49
- 14 Weisgraber, K. H. and Mahley, R. W. (1980) *J. Lipid Res.* **21**, 316–325
- 15 Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- 16 Slotte, J. P., Oram, J. F. and Bierman, E. L. (1987) *J. Biol. Chem.* **262**, 12904–12907
- 17 Dale, I. L., Bradshaw, T. D., Gescher, A. and Pettit, G. R. (1989) *Cancer Res.* **49**, 3242–3245
- 18 Gescher, A. and Reed, D. J. (1985) *Cancer Res.* **45**, 4315–4321
- 19 Keith, C. H., Bajer, A. S., Rotan, R., Maxfield, F. R. and Shelanski, M. I. (1986) *Ann. N.Y. Acad. Sci.* **466**, 375–391
- 20 Vandewaelle, B., Hornez, L., Lassalle, B., Revillon, F., Bertout, M. and Lefebvre, J. (1993) *Int. J. Oncol.* **2**, 613–620
- 21 Ferreri, K. and Menon, K. M. J. (1992) *Biochem. J.* **287**, 841–848
- 22 Darbon, J. M., Tournier, J. F., Tauber, J. P. and Bayard, F. (1986) *J. Biol. Chem.* **261**, 8002–8008
- 23 Wu, Y. Q. and Handwerger, S. (1992) *Endocrinology* **131**, 2935–2940
- 24 Murray, N. R., Baumgardner, G. P., Burns, D. J. and Fields, A. P. (1993) *J. Biol. Chem.* **268**, 15847–15853
- 25 Yeagle, P. L. (1985) *Biochim. Biophys. Acta* **822**, 267–287
- 26 Porn, M. I., Akerman, K. E. and Slotte, J. P. (1991) *Biochem. J.* **279**, 29–33
- 27 Stanwell, C., Gescher, A., Bradshaw, T. D. and Pettit, G. R. (1994) *Int. J. Cancer* **56**, 585–592
- 28 Ferraroni, M., Gerber, M. M., Decarli, A. et al. (1993) *Int. J. Epidemiol.* **22**, 772–780
- 29 Reichl, D., Simons, L. A., Myant, N. B., Plug, J. J. and Mills, G. L. (1973) *Clin. Sci. Mol. Med.* **45**, 313–329
- 30 Weisgraber, K. H., Innerarity, T. L. and Mahley, R. W. (1978) *J. Biol. Chem.* **253**, 9053–9062