A novel sterol-regulated surface protein on chicken fibroblasts

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Abstract In the laying hen, two different receptors for apolipoprotein B (apoB)-containing lipoproteins are expressed on somatic cells and oocytes, respectively. The somatic protein has an apparent M_r of 130,000, while the oocyte receptor is a 95-kDa protein (1989. K. Hayashi, J. Nimpf, and W. J. Schneider. *J. Biol. Chem.* **264:** 3131–3139). In order to investigate the yet unresolved relationship between these two proteins, we applied immunoblotting with anti-receptor antibodies to extracts of oocytes and chicken embryo fibroblasts. IgG fractions that recognize the 95-kDa oocyte receptor did not cross-react with the somatic receptor; however, chicken fibroblasts as well as ovarian granulosa cells that had been exposed to sterols (cholesterol and 25-OH-cholesterol) or low density lipoprotein (LDL) were shown to express a novel immunoreactive protein with an apparent M_r of 110,000. This protein is localized on the cell surface, and is unable to bind apoB-containing lipoproteins. The formation of the 110-kDa protein in fibroblasts is induced in time- and concentration-dependent fashion by sterols, concomitant with a progressive decrease in the amount of functional 130-kDa receptor protein. Following its induction, exposure of cells to LDL, but not to high density lipoprotein, caused the disappearance of the immunoreactive protein. Furthermore, the production of the 110-kDa protein did not require protein synthesis. These data are compatible with the notion that this novel receptor-related, nonfunctional protein is a truncated intermediate in the degradation pathway for the 130-kDa apoB receptor, and that the truncation generates antigenic epitope(s) shared by the 95-kDa oocyte receptor and the 110-kDa protein, but not expressed on the somatic receptor. —**Hayashi, K., S. Ando, S. Stifani, and W. J. Schneider.** A novel sterol-regulated surface protein on chicken fibroblasts. *J. Lipid Res.* 1989. **30:** 1421–1428.

Supplementary key words oocyte • receptor • sterol-regulation • apoB • cell surface

The laying hen provides an excellent model for investigations into the mechanisms underlying the maintenance of somatic lipid homeostasis with simultaneous massive lipid transport into the growing oocytes. On one hand, recent studies from our laboratory have shown that cholesterol homeostasis in somatic cells such as fibroblasts is afforded, at least in part, by a regulatory sequence analogous to the low density lipoprotein receptor pathway in mammalian cells (1). The chicken fibroblast receptor is specific for apolipoprotein (apo)B, and has an apparent molecular mass of 130,000 daltons (1). On the other hand, we have demonstrated that transport of low and very low density lipoproteins into chicken oocytes is mediated by a 95-kDa apoB-specific receptor (2,3). These two different chicken receptors appear to be expressed in cell-specific fashion in that the oocyte protein (95-kDa) has not been detected in somatic cells, and the 130-kDa fibroblast receptor is absent from oocytes (1). Furthermore, the biochemical defect in the mutant nonlaying "Restricted Ovulator" hen has been shown to be the absence of the 95-kDa receptor from oocytes (4). The nonlaying phenotype is due to a single gene defect (5), and the somatic metabolism of affected hens and carrier roosters is normal, lending further support to the existence of a zygotic/somatic receptor dichotomy in the laying hen.

The relationship between the two receptors has not been resolved. Clearly, both receptors bind apoB (1–3), suggesting that their ligand-binding domains share structural features. Yet, polyclonal antibodies that recognize the oocyte receptor do not cross-react with the fibroblast receptor, and the 95-kDa protein, but not the 130-kDa receptor, interacts with rabbit β-VLDL, an apoE-containing class of lipoproteins (1). In the studies reported here, we have obtained further evidence for limited structural homology between the two chicken receptors.

MATERIALS AND METHODS

Materials

Eagle's minimum essential medium (MEM) with nonessential amino acids (Cat. No. 410-1500) was purchased from

Abbreviations: apo, apolipoprotein; CEF, chicken embryo fibroblasts; LPDS, lipoprotein-deficient serum; FBS, fetal bovine serum; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

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Gibco; we obtained fetal bovine serum (Cat. No. 29-101-54) from Flow Laboratories; Triton X-100, 17α-ethinylestradiol, bovine serum albumin (Cat. No. A 7030), cholesterol, L-glutamine, PMSF, leupeptin, collagenase (type IV), penicillin, streptomycin, and DL-aminoglutethimide (Cat. No. A-9657) were from Sigma. Fertile chicken eggs were obtained from the University Poultry Farm, University of Alberta. Polyclonal rabbit anti-bovine LDL receptor IgG was prepared as described (2), except that the receptor was co-precipitated with egg phosphatidylcholine (6) before injection. Other materials were obtained from previously reported sources (1).

Animals and diets

Eight- to 18-month-old White Leghorn layers and 5- to 8-week-old White Leghorn roosters were kindly provided by Dr. F. Robinson, Department of Animal Sciences, University of Alberta. They were maintained at controlled temperature (22-24°C) with a light period of 12 h. Male animals received a single intramuscular injection of 17α-ethinylestradiol dissolved in propyleneglycol (10 mg/kg body weight) 72 h prior to being killed. Adult female New Zealand White rabbits were used for raising antibodies.

Lipoproteins

Plasma from roosters that had been treated with 17α-ethinylestradiol was centrifuged for 24 h at 4°C and 200,000 *g*, and the floating lipoprotein fraction was separated from the infranate. VLDL was prepared by subjecting the floating fraction to a second ultracentrifugation step (ϱ 1.006 g/ml) as described (2). LDL was obtained from the infranate of the initial centrifugation by further centrifugation at ϱ 1.060 g/ml (top recovered), followed by equilibrium density gradient centrifugation (3). HDL ($1.060 < \varrho < 1.21$ g/ml) was prepared from the plasma of untreated roosters by sequential ultracentrifugation steps at 200,000 *g* and 4°C at densities 1.006, 1.060, and 1.21 g/ml for 24 h, 24 h, and 36 h, respectively (2). All lipoproteins were extensively dialyzed against a buffer containing 0.15 M NaCl, 0.2 mM EDTA, pH 7.4, prior to use and stored at 4°C. Lipoprotein concentrations are expressed in terms of protein content. ^{125}I-Labeled lipoproteins were obtained by the iodine monochloride method (7).

Cell culture

Chicken embryo fibroblasts were isolated as previously described (1) and grown in monolayer culture at 37°C in an atmosphere of 95% air/5% CO_2. Growth conditions were as reported earlier (1) and as indicated in the figure legends. Chicken granulosa cells were isolated according to a modified version of the procedure first described by Gilbert et al.(8). In brief, the granulosa cell layers isolated from the largest preovulatory follicles were quickly placed into ice-cold, sterile medium A (140 mM NaCl, 5 mM KCl, 0.5 mM EGTA, 10 mM HEPES, 0.5 mM NaH_2PO_4, 0.5 mM Na_2HPO_4, 4 mM $NaHCO_3$, 5 mM glucose, 0.0006% phenol red) and centrifuged at 4°C at 200 *g* for 5 min. After recovery, cells were washed twice (200 *g* for 5 min) with medium A and then incubated in 10 ml of the same medium containing 500 μg/ml of collagenase. After incubation for 15 min at 37°C with constant shaking (100 cycles/min), granulosa cells were collected by centrifugation at 200 *g* for 5 min, washed twice with medium B (MEM containing 2 mM glutamine, 100 units/ml of penicillin, and 100 μg/ml of streptomycin), and then resuspended in 20 ml of medium B supplemented with 10% (v/v) FBS. Cells in suspension were counted and assessed for viability by trypan blue dye exclusion. Approximately 5×10^5 cells/ml (3 ml) were seeded in 60-mm dishes and cultured at 37°C under an atmosphere of 5% CO_2 and 95% air. Medium was changed on days 2 and 5, and experiments were initiated on day 6. Experimental conditions are indicated in the figure legends.

Preparation of cell extracts

After washing the monolayers twice with 3 ml each of phosphate-buffered saline, cells were collected by scraping the monolayers with a rubber policeman, followed by solubilization in the presence of Triton X-100 and a mixture of protease inhibitors (1). Cell extracts were subjected to ultracentrifugation at 300,000 *g* for 40 min at 4°C and the supernatants were subjected to one-dimensional SDS gel electrophoresis as described below.

Electrophoresis, transfer to nitrocellulose and blotting procedures

Nonreducing SDS gel electrophoresis was carried out on 4.5–18% polyacrylamide gradient slab gels, according to the method of Laemmli (9). Gels were run and calibrated as previously described (1). Electrophoretic transfer of proteins to nitrocellulose was performed according to Beisiegel et al. (10). In ligand blotting experiments, nitrocellulose was incubated with ^{125}I-labeled lipoproteins in the presence of 5% (w/v) BSA as blocking agent (11). In Western blots, the bound antibodies were detected by using ^{125}I-labeled goat anti-rabbit IgG as second antibody. The concentrations and specific radioactivities of the ligands and antibodies used in the incubation mixtures are indicated in the figure legends. Autoradiographs were obtained by exposing the dried nitrocellulose paper to Kodak XAR-5 film for 16–36 h at room temperature.

Other methods

Chicken oocyte membranes were prepared and detergent extracts were obtained as described in (2). Lipoprotein-deficient serum (LPDS) was prepared from fetal bovine serum as previously reported (1). ^{125}I-Labeled goat anti-rabbit IgG

was obtained by the Iodogen method (12). The protein content of samples containing no Triton X-100 was determined by the method of Lowry et al.(13). The protein contents of samples containing Triton X-100 and of lipoproteins were measured by a modification of the Lowry procedure as previously described (14) with bovine serum albumin as standard. Treatment of cell monolayers with Pronase was performed as described previously (1).

RESULTS

When chicken embryo fibroblasts (CEF) were cultured in the absence of sterols, ligand blotting with ^{125}I-labeled VLDL revealed the receptor as a 130-kDa protein under nonreducing conditions; as expected (1), the receptor completely disappeared upon exposure of the cultured cells to 12 μg/ml of cholesterol for 24 h (**Fig. 1**, right panel). Polyclonal rabbit IgG raised against the purified bovine LDL receptor that had been precipitated by acetone in the presence of phosphatidylcholine (6) reacted with the chicken oocyte receptor, a 95-kDa protein (Fig. 1, left panel). However, as observed previously (1) with rabbit antibodies obtained by immunization with bovine LDL receptor that had not been coprecipitated with phosphatidylcholine, this IgG fraction failed to react with the receptor expressed in fibroblasts grown in the absence of sterols. Surprisingly, the IgG bound specifically to a protein of approximately 110 kDa which was present only in extracts of CEF exposed to cholesterol. As Fig. 1 shows, this protein did not bind ^{125}I-labeled VLDL, and was absent from oocyte membrane extracts and from CEF grown in sterol-depleted medium. Control IgG did not react with any proteins under these conditions (not shown). Thus, it appeared that exposure to sterols induces an immunologically cross-reactive protein in CEF with an apparent M_r of 110,000 that had not been observed previously.

In order to study this phenomenon in greater detail, we tested whether the level of expression of the 110-kDa protein was dependent on the concentration of cholesterol in the culture medium. Cells were cultured in the absence of sterols, and for the last 24 h before solubilization were switched to medium containing increasing concentrations of cholesterol (**Fig. 2**). There was a progressive increase in the intensity of the 110-kDa band as visualized by immunoblotting with the anti-receptor IgG when cells had been exposed to 0,6,12,24, or 48 μg/ml cholesterol (Fig. 2, lanes 2–6). Essentially identical results were obtained by incubating cells with medium containing increasing concentrations of 25-OH-cholesterol in the range of 1–8 μg/ml, or chicken LDL (10–100 μg/ml, data not shown).

Next, the time-course for the appearance of the novel 110-kDa protein was investigated. As shown in **Fig. 3**, there was a progressive increase in the amount of the protein during exposure of the cells to 4 μg/ml 25-OH-cholesterol (lanes A–C). The 110-kDa band was absent from cells that had been grown in the absence of sterol, but could easily be detected by Western blotting following growth for 12 h in sterol-containing medium, and became prominent by 24 h. Concomitant with the appearance of the 110-kDa immunoreactive protein, a decrease in the amount of the 130-kDa receptor, detected by ligand blotting, was observed (Fig. 3, lanes E–G). These data were compatible with a conversion of the 130-kDa receptor into the 110-kDa protein. However, because of the different reagents required for the visualization of the two proteins—anti-receptor IgG/^{125}I-labeled goat anti-rabbit IgG for the 110-kDa protein, and ^{125}I-labeled VLDL for the 130-kDa receptor—a precursor–product relationship could not be established unambiguously.

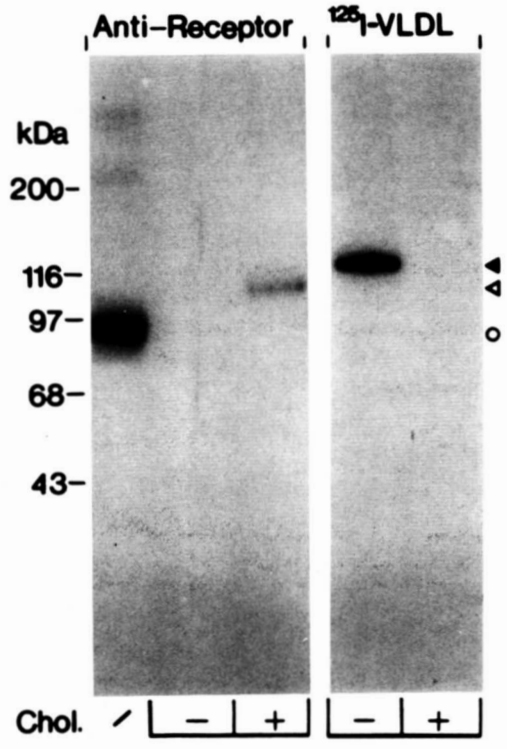

Fig. 1. Detection of lipoprotein receptors and a related protein by ligand- and immunoblotting. Chicken embryo fibroblasts were cultured as described in Materials and Methods. For the last 24 h before harvest, cells received medium containing 10% LPDS alone (–) or 10% LPDS in the presence of 12 μg/ml of cholesterol (+). Cell pellets from five dishes each and chicken oocyte membranes (far left lane) were solubilized as described in Materials and Methods. Aliquots (oocyte membranes, 35 μg; fibroblasts, 260 μg of protein) were subjected to SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and incubated with ^{125}I-labeled VLDL (287 cpm/ng protein, 10^6 cpm/ml; right panel) or rabbit anti-bovine LDL receptor IgG (10 μg/ml; left panel), followed by ^{125}I-labeled goat anti-rabbit IgG (0.5 μg/ml; 10^4 cpm/ng protein) as described in Materials and Methods. Autoradiography was for 16 h (left panel) or 36 h (right panel), respectively. Molecular mass standards and the position of migration of the fibroblast receptor (solid arrowhead), the oocyte receptor (○), and the 110-kDa protein (open arrowhead) are indicated.

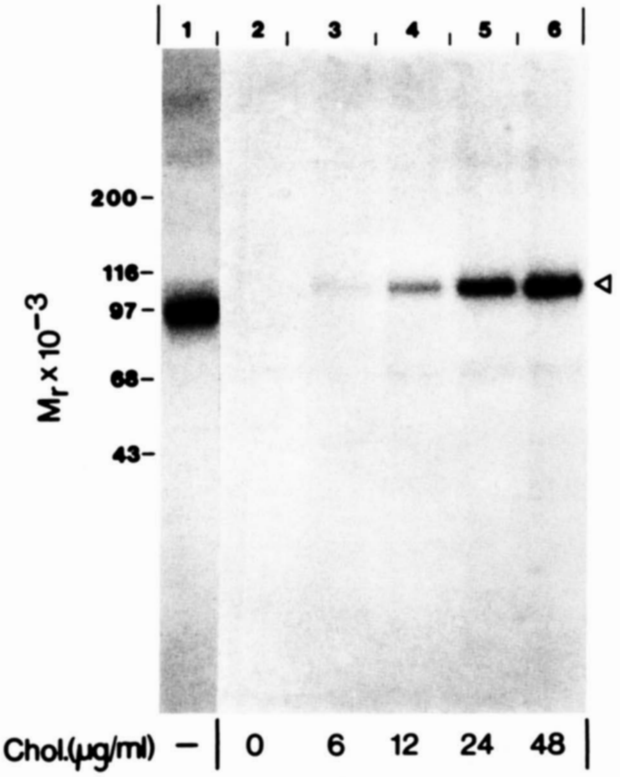

Fig. 2. Expression of the immunoreactive 110-kDa protein as a function of cholesterol concentration in culture medium. Chicken embryo fibroblasts were cultured in the absence of sterols, and for the last 24 h before harvest received medium containing 10% LPDS in the presence of the indicated concentrations of cholesterol (lanes 2–6). Cell pellets from five dishes each and chicken oocyte membranes (lane 1) were solubilized and subjected to immunoblotting analysis exactly as described in the legend to Fig. 1. Lane 1 received 35 μg, and lanes 2–6, 270 μg of protein each, respectively. The open arrowhead denotes the position of migration of the 110-kDa fibroblast protein, and molecular mass standards are indicated on the left. Autoradiography was for 18 h.

To further test the relationship between the two proteins, we traced the fate of the 110-kDa protein in cells exposed to various lipoproteins following its induction by cholesterol (**Fig. 4**). After culture in the absence of sterols for 24 h, the cells were grown in medium containing 50 μg/ml cholesterol for 24 h, and then were either harvested (Fig. 4, lane A) or incubated in the presence of 10% LPDS, 10% LPDS plus 100 μg/ml LDL, or 10% LPDS plus 100 μg/ml HDL for a further period of 24 h (Fig. 4) or 48 h (not shown). In both the absence of sterols and the presence of HDL, the 110-kDa protein continued to be present at high levels, but incubation of the cells with LDL reduced the amount of 110-kDa protein significantly (Fig. 4, lane C). Thus, under conditions that promote the synthesis of the 130-kDa receptor (1), the 110-kDa protein persists. In contrast, it disappears when the cells do not require the apoB receptor, such as in the presence of LDL.

Does expression of the 110-kDa protein require protein synthesis? To address this question, cells were grown in the absence of sterols for 24 h, and then exposed to sterols for 6 or 12 h in the absence or presence of 0.5 and 5 mM cycloheximide. There was no difference in the amount of 110-kDa protein formed in the presence or absence of the protein synthesis inhibitor (**Table 1**).

Taken together, these data suggest that the 110-kDa protein (which fails to recognize apoB) is an intermediate in the regulated breakdown of the functional 130-kDa apoB receptor. It was of interest to determine whether or not such a nonfunctional receptor fragment would localize to the cell surface. Previously, we had shown that the 130-kDa receptor on intact CEF was accessible to Pronase (1); **Fig. 5** shows that the same is true for the 110-kDa protein, demonstrating that the immunoreactive protein is expressed on the plasma membrane.

Finally, we tested whether the formation of the novel protein is confined to chicken fibroblasts. To this end, we cultured chicken ovarian granulosa cells and performed immunoblotting under the conditions described in **Fig. 6**. A 110-kDa protein was detected in cells exposed to a mixture of 25-OH-cholesterol and cholesterol, but it was absent from granulosa cells grown in sterol-free medium. The addition of aminoglutethimide, which prevents cholesterol utilization for steroidogenesis (15) had no effect on

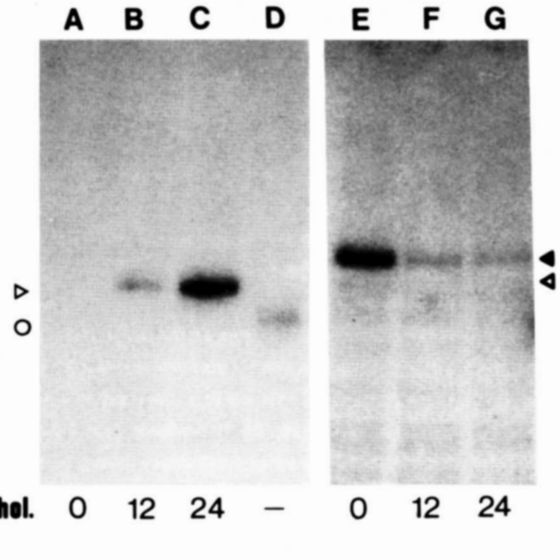

Fig. 3. Time-course of appearance of the 110-kDa protein in chicken embryo fibroblasts. Cells were cultured in medium containing 10% LPDS, and then received 4 μg/ml of 25-OH-cholesterol for the indicated times (lanes A–C and E–G). Cell pellets from five dishes each and chicken oocyte membranes (lane D) were solubilized and subjected to analysis by immunoblotting (lanes A–D) or ligand blotting (lanes E–G) as described in the legend to Fig. 1. Lanes A–C and E–G received 270 μg each, and lane D, 20 μg of protein, respectively. The positions of migration of the 95-kDa oocyte receptor (○), the 110-kDa protein (open arrowhead), and the 130-kDa fibrobalst receptor (closed arrowhead) are indicated. Autoradiography was for 16 h (immunoblot) and 36 h (ligand blot), respectively.

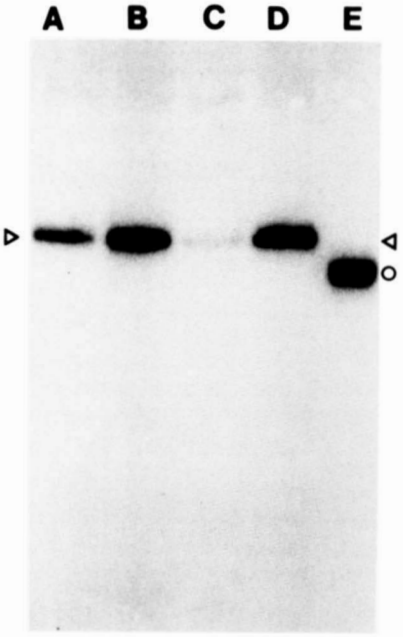

Fig. 4. Expression of the 110-kDa fibroblast protein under various culture conditions. Following growth in sterol-free medium, cells were cultured in medium containing 10% LPDS in the presence of 50 μg/ml of cholesterol for 24 h, and then were either harvested immediately (lane A), or incubated for a further 24 h in medium containing 10% LPDS in the presence of no addition (lane B), 100 μg/ml of LDL (lane C), or 100 μg/ml of HDL (lane D). Cell pellets from five dishes each (lanes A–D, 265 μg of protein each) and chicken oocyte membranes (lane E, 35 μg of protein) were solubilized and subjected to immunoblot analysis as described in the legend to Fig. 1. The positions of migration of the 110-kDa protein (open arrowhead) and of the oocyte receptor (○) are indicated.

the sterol-induced formation of the 110-kDa protein in cultured granulosa cells (Fig. 6, lanes B and C). In studies to be reported elsewhere (Hayashi, K., S. Stifani, and W. J. Schneider, unpublished results), the 110-kDa protein was also identified in sterol-treated cultured chicken hepatocytes, and both granulosa cells and hepatocytes were shown to synthesize the 130-kDa apoB receptor in the absence of sterols.

DISCUSSION

In the studies presented here, we have identified a novel sterol-regulated surface protein expressed by chicken somatic cells. Its apparent M_r of 110,000 lies between those of the previously identified apoB-specific receptors on chicken embryo fibroblasts (130,000) and chicken oocytes (95,000), respectively (1, 2). These different functional receptors appear to be expressed in cell-specific fashion so as to ensure lipid transport into the oocyte (via the 95-kDa receptor) with concomitant maintenance of somatic cholesterol homeostasis (involving the 130-kDa receptor; cf. ref. 1). We do not know what structural relationships, if any, exist between these two receptors. Both bind apoB,

TABLE 1. Effect of cycloheximide on expression of the 110-kDa protein

Duration of Culture with Cycloheximide Plus Sterols	Concentration of Cycloheximide		
	0 mM	0.5 mM	5 mM
h	*^{125}I-radioactivity (cpm)*		
6	1673	1686	1702
12	3787	3753	3807

Chicken embryo fibroblasts were cultured in sterol-free medium for 24 h, and then received 4 μg/ml 25-OH-cholesterol plus 25 μg/ml cholesterol for 0 (control), 6, or 12 h in the absence or presence of 0.5 or 5 mM cycloheximide as indicated. Immunoblots were performed, and following visualization by autoradiography, the 110-kDa bands were cut from the nitrocellulose. ^{125}I Radioactivity was determined; each value is the average of results from two independent experiments. The control value, determined by counting an area corresponding to the 110-kDa band derived from cells not exposed to sterols, was 153 cpm.

but only the oocyte receptor binds apoE-rich lipoproteins such as rabbit β-VLDL (1). Surprisingly, we found that antibodies raised against the pure bovine LDL receptor (M_r, 130,000) recognize the 95-kDa oocyte receptor, but not the 130-kDa chicken fibroblast receptor.

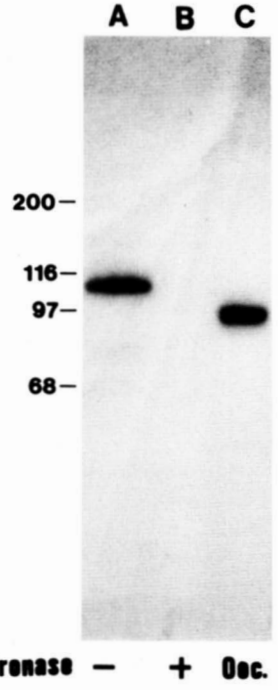

Fig. 5. Localization of the 110-kDa protein. Chicken embryo fibroblasts were cultured in medium containing 10% LPDS plus 50 μg/ml of cholesterol for 24 h before the experiment. Monolayers (three dishes each) were treated with (+) or without (–) 20 μg/ml Pronase as described in Materials and Methods. The cell pellets (lanes A and B) and oocyte membranes (lane C) were solubilized and subjected to immunoblot analysis with the anti-bovine LDL receptor IgG as described in the legend to Fig. 1. Molecular mass standards are indicated on the left. Autoradiography was for 24 h.

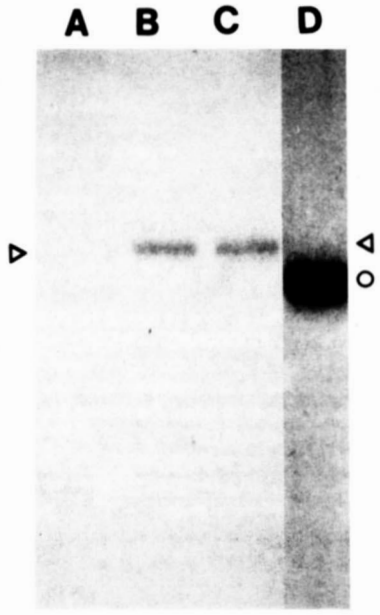

Fig. 6. Granulosa cells express the 110-kDa protein in sterol-regulated fashion. Chicken ovarian granulosa cells were prepared as described in Materials and Methods, and for the last 24 h before harvest cultured in medium containing 10% LPDS alone (lane A), or 10% LPDS in the presence of 4 μg/ml 25-O-cholesterol (B) or 4 μg/ml 25-O-cholesterol + 100 μg/ml aminoglutethimide (lane C). Cell pellets from six dishes each and chicken oocyte membranes (lane D) were solubilized and subjected to immunoblot analysis as described in the legend to Fig. 1. Lanes A–C received 210 μg and lane D, 35 μg of protein, respectively. The positions of migration of the 110-kDa protein (arrowhead) and the oocyte receptor (O) are indicated. Autoradiography was for 20 h.

In our search for a tool to investigate the molecular basis for the somatic–zygotic receptor dichotomy in the laying hen, we discovered the 110-kDa protein described herein. This protein, visualized by immunoblotting with antibodies that recognize the 95-kDa receptor, but not the 130-kDa receptor (Fig. 1), is present only in fibroblasts or other somatic cells such as ovarian granulosa cells (Fig. 6) after exposure to sterols (cholesterol and/or 25-OH-cholesterol). The 110-kDa protein could not be detected in extracts of oocyte membranes. Since exposure to sterols raises cellular cholesterol levels, and the growing oocyte accumulates massive amounts of cholesterol-carrying lipoproteins, but the 110-kDa protein is absent from oocytes, its expression appears to depend on the cells' ability to express the 130-kDa receptor. These findings suggest a relationship between the 130-kDa and 110-kDa proteins. Several possibilities exist for the accumulation of this novel sterol-regulated protein. First, there could be a post-translational processing event during the synthesis of the 130-kDa receptor; if the 110-kDa protein were the substrate for the rate-limiting step(s) in the synthetic pathway, and the rate of processing were sterol-suppressed, the amount of 110-kDa protein would increase upon exposure of cells to sterols. However, the fact that the 110-kDa protein accumulates on the cell surface (Fig. 5) is hard to reconcile with the known intracellular itinerary of the LDL receptor during biosynthesis (16). Second, a novel type of modification on the receptor could occur in chicken somatic cells under the influence of sterols which, in turn, affects its size and/or degradation rate, leading to production and accumulation of the 110-kDa protein. We have obtained no evidence for or against a novel type of posttranslational modification of the 130-kDa receptor. A third possibility, and the one favored by us, is that the 110-kDa protein is an intermediate in the degradative pathway of the LDL receptor. There are several lines of evidence in support of this notion.

First, this protein is absent from cells that had been maintained in sterol-free medium, when receptor expression is at a maximum. The 110-kDa protein is produced in time- and concentration-dependent fashion only in cells exposed to sterols, when receptor numbers decline. Second, following its induction by sterols for 24 h, the level of the 110-kDa protein remains unchanged when cells are switched to medium containing no sterol or HDL for an additional 24-h period (i.e., when the receptor function is required); in contrast, when changed to LDL-containing medium, both the 130-kDa receptor and the 110-kDa protein disappear (Fig. 4). In this context, it is important to note that exposure to medium containing sterols for 24 h does not abolish expression of the 130-kDa receptor completely (see Fig. 3, lane G, and ref. 1), allowing receptor-mediated uptake of LDL and thus further cholesterol import. We have previously shown that HDL does not trigger regulatory responses such as changes in apoB receptor number and activity of 3-hydroxy-3-methylglutaryl CoA reductase in chicken embryo fibroblasts, but that chicken LDL is an effective regulator of both (1). Thus, the levels of the 110-kDa protein, like those of the 130-kDa receptor, are regulated by LDL, but not HDL.

Third, when the time course of expression of the 110-kDa and 130-kDa proteins was compared (Fig. 3), the appearance of the smaller protein correlated with the decline in apoB receptor expression, suggesting a precursor–product relationship. To establish such relationship unambigiously, it would be necessary to quantitate both proteins with the same reagent. Unfortunately, we are not in a position to do so, because *i*) the 130-kDa receptor, but not the 110-kDa protein binds LDL; and *ii*) the 110-kDa protein, but not the receptor, is recognized by the available antibodies. Thus, a pulse/chase experiment using the same quantification procedure for both proteins cannot be performed. Nevertheless, while these results do not prove, they are certainly compatible with the following scenario: exposure of somatic chicken cells to sterols or LDL causes degradation of the 130-kDa apoB receptor; its conversion to a 110-kDa intermediary product is an early (and possibly rate-limiting) step in the degradative pathway. Alternatively, the 110-kDa protein could be newly synthesized

when cells accumulate sterols. However, inasmuch as cycloheximide does not suppress the increase of 110-kDa protein levels upon exposure of cells of sterols (Table 1), de novo synthesis of the immunoreactive protein is highly unlikely. The results shown in Fig. 4 further support the interpretation of the 110-kDa protein as an intermediate in the degradation of the 130-kDa receptor: further degradation is halted by culture in LPDS or HDL (when cells require the intact receptor), but promoted by the addition of LDL to the medium.

Of particular interest is our finding that the 110-kDa protein is localized to the cell surface (Fig. 5). There are previous reports in the literature describing proteins that react with anti-LDL receptor antibodies, and which likely represent receptor breakdown products that have lost all or parts of the amino-terminal LDL binding domain (17–20). The apparent M_r of these immunoreactive, but LDL-binding-deficient proteins is in the range of 105,000 to 125,000 (depending on the presence or absence of reducing agents during SDS-polyacrylamide gel electrophoresis). Unfortunately, neither the localization nor the possible regulation of these proteins has been investigated, and it was assumed that they were artifactual proteolytic breakdown products of the LDL receptor (17,18). The 110-kDa protein in chicken somatic cells cannot be a procedural artifact, because we observed in all experiments a negative, and not a positive, correlation between the amounts of LDL receptor (130-kDa) and of the 110-kDa protein. Thus, the 110-kDa protein is most likely a cell-generated receptor fragment that has lost its ability to bind apoB-containing lipoproteins but remains localized to the plasma membrane. At present, we do not know how cells do produce this protein; in preliminary experiments, we found that lysosomotropic agents do not alter the expression of the 110-kDa protein, indicating that lysosomal enzymes may not be involved.

Finally, it is intriguing that the anti-LDL receptor antibody recognizes the 95-kDa oocyte receptor and the 110-kDa protein, but not the fibroblast 130-kDa receptor. In studies to be reported elsewhere, we have raised polyclonal antibodies against the oocyte receptor as the antigen; none of the antibodies obtained to date recognize the 110-kDa protein or the 130-kDa apoB receptor on fibroblasts. Since both the oocyte and the somatic receptors bind apoB, their ligand binding domains would be expected to be structurally related. However, differences in binding characteristics exist: the oocyte receptor, but not the somatic cell receptor binds rabbit β-VLDL, a class of lipoproteins thought to interact with the LDL receptor via apoE (1,21). We conclude from these and the current findings that the truncation of the 130-kDa receptor (which abolishes apoB binding) generates an antigenic epitope(s) shared by the 95-kDa oocyte receptor and the 110-kDa protein, but not displayed on the 130-kDa receptor of somatic cells.

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