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Loss of Transcriptional Activation of Three Sterol-Regulated Genes in Mutant Hamster Cells

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Cholesterol biosynthesis and uptake are controlled by a classic end product-feedback mechanism whereby elevated cellular sterol levels suppress transcription of the genes encoding 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase, HMG-CoA reductase, and the low-density lipoprotein receptor. The 5′-flanking region of each gene contains a common *cis*-acting element, designated the sterol regulatory element (SRE), that is required for transcriptional regulation. In this report, we describe mutant Chinese hamster ovary (CHO) cell lines that lack SRE-dependent transcription. Mutant cell lines were isolated on the basis of their ability to survive treatment with amphotericin B, a polyene antibiotic that kills cells by interacting with cholesterol in the plasma membrane. Four mutant lines (SRD-6A, -B, -C, and -D) were found to be cholesterol auxotrophs and demonstrated constitutively low levels of mRNA for all three sterol-regulated genes even under conditions of sterol deprivation. The mutant cell lines were found to be genetically recessive, and all four lines belonged to the same complementation group. When transfected with a plasmid containing a sterol-regulated promoter fused to a bacterial reporter gene, SRD-6B cells demonstrated constitutively low levels of transcription, in contrast to wild-type CHO cells, which increased transcription under conditions of sterol deprivation. Mutation of the SREs in this plasmid prior to transfection reduced the level of expression in wild-type CHO cells deprived of sterols to the level of expression found in SRD-6B cells. The defect in SRD-6 cells is limited to transcriptional regulation, since posttranscriptional mechanisms of sterol-mediated regulation were intact: the cells retained the ability to posttranscriptionally suppress HMG-CoA reductase activity and to stimulate acyl-CoA:cholesterol acyltransferase activity. These results suggest that SRD-6 cells lack a factor required for SRE-dependent transcriptional activation. We contrast these cells with a previously isolated oxysterol-resistant cell line (SRD-2) that lacks a factor required for SRE-dependent transcriptional suppression and propose a model for the role of these genetically defined factors in sterol-mediated transcriptional regulation.

Mammalian cells demonstrate exquisite control over the level of free cholesterol within the cell (reviewed in reference 17). This control is achieved through the concerted action of a number of mechanisms, including the coordinate transcriptional suppression of the genes encoding 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase, HMG-CoA reductase, and the low-density lipoprotein (LDL) receptor. HMG-CoA synthase and HMG-CoA reductase are the first two committed steps in the pathway of cholesterol biosynthesis, and transcriptional suppression of these genes decreases the rate of cholesterol synthesis. The LDL receptor mediates endocytosis of cholesterol-rich lipoprotein particles such as LDL, and transcriptional suppression of the LDL receptor results in a decreased rate of cholesterol uptake. Transcriptional suppression of these three genes appears to be mediated through a common *cis*-acting sequence, termed the sterol regulatory element (SRE), that resides in the 5′-flanking region of each gene. A protein that interacts with the SRE of the LDL receptor promoter has recently been identified and purified (1, 40).

Cholesterol metabolism is also regulated by at least three posttranscriptional mechanisms. When cellular cholesterol levels rise, the rate of degradation of HMG-CoA reductase is enhanced. This increased degradation requires the membrane-spanning domain of the protein, which anchors it to the endoplasmic reticulum (14). This increased degradation results in decreased HMG-CoA reductase activity and a consequent decrease in the rate of cholesterol synthesis. Sterols also inhibit translation of HMG-CoA reductase mRNA (28), which further decreases the rate of cholesterol synthesis. In addition, sterols stimulate acyl-CoA:cholesterol acyltransferase (ACAT) activity by a mechanism that does not require new protein synthesis (4, 16). ACAT resides in the endoplasmic reticulum and catalyzes the transfer of long-chain fatty acyl residues from acyl-CoA to the 3-hydroxyl group of cholesterol to form cholesteryl esters. While free cholesterol can be deleterious to cells, cholesteryl esters are innocuous and can accumulate to relatively high levels as cytosolic lipid droplets. Sterol-mediated stimulation of ACAT activity reduces the level of free cholesterol by enhancing the conversion of cholesterol to cholesteryl esters.

Somatic cell genetic studies have provided insight into the mechanisms that control cholesterol metabolism. Mutant cell lines that grow in the presence of 25-hydroxycholesterol, a potent repressor of cholesterol biosynthesis, have been isolated (5, 7, 12, 25, 36). Normally, 25-hydroxycholesterol prevents cell growth by suppressing the synthesis of cholesterol and other important products of this biosynthetic pathway. In general, the mutant cell lines show a coordinate resistance to suppression of HMG-CoA synthase and HMG-

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CoA reductase activity when incubated with either 25-hydroxycholesterol or plasma LDL (5). Sinensky et al. (37) showed that some oxysterol-resistant cells fail to suppress the incorporation of [^{35}S]methionine into immunoprecipitable HMG-CoA reductase in the presence of 25-hydroxycholesterol, suggesting that the defect in these cells is in sterol-mediated transcriptional control. More recently, we (12, 25) demonstrated that oxysterol-resistant cells fail to suppress the mRNA for HMG-CoA synthase, HMG-CoA reductase, and the LDL receptor in the presence of 25-hydroxycholesterol. In addition, these cells fail to repress transcription of transfected chimeric plasmids containing the promoters of any of these genes, indicating that the defect in these cells is at the transcriptional level. The loss of transcriptional regulation of three sterol-regulated genes in these cells demonstrates that a common mechanism controls both the synthesis and the uptake of cholesterol. Cell fusion experiments showed that mutations resulting in oxysterol resistance are genetically recessive and belong to several complementation groups (12, 36), indicating that more than one factor is required for sterol-mediated transcriptional suppression. The defects in these cells lines are, however, limited to transcriptional regulation, since these cells respond to sterols by (i) enhancing the rate of degradation of HMG-CoA reductase protein (12) and (ii) stimulating ACAT activity (25). These findings suggest that sterol-dependent HMG-CoA reductase protein turnover and sterol-dependent ACAT activation are achieved by mechanisms that are genetically distinct from that of sterol-dependent transcriptional regulation.

Mutant cell lines auxotrophic for cholesterol have also provided insight into the mechanisms that control cholesterol metabolism (34). Many of these mutant cell lines fail to synthesize cholesterol due to defects in enzymes of the cholesterol biosynthetic pathway. These defects include the loss of (i) HMG-CoA synthase activity (33), (ii) HMG-CoA reductase activity (27), and (iii) the 4α-methylsterol oxidase system involved in the demethylation of lanosterol (6, 32). In addition, some cholesterol auxotrophs result from mutations that affect the regulation of HMG-CoA reductase activity. Sinensky et al. (35) isolated a mutant cell line that has normal HMG-CoA reductase activity in the presence of sterols but fails to stimulate activity when exogenous sterols are removed. Limanek et al. (23) isolated a cell line that requires exogenous sources of both cholesterol and unsaturated fatty acid. This cell line appears to be defective in the regulation of HMG-CoA reductase and, interestingly, also appears to be defective in the regulation of the LDL receptor (8).

In this report, we describe the use of a selection scheme to specifically isolate mutant Chinese hamster ovary (CHO) cell lines (SRD-6 cells; SRD stands for sterol regulation-defective phenotype [25]) that have reduced capacity to synthesize cholesterol and to utilize LDL-derived cholesterol. We show that SRD-6 cells are cholesterol auxotrophs and fail to induce LDL receptor, HMG-CoA reductase, and HMG-CoA synthase mRNAs under conditions of sterol deprivation. Cell fusion experiments demonstrate that the mutations in SRD-6 cells are genetically recessive and that all four cell lines belong to the same complementation group. The defect in these cells is limited to transcriptional regulation, since posttranscriptional mechanisms of sterol-mediated regulation are intact. In addition, SRD-6 cells lack SRE-dependent transcriptional activation of a transfected chimeric reporter plasmid. We contrast these mutant cells with a previously isolated oxysterol-resistant cell line (SRD-2 [25]) that fails to suppress transcription of sterol-regulated genes in the presence of sterols and suggest a model for sterol-mediated transcriptional regulation in which SRD-6 cells lack a functional sterol-dependent transcriptional activator.

MATERIALS AND METHODS

Materials. Human LDL (ρ 1.019 to 1.063 g/ml) and newborn calf lipoprotein-deficient serum (ρ > 1.215 g/ml; cholesterol content of 33 to 61 μg/ml) were prepared by ultracentrifugation as described previously (15). LDL was radiolabeled with ^{125}I as described previously (15). Compactin was kindly provided by Akira Endo (Tokyo Noko University, Tokyo, Japan). DL-Mevalonic acid lactone was purchased from Fluka Chemical Co. and was converted to the sodium salt as described previously (2). Methanesulfonic acid ethyl ester was purchased from Sigma Chemical Co. [1-^{14}C]oleic acid (55 to 58 mCi/mmol) and [2-^{14}C]acetic acid (55 mCi/mmol) were purchased from Du Pont-New England Nuclear. Plasmid pRc/CMV was obtained from Invitrogen, and plasmids pF and pG (1) were obtained from Mike Briggs (University of Texas Southwestern Medical Center, Dallas). pCMV7S is a derivative of pRc/CMV and contains the bacterial neomycin resistance gene inserted downstream of the simian virus 40 early promoter. pSV3 Hyg (12), antireductase monoclonal antibody IgG-A9 (24), and the CHO-7 subline of CHO-K1 cells (25) were previously described in the indicated references. *ldlA*-7 cells, a mutant CHO cell line lacking functional LDL receptors (20), were provided by Monty Krieger (Department of Biology, Massachusetts Institute of Technology, Cambridge). Other materials were obtained from previously reported sources (12, 25, 26).

Cell growth. All cells were grown in monolayer at 37°C in an atmosphere of 9% CO_2. CHO-7 cells were maintained in medium A (a 1:1 mixture of Ham's F12 medium and Dulbecco's modified Eagle's minimum essential medium containing 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 4.5 to 10% [vol/vol] newborn calf lipoprotein-deficient serum). SRD-2 cells (25) were maintained in medium A containing 1 μg of 25-hydroxycholesterol per ml. *ldlA*-7 cells were maintained in medium B (a 1:1 mixture of Ham's F12 medium and Dulbecco's modified Eagle's minimum essential medium containing 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 5% [vol/vol] fetal calf serum [FCS]). SRD-6A, -B, -C, and -D cells (see below) were maintained in medium B containing 5 μg of cholesterol per ml and 1 mM sodium mevalonate. Cholesterol was added to culture media in ethanol; the final ethanol concentration did not exceed 0.5% (vol/vol).

Mutagenesis and isolation of amphotericin B-resistant cells. SRD-6A cells were derived from CHO-7 cells that had been mutagenized with methanesulfonic acid ethyl ester. On day 0, CHO-7 cells were divided into 13 pools of 3×10^5 cells per pool, and each pool was plated into a 75-cm^2 flask in medium A. On day 1, the medium was replaced with fresh medium containing 0.6 mg of methanesulfonic acid ethyl ester per ml. After 24 h, the cells were washed three times with phosphate-buffered saline (PBS) and refed medium A containing 200 μM sodium mevalonate. On day 4, the cells were replated in medium A containing 10 mM sodium mevalonate. On day 7, the mutagenized cells were frozen as independent pools. SRD-6B, -C, and -D cells were derived from CHO-7 cells that had been mutagenized with γ irradiation as previously described (25). Immediately after irradiation, the mutagenized CHO cells were subdivided into 18 pools of 3×10^5 cells per pool.

Prior to selection for amphotericin B resistance, pools of mutagenized cells were grown for 5 days in medium A containing 10 mM sodium mevalonate. Selection for amphotericin B resistance was then carried out as follows. On day 0, the mutagenized pools of CHO-7 cells were plated into four dishes at 5×10^5 cells per 100-mm-diameter dish in medium A containing 10 mM sodium mevalonate (SRD-6A cells) or medium B containing 5 μg of cholesterol per ml and 200 μM sodium mevalonate (SRD-6B, -C, and -D cells). On day 2, the cells were fed medium A containing LDL at 30 μg of protein per ml. On day 3, cells were subjected to amphotericin B killing as described below. On day 5, cells were again fed medium A containing LDL at 30 μg of protein per ml and subjected to amphotericin B killing on day 6. Surviving cells were grown in either medium A supplemented with 10 mM sodium mevalonate (SRD-6A cells) or medium B containing 5 μg of cholesterol per ml and 200 μM sodium mevalonate (SRD-6B, -C, and -D cells) for 7 to 10 days with refeeding every 2 to 3 days.

A total of 8 of 31 pools from the two mutagenesis experiments produced 20 to 60 surviving colonies per dish, whereas the remaining 23 pools produced fewer than 3 amphotericin B-resistant colonies per dish. Clonal cell lines were established by harvesting individual amphotericin B-resistant colonies by using cloning cylinders and transferring these clones to 24-well Linbro plates in 1 ml of medium per well. Cell lines established from five of the eight positive pools were chosen for futher characterization. Once optimal growth conditions were established (see Results), the mutant cell lines were grown in medium B containing 5 μg of cholesterol per ml and 1 mM sodium mevalonate.

Amphotericin B killing. On day 0, cells were plated at 2×10^5 cells per 100-mm-diameter dish or 5×10^4 to 15×10^4 cells per 60-mm-diameter dish in their respective growth media. On day 2, cells were washed with PBS and refed medium A containing various additions as indicated. On day 3, 10 to 14 h following the additions, cells were rinsed once with PBS and refed a 1:1 mixture of Ham's F12 medium and Dulbecco's modified Eagle's minimum essential medium containing 1% lipoprotein-deficient serum, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 50 μg of amphotericin B per ml in dimethyl sulfoxide. (The final concentration of dimethyl sulfoxide was 0.5% [vol/vol].) The cells were incubated 3 to 5 h at 37°C, washed twice with PBS, and refed their respective growth media.

Assays. The proteolytic degradation of ^{125}I-LDL and the incorporation of [^{14}C]oleate into cholesteryl [^{14}C]oleate by cell monolayers were measured as previously described (15). The incorporation of [^{14}C]acetate into lipids by cell monolayers was measured as described elsewhere (2). HMG-CoA reductase mRNA and LDL receptor mRNA were measured by quantitative S1 nuclease analysis of total cellular RNA as described previously (25). Primer extension analyses for HMG-CoA synthase mRNA, ribosomal protein S17 mRNA, and chloramphenicol acetyltransferase (CAT) mRNA were performed as described previously (25). S1 nuclease-resistant or primer-extended products were subjected to electrophoresis on denaturing polyacrylamide gels, which were dried and exposed to Kodak XAR film at −70°C with intensifying screens. Quantitation was performed by direct radiometry, using the Ambis radioanalytical imaging system (Ambis Systems, San Diego, Calif.).

For immunoblot analysis, cell pellets were solubilized in 15% (wt/vol) sodium dodecyl sulfate (SDS)–8 M urea–10% (wt/vol) sucrose–62.5 mM Tris-HCl–10 mM EDTA–5 mM dithiothreitol at pH 6.8 by 20 passes through a 23-gauge needle. Immunoblot analysis was performed as previously described, using the HMG-CoA reductase-specific monoclonal antibody IgG-A9 (28). Quantitation of immunoblots was performed by direct radiometry as described above.

Genetic complementation analyses. G418-resistant (G418^r) and hygromycin B-resistant (Hyg^r) cell lines were established by calcium phosphate-mediated transfection with plasmids pCMV7S and pSV3 Hyg, respectively. Paired fusion of G418^r and Hyg^r cells was performed by plating 8×10^5 cells of each cell type in single wells of six-well Linbro plates (surface area, 9.6 cm^2) on day 0 in medium B containing 5 μg of cholesterol per ml and 1 mM sodium mevalonate. On day 1, the cells were fused with polyethylene glycol as previously described (12) and refed medium B supplemented with 5 μg of cholesterol per ml and 1 mM sodium mevalonate. On day 2, the cells in each well were replated into six 100-mm-diameter dishes in medium B containing 5 μg of cholesterol per ml and 1 mM sodium mevalonate. On day 3, the cells were refed medium B containing 5 μg of cholesterol per ml, 1 mM sodium mevalonate, 700 μg of G418 per ml, and 500 μg of hygromycin B per ml. On days 14 to 20, G418^r Hyg^r cells (approximately 1,000 colonies per fusion) were set up for amphotericin B killing and RNA analysis as described above.

Promoter construct analysis. CHO-7 and SRD-6B cells were transfected by the calcium phosphate-mediated gene transfer method, using coprecipitates of 7.5 μg of plasmid pF or pG (1) per dish and 2.5 μg of pRc/CMV per dish. G418^r colonies (450 to 900 individual clones) were pooled, expanded in mass culture in the presence of G418, and used for RNA analysis experiments as described above.

RESULTS

Isolation of amphotericin B-resistant CHO cells. To isolate mutant CHO cells that fail to induce sterol-regulated mRNAs in response to sterol deprivation, we used a modified version of the selection scheme for cholesterol auxotrophs that was developed by Saito et al. (32) and used previously by others (3, 19, 22). The scheme takes advantage of the fact that amphotericin B, a polyene antibiotic, kills cells by binding to cholesterol in the outer leaflet of the plasma membrane (21). Cells with cholesterol deficiency are resistant to amphotericin B killing, apparently because they lack sufficient binding sites for the antibiotic.

CHO cells cultured in the absence of LDL were sensitive to amphotericin B killing (Fig. 1, column 1) as a result of endogenous cholesterol synthesis. Inhibition of endogenous synthesis with compactin (column 2), a competitive inhibitor of HMG-CoA reductase, or suppression of cholesterol synthesis with 25-hydroxycholesterol (column 3) protected the cells from amphotericin B killing. CHO cells cultured in the presence of LDL were also sensitive to amphotericin B killing (column 4) as a result of the availability of both endogenously synthesized and LDL-derived cholesterol. Inhibition of cholesterol synthesis with compactin failed to protect the cells (column 5), whereas treatment with 25-hydroxycholesterol did provide protection (column 6) as a result of suppression of both cholesterol synthesis and LDL uptake.

Using the amphotericin B selection procedure, we isolated five independent lines of mutant cells that are resistant to amphotericin B killing in the presence of LDL. One of the mutant cell lines was similar to previously reported mutant cell lines (6, 32) in that it failed to convert lanosterol to cholesterol. The remaining four cell lines are designated

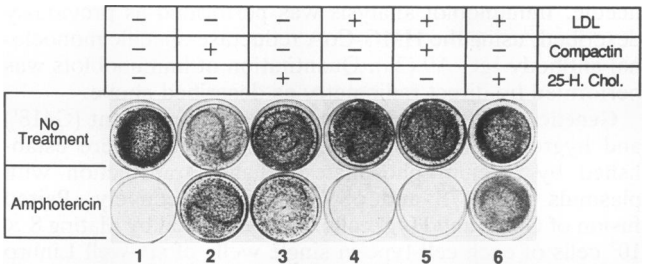

FIG. 1. Sensitivity of CHO cells to amphotericin B treatment. On day 0, cells were plated at 5×10^4 cells per 60-mm-diameter dish in medium A. On day 1, cells were refed medium A containing 100 μM sodium mevalonate supplemented with either LDL (30 μg of protein/ml), compactin (100 μM), or 25-hydroxycholesterol (25-H. Chol.; 1 μg/ml) as indicated. On day 3, the cells were treated with amphotericin B as described in Materials and Methods. Cells were subsequently grown for 7 to 10 days in medium A supplemented with 10 mM sodium mevalonate, washed, fixed, and stained with crystal violet.

SRD-6A, SRD-6B, SRD-6C, and SRD-6D. SRD-6A cells were isolated from CHO-7 cells that had been mutagenized chemically with methanesulfonic acid ethyl ester, while the other three mutant lines were isolated from CHO-7 cells mutagenized with γ irradiation. Unlike CHO cells, which were resistant to amphotericin B killing only when cultured in the presence of 25-hydroxycholesterol (Fig. 2), SRD-6 cells were resistant to amphotericin B killing when cultured in either the absence (column 2) or presence (column 3) of 25-hydroxycholesterol. In contrast, SRD-2 cells, which show no suppression of cholesterol synthesis or LDL receptor activity in the presence of 25-hydroxycholesterol (25), were sensitive to amphotericin B killing when cultured in

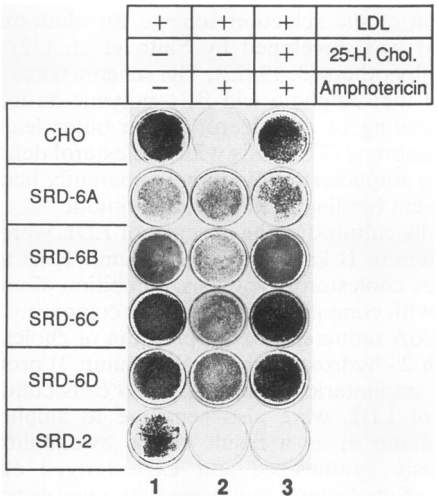

FIG. 2. Sensitivity of CHO, SRD-6A, SRD-6B, SRD-6C, SRD-6D, and SRD-2 cells to amphotericin B treatment. On day 0, cells were plated at 3×10^4 cells per 60-mm-diameter dish in their respective growth media. On day 1, cells were refed medium A containing LDL (30 μg of protein/ml) in the absence or presence of 1 μg of 25-hydroxycholesterol (25-H. Chol.) per ml as indicated. On day 2, cells were treated with amphotericin B as indicated and refed their respective growth media. On day 4, the cells were subjected to a second amphotericin B treatment. Cells were subsequently grown for 6 days in their growth media, washed, fixed, and stained with crystal violet.

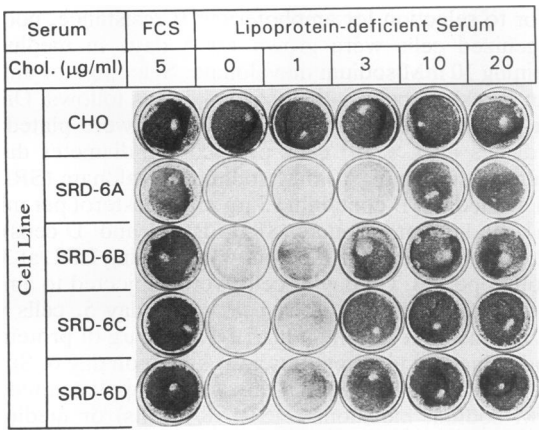

FIG. 3. Growth of CHO, SRD-6A, SRD-6B, SRD-6C, and SRD-6D cells in cholesterol. On day 0, cells were plated at 3×10^4 cells per 60-mm-diameter dish in medium B supplemented with 5 μg of cholesterol (Chol.) per ml and 1 mM sodium mevalonate. On day 1, the cells were washed three times with PBS and refed either medium B supplemented with 5 μg of cholesterol per ml and 1 mM sodium mevalonate (FCS) or medium A supplemented with 0.1 mM sodium mevalonate (lipoprotein-deficient serum) and the indicated concentration of cholesterol. The cells were refed every 2 to 3 days with medium of identical composition. On day 10, the cells were washed, fixed, and stained with crystal violet.

either the absence (column 2) or presence (column 3) of 25-hydroxycholesterol.

Figure 3 shows that all four SRD-6 cell lines grew well when cultured in 5% FCS supplemented with 1 mM mevalonate and 5 μg of cholesterol per ml. When the cholesterol and lipoproteins were removed from the medium and the concentration of mevalonate was reduced to 0.1 mM (designated lipoprotein-deficient serum in Fig. 3), CHO cells continued to grow well, but all four of the SRD-6 cell lines failed to grow. Restoration of normal growth required the addition of at least 3 μg of cholesterol per ml to the lipoprotein-deficient medium. These data demonstrate that all four of the amphotericin B-resistant SRD-6 cell lines are cholesterol auxotrophs.

Defective sterol synthesis in SRD-6A cells. The resistance of the SRD-6 cells to amphotericin B in the presence of LDL suggested that the cells have both a low rate of cholesterol synthesis and a low rate of LDL cholesterol uptake. To test whether cholesterol synthesis was impaired in these cells, we measured the rate of incorporation of [^{14}C]acetate into [^{14}C]lanosterol and [^{14}C]cholesterol. As shown in Fig. 4, SRD-6A cells synthesized these sterols at a rate that was only 15% of the rate in CHO cells. Addition of 25-hydroxycholesterol reduced the rate of sterol synthesis in CHO cells by 97%. Similarly, sterols caused a significant, further reduction of the already low rate of sterol synthesis in SRD-6A cells. In the same experiment, the incorporation of [^{14}C]acetate into ^{14}C-labeled fatty acids was similar in CHO and SRD-6A cells cultured in the absence of sterols (334 and 476 pmol/h/mg of protein, respectively). Similar results were obtained when [^{14}C]pyruvate was used as the substrate for incorporation into sterols in SRD-6A and CHO cells (data not shown).

Defective LDL receptor activity in SRD-6A cells. To estimate the activity of LDL receptors in SRD-6A cells, we measured the ability of LDL to stimulate the incorporation of [^{14}C]oleate into cholesteryl [^{14}C]oleate. This reesterifica-

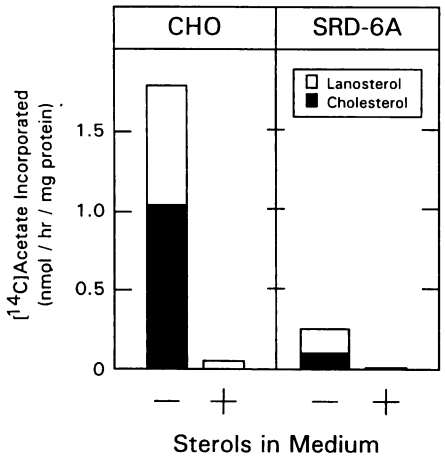

FIG. 4. Incorporation of [^{14}C]acetate into cellular sterols in CHO and SRD-6A cells. On day 0, cells were plated at 6 × 10^4 to 10 × 10^4 cells per 60-mm-diameter dish in their respective growth media. On day 1, the cells were washed twice with PBS and refed medium A (CHO) or medium A supplemented with 0.5% FCS plus 200 μM mevalonate (SRD-6A). On day 2, the duplicate dishes were refed medium A containing 100 μM mevalonate in either the absence (−) or presence (+) of sterols (a mixture of 1 μg of 25-hydroxycholesterol per ml plus 10 μg of cholesterol per ml). On day 3, the cells were washed twice with PBS and refed a 1:1 mixture of Ham's F12 medium and Dulbecco's modified Eagle's minimum essential medium supplemented with penicillin, streptomycin, 2 mg of bovine serum albumin per ml, and 0.5 mM [^{14}C]acetate (22 dpm/pmol). After incubation at 37°C for 2 h, the cells were harvested for measurement of [^{14}C]cholesterol, [^{14}C]lanosterol, and ^{14}C-labeled fatty acid content as previously described (2). Incorporation of [^{14}C]acetate in ^{14}C-labeled fatty acids was 334 pmol/h/mg of protein for CHO cells grown in the absence of sterols and 476 pmol/h/mg of protein for SRD-6A cells. Each value represents the average of duplicate incubations. The average protein contents per dish were 148 μg (CHO) and 123 μg (SRD-6A).

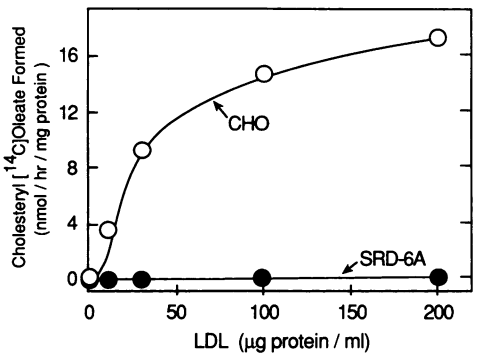

FIG. 5. LDL-stimulated incorporation of [^{14}C]oleate into cholesteryl [^{14}C]oleate in CHO and SRD-6A cells. On day 0, cells were plated at 7 × 10^4 to 15 × 10^4 cells per 60-mm-diameter dish in medium A supplemented with 1% (vol/vol) FCS and 200 μM sodium mevalonate. On day 2, cells were refed with medium of identical composition. On day 3, monolayers were washed with PBS and refed medium A supplemented with 100 μM sodium mevalonate and 10 μm compactin. On day 4, the cells were refed 2 ml of Dulbecco's modified Eagle's minimum essential medium (without glutamine) containing 2 mg of bovine serum albumin per ml, 10 μM compactin, and the indicated additions of human LDL. After incubation for 5 h at 37°C, each monolayer was pulsed for 2 h with 0.2 mM [^{14}C]oleate/albumin (9,736 dpm/nmol), after which the cells were harvested for measurement of cholesteryl [^{14}C]oleate content. Each value was determined from duplicate or triplicate incubations. The levels of incorporation of [^{14}C]oleate into cellular ^{14}C-labeled triglycerides were similar in the two cell lines. These values were, respectively, 39 and 24 nmol/h/mg of protein for CHO and SRD-6A cells incubated in the absence of LDL and 34 and 29 nmol/h/mg of protein for CHO and SRD-6A cells incubated in the presence of 200 μg of LDL per ml.

tion reaction, catalyzed by ACAT, is enhanced when cells take up significant amounts of LDL-derived cholesterol via the LDL receptor (18). Whereas CHO cells showed the expected stimulation of cholesteryl ester synthesis in the presence of LDL, SRD-6A cells showed no response (Fig. 5). As a control, the rates of incorporation of [^{14}C]oleate into ^{14}C-labeled triglycerides were similar in CHO and SRD-6A cells and were not affected by LDL (see the legend to Fig. 5). To make certain that the failure of SRD-6A cells to respond to LDL was not secondary to a defect in the ACAT enzyme, we measured the ability of 25-hydroxycholesterol to stimulate the incorporation of [^{14}C]oleate into cholesteryl [^{14}C] oleate (Table 1). CHO and SRD-6A cells had similar basal rates of cholesteryl ester synthesis, and both responded similarly to sterols, indicating that the ACAT enzyme was intact. These results suggest that ACAT activity is normal in SRD-6A cells and that LDL fails to deliver cholesterol to the cells, possibly because of a deficiency in LDL receptor activity.

To assess LDL receptor function more directly, we measured the rate of receptor-dependent degradation of ^{125}I-LDL (Table 2). In the presence of sterols, CHO cells degraded ^{125}I-LDL at a low rate that increased by more than sixfold when sterols were removed. SRD-6A cells also showed a relatively low rate of ^{125}I-LDL degradation in the presence of sterols, but these cells showed no induction when sterols were removed. The low level of ^{125}I-LDL degradation in SRD-6A cells is attributable to LDL receptor activity, since it was much higher than the rate of degradation in *ldlA*-7 cells, a mutant line of CHO cells that lacks functional LDL receptors (20). These data suggest that SRD-6A cells have a basal constitutive rate of LDL receptor synthesis, but they are unable to increase this synthesis in response to cholesterol deprivation. A similar conclusion was reached when the LDL receptors were visualized directly by ligand blotting with ^{125}I-LDL on nitrocellulose blots (data not shown) by the method of Daniel et al. (10).

Constitutive suppression of HMG-CoA reductase, HMG-CoA synthase, and LDL receptor mRNA in SRD-6A cells. The data presented so far indicate that SRD-6A cells fail to

TABLE 1. Sterol-stimulated incorporation of [^{14}C]oleate into cholesteryl [^{14}C]oleate in CHO and SRD-6A cells

Cell line	Cholesteryl [^{14}C]oleate formed[a] (nmol/h/mg of protein)	
	Without sterols	With sterols
CHO	0.29	2.9
SRD-6A	0.29	2.4

[a] Cells were set up for experiments on day 0 and grown through day 3 as described in the legend to Fig. 5. On day 4, cells were refed 2 ml of Dulbecco's modified Eagle's minimum essential medium (without glutamine) containing 2 mg of bovine serum albumin per ml and 10 μM compactin in the absence or presence of sterols (1 μg of 25-hydroxycholesterol per ml plus 10 μg of cholesterol per ml). After incubation for 5 h at 37°C, each monolayer was pulsed for 2 h with 0.2 mM [^{14}C]oleate–albumin (9,736 dpm/nmol), after which the cells were harvested for measurement of cholesteryl [^{14}C]oleate content. Each value is the mean of triplicate incubations.

TABLE 2. Regulation of LDL receptor activity in CHO, SRD-6A, and *ldlA*-7 cells

Cell line	^{125}I-LDL degraded[a] (ng/5 h/mg of protein)	
	Without sterols	With sterols
CHO	1,172	186
SRD-6A	235	358
ldlA-7	13	11

[a] On day 0, cells were plated at 4×10^4 (CHO and *ldlA*-7 cells) or 8×10^4 (SRD-6A cells) cells per 60-mm-diameter dish in medium A supplemented with 1% (vol/vol) FCS and 200 μM sodium mevalonate. On day 2, cell monolayers were washed with PBS and refed medium A containing 100 μM sodium mevalonate in the absence or presence of sterols (a mixture of 1 μg of 25-hydroxycholesterol per ml plus 10 μg of cholesterol per ml). On day 3, the cells were refed medium of identical composition. On day 4, the cells were refed 2 ml of Dulbecco's modified Eagle's minimum essential medium (without glutamine) containing 2 mg of bovine serum albumin per ml, and ^{125}I-LDL (10 μg of protein/ml; 153 cpm/ng of protein) in the absence or presence of unlabeled LDL (500 μg of protein per ml). After incubation for 5 h at 37°C, high-affinity degradation of ^{125}I-LDL was determined by subtracting the values for nonspecific degradation (in the presence of unlabeled LDL) from the values for total degradation (in the absence of unlabeled LDL). Each value is the mean of triplicate incubations.

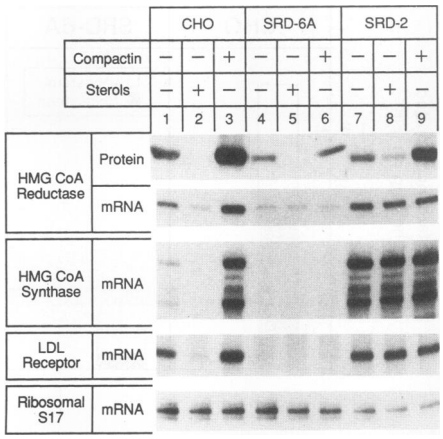

FIG. 6. Regulation of HMG-CoA reductase, HMG-CoA synthase, and LDL receptor mRNA levels and HMG-CoA reductase protein levels in CHO, SRD-6A, and SRD-2 cells. On day 0, cells were plated at 5×10^5 cells per 100-mm-diameter dish in their respective growth media. On day 2, cells received medium A containing 100 μM sodium mevalonate and the indicated additions of 100 μM compactin or sterols (10 μg of cholesterol per ml plus 1 μg of 25-hydroxycholesterol per ml). After incubation for 12 to 16 h, the cells were harvested for total cellular protein and total RNA. Aliquots of solubilized cell extract (40 μg) were subjected to SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and incubated with 10 μg of antireductase monoclonal antibody IgG-A9 per ml. Primary antibody interactions were detected by using ^{125}I-labeled rabbit anti-mouse immunoglobulin G (2×10^6 cpm/ml; 4,500 cpm/ng). The nitrocellulose filters were exposed to Kodak XAR film for 24 h at −70°C with intensifying screens. Aliquots of isolated RNA (20 to 40 μg) were subjected either to quantitative S1 nuclease analysis for HMG-CoA reductase mRNA and LDL receptor mRNA or to primer extension analysis for HMG-CoA synthase mRNA and ribosomal protein S17 mRNA as described in Materials and Methods. The dried gels were exposed to Kodak XAR film with intensifying screens for 4 to 24 h at −70°C.

increase the rates of sterol biosynthesis and LDL receptor activity in response to sterol deprivation. To determine whether this defect results from a failure to increase the production of sterol-regulated mRNAs, we compared the amounts of mRNA for HMG-CoA reductase, HMG-CoA synthase, and the LDL receptor in SRD-6A cells and CHO cells incubated under conditions of sterol excess or deficiency. mRNA levels were measured by S1 nuclease analysis (HMG-CoA reductase and LDL receptor) or by primer extension (HMG-CoA synthase), and the amount of mRNA for a control nonregulated protein (ribosomal protein S17) was measured by primer extension. Autoradiograms from one experiment are shown in Fig. 6, and the average results of quantitative scans of autoradiograms from several experiments are presented in Table 3. In CHO cells, the level of HMG-CoA reductase mRNA was suppressed 2.5-fold when excess 25-hydroxycholesterol and cholesterol were provided and induced 3.7-fold when sterol synthesis was inhibited by compactin. SRD-6A cells produced the same amount of mRNA as did CHO cells in the suppressed state, and there was no induction by compactin. HMG-CoA synthase mRNA showed an even greater relative degree of suppression and induction than did HMG-CoA reductase mRNA in CHO cells (total change of greater than 100-fold). In SRD-6A cells, HMG-CoA synthase mRNA was not detectable under any condition. For comparison, we also studied SRD-2 cells in the same experiments. These cells had constitutively high levels of all three mRNAs as previously reported (25), and again there was no regulation by sterol manipulation.

Posttranscriptional regulation of HMG-CoA reductase is intact in SRD-6A cells. In addition to regulation at the level of mRNA transcription, HMG-CoA reductase is regulated posttranscriptionally. Addition of sterols decreases the rate of translation and increases the rate of degradation of the protein (17). This regulation is evident in Fig. 6 and Table 3, in which we measured the amount of HMG-CoA reductase protein by immunoblotting. In CHO cells, the addition of sterols decreased the amount of reductase protein by more than 90% while decreasing the amount of mRNA by only 40%. The ratio of protein to mRNA was decreased by more than 90%, reflecting the existence of posttranscriptional control mechanisms. At least one of the posttranscriptional mechanisms that controls HMG-CoA reductase activity must be intact in the SRD-6A cells; the addition of sterols reduced the HMG-CoA reductase protein by more than 90% even though the amount of mRNA did not decline. Interestingly, the addition of compactin did not alter the protein/mRNA ratio in either CHO or SRD-6A cells. This posttranscriptional regulation of HMG-CoA reductase activity is likely to be at least partly responsible for the reduction in the rate of cholesterol synthesis observed in SRD-6A cells grown in the presence of sterols (Fig. 4).

SRD-6A, -B, -C, and -D cells are genetically recessive and belong to the same complementation group. Although most of the phenotypic data presented so far have been for SRD-6A cells, essentially identical results were obtained for SRD-6B, -C, and -D cells in all assays in which they were tested (data not shown). To determine whether the mutations in the SRD-6A, -B, -C, and -D cells are allelic, we performed a series of somatic cell hybridization experiments. To select for hybrid cells, Hyg^r and G418^r derivatives of each cell line were created by transfection with plasmids expressing either the hygromycin B phosphotransferase (*hyg*) gene or the aminoglycoside 3γ-phosphotransferase (*neo*) gene. The latter gene renders cells resistant to the antibiotic G418. Pools of stable transformants were isolated, and paired fusions were performed by using polyethylene glycol. Pools of hybrid colonies were selected by growth in the simultaneous presence of hygromycin and G418 and assayed for ampho-

TABLE 3. Regulation of HMG-CoA reductase, HMG-CoA synthase, and LDL receptor mRNA in CHO, SRD-6A, and SRD-2 cells

Cell line	Addition	Relative level				
		HMG-CoA reductase			HMG-CoA synthase mRNA[a]	LDL receptor mRNA[a]
		Protein[b]	mRNA[a]	Protein/mRNA		
CHO	None	1.0	1.0	1.0	1.0	1.0
	Sterols	<0.05	0.6	<0.08	<0.05	0.3
	Compactin	3.8	3.7	1.0	5.6	3.3
SRD-6A	None	0.5	0.6	0.8	<0.05	<0.2
	Sterols	<0.05	0.6	<0.08	<0.05	<0.2
	Compactin	0.6	0.7	0.9	<0.05	<0.2
SRD-2	None	0.7	4.7	0.1	22.0	5.2
	Sterols	0.4	5.2	0.1	23.1	5.1
	Compactin	2.4	4.9	0.5	20.7	5.0

[a] Assayed as described in the legend to Fig. 6 and quantitated by direct radiometry of the dried gels, using the Ambis radioanalytical imaging system. The data were normalized to the signal produced from the primer extension of ribosomal protein S17 mRNA. Values are relative to levels observed in CHO cells that received no additions and represent means of four to six experiments.

[b] Measured by immunoblotting with monoclonal antibody IgG-A9 as described in Materials and Methods. The relative levels of protein were determined by direct radiometry of the nitrocellulose filters. Values are relative to levels observed in CHO cells that received no additions and represent means of four to six experiments.

tericin B sensitivity. As a control, we showed that each cell line maintained the parental phenotype when fused with itself (Fig. 7). For example, fused CHO cells that were doubly resistant to hygromycin and G418 escaped amphotericin B killing only when cultured in the presence of 25-hydroxycholesterol, while fused SRD-6B cells that were doubly resistant to hygromycin and G418 were resistant to amphotericin B in either the absence or presence of 25-hydroxycholesterol.

When Hyg^r CHO cells were fused with G418^r SRD-6A, -B, -C, or -D cells, the resulting hybrids were similar to CHO cells in their sensitivity to amphotericin B. Identical results were obtained when the resistance markers were switched, i.e., when G418^r CHO cells were fused with Hyg^r SRD-6B, -C, or -D cells. (For unknown reasons, we were unable to isolate a Hyg^r line of SRD-6A cells.) These findings suggest that all four mutations are genetically recessive with respect to the wild type. Fusions between any pair of Hyg^r and G418^r SRD-6 cell lines always yielded hybrids that maintained the mutant phenotype; i.e., they resisted killing by amphotericin B. To confirm that these mutant cells did not complement each other at the level of mRNA regulation, the hybrid cells were incubated under three conditions: (i) absence of sterols, (ii) presence of sterols, and (iii) presence of compactin. The amounts of HMG-CoA synthase mRNA and the control mRNA encoding the ribosomal S17 protein were measured by primer extension (Fig. 8). When any of the cell lines were fused with CHO cells, the hybrids showed the normal pattern of mRNA regulation, i.e., suppression by sterols and induction by compactin. When any of the SRD-6 cell lines were fused with any other SRD-6 cell line, the mutant phenotype, i.e., low level expression of HMG-CoA synthase mRNA with no induction by compactin was retained. The results were the same irrespective of which partner had the hygromycin resistance or G418 resistance gene. These findings indicate that all four lines of SRD-6 cell lines belong to the same complementation group and suggest that all four lines have mutations in the same gene.

Previous cell hybridization studies demonstrated that the mutation in the sterol-resistant SRD-2 cells is recessive with respect to the wild type (12). Fusion of SRD-2 cells with SRD-6A, -B, -C, and -D cells produced the wild-type pattern of amphotericin survival (Fig. 7), demonstrating that the SRD-2 and SRD-6 mutations are not allelic.

SRD-6B cells lack SRE-dependent transcription. In the final experiment, we sought to determine whether the failure of

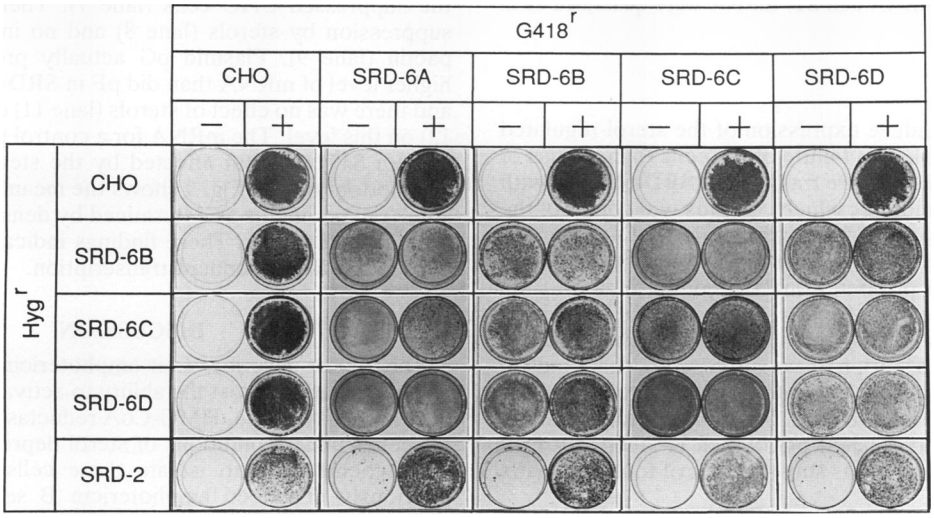

FIG. 7. Genetic complementation analysis: sensitivity of fused cells to amphotericin B treatment. Pools of G418^r Hyg^r cells were isolated following paired fusions between G418^r (horizontal axis) and Hyg^r (vertical axis) cell lines as described in Materials and Methods. On day 0, G418^r Hyg^r cells were seeded at 5×10^5 cells per 100-mm-diameter dish in medium B containing 5 μg of cholesterol per ml, 1 mM sodium mevalonate, 700 μg of G418 per ml, and 500 μg of hygromycin B per ml. On day 1, the cells were washed with PBS and refed medium A containing 30 μg of LDL per ml in the absence (−) or presence (+) of 1 μg of 25-hydroxycholesterol per ml. On day 2, the cells were subjected to amphotericin B killing as described in Materials and Methods and refed medium B containing 5 μg of cholesterol per ml, 1 mM sodium mevalonate, 700 μg of G418 per ml, and 500 μg of hygromycin B per ml. On day 9, the cells were washed, fixed, and stained with crystal violet.

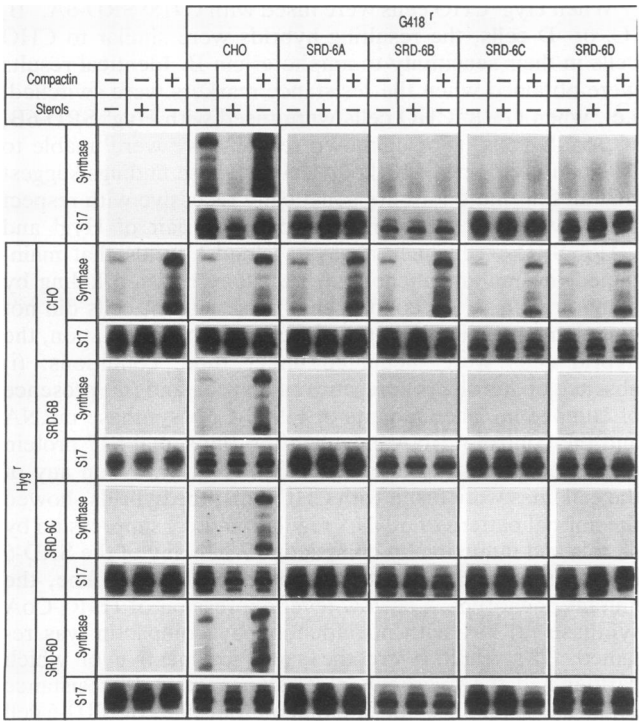

FIG. 8. Genetic complementation analysis: regulation of HMG-CoA synthase mRNA in cell fusions. Pools of G418^r Hyg^r cells were isolated following paired fusions between G418^r and Hyg^r cell lines as described in Materials and Methods. On day 0, cells were plated at 5×10^5 cells per 100-mm-diameter dish in medium B containing 5 μg of cholesterol per ml, 1 mM mevalonate, and the appropriate antibiotics (700 μg of G418 per ml for G418^r cell lines, 500 μg of hygromycin per ml for Hyg^r cell lines, and 700 μg of G418 per ml plus 500 μg of hygromycin per ml for the fusion cell lines). On day 1, cells were washed with PBS and refed medium A. On day 2, cells received medium A containing 100 μM sodium mevalonate and the indicated additions of 100 μM compactin or sterols (10 μg of cholesterol per ml and 1 μg of 25-hydroxycholesterol per ml). HMG-CoA synthase mRNA and S17 mRNA were quantified as in Fig. 6.

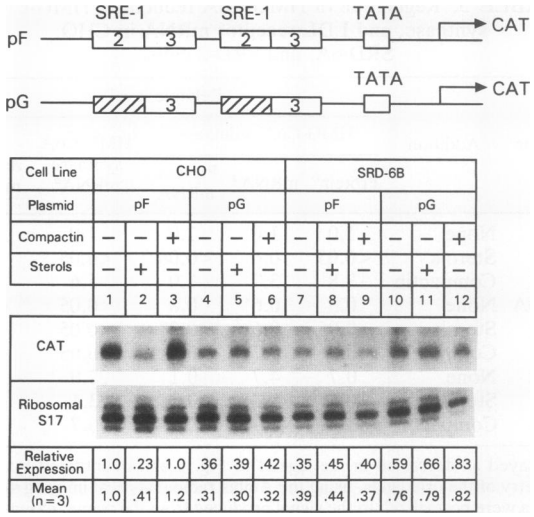

FIG. 9. Sterol-mediated suppression of transfected promoter-CAT plasmids containing repeats 2 and 3 of the LDL receptor promoter. A schematic diagram of the LDL human receptor promoter-CAT chimeric constructs (pF and pG [22]) is shown at the top. CHO and SRD-6B cells were transfected with pRc/CMV and the pF and pG plasmids containing the indicated insertion from the LDL receptor promoter. Total RNA was prepared from pools of stably transfected cells grown in the absence (−) or presence (+) of sterols and compactin as described in the legend to Fig. 6. Total RNA was subjected to primer extension analysis using oligonucleotides specific for either CAT mRNA or ribosomal protein S17 mRNA as previously described (25). The relative expression of CAT mRNA was normalized in reference to the signal produced by the S17 mRNA and is shown for a representative experiment. The values reported represent the means of three independent experiments, with each RNA quantified twice in each experiment.

the SRD-6 cells to induce expression of the sterol-regulated mRNAs is attributable to a failure of the cells to make use of the SRE. For this purpose we transfected SRD-6B cells with a recombinant plasmid, pF, which contains two copies of the tandem repeats 2 and 3 from the human LDL receptor promoter positioned upstream of a TATA box obtained from the adenovirus E1B promoter and the coding region of the bacterial CAT gene (1). Repeat 3 contains a weak Sp1 binding site, but it is insufficient to drive high-level transcription without the contribution of a positive factor that binds to the adjacent SRE, and the SRE-dependent factor functions only in the absence of sterols (1). As a control for the effect of the SRE, we used plasmid pG, which is identical to pF except that the SRE contains substitutions of four base pairs that inactivate it (1).

In wild-type CHO cells incubated in the absence of sterols, plasmid pF produced mRNA encoding CAT, as determined by primer extension (Fig. 9, lane 1). Transcription was suppressed by about 75% in the presence of sterols (lane 2), but compactin had little effect (lane 3). When the SRE was inactivated (plasmid pG), the amount of transcription was reduced to suppressed levels (lane 4), and there was no further suppression with sterols or induction with compactin (lanes 5 and 6). In SRD-6B cells, plasmid pF produced levels of mRNA that were similar to the levels produced in the suppressed CHO cells (lane 7). There was no further suppression by sterols (lane 8) and no increase with compactin (lane 9). Plasmid pG actually produced a slightly higher level of mRNA than did pF in SRD-6B cells (lane 10), and there was no effect of sterols (lane 11) or compactin (lane 12) on this level. The mRNA for a control protein (ribosomal protein S17) was not affected by the sterol manipulations. The bottom row of Fig. 9 shows the mean results from three similar experiments as determined by densitometric scans of the autoradiograms. These findings indicate that the SRD-6 cells lack SRE-dependent transcription.

DISCUSSION

This report describes four amphotericin B-resistant CHO cell lines that have lost the ability to activate transcription of HMG-CoA synthase, HMG-CoA reductase, and LDL receptor genes under conditions of sterol deprivation. The selection scheme used to isolate these cells was based on a previously described amphotericin B selection procedure (32) and was specifically designed to isolate cells with defects in both cholesterol synthesis and LDL uptake. Amphotericin B is a polyene antibiotic that kills cells by binding to cholesterol in the plasma membrane (21). Cells depleted of sterols survive amphotericin B killing, presumably because of the lack of cholesterol targets in the membrane. Endogenously synthesized cholesterol provides a

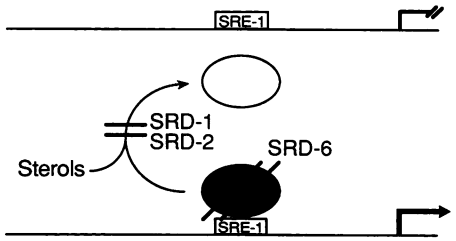

FIG. 10. Suggested role for SRD mutations in sterol-mediated transcriptional regulation. See text for details.

target for amphotericin B, as demonstrated by the sensitivity of CHO cells when grown in the absence of LDL. Suppression of cholesterol synthesis with oxysterols renders the cells resistant to killing. LDL-derived cholesterol also provides a target for amphotericin B killing, as demonstrated by the sensitivity of CHO cells to amphotericin B when grown in the presence of LDL but in the absence of endogenous cholesterol synthesis. These results demonstrate that in order for cells to survive amphotericin B treatment in the presence of LDL, the cells must possess both a reduced capacity to synthesize cholesterol and a reduced capacity to utilize LDL-derived cholesterol. All of the cell lines isolated by this procedure did in fact exhibit both a decreased capacity to synthesize cholesterol and a decreased capacity to utilize LDL-derived cholesterol.

The phenotype of the SRD-6 cells is reciprocal to that of previously described oxysterol-resistant cell lines SRD-1 and SRD-2 (25) that fail to suppress transcription of sterol-regulated genes in the presence of sterols. Each of the three sterol-regulated genes is under the control of a common SRE located in the 5'-flanking region of the gene. Chimeric promoter plasmids containing the SRE direct regulated expression of reporter genes when transfected into CHO cells. In the case of HMG-CoA synthase and the LDL receptor, mutation of the SRE prior to transfection eliminates regulated expression, with the level of expression being comparable to that of the wild-type promoter in sterol-suppressed cells (11, 38). These studies indicate that the SRE functions as a conditional activator of transcription and suggest a model in which (i) a transcription factor binds to the SRE to promote transcription in the absence of sterols and (ii) sterols inactivate this factor (Fig. 10). The high rates of transcription observed in SRD-1 and SRD-2 cells are dependent on the SRE; mutation of the SRE in chimeric promoter constructs prior to transfection eliminates overexpression (25). The defects in SRD-1 and SRD-2 cells are genetically recessive (12), suggesting that SRD-1 and SRD-2 cells have loss-of-function mutations in cellular factors required for sterol-mediated inactivation of the transcriptional activator. According to this model, mutations in the transcriptional activator itself which prevent sterol-mediated inactivation could also produce oxysterol-resistant cells; however, this type of mutation should be dominant. Dominant oxysterol-resistant cell lines have been described (31).

Loss-of-function mutations in the transcriptional activator should produce cells with low mRNA levels for sterol-regulated genes even under conditions of sterol deprivation. The SRD-6 cell lines described in this report fulfill this criterion, suggesting that these cells lack a functional transcriptional activator. This could be due to mutation of the activator itself or to mutation in another cellular factor required for the production of a functional activator. The finding that all four of the SRD-6 cell lines belong to the same complementation group suggests that there are a limited number genes (possibly one) that when mutated produce this phenotype.

Several lines of evidence indicate that the defect in SRD-6 cells is limited to transcriptional mechanisms of sterol-mediated regulation. We have shown previously that post-transcriptional mechanisms of cholesterol homeostasis are retained in cells of the SRD-1 and SRD-2 complementation groups (12, 25); these cells retain the ability to stimulate ACAT activity and to enhance the rate of HMG-CoA reductase degradation in response to sterols. SRD-6 cells also retain the ability to suppress HMG-CoA reductase activity and to enhance cholesterol esterification in response to sterols. SRD-6 cells stimulate cholesterol esterification normally in response to the direct addition of sterols to the culture media (Table 1), and although we did not directly measure the rate of HMG-CoA reductase protein synthesis or degradation, HMG-CoA protein levels did not directly parallel changes in the mRNA level (Table 3), suggesting that posttranscriptional mechanisms controlling HMG-CoA reductase activity must also be retained in these cells. In SRD-6A cells, the addition of sterols to the media reduces the level of HMG-CoA reductase protein by >10-fold, with no apparent change in mRNA level (Fig. 6; Table 3). These findings support previous studies (12, 25, 31) which demonstrate that transcriptional and posttranscriptional control of cholesterol metabolism are achieved by genetically distinct mechanisms.

The role of the SRE in the HMG-CoA reductase promoter is not as well defined as its role in the HMG-CoA synthase and the LDL receptor promoters. Detailed single-nucleotide substitution analyses of the HMG-CoA reductase regulatory domain indicated that two separate regions are essential for regulation (29). Although one of these regions overlaps the 5' portion of the SRE, the 3' portion of the SRE was not required. This unique requirement for *cis* elements has led to the speculation that sterol-mediated transcriptional regulation of HMG-CoA reductase is fundamentally different from that of other sterol-regulated genes. A DNA-binding protein (Red25) that interacts with the 5' portion of the HMG-CoA reductase SRE has recently been purified (30). However, since the nucleotides required for Red25 binding and the nucleotides required for transcriptional regulation differ, Osborne et al. (30) have suggested that Red25 acts in concert with other transcription factors that bind to adjacent or overlapping sites within the regulatory region. Red25 is specific for the HMG-CoA reductase promoter and appears not to bind to the HMG-CoA synthase promoter or the LDL receptor promoter (30). Our findings that regulation of HMG-CoA reductase, HMG-CoA synthase, and the LDL receptor is defective in SRD-1, SRD-2, and SRD-6 cells indicate that at least three cellular factors required for transcriptional regulation of HMG-CoA reductase are the same as those required for transcriptional regulation of other sterol-regulated genes. Although these results argue against a general role of Red25 in sterol-mediated transcriptional regulation, it is possible that the genetically defined regulatory mechanism acts upon gene-specific transcription factors, such as Red25, to affect transcriptional regulation. Mutations in these gene-specific factors would not have been identified in the current studies since our selection scheme was specifically designed to isolate cell lines with defects in both cholesterol synthesis and LDL uptake.

The SRD-1, SRD-2, and SRD-6 cell lines provide a useful system for addressing several questions regarding the mech-

anisms of cholesterol homeostasis. For example, a number of other enzymes involved in cholesterol biosynthesis are regulated by sterols. These enzymes include mevalonate kinase (39), farnesyl-pyrophosphate synthase (9), and squalene synthase (13). If regulation of these enzymes is defective in SRD-1, SRD-2, and SRD-6 cells, then it is likely that these enzymes are regulated by the same mechanism that controls HMG-CoA reductase, HMG-CoA synthase, and the LDL receptor. If, however, these enzymes retain normal regulation in the mutant cells, then a genetically distinct regulatory mechanism must be involved. These cells will also be useful in evaluating the role and function of the SRE-binding protein that has recently been identified by Briggs et al. (1) and purified by Wang et al. (40).

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