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Receptor-mediated endocytosis of low density lipoprotein: Somatic cell mutants define multiple genes required for expression of surface-receptor activity

(complementation of Chinese hamster ovary cell mutants/revertant selection/hamster–human cell fusion/familial hypercholesterolemia/low-density lipoprotein receptor gene)

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ABSTRACT **We have used cell fusion and mutant reversion analysis to study a collection of Chinese hamster ovary (CHO) cell mutants that are unable to bind and internalize low density lipoprotein (LDL). Pairwise cell fusions show that these LDL receptor-deficient mutants fall into three recessive complementation groups, *ldlA*, *ldlB*, and *ldlC*. Complementation was detected by observing the uptake of fluorescent LDL and was quantitated by measuring the degradation of ^{125}I-labeled LDL by isolated hybrid cells. Previous studies had defined a fourth recessive complementation group, *ldlD*. Complementation tests between CHO cells and human fibroblasts suggested that the defects in mutants of the *ldlA* complementation group are analogous to those in a patient with homozygous familial hypercholesterolemia. A revertant of an *ldlA* mutant was isolated and appeared to be heterozygous at the *ldlA* locus. The phenotype of this revertant was similar to that of cells from patients with the heterozygous form of familial hypercholesterolemia. Together with recent DNA transfection studies, these results suggest that the *ldlA* locus is the structural gene for the LDL receptor in CHO cells. Mutants in the *ldlB*, *ldlC*, and *ldlD* complementation groups must have defects in genes that are required for either the regulation, synthesis, transport, recycling, or turnover of LDL receptors.**

Receptor-mediated endocytosis is a process by which cells take up a wide variety of physiologically active macromolecules, including transport proteins, viruses, toxins, and peptide hormones (1). These macromolecules bind to specific cell-surface receptors with high affinity, are internalized through coated pits and coated vesicles, and in many cases are subsequently degraded in lysosomes. One of the best understood systems of receptor-mediated endocytosis is the low density lipoprotein (LDL) pathway of cholesterol metabolism. LDL is the major cholesterol transport protein in human plasma. Lysosomal hydrolysis of cholesterol esters in the core of internalized LDL particles supplies cells with cholesterol required for membrane synthesis and growth. Extensive work by Brown, Goldstein, and their colleagues suggests that defects in the structural gene for the human LDL receptor are responsible for familial hypercholesterolemia, a relatively common human genetic disease characterized by elevated serum cholesterol levels and atherosclerosis (2). These naturally occurring mutations in the LDL receptor have been invaluable in delineating the role of receptor-mediated endocytosis in delivering cholesterol to cells.

To characterize in greater detail the cellular components and processes involved in receptor-mediated endocytosis, we have developed methods for additional genetic analysis of the LDL pathway in somatic cells (3, 4). The LDL pathway was chosen for these studies, because the unique structure and function of LDL can be exploited for the selection of mutants and revertants. For example, LDL pathway mutants can be isolated in Chinese hamster ovary (CHO) cells by incubating mutagen-treated cells with LDL that has been reconstituted so that the cholesterol esters in the core of the lipoprotein are replaced with either toxic 25-hydroxycholesteryl oleate or fluorescent cholesterol ester (3). Rare mutants that grow in the presence of toxic LDL and that fail to accumulate fluorescence from fluorescent LDL are markedly deficient in LDL receptor activity. As an alternative mutant selection technique (4), cells can be incubated overnight in medium containing LDL, trace amounts of mevalonate, and compactin, a potent inhibitor of endogenous cholesterol biosynthesis. In this medium (Mev/LDL/Com), cells that cannot utilize the cholesterol in LDL become cholesterol deficient, while cells with a normal LDL pathway remain cholesterol replete. Subsequent treatment with amphotericin B, a polyene antibiotic that forms toxic complexes with sterols in cell membranes, kills normal but not cholesterol-deficient mutant cells (5, 6). The timing of the incubation in Mev/LDL/Com medium is crucial. If incubation is continued for more than 2 days, mutant cells that cannot obtain cholesterol from LDL will stop growing and will eventually die of cholesterol deprivation, while cells with an intact LDL pathway will grow continuously (3, 7, 8). Thus, Mev/LDL/Com medium can be used to select either mutants (when used in conjunction with amphotericin B), or revertants of mutants (when used alone).

In the current studies, we have extended our genetic analysis of endocytosis by using a rapid complementation assay to characterize further the defects in LDL receptor-deficient CHO cell mutants. The current complementation analysis and previous studies (9) suggest that at least four distinct types of genetic defect can disrupt functional expression of LDL receptors in CHO cells. Fusions between hamster and human mutants allow us to compare the defects in the CHO cell mutants with those in a familial hypercholesterolemia (FH) patient.

MATERIALS AND METHODS

Materials. LDL labeled with the fluorescent probe 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine iodide

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Abbreviations: LDL, low density lipoprotein; DiI-LDL, LDL labeled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine iodide; Mev/LDL/Com, medium A containing newborn bovine lipoprotein-deficient serum (3%), mevalonate (0.25 mM), LDL (3–30 μg of protein per ml), and compactin (40 μM); FH, familial hypercholesterolemia.

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(Molecular Probes, Junction City, OR), DiI-LDL, was prepared by the method of Pitas *et al.* (10). Polyethylene glycol 1000 was obtained from Baker. All other reagents and supplies were purchased from Sigma or were obtained as described (9).

Cell Culture. Parental and mutant CHO-K1 cells, and FH human fibroblasts (M.C., GM 2000) were grown as described (9). Mutant 7 refers to mutant 320-7a-1 (4), mutant 11 refers to a subclone of mutant 11-1a1 (3), mutant 475 refers to mutant 475-11-1 (3), and the *ldlD* mutant refers to mutant 14-1a1 (3, 9). All of the mutants isolated by reconstituted LDL selection and seven mutants isolated by Mev/LDL/Com–amphotericin B selection have been described (3, 4). Fifty additional mutants have been isolated by using minor variations (e.g., preincubation for 36 hr) of the Mev/LDL/Com–amphotericin B procedure (4). Three of these mutants were isolated after γ irradiation of wild-type cells (800 rad; cobalt source). All of the new mutants were unable to accumulate fluorescent LDL or were unable to degrade ^{125}I-labeled LDL. Stock cultures were grown in medium A [Ham's F-12 medium containing penicillin (100 units/ml), streptomycin (100 μg/ml), and glutamine (2 μM)] supplemented with 10% (vol/vol) newborn bovine serum (medium B). All incubations were at 37°C in a humidified 5% CO_2/95% air incubator unless otherwise noted.

Complementation Assay. Mixtures of cells were seeded into 24-well culture dishes in medium C [medium A supplemented with 3% (vol/vol) newborn bovine lipoprotein-deficient serum] and fused 1 day later with 50% (wt/vol) polyethylene glycol 1000 (11) in Ham's F-12 medium. Polyethylene glycol was omitted for mock fusions. One day after fusion, cells were dispersed with trypsin and replated in 24-well dishes in medium C to prevent subsequent overgrowth. After a 3-day expression period, cells were assayed for LDL receptor activity by incubation for 3–5 hr in medium C containing DiI-LDL (1 μg of protein per ml) (10). Accumulation of fluorescence was examined *in situ*, using a Leitz inverted fluorescence microscope (rhodamine filter package: exciter filter, 560/30 nm; chromatic beam splitter, 580 nm; barrier filter, 580 nm).

Isolation of Revertants. Mutant 7 cells were plated (750,000 cells per 100-mm dish) in Mev/LDL/Com medium (medium C supplemented with 250 μM mevalonate, 3 μg of protein per ml of LDL, and 40 μM compactin). Dishes were re-fed with Mev/LDL/Com medium every 3 days for 3 weeks. A high background of small slowly growing LDL receptor-deficient colonies often appears after prolonged Mev/LDL/Com selection, presumably because of low levels of nonspecific lipoprotein uptake. Surviving colonies were incubated with DiI-LDL as described above. Approximately 50% of the large healthy colonies accumulated fluorescence. Some of these were isolated, grown in Mev/LDL/Com medium, cloned by dilution plating, and subsequently maintained in medium B.

Isolation of Hybrid Cells. Cells were fused as described above. One day later, cell mixtures were replated (20,000–200,000 cells per 100-mm dish) in Mev/LDL/Com medium. Dishes were re-fed every 3 days and colonies that appeared after 10–14 days were incubated with DiI-LDL (see above). Most large colonies examined accumulated fluorescence. Some of these were isolated and maintained as stock cultures in Mev/LDL/Com medium. The modal number of chromosomes in each hybrid was determined from at least 20 well-spread metaphase cells (12).

Other Procedures. Cellular binding of ^{125}I-labeled LDL (4°C) and degradation of ^{125}I-labeled LDL (37°C) were measured in medium D [medium A supplemented with 5% (vol/vol) human lipoprotein-deficient serum] as described (9). The specific, high-affinity values presented are the differences between determinations made in the presence (single incubations) and absence (duplicate incubations) of excess unlabeled LDL.

RESULTS

The binding and uptake of fluorescent DiI-LDL (10) was used as a rapid assay to detect complementation of fused pairs of LDL receptor-deficient CHO cell mutants. Parental CHO cells accumulated fluorescent dye when incubated with DiI-LDL (Fig. 1 *A* and *B*), and this accumulation was inhibited when excess unlabeled LDL was included in the incubation medium (Fig. 1*C*). In contrast, receptor-deficient mutant CHO cell clones did not accumulate fluorescent dye from DiI-LDL (see below). When LDL receptor-deficient clone 7 was fused with LDL receptor-deficient clone 11 and the culture was subsequently incubated with DiI-LDL, accumulation of fluorescence was observed in 1%–10% of the cells, primarily in large multinucleated cells (Fig. 1 *D* and *E*). The fusion efficiency (fusion-induced multinucleated cells including homo- and heterokaryons) was 10%–20%. Essentially no cellular accumulation of fluorescence was detected when mixtures of clone 7 and 11 were subjected to mock fusion (Fig. 1*F*). Fusion-dependent complementation was also observed between mutants 7 and 475 and between mutants 11 and 475 (Fig. 1 *G–L*). The fusion procedure itself did not induce LDL receptor activity in homogeneous populations of any of the mutants (data not shown). In all cases, accumulation of fluorescence was inhibited by an excess of unlabeled LDL (data not shown).

To quantitate the level of LDL receptor activity in the fused cells, we isolated hybrids by using Mev/LDL/Com selection medium. Cells can grow rapidly in this medium only if they express the LDL pathway of receptor-mediated endocytosis (3, 7, 8). After incubation of fused mutants for 10–14 days in Mev/LDL/Com medium, large DiI-LDL-accumulating colonies appeared at a frequency of $\approx 10^{-3}$, and several were isolated for further study. In control experiments with

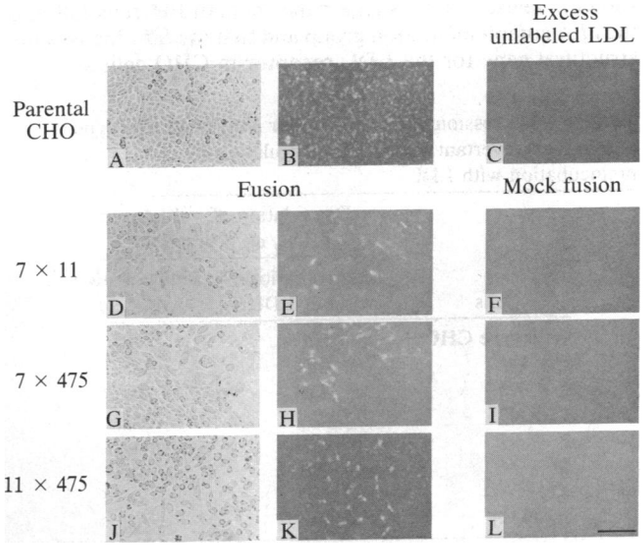

FIG. 1. Accumulation of fluorescent LDL by receptor-deficient CHO mutants after cell fusion. On day 0, equal mixtures of the indicated cell types were seeded into 24-well plates at a total concentration of 200,000 cells per well in 1.5 ml of medium C. On day 1, cells were fused or mock-fused. On day 2, monolayers were dispersed with trypsin and seeded into 24-well plates at ≈60,000 cells per well in 1.5 ml of medium C. On day 5, LDL receptor activity was measured by incubating cells for 3 hr in medium C containing DiI-LDL (1 μg of protein per ml) in the presence (*C*) or absence (*A*, *B*, and *D–L*) of excess unlabeled LDL (400 μg of protein per ml). Accumulation of fluorescence was examined *in situ* using bright-field (*A*, *D*, *G*, and *J*) and epifluorescence (*B*, *C*, *E*, *F*, *H*, *I*, *K*, and *L*) microscopy. (Bar = 100 μm.)

untreated or mock-fused mutants, no large DiI-LDL-accumulating colonies were detected (frequency, $<10^{-5}$). The isolated colonies appeared to be hybrids, because they were pseudotetraploid (modal chromosome numbers: 37–38). Pure clones of wild-type CHO cells and mutants 7, 11, and 475 were all pseudodiploid (modal chromosome numbers: 19–20). As shown in Table 1, the ability of the hybrid cells to degrade ^{125}I-labeled LDL was intermediate between that of wild-type CHO cells and the mutant clones from which the hybrids were derived. LDL-mediated suppression of receptor activity, which is a hallmark of the normal LDL pathway (2), was observed in the hybrids (Table 1).

The fusion-dependent induction of LDL-receptor activity in otherwise receptor-deficient mutants suggested that clones 7, 11, and 475 represent members of three distinct complementation groups, which we designate *ldlA* (mutant 7), *ldlB* (mutant 11), and *ldlC* (mutant 475). Sixty-three additional LDL pathway mutants isolated by two different selection techniques were fused to clones 7 and 11, and the ability of the fused cells to accumulate DiI-LDL was determined (Table 2). All mutants isolated using Mev/LDL/Com–amphotericin B selection were recessive and fell into the *ldlA* complementation group (accumulation of DiI-LDL after fusion with clone 11 but not clone 7; data not shown). Mutants isolated by reconstituted LDL selection fell predominately but not exclusively into the *ldlB* complementation group. At least 31 of the *ldlA* mutants and 2 of the *ldlB* mutants came from separate populations of mutagen-treated cells and, hence, are derived from independent mutational events.

To compare the defects in CHO mutants with the LDL receptor defects in human fibroblasts, we fused representative members of each complementation group to diploid fibroblasts from a patient with the homozygous receptor-negative form of FH. The defects in *ldlB* mutant 11 or *ldlC* mutant 475 were complemented by the FH cells, whereas the defects in *ldlA* mutant 7 and three other independent *ldlA* mutants were not complemented (Fig. 2 and data not shown). These results suggest that human FH cells fall into the *ldlA* complementation group and that the *ldlA* locus is the structural gene for the LDL receptor in CHO cells.

Table 1. Expression of LDL receptor activity in wild-type, hybrid, and revertant CHO cells: Regulation by preincubation with LDL

Cells	Degradation of ^{125}I-labeled LDL, ng/5 hr per mg	
	Preincubation without LDL	Preincubation with LDL
Wild-type CHO	2320*	490
7 × 11	704	245
7 × 475	1110	304
11 × 475	1210	245
7	0	—
11	49	—
475	103	—
RevA7-1	1485	294

On day 0, cells were seeded into 24-well dishes (60,000 or 120,000 cells per well) in 1.5 ml of medium C. On day 1, cells were divided into two groups. One group was re-fed with 1.5 ml of medium B and the other group was re-fed with 1.5 ml of medium B containing LDL (300 μg of protein per ml). On day 2, all cells were washed once with phosphate-buffered saline and incubated in 0.5 ml of medium D containing ^{125}I-labeled LDL (10 μg of protein per ml) in the presence or absence of unlabeled LDL (400 μg of protein per ml). After incubation for 5 hr at 37°C, the amounts of ^{125}I-labeled LDL degradation products excreted into the medium were determined. Each cell type was tested in one of three separate experiments, each of which included control wild-type cells.

*Average of three experiments (range, 2070–2450 ng/5 hr per mg).

Table 2. Distribution of mutants among the *ldl* complementation groups

Selection method	Complementation group, no. of isolates			
	ldlA	*ldlB*	*ldlC*	*ldlD*
Mev/LDL/Com–amphotericin B	57	—	—	—
Reconstituted LDL	1	7	1	1

In human patients with FH, mutant alleles at the LDL receptor locus are expressed in codominant fashion (2). Fibroblasts from heterozygous FH individuals express ≈50% of the normal number of LDL receptors, whereas cells from receptor-negative FH homozygotes express <2%. If the *ldlA* locus represents the structural gene for the hamster LDL receptor, CHO cells heterozygous at the *ldlA* locus might show similar gene dosage effects. To test this prediction, we used Mev/LDL/Com selection to isolate revertants of an apparently homozygous *ldlA* mutant. Spontaneous revertants of *ldlA* mutant 7 appeared at a frequency of $\approx 10^{-7}$. One revertant, RevA7-1, was examined in detail. As previously observed for heterozygous FH fibroblasts, the rate of degradation of ^{125}I-labeled LDL at 37°C by RevA7-1 cells was ≈50% of that seen in wild-type cells and was subject to normal regulation by sterols (Table 1). The binding of ^{125}I-labeled LDL at 4°C to RevA7-1 cells exhibited a normal K_m, although the extent of binding was 50% of that seen for wild-type cells (Fig. 3).

While it is possible that RevA7-1 cells carry an extragenic suppressor of the *ldlA* mutation, the half-normal levels of LDL receptor activity in the cells suggested that RevA7-1 was a heterozygote at the *ldlA* locus itself. The lack of convenient segregation systems for mammalian somatic cells makes it difficult to establish this point directly. It has been observed, however, that heterozygous mutants of somatic cells give rise to homozygous mutants at frequencies two to three orders of magnitude higher than the frequencies seen for conversion of wild-type cells to homozygous mutants (reviewed in ref. 13). When RevA7-1 cells were treated with the mutagen ethyl methanesulfonate and subjected to Mev/LDL/Com–amphotericin B selection, surviving LDL pathway mutants appeared at a frequency that was 250 times greater than that for control wild-type cells (Table 3). Three

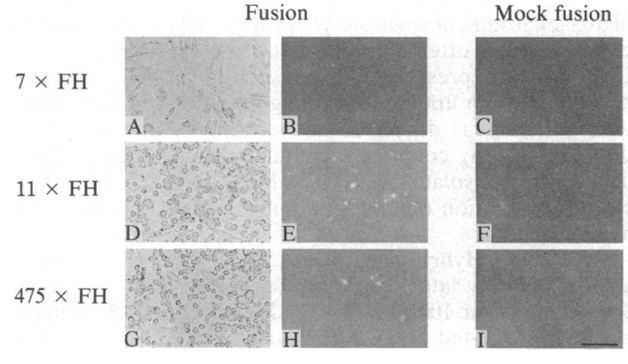

FIG. 2. Comparison of CHO and human cell mutants by cell fusion. Mixtures of the indicated cell types were seeded at a concentration of 100,000 CHO cells and 20,000 human cells per well in 24-well culture dishes in 1.5 ml of medium C. One day later, cells were fused or mock-fused. The following day, cells were dispersed with trypsin and seeded into 24-well dishes at a concentration of ≈60,000 cells per well in 1.5 ml of medium C. Three days later, LDL receptor activity was measured by incubating populations of cells for 5 hr in medium C containing DiI-LDL (1 μg of protein per ml). Accumulation of fluorescence was examined *in situ* using bright-field (*A*, *D*, and *G*) and epifluorescence (*B*, *C*, *E*, *F*, *H*, and *I*) microscopy. (Bar = 100 μm.)

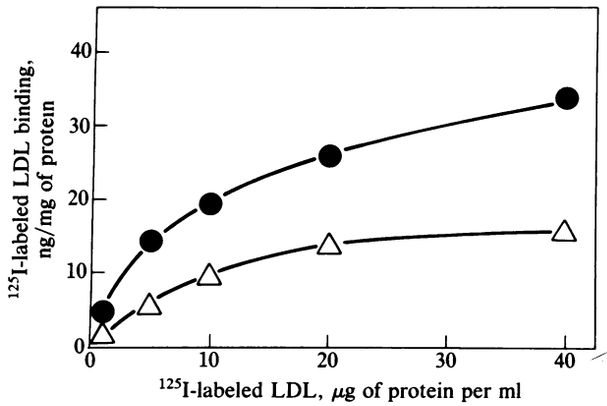

FIG. 3. Binding of ^{125}I-labeled LDL to the cell surface of wild-type and revertant cells at 4°C. On day 0, wild-type (●) and RevA7-1 (△) cells were seeded into 6-well plates (100,000 cells per well) in 3 ml of medium C. On day 2, cells were re-fed with 3 ml of medium C. On day 3, the medium was replaced with 1 ml of medium D containing the indicated amounts of ^{125}I-labeled LDL in the presence or absence of unlabeled LDL (400 μg of protein per ml). After incubation for 2 hr at 4°C, the amount of high-affinity surface-bound ^{125}I-labeled LDL was determined.

LDL pathway-defective colonies that arose from mutagen-treated RevA7-1 cells were isolated and shown to be members of the *ldlA* complementation group (data not shown). While we cannot rule out the possibility of extragenic suppression in the RevA7-1 cells, our failure to isolate receptor-negative suppressor-positive cells from RevA7-1 makes this possibility less likely.

DISCUSSION

As part of an ongoing genetic analysis of receptor-mediated endocytosis, we have used cell fusion and revertant selection techniques to characterize a collection of CHO cell mutants that are unable to bind and internalize LDL. Although all CHO cell mutants examined in detail exhibit an LDL receptor-deficient phenotype, these mutants can be separated into four distinct genetic groups by complementation analysis. The studies reported here have defined three recessive fusion-dependent complementation groups, *ldlA*, *ldlB*, and *ldlC*. These groups were initially detected by using a rapid fluorescence assay and were confirmed by analysis of LDL receptor activity in isolated hybrid cells. Previous studies with mixtures of cells demonstrated a fourth recessive complementation group, *ldlD* (previously called *cbc*; ref. 9). LDL receptor activity in *ldlD* mutant cells can be induced by cocultivating *ldlD* cells with either wild-type cells or LDL receptor-deficient mutants from several species. This fusion-independent form of complementation depends on the close proximity of complementing cells and cannot be mimicked by conditioned medium (9).

Table 3. Mutagen-dependent frequency of appearance of LDL pathway mutants after Mev/LDL/Com–amphotericin B selection: Comparison of wild-type and RevA7-1 cells

Cells	Colonies surviving selection, no. per 10^6 cells	
	Without ethyl methanesulfonate	With ethyl methanesulfonate
Wild-type CHO	0.0	1.3
RevA7-1	2.0	324.0

On day 0, control cells and cells treated with ethyl methanesulfonate (3) were seeded into 100-mm dishes (10^6 cells per dish) in medium C. On day 1, plates were re-fed with modified Mev/LDL/Com medium (LDL concentration increased to 30 μg of protein per ml). On day 3, cells were treated with amphotericin B (300 μg/ml) (4) and were re-fed medium C. On days 7 and 8, cells were again subjected to Mev/LDL/Com–amphotericin B selection. On day 11, surviving colonies were incubated with DiI-LDL. None of the surviving colonies accumulated fluorescence, and colonies were subsequently fixed, stained, and counted. The total numbers of cells subjected to selection were as follows: CHO (without ethyl methanesulfonate), 2×10^6; CHO (with ethyl methanesulfonate), 6×10^6; RevA7-1 (without ethyl methanesulfonate), 2×10^6; and RevA7-1 (with ethyl methanesulfonate), 9×10^6. Similar results were observed in two additional mutagenesis-selection experiments (data not shown).

The striking genetic diversity of the mutants we have isolated using reconstituted LDL selection (Table 2) strongly suggests that additional selections with this technique will uncover additional types of mutation that disrupt the LDL pathway. In contrast, the Mev/LDL/Com–amphotericin B selection appears to be genotype specific (Table 2). This specificity was unanticipated and may be related to either the stringency or toxicity of the Mev/LDL/Com–amphotericin B selection. For example, despite its low receptor activity (Table 1), the *ldlC* mutant apparently takes up enough LDL under Mev/LDL/Com conditions to become sensitive to amphotericin B (data not shown). The *ldlB* and *ldlD* mutants, perhaps because of changes in membrane structure, are unusually sensitive to high amphotericin B concentrations (300 μg/ml), regardless of preincubation conditions (data not shown). Subtle differences in wild-type CHO cells also may contribute to the mutant distributions in Table 2, because most of the Mev/LDL/Com–amphotericin B mutants were selected from a subclone of the CHO cells used for the reconstituted LDL selections.

Very few genetic studies of cultured mammalian cells have identified multiple complementation groups that affect the activity of a single protein (13–15). The diversity of genetic defects that disrupt LDL-receptor activity in CHO cells is probably due to the complex structure and life cycle of LDL receptors. The LDL receptor is a 160-kDa membrane glycoprotein that is synthesized from a 120-kDa glycoprotein precursor by addition of N- and O-linked oligosaccharide chains (16, 17). The mature form of the receptor recycles continuously between the inside and surface of the cell but is found predominately on the surface, where it is preferentially associated with coated pits (16). Expression of LDL-receptor activity and the abundance of LDL receptor mRNA are subject to sterol-mediated feedback regulation (2, 18). Mutations that affect any step in the life cycle of the LDL receptor, including regulation, synthesis, transport, recycling, or degradation, could result in a LDL receptor-deficient phenotype.

In human patients with FH, LDL receptor deficiencies are due to mutations at *ldlr*, the structural gene for the LDL receptor (2, 19, 20). The CHO cell *ldlA* locus appears to be homologous to the human *ldlr* locus, because *ldlA* mutants cannot complement the defects in fibroblasts from a homozygous FH patient. Also, a revertant of an *ldlA* mutant appears to be heterozygous at the *ldlA* locus and has a phenotype similar to that seen in fibroblasts from heterozygous FH patients (50% of normal receptor activity). The genotypic and phenotypic similarity between *ldlA* mutants and FH mutants suggests that the *ldlA* locus is the structural gene for the LDL receptor in CHO cells. This conclusion is strongly supported by recent DNA transfection studies (8). In these studies, the LDL receptor-deficient phenotype of an *ldlA* mutant CHO clone was corrected (complemented) by transfection with normal human DNA. Complementation of the *ldlA* mutation occurred because of the synthesis and expression of functional human LDL receptors in the transfected hamster cells.

In combination with the mutations identified in human FH cells, a large collection of *ldlA* mutations in CHO cells should be extremely useful for a detailed analysis of the rela-

tionship between LDL receptor structure and function. It is already clear that mutant alleles at the *ldlr* locus in humans can affect the amount, size, post-translational modification, internalization, and binding activity of the human LDL receptor (2, 19). CHO cell *ldlA* mutations have not yet been studied as extensively but should provide an even more diverse group of alleles, because they can be induced with a variety of mutagens and can be isolated routinely in large numbers (Table 2).

The nature of the defects that disrupt LDL receptor activity in the *ldlB*, *ldlC*, and *ldlD* mutants has not yet been elucidated. The LDL receptor (16, 21, 22) is apparently a monomeric protein, so it is unlikely that these mutants can be explained by intra-allelic complementation at the *ldlA* locus. It also seems unlikely that these mutations pleiotropically disrupt the coated pit-coated vesicle pathway of endocytosis. Mutants from the *ldlB* and *ldlD* complementation group do not exhibit altered lysosomal enzyme receptor activity (23) or altered susceptibility to infection by vesicular stomatitis virus (unpublished data), an animal virus thought to enter cells via coated pits and coated vesicles (24). Stable, cryptic, internal pools of LDL receptor activity have not been detected in soluble extracts of members of the *ldlB* and *ldlC* complementation groups (unpublished data). Nucleic acid and immunological probes specific for the LDL receptor of CHO cells will help to characterize further the defects in the *ldlB*, *ldlC*, and *ldlD* mutants.

The ability to select both mutants and revertants in the LDL pathway is one of the important attributes of this genetic system. The Mev/LDL/Com reversion selection was critical for the isolation of the hybrid cell lines and the apparently heterozygous *ldlA* mutant reported in this paper. The same selection has been used to isolate an *ldlA* mutant that regained an intact LDL pathway after transfection with normal human DNA (ref. 8; see above). It should be possible to isolate similar DNA-mediated revertants from mutants in the other complementation groups. Since human-specific repetitive DNA can be used to isolate functional human genes from transfected rodent cells (25), Mev/LDL/Com selection may provide the basis for identifying the normal counterparts of the defective genes in each of the four existing types of LDL receptor-deficient CHO mutants. As additional selections identify additional types of mutations that affect the LDL pathway, biochemical and reversion analysis may help characterize many of the cellular components required for the correct regulation, expression, and function of receptor-mediated endocytosis.

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