Three Types of Low Density Lipoprotein Receptor-deficient Mutant Have Pleiotropic Defects in the Synthesis of N-linked, O-linked, and Lipid-linked Carbohydrate Chains

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Abstract. Biochemical, immunological, and genetic techniques were used to investigate the genetic defects in three types of low density lipoprotein (LDL) receptor-deficient hamster cells. The previously isolated ldlB, ldlC, and ldlD mutants all synthesized essentially normal amounts of a 125,000-D precursor form of the LDL receptor, but were unable to process this receptor to the mature form of 155,000 D. Instead, these mutants produced abnormally small, heterogeneous receptors that reached the cell surface but were rapidly degraded thereafter. The abnormal sizes of the LDL receptors in these cells were due to defective processing of the LDL receptor's N- and O-linked carbohydrate chains. Processing defects in these cells appeared to be general since the ldlB, ldlC, and ldlD mutants also showed defective glycosylation of a viral glycoprotein, alterations in glycolipid synthesis, and changes in resistance to several toxic lectins. Preliminary structural studies suggested that these cells had defects in multiple stages of the Golgi-associated processing reactions responsible for synthesis of glycolipids and in the N-linked and O-linked carbohydrate chains of glycoproteins. Comparisons between the ldl mutants and a large number of previously isolated CHO glycosylation defective mutants showed that the genetic defects in ldlB, ldlC, and ldlD cells were unique and that only very specific types of carbohydrate alteration could dramatically affect LDL receptor function.

Materials and Methods

Materials

Human and newborn calf lipoprotein-deficient sera, human LDL, and 125I-labeled LDL were prepared as previously described (Krieger, 1983). Ham's F-12 medium (30 μM methionine), methionine-free Ham's F-12 medium, minimal essential medium, and alpha minimal essential medium were obtained from Gibco Laboratories (Grand Island, NY) or KC Biological Inc. (Lenexa, KS). [35S]Methionine (>800 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Endoglycosidase H was provided by Phillips Robbins and Catherine Hubbard (Massachusetts Institute of Technology [MIT]). Sialidase and pronase were purchased from Calbiochem/Behring Diagnostics Corp. (La Jolla, CA). Lectins and all other reagents and supplies were purchased from...
Cell Culture and Selection of Somatic Cell Hybrids

Proline requiring (Pro−) parental and mutant CHO-K1 cells were grown in medium I (Ham's F-12 medium containing penicillin (100 U/ml), streptomycin (100 µg/ml), and glutamine (2 mM)) supplemented with 5% (vol/vol) fetal bovine serum (medium II). The lldA, lldB, lldC, and lldD mutants used in this paper refer to mutant 7, mutant 11, mutant 475, and mutant 14-1α, respectively (Krieger, et al., 1981; Kingsley and Krieger, 1984). Previously isolated lectin-resistant (Lee) CHO mutants and their corresponding parental cells were obtained from Dr. Pamela Stanley (Albert Einstein College of Medicine, New York). The properties of these cells have been reviewed recently (Stanley, 1984, 1985a, b). Pro− Lec mutants representing recessive complementation groups 1, 2, 2B, 3, 4, 5, 8, 13, 13A, and 15; a double Lee1.Lee2 mutant; and the dominant LEC mutants 10, 11, 12, 14, and 17 were maintained in medium II. The Lee1.Lee2 mutant was originally isolated by Dr. Carlos Hirschberg (St. Louis University, St. Louis), who provided us with corresponding parental cells. Glycine, adenosine, and thymidine requiring (Gat+) Lee mutants representing recessive complementation groups 1, 2, 3, and 8; the double mutants Lee1.Lee6 and Lee2.Lee7; and the dominant LEC16 mutant were maintained in medium II formulated with alpha minimal essential medium instead of Ham's F-12. Hybrids between (Gat+) Lee mutants representing complementation groups 1, 2, 3, and 6, and the (Pro−) lld mutants representing complementation groups B, C, and D were isolated by cell fusion (Kingsley and Krieger, 1984) and growth in minimal essential medium lacking proline, glycine, adenosine, and thymidine which was supplemented with 3% (vol/vol) newborn calf lipoprotein-deficient serum. All incubations were at 37°C in a humidified 5% CO2 incubator unless otherwise indicated. Stocks of Lecl, Lee9, and LEC16 cells were maintained at 34°C.

Wheat Germ Agglutinin Selections

Forty million CHO cells were plated into 40 100-mm dishes in medium I supplemented with 10% (vol/vol) newborn calf serum and wheat germ agglutinin (WGA; 20 µg protein/ml). After 7 d, cells were washed once with PBS and refed medium III (medium I supplemented with 3% [vol/vol] newborn calf lipoprotein-deficient serum) to induce LDL receptor activity. 2 d later, all surviving colonies were screened in situ for expression of LDL receptor activity using DiI-LDL, (LDL labeled with 1,1'-dioctadecyl-3,3',3',3'-tetramethyl indocarbocyanine iodide; Pitas et al., 1981; Kingsley and Krieger, 1984). Colonies that failed to accumulate fluorescence were isolated and tested for complementation with existing lld mutants as previously described (Kingsley and Krieger, 1984).

Viral Infection and Metabolic Labeling

Stocks of vesicular stomatitis virus (VSV) (Indiana Serotype) were grown and titered on wild-type CHO cells (Krieger et al., 1983). For metabolic labeling experiments, 300,000–400,000 cells/well were seeded into the wells of a six-well dish in 3 ml of medium III. 2 d later, cells were infected with 5–10 plaque-forming units of VSV per cell in 0.5 ml of medium III for 1 h then refed with medium III. 3–4 h later, cells were washed twice with methionine-free medium III, pulse-labeled for 5–10 min with [35S]methionine (20 µCi/ml) in methionine-free medium III, and chased for 1 h in complete medium III. Cells were then washed twice with PBS and lysed in 0.25 ml of either buffer A (PBS without calcium and magnesium and supplemented with 1% [vol/vol] Triton X-100, 1% [vol/vol] Nonidet P-40, and 1 mM phenylmethylsulfonyl fluoride) or buffer B (150 mM Tris, pH 8.8, 1% [vol/vol] SDS, 1% [vol/vol] β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride).

Immunoprecipitations

The conditions for setting cells, labeling with [35S]methionine, treating with tunicamycin or pronase, harvesting, and immunoprecipitating LDL receptors with the anti-C polyclonal antibody are described in the accompanying paper (Kozarsky et al., 1986). Endoglycosidase H and sialidase digestions also were performed as described (Kozarsky et al., 1986).

Polyacrylamide Gel Electrophoresis

Immunoprecipitates of LDL receptors and lysates of VSV-infected cells were analyzed in 6 or 10% SDS-polyacrylamide gels by the method of Laemmli (1970; Kozarsky et al., 1986).

Glycolipid Analysis

Glycolipids were isolated from cells grown in medium III and analyzed by thin-layer chromatography, using minor modifications of previously described methods (Stanley et al., 1980; Kundo, 1981). Glycolipid standards were gifts from Dr. Gary Schwarting (E. K. Shriver Center, Waltham, MA).

Other Assays

The sensitivity of cells to toxic lectins was determined by seeding cells on day 0 into 96-well dishes at a concentration of 2,000 cells/well in 0.1 ml of medium III. On day 1, various concentrations of lectins were added to the cells in medium III (0.1 ml/well). On day 3, cells were washed, fixed, and stained (Krieger et al., 1981). Lectin sensitivities are expressed as the lowest lectin concentration that reduced cell density to 0–10% of that seen in wells without lectins (Stanley, 1981).

The receptor-mediated uptake and degradation of [125I]-labeled LDL was measured at 34°C (Lee9 cells) or 37°C (all other cells) as previously described (Krieger, 1983). The values presented are the differences between determinations made in the absence (duplicate determinations) and presence (single determinations) of excess unlabeled LDL. For experiments with the Lee9 cells and the Gat+ cell types, alpha minimal essential medium was used instead of Ham's F-12. The protein concentrations of cells and lipoproteins were determined by the method of Lowry et al. (1951).

| Pulse | Chase |
|-------|-------|
|       | 0 15' | 30' | 1h | 3h | 6h |
| CHO   | m-    | p-  | 1 2 | 3 4 | 5 6 |
| lldB  | m-    | m-  | 7 8 | 9 10| 11 12|
| lldC  | m-    | m-  | 13 14| 15 16| 17 18|
| lldD  | m-    | m-  | 19 20| 21 22| 23 24|

Figure 1. Structure and processing of LDL receptors in wild-type and lld mutant cells. The indicated cell types were pulse-labeled with 180 µCi/ml of [35S]methionine for 30 min, chased for the indicated times in medium III, and subjected to immunoprecipitation, electrophoresis, and autoradiography as described in Materials and Methods. The letters p and m refer to the 125,000-D precursor and the 155,000-D mature form of the LDL receptor protein, respectively.
Results

Altered LDL Receptors in ldID Mutants

An anti-LDL receptor antibody (see accompanying paper, Kozarsky et al., 1986) was used to compare the synthesis and structure of LDL receptors in wild-type and ldID mutant cells. Cells were pulse-labeled with $[^{35}S]$-methionine, chased for various lengths of time, and analyzed by immunoprecipitation, electrophoresis, and autoradiography (Fig. 1). Wild-type cells synthesized a 125,000-D precursor form ($p$) of the LDL receptor that was rapidly converted to a mature form ($m$) of 155,000 D (Fig. 1, lanes 1-6). This mature form was stable for at least 6 h and could still be detected in wild-type cells after chase periods as long as 20-30 h (Kozarsky et al., 1986). The ldID mutants all produced approximately normal amounts of a 125,000-D precursor form of the LDL receptor but failed to process these receptors to the mature form (Fig. 1, lanes 7-24). Instead these mutants produced small, heterogeneous LDL receptors that were almost completely degraded within 3-6 h of synthesis.

Previous experiments have shown that the conversion of the 125,000-D precursor form of the LDL receptor to a mature form of 155,000 D is the result of extensive processing of N- and O-linked carbohydrate chains (Kozarsky et al., 1986; Cummings et al., 1983; see Fig. 2 for review of these processing pathways). When cells were treated with tunicamycin to block addition of N-linked chains (Hubbard and Ivatt, 1981), the ldID mutants still produced receptors which were substantially smaller and more heterogeneous than receptors in wild-type cells (Fig. 3). This suggests that the production of abnormal forms of the LDL receptor in ldID mutants is at least in part due to defective synthesis of O-linked chains.

The LDL receptors made by ldID mutants were more heterogeneous in size than receptors produced by wild-type cells. Most of this size heterogeneity was eliminated when LDL receptors from ldIB, ldID, and ldID mutants were treated with sialidase after immunoprecipitation (Fig. 4). Heterogeneity in sialic acid content thus appears to be a major source of the heterogeneous mobility of LDL receptors from ldIB, ldID, and ldID cells. In addition, sialidase-treated LDL receptors from tunicamycin-treated ldID mutants were substantially smaller than receptors from similarly treated wild-type cells (Fig. 4, compare lanes 10, 12, 14, and 16). These results suggest that ldID mutants have alterations in O-linked chains that are more extensive than simple variability in sialic acid content.

A pronase assay was used to determine if abnormal glycosylation of the LDL receptor affected its localization in ldID mutants (Kozarsky et al., 1986). When wild-type and ldID mutant cells were pulse-labeled with $[^{35}S]$-methionine for 30 min and immediately treated with pronase, a substantial fraction of the LDL receptors were resistant to digestion.

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Figure 2. Structure and synthesis of different classes of glycoconjugates. (top) High mannose N-linked carbohydrate chains are added cotranslationally to glycoproteins in the rough endoplasmic reticulum. These chains can be trimmed and subsequently converted to the complex type by a series of Golgi processing steps (Hubbard and Ivatt, 1981). The partially processed structure in the middle is one of the earliest processing intermediates that should be resistant to digestion with endoglycosidase H (Tai et al., 1977). The complex structure shown is the major form of N-linked carbohydrate found on mature VSV G protein in CHO cells (Stanley, 1985a). The N-linked chains of the mature LDL receptor are also of the complex type (Cummings et al., 1983; Kozarsky et al., 1986). (middle) A wide variety of O-linked oligosaccharides attached to serine or threonine side chains has been observed (Kornfeld and Kornfeld, 1980). In most O-linked chains determined to date, an N-acetylgalactosamine residue is linked directly to the hydroxyl group of the serine or threonine side chains. Structures I and II have been observed on the precursor and mature forms, respectively, of the LDL receptor in human A431 cells in culture (Cummings et al., 1983). (bottom) The pathway of glycolipid synthesis in CHO cells involves the sequential addition of three different monosaccharides to ceramide (Yogeeswaran et al., 1974; Briles et al., 1977; Stanley et al., 1980, Stanley, 1980). abbreviations: Fuc, fucose; Gal, galactose; Glc, glucose; Man, mannose; GlcNAc, N-acetylgalactosamine; GalNAc, N-acetylgalactosamine; SA, sialic acid; Asn, asparagine; Ser/Thr, serine or threonine; cer, ceramide; GC, glucosylceramide; LC, lactosylceramide; GM3, sialyl-galactosylglycosylceramide.

Figure 3. Effect of tunicamycin on LDL receptor structure in wild-type and ldID mutant cells. The indicated cell types were pulse-labeled with 200 $\mu$Ci/ml $[^{35}S]$-methionine for 1 h, chased for 1 h, and subjected to immunoprecipitation, electrophoresis, and autoradiography as described in Materials and Methods. Tunicamycin (2 $\mu$g/ml) was present continuously in the preincubation, labeling, and chase media for the cells shown in lanes 5-8.
When pulse-labeled cells were chased for 1 h and then treated with pronase, almost all of the LDL receptors in wild-type and mutant cells were accessible to pronase digestion (Fig. 5, lanes 1-8). Similar pronase sensitivities were observed after labeling for 4.5 h (data not shown). This suggests that the abnormal LDL receptors in wild-type cells were accessible to pronase digestion. Similar pronase sensitivity was observed after labeling for 4.5 h (data not shown). This suggests that the abnormal LDL receptors in wild-type and mutant cells were accessible to pronase digestion (Fig. 5, lanes 9-16). Similar endoglycosidase H results were seen for the apparently normal precursor forms of the LDL receptor produced by all of the ldl mutants (data not shown). These results suggest that all of the mutants were able to assemble high mannose N-linked carbohydrate chains and to transfer them to proteins normally (Fig. 2).

During a 1-h chase, the apparent molecular weight of the G protein in wild-type cells increased (Fig. 7, compare lanes 1 and 9). Simultaneously, the G protein became resistant to sialidase (Fig. 8, lanes 1-2). This behavior is typical of glycoproteins whose high mannose N-linked carbohydrate chains have been trimmed and then processed to a complex form (Fig. 2). In contrast, the G proteins in ldlB, ldlC, and ldlD cells showed a slight decrease in apparent size during a 1-h chase (Fig. 7, and data not shown) and did not show further changes after 2 h of chase (data not shown). Unlike the mature G protein in wild-type cells, the mature forms in these mutants were resistant to sialidase (Fig. 8, lanes 3-8), indicating that ldlB, ldlC, and ldlD cells cannot completely process high mannose chains to the fully complex form. The different mutants apparently process the high mannose oligosaccharides to different extents. In ldlB, ldlC, and ldlD mutants, the mature G protein was fully resistant to endoglycosidase H digestion (Fig. 7, lanes 1-8). Similar endoglycosidase H results were seen for the apparently normal precursor forms of the LDL receptor produced by all of the ldl mutants (data not shown). These results suggest that all of the mutants were able to assemble high mannose N-linked carbohydrate chains and to transfer them to proteins normally (Fig. 2).

A variety of assays were used to determine if abnormal processing of LDL receptors in ldlB, ldlC, and ldlD mutants.

**Altered VSV G Protein in ldlB, ldlC, and ldlD Mutants**

To determine if other membrane glycoproteins were abnormally processed in the ldl mutants, we examined the synthesis of the N-glycosylated G protein of VSV. Wild-type and mutant cells were infected with VSV, labeled with [35S]methionine, and the proteins in detergent lysates were separated by SDS-gel electrophoresis and visualized by autoradiography (Fig. 6). In all cell types, the unglycosylated L and N virus-encoded proteins exhibited identical electrophoretic mobilities. In wild-type cells, the VSV G protein appeared as a prominent 69,000-D band (G form) whose apparent size was reduced to ~60,000 D (Go form) after treatment with tunicamycin (Fig. 6, compare lanes 2 and 3). As anticipated, the VSV G protein produced by an ldlA (receptor structural gene) mutant (lane 4) was similar in apparent size to that produced by wild-type cells. However, the electrophoretic mobilities of the VSV G proteins produced in ldlB, ldlC, and ldlD mutants were greater than the mobility of G protein made in wild-type cells (Fig. 6, lanes 5-7). Treatment with tunicamycin eliminated these differences (data not shown), suggesting that N-linked carbohydrate processing was abnormal in these mutants.

The VSV G proteins produced by wild-type and ldl mutant cells during a short pulse labeling with [35S]methionine all showed identical electrophoretic mobilities and were all sensitive to endoglycosidase H digestion (Fig. 7, lanes 1-8). Similar endoglycosidase H results were seen for the apparently normal precursor forms of the LDL receptor produced by all of the ldl mutants (data not shown). These results suggest that all of the mutants were able to assemble high mannose N-linked carbohydrate chains and to transfer them to proteins normally (Fig. 2).

A variety of assays were used to determine if abnormal
glycosylation of the VSV G protein affected virus production. Pronase digestion showed that the abnormal forms of the VSV G protein in ldlB, ldlC, and ldlD reached the cell surface (data not shown). All of the mutants developed similar numbers of viral plaques when monolayers were exposed to dilute VSV stocks (Kingsley and Krieger, 1984; and data not shown). Plaques on ldlC and ldlD cells were larger than those on wild-type cells; plaques on ldlB were smaller. All of the mutants produced amounts of infectious VSV during an 8-h period after initial VSV infection that were equal to or greater than that produced by wild-type cells (data not shown).

**Altered Glycolipids and Lectin-resistance in ldlB, ldlC, and ldlD Mutants**

To determine if the defects in ldlB, ldlC, and ldlD cells affected additional classes of glycoconjugates, glycolipids were extracted from wild-type and ldl mutant cells and analyzed by thin-layer chromatography (Fig. 9). Previous studies (Yogeeswaran et al., 1974; Briles et al., 1977; Stanley et al., 1980, Stanley, 1980) have shown that the predominant glycolipid species made in CHO cells is sialyl-galactosyl-glucosylceramide (Fig. 2). Sialyl-galactosyl-glucosylceramide was present in wild-type CHO cells but was strikingly reduced in ldlB, ldlC, and ldlD mutants (Fig. 9). Lactose ceramide was also present in wild-type cells and the ldlB and ldlC mutants but was reduced in the ldlD mutant. The ldlC and ldlD cells contained slightly more material that comigrated with a glucosylceramide standard than did wild-type cells. The glycolipids in an ldlA mutant were indistinguishable from those in wild-type cells (data not shown). These studies suggest that the defects in ldlB, ldlC, and ldlD cells affect addition of galactose and sialic acid residues to glycolipids.

To detect possible changes in a broad spectrum of glycoconjugates in the ldl mutants, we examined the sensitivity of various cells to five different toxic plant lectins that bind to cell surfaces with different sugar specificities (Table I). Mutants in the ldlB, ldlC, and ldlD complementation groups showed up to 200-fold changes in sensitivity to these lectins relative to wild-type cells. For example, the ldlB, ldlC, and ldlD mutants all show marked resistance to WGA (Table I), a lectin that binds to sialic acid and N-acetylglucosamine residues (Goldstein and Hayes, 1978; Bhavanandan and Katlic, 1979). In contrast, a mutant in the ldlA complementation group showed no significant differences from wild-type CHO cells.

**Comparison of ldl Mutants and Previously Isolated Glycosylation Mutants**

At least 17 different types of CHO glycosylation mutant have been isolated by other laboratories, primarily on the basis of changes in sensitivity to toxic plant lectins (reviewed by...
were washed with Hepes-buffered saline and refed either 0.5 ml of Hepes-buffered saline (lanes 1, 3, 5, and 7) or 0.5 ml of Hepes and labeled with \(^{35}\)S)methionine for 5 min. After a 1.5-h chase, cells and analyzed by electrophoresis and autoradiography as described in Materials and Methods. Control experiments with a pronase assay present in the cells after a 1.5-h chase was on the cell surface (data not shown).

After 20 min at 37°C, cells were washed, lysed in 0.25 ml of buffer in Materials and Methods. Control experiments with a pronase assay showed that all of the radiolabeled G protein material that comigrated with these standards, and the material in four of these previously described mutants (Lee 1, Lee2, Lee3, and Lee8 cells) were somewhat similar to those seen in wild-type cells and the Lecl, Lee2, and Lee8 mutants are for the most part similar to those previously reported (Stanley, 1985a). Differences between absolute lectin sensitivities in these and previous experiments, as well as a substantially higher relative sensitivity of Lee8 cells to ricin in our experiments, may be due to the different growth conditions, serum sources and concentrations, and lectin preparations used in different laboratories. Although the binding specificities of lectins are complex and incompletely understood, the following carbohydrate structures are likely components of the surface binding sites for the five lectins shown in this table: WGA, terminal sialic acid or N-acetylgalactosamine residues; ricin (RIC), terminal galactose or N-acetylgalactosamine residues; phytohemagglutinin (PHA), galactose in \(\beta\)-1-4 branches of complex N-linked carbohydrate chains; concanavalin A (Con A), mannose residues; lens culinaris agglutinin (LCA), mannose residues in fucosylated N-linked carbohydrate chains (Goldstein and Hayes, 1978; Stanley, 1985a).

Somatic cell hybrids were isolated between these four Lec mutants and the ldlB, ldlC, and ldlD cells. Unlike all of the original mutant cells, all of the hybrids produced VSV G proteins with wild-type electrophoretic mobilities (data not shown). Thus, these Lec and ldl mutants represent distinct genetic complementation groups.

Although ldlB, ldlC, and ldlD mutants expressed only 5-10% of wild-type LDL receptor activity, all of the 17 previously classified Lec mutants expressed 30-170% of the receptor activity in their respective parental cells, Pro-5 or Gat-2 (Table II). In addition, other isolates of Lec mutants (Lec1A, Lec2B, Lec13A, and Lec1.Lec2 cells) were examined and found to express 71-102% of parental LDL receptor activity. Taken together, these data indicated that the ldlB, ldlC, and ldlD mutants were unrelated to previously described CHO mutants. Four of the Lec mutants were examined by immunoprecipitation with the anti-C peptide antibody (Lec1, Lec2, Lec3, and Lec8 cells). Each of these mutants synthesized an abnormal form of the LDL receptor protein that differed in size or heterogeneity from the receptors observed in ldlB, ldlC, and ldlD cells (data not shown).

**Genetic Linkage between Structural and Functional Phenotypes**

Because the ldlB, ldlC, and ldlD mutants were isolated after heavy mutagenesis, we conducted a series of experiments to determine if the glycosylation defects and the LDL receptor-deficient phenotypes of these cells were due to single or to multiple mutations. First, CHO cells (no mutagen) were incubated with concentrations of WGA (20 \(\mu\)g/ml) that are toxic to wild-type cells but not the ldlB, ldlC, or ldlD cells. WGA-resistant colonies appeared at a frequency of \(5 \times 10^{-6}\) (45 million cells tested in two independent experiments).
Approximately 1% of the WGA-resistant (WGA) colonies (3 of 215) proved to have a dramatic deficiency in LDL receptor activity based on the uptake of fluorescent LDL and the degradation of \( ^{125} \)I-LDL (see below and data not shown). The overall frequency of isolating WGA-resistant, LDL receptor-negative colonies in these experiments (7 \times 10^{-9}) is at least five orders of magnitude higher than the frequency expected if WGA resistance and LDL receptor deficiency are due to defects in two independent genes (overall frequency estimated as the product of the spontaneous frequency of each event, \( \times 5 \times 10^{-9} \) [WGA resistance, this paper] \times < 1 \times 10^{-11} [LDL receptor deficiency, Krieger et al., 1981 and 1983] = < 5 \times 10^{-15} ). Complementation tests (Kingsley and Krieger, 1984)
established that the three new isolates were members of the
ldlB complementation group (data not shown). The LDL
receptor activity, LDL receptor structure, VSV G protein
structure, and lectin sensitivity of one of the WGA-selected
colonies (WGA-2) were tested and found to be essentially
identical to that seen for ldlB cells (Fig. 10, lectin-selection,
A; and Table I).

Functional linkage between the altered processing and al-
ter receptor activity in the ldl mutants was also tested by a
biochemical reversion test. LDL receptor activity in ldlD cells
can be partially restored by cocultivation with other cells or
by treatment of ldlD cells with high concentrations of serum
(Krieger, 1983). The electrophoretic mobilities of both the
LDL receptor and the VSV G protein produced in ldlD cells
were also restored to nearly wild-type mobilities after the cells
had been treated with high concentrations of serum (Fig. 10,
serum induction, B). These and other recent data (Kingsley
et al., 1986; see below) show that the various functional and
structural phenotypes of ldlD cells are due to a single defect.

Discussion

Anti-LDL receptor antibodies (Kozarsky et al., 1986) have
helped define the nature of the genetic defect in three types of
CHO mutant. These mutants from the previously defined
ldlB, ldlC, and ldlD complementation groups are deficient in
LDL receptor activity but are genetically distinct from the
ldlA mutants that have defects in the structural gene for the
LDL receptor (Kingsley and Krieger, 1984). In wild-type CHO
cells the LDL receptor is initially synthesized as a 125,000-D
precursor that is subsequently processed to a mature form of
135,000 D by extensive glycosylation (Kozarsky et al., 1986).
The ldlB, ldlC, and ldlD mutants all produce substantial
amounts of a 125,000-D precursor form of the LDL receptor
protein. Therefore these mutants do not have defects in the
production, accumulation, or translation of receptor mes-
enger RNA. The ldlB, ldlC, and ldlD mutants produce mature
forms of the LDL receptor that are abnormally small and
heterogeneous in size. The abnormal sizes of the LDL recep-
tors in these cells are due to defects in processing of the LDL
receptor’s N- and O-linked carbohydrate chains. Glycosyla-
tion defects in these cells also affect the N-linked chains of
the VSV G protein and the lipid-linked carbohydrates of
cellular glycolipids. The carbohydrate structures of many
different cell surface molecules are probably altered in the
ldlB, ldlC, and ldlD cells since all of these mutants show
dramatic changes in sensitivity to five different toxic plant
lectins.

Our results and previous experiments suggest that glyco-
sylation mutations can make up a significant fraction of the
mutants that are isolated after selection for altered function
of a particular surface molecule (reviewed by Stanley,
1985a,b). A CHO mutant that was isolated because of de-
creased levels of mannose-6-phosphate receptor activity (Rob-
bins et al., 1981) has been shown to have a general defect in
synthesis of high mannose N-linked carbohydrate chains. This
defect results from a marked deficiency in the enzyme dolich-
ol-mannose-phosphate-synthetase (Stoll et al., 1982). In
addition, four of five types of mutant lymphoma cells isolated
because of decreased expression of Thy-1 antigen show altered
glycosylation of this molecule (Trowbridge et al., 1978). One
of these classes of mutant, Thy-1E, has a defect in dolichol-
mannose-phosphate synthetase activity that is very similar to
that in the mannose-6-phosphate receptor defective CHO
mutant (Chapman et al., 1980). The close associations be-
tween glycosylation defects and altered expression of the LDL
receptor, the mannose-6-phosphate receptor, and the Thy-1
antigen emphasize the importance of carbohydrate chains for
the expression and function of some cell surface glycoproteins.

Although we have seen a strong association between altered
glycosylation and altered LDL receptor activity in ldlB, ldlC,
and ldlD mutants, there are many carbohydrate alterations
that appear to have relatively little effect on LDL receptor
function. For example, previous studies have shown that
treatment of LDL receptors with sialidase has little effect on
LDL binding activity (Schneider et al., 1982). In addition, we
have shown that 17 different types of previously isolated CHO
glycosylation mutant have substantial levels of LDL receptor
activity. One of these mutants (Lecl5) is the mannose-6-
phosphate receptor-deficient CHO mutant described above.
A different mannose-6-phosphate receptor-deficient CHO
mutant with alterations in endosome acidification, Golgi
glycosylation patterns, and lectin sensitivity (Robbins et al.,
1984) has been reported to have essentially normal LDL
receptor activity (Robbins et al., 1983). The finding of essen-
tially normal LDL receptor activity in a large number of
different glycosylation mutants suggests that only very specific
types of glycosylation defects can dramatically affect LDL
receptor function. The alterations in O-linked carbohydrate
chains in ldlB, ldlC, and ldlD cells may be particularly im-
portant in this regard (see below). Conversely, the glycosyla-
tion defects that affect LDL receptor function do not neces-
arily affect the expression of other glycoproteins. For exam-
ple, previous studies have shown that both ldlB and ldlD
mutants express significant levels of mannose-6-phosphate
receptor activity (Leichtman and Krieger, 1984). These results
emphasize the variety of effects that particular carbohydrate
alterations can have on the functions of different glycopro-
teins.

The current experiments provide insight into the nature of
the glycosylation defects in ldlB and ldlC mutants, but the
molecular bases for these defects are not yet known. The
structural alterations in these two mutants are strikingly sim-
ilar. Both mutants are able to add normal high mannose N-
linked carbohydrate chains to the precursor forms of the VSV
G protein and the LDL receptor. Neither mutant can convert
the VSV G chains to a completely endoglycosidase H-resistant
form and neither mutant appears to subsequently add galactose
(unpublished data) or sialic acid residues to these chains.
Both mutants produce O-linked chains on the LDL receptor
that are only partially processed and that show heterogeneity
in sialic acid content. Both mutants can add glucose and some
galactose to glycolipids but do not add significant amounts of
sialic acid. Thus both the ldlB and ldlC mutants appear to
have defects in a number of different processing reactions that
previously have been shown to take place primarily in the
Golgi apparatus (Dunphy and Rothman, 1985). The hetero-
genous defects in N-linked trimming are consistent with
disruptions of cis or medial Golgi mannosidase or N-acetyl-
glucosamine transferase activities (Dunphy et al., 1985). The
heterogeneity or absence of sialic acid residues in glycolipids
and the N-linked and O-linked chains of glycoproteins sug-
gests additional defects in activities, probably associated with
the trans Golgi (Bennett and O’Shaughnessy, 1981). Overall
transport between different Golgi compartments is probably not disrupted in the ldlB and ldlC mutants since both the VSV G protein and the LDL receptor protein reach the cell surface.

Because of the complicated nature of the processing defects in ldlB and ldlC cells, we presume that both of these mutants have alterations that affect the regulation, compartmentalization, or activity of several different Golgi enzymes or enzyme substrates. Nevertheless, genetic evidence strongly suggests that the various processing changes seen in ldlB cells are due to defects in a single gene. Independent isolates of ldlB mutants show similar phenotypes, new ldlB mutants can be isolated in the absence of mutagen treatment by direct selection for changes in lectin resistance (see Results), and DNA-mediated-reversion of the LDL receptor defects in ldlB mutants is always accompanied by the simultaneous correction of the glycosylation abnormalities (Kingsley, D., R. S. Sege, K. F. Kozarsky, and M. Krieger, manuscript in preparation). We have not yet identified the ldlB defect, but its consequences are very similar to those of an independent defect in ldlC cells. Although the structural phenotypes of the ldlB and ldlC mutants are almost indistinguishable, the two mutants belong to distinct complementation groups and hybrids between the mutants show essentially normal levels of LDL receptor activity and essentially normal patterns of glycoprotein processing (unpublished data). This suggests either that defects in two distinct genes give rise to a very similar processing phenotype or that complementation between ldlB and ldlC is actually intra-allelic.

In contrast to the complicated structural changes in ldlB and ldlC cells, most of the structural changes in ldlD cells can be accounted for by a general defect in addition of galactose and N-acetylgalactosamine (GalNAc) residues to glycoconjugates. We have recently shown that this is due to a severe deficiency in UDP-Gal/UDP-GalNAc-4-epimerase activity (Kingsley et al., 1986). Without this enzyme, ldlD cells are unable to synthesize normal amounts of UDP-galactose and UDP-N-acetylgalactosamine from their corresponding glucose precursors. Addition of galactose and GalNAc to the culture medium allows the ldlD mutant to synthesize these UDP-sugars via salvage pathways and fully corrects all of the structural and functional defects in the cells. Galactose alone fully corrects N-linked but not O-linked processing defects and does not induce LDL receptor activity. In contrast, N-acetylgalactosamine alone partially corrects O-linked but not N-linked processing and substantially increases LDL receptor activity (Kingsley et al., 1986). It thus appears that the defects in O-linked processing may be the primary cause of the LDL receptor-deficient phenotype of the ldlD mutant. We do not yet know if this is also true for the ldlB and ldlC mutants, but both of these mutants do have severe defects in O-linked processing, and in this respect are different from many of the previously described N-linked CHO glycosylation mutants which express substantial LDL receptor activity (Table II).

The fate of the abnormally glycosylated LDL receptors is quite similar in the ldlB, ldlC, and ldlD cells. In each of these mutants, LDL receptors reach the cell surface but are degraded at least 5–10-fold more quickly than receptors in wild-type cells. It appears that the altered stability or function of LDL receptors in ldl mutants can largely account for their LDL receptor-deficient phenotype. Given approximately equal rates of receptor synthesis, a 5–10-fold reduction in receptor stability should lead to a 5–10-fold reduction in the steady-state levels of LDL receptors in the mutant cells. Actual residual levels of LDL receptor activity in ldlB, ldlC, and ldlD cells are ~5–10% of normal (Table II and Krieger et al., 1981).

Enhanced degradation of LDL receptors in ldlB, ldlC, and ldlD mutants could be the direct result of the altered structure of the LDL receptors in these cells. Alternatively, enhanced degradation of LDL receptors could be an indirect result of changes in the structure or function of other components in the ldlB, ldlC, and ldlD mutants. Studies with other systems have shown that some carbohydrate alterations can grossly disrupt the conformation, solubility, and intracellular transport of certain glycoproteins (e.g., the VSV G protein, Gibson et al., 1980). It seems unlikely that the glycosylation defects in ldlB, ldlC, and ldlD mutants have such a drastic effect on the structure and solubility of LDL receptors. A significant fraction of LDL receptors in these cells reaches the cell surface, and these mutants express small but detectable levels of LDL binding and internalization activity. The carbohydrates that are normally found on wild-type LDL receptors may help stabilize the receptor during multiple rounds of endocytosis. For example, these oligosaccharides may help protect the protein from denaturation in the acidic environment of endosomes (Tyko and Maxfield, 1982). Alternatively, carbohydrate chains on LDL receptors or other molecules may be essential for some aspect of LDL receptors' recycling to the cell surface after endocytosis. We are currently investigating these possibilities and are also attempting to identify the nature of the cellular components responsible for rapid degradation of the altered LDL receptors in ldlB, ldlC, and ldlD mutants.

Hilarie Brush and Marsha Penman provided excellent technical assistance. We are grateful to R. Rosenberg for access to the gamma spectrophotometer; G. Schwarting for glycolipid standards; P. Robbins and C. Hubbard for endoglycosidase H; H. Lodish for VSV stocks; P. Stanley and C. Hirschberg for Lec mutant and parental controls; and H. Lodish, P. Stanley, A. Mercurio, L. Hobbie, and R. Sege for useful discussions. Parke-Davis provided thrombin used to isolate human lipoprotein-deficient serum.

Grants from the National Institutes of Health supported this research. David M. Kingsley was supported by a Whitaker Health Sciences Fund fellowship, Karen F. Kozarsky by an Exxon fellowship, Mark Segal by funds from the MIT Undergraduate Research Opportunities Program and the MIT chapter of Sigma Xi, and Monty Krieger was the recipient of a National Institutes of Health Career Development Award.

Received for publication 18 October 1985, and in revised form 14 January 1986.

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