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DNA-Mediated Transfer of a Human Gene Required for Low-Density Lipoprotein Receptor Expression and for Multiple Golgi Processing Pathways

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Transfection of a hamster cell mutant with human DNA corrected both the low-density lipoprotein receptor-deficient phenotype and the multiple glycosylation defects of the cells. Independently transfected colonies contained a small set of common human DNA fragments. These fragments may correspond to the human analog of a single gene required for several different Golgi processing pathways.

We previously described (6) four types of Chinese hamster ovary (CHO) cell mutants that fail to express significant levels of low-density lipoprotein (LDL) receptor activity (*ldlA*, *ldlB*, *ldlC*, and *ldlD* mutants). The *ldlA* mutants have defects in the structural gene for the LDL receptor (6, 6a, 8, 12; R.D. Sege, K. F. Kozarsky, and M. Krieger, submitted for publication) and are thus analogous to fibroblasts from patients with familial hypercholesterolemia (3). The *ldlD* mutant is deficient in UDP-galactose/UDP-*N*-acetylgalactosamine 4-epimerase activity and consequently has defects in the glycosylation of the LDL receptor and many other glycoconjugates (5). The *ldlB* and *ldlC* mutants also express pleiotropic glycosylation defects, but the primary biochemical defects in these cells are not yet known (5a). Existing structural data suggest that both the *ldlB* and *ldlC* mutants have defects in multiple stages of several different Golgi processing pathways, including the pathways responsible for the synthesis of both the N- and O-linked chains of glycoproteins and the ceramide-linked chains of glycolipids (5a). To determine whether this complex phenotype is the result of a single mutation, we used DNA transfection techniques to isolate phenotypic revertants of an *ldlB* mutant.

LDL receptor-deficient *ldlB* clone 11 cells were transfected with human liver DNA (12) and incubated in a previously described nutritional selection medium which only allows rapid growth of cells that are able to obtain cholesterol by the receptor-mediated endocytosis of LDL (4, 6, 12). LDL endocytosis transfectants of *ldlB* cells (LET-B cells) appeared at a frequency of approximately 10^{-7} from 3 $\times$ 10^7 treated cells. Unlike the original *ldlB* mutant, the primary LET-B colonies expressed essentially wild-type levels of LDL receptor activity (data not shown). DNA from the original *ldlB* mutant and from one of the transfectants (LET-B-10) was isolated, digested with *Eco*RI, resolved by electrophoresis in a 1% agarose gel, transferred (14, 18) to a nylon membrane (Zetabind; AMF-Cuno), and hybridized with ^{32}P-labeled (11) total human fetal liver DNA (10^7 cpm, 4 $\times$ 10^8 cpm/μg). Unlike *ldlB* cells, the LET-B-10 cells contained substantial amounts of human-specific repetitive DNA sequences (Fig. 1).

To isolate *ldlB* transfectants that contain only one or a few human genes (13), we used DNA from the primary transfectant LET-B-10 for another round of transfection of *ldlB* cells followed by selection for LDL receptor expression. Secondary transfectants were isolated at a frequency of 2 $\times$ 10^{-7}. The transfected colonies were tested for the ability to internalize and degrade ^{125}I-labeled LDL (7) and were all found to express essentially wild-type levels of LDL receptor activity (see Fig. 3; data not shown). DNA samples from the secondary transfectants were isolated and analyzed by Southern blot hybridization with nick-translated total human liver DNA, as described above. Independent secondary transfectants contained small amounts of human repetitive

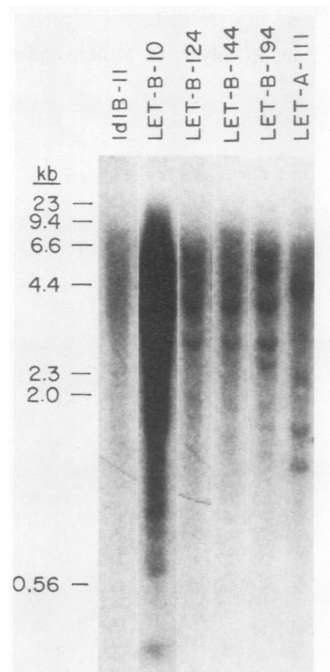

FIG. 1. Detection of human DNA sequences in *ldlB* mutant cells after transfection and selection. DNA (10 μg) from the indicated cell types was digested with *Eco*RI and analyzed by Southern blot hybridization with nick-translated total human liver DNA to detect human-specific repetitive DNA sequences. Cell types: ldlB-11, *ldlB* mutant 11; LET-B-10, primary transfectant of *ldlB* mutant 11; LET-B-100 series, secondary transfectants of *ldlB* mutant 11; LET-A-111, secondary transfectant of *ldlA* mutant 7. kb, Kilobases.

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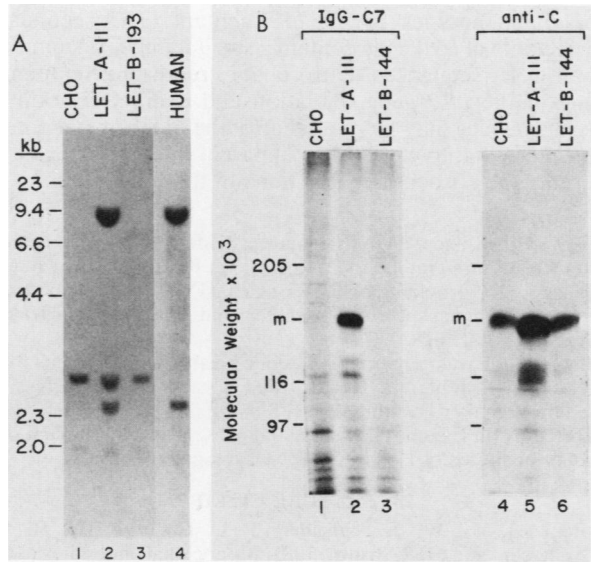

FIG. 2. Analysis of LDL receptors in secondary transfectants of *ldlA* and *ldlB* cells. (A) Gene structure. DNA (10 μg) from the indicated cell types and a specimen of human liver DNA were digested with *Eco*RI and analyzed by Southern blot hybridization with a fragment of human LDL receptor cDNA (see text). kb, Kilobases. (B) Protein structure. The indicated cell types were labeled with [^{35}S]methionine and subjected to immunoprecipitation, electrophoresis, and autoradiography (6a). The IgG-C7 antibody recognizes human but not hamster LDL receptors (1, 12). The anti-C antibody recognizes receptors from both species (6a). The prominent lower band in lane 5 includes the abnormally processed mutant form of the hamster receptor synthesized in *ldlA-7* cells (6a). m, 155,000-dalton mature form of the receptor.

DNA which shared common restriction fragments (Fig. 1; LET-B-100 series).

We previously isolated primary transfectants (now called LET-A cells) of a mutant from the *ldlA* complementation group (12). When DNA from a primary LET-A colony was used for another round of transfection of *ldlA* cells followed by selection for functional LDL receptors, secondary LET-A transfectants (e.g., LET-A-111 cells) appeared at frequencies similar to those seen for the isolation of LET-B cells (four colonies from 2×10^7 cells). LET-A-111 cells expressed approximately 300% of wild-type LDL receptor activity (data not shown) and contained a small number of *Eco*RI restriction fragments that hybridized to human repetitive DNA sequences (Fig. 1). These fragments were distinct from the common fragments seen in the LET-B cells, suggesting that DNA-mediated reversion of the *ldlA* and *ldlB* cells took place by transfer of different human genes.

The nature of the human DNA in the LET-A and LET-B transfectants was further analyzed by Southern blot hybridization with a human LDL receptor cDNA probe (Fig. 2A). DNA from the different cell types and from a specimen of human liver was digested with *Eco*RI, resolved by electrophoresis in a 1% agarose gel, and transferred to a nylon membrane. LDL receptor gene sequences were detected by hybridization to a ^{32}P-labeled (2) *Eco*RI fragment of pLDLR3 (19). This probe includes bases −13 to 718 of the human LDL receptor cDNA (19). DNA from all three types of hamster cells examined and from the original *ldlA* mutant contained a fragment of 2.7 kilobases which hybridized strongly to the probe (Fig. 2A, lanes 1 through 3) (R. D. Sege, K. F. Kozarsky, and M. Krieger, submitted for publication). DNA from human liver contained two different fragments which hybridized strongly to the probe (Fig. 2A, lane 4). LET-A-111 DNA contained a combination of both hamster and human bands (lane 2). In contrast, LET-B-193 DNA contained only the hamster LDL receptor gene fragments (lane 3). These results demonstrate that transfection-mediated reversion of *ldlA* cells (12), but not *ldlB* cells, took place by transfer of the structural gene for the human LDL receptor.

LET-A cells, LET-B cells, and wild-type CHO cells also were labeled with [^{35}S]methionine (120 μCi/ml) for 4.5 h, washed, lysed, and subjected to immunoprecipitation with antibodies that recognize LDL receptors from different species (6a). The immunoprecipitates were then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (6a, 9) and autoradiography (10). A monoclonal antibody that recognizes human but not hamster LDL receptors

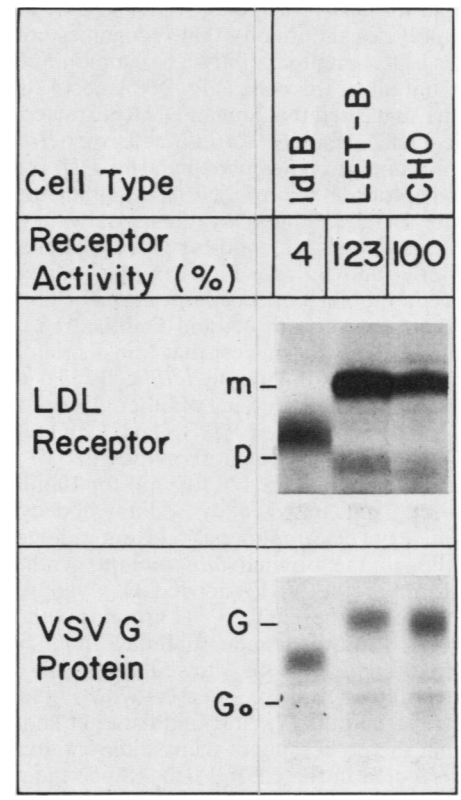

FIG. 3. Correction of abnormal *ldlB* phenotypes by transfection. For LDL receptor activity, the indicated cells were grown for 2 days in medium containing lipoprotein-deficient serum and were then incubated in medium containing ^{125}I-labeled LDL (10 μg of protein per ml; 85 cpm/ng). After incubation for 5 h at 37°C, the amounts of specifically degraded ^{125}I-labeled LDL products released into the medium were determined as previously described (7). LET-B refers to the secondary transfectant LET-B-144. For LDL receptor structure, the indicated cells were labeled with [^{35}S]methionine and subjected to immunoprecipitation (anti-C antibody), electrophoresis, and autoradiography (6a). p and m refer to the 125,000-dalton precursor and the 155,000-dalton mature forms of the wild-type hamster LDL receptor, respectively (6a). For VSV G protein structure, the indicated cells were infected with VSV, labeled with [^{35}S]methionine, and washed, lysed, and analyzed by electrophoresis and autoradiography as previously described (5a). G_o refers to the 60,000-dalton unglycosylated form of the VSV G protein synthesized in the presence of tunicamycin, and G refers to the 65,000-dalton fully processed form (5a).

TABLE 1. Lectin sensitivities of wild-type, *ldlB*, and LET-B-144 cells

Cells	Lectin sensitivity (LD_{10})[a]				
	WGA (μg/ml)	RIC (ng/ml)	PHA (μg/ml)	ConA (μg/ml)	LCA (μg/ml)
CHO	3	240	15	25	60
ldlB	120	5	>240	5	>240
LET-B-144	3	120	20	25	60

[a] The lectin concentrations required to reduce cell density to 0 to 10% of that seen for untreated cells (LD_{10}) were determined by a semiquantitative 3-day growth test (5a, 15). Abbreviations: WGA, wheat germ agglutinin; RIC, ricin; PHA, phytohemagglutinin; ConA, concanavalin A; LCA, *Lens culinaris* agglutinin.

(IgG-C7; 1, 12) immunoprecipitated human LDL receptors from the *ldlA* transfectant LET-A-111 (Fig. 2B, lane 2) but did not detect the LDL receptors in wild-type CHO cells (lane 1) or in an *ldlB* transfectant (LET-B-144; lane 3). In contrast, a polyclonal antibody that recognizes both human and hamster LDL receptors (anti-C; 6a) immunoprecipitated receptors from all of the cells (Fig. 2B, lanes 4 through 6). These results suggested that human DNA corrected the LDL receptor-deficient phenotype of *ldlB* cells by permitting the normal expression of endogenous hamster LDL receptors.

Unlike receptors in the original *ldlB* mutant, the mature forms of the LDL receptors synthesized by primary and secondary LET-B cells had wild-type electrophoretic mobilities (Fig. 2B, lane 6, and Fig. 3). Since the abnormal electrophoretic mobility of receptors in *ldlB* cells is due to defective synthesis of both N- and O-linked carbohydrate chains (5a), these results suggest that transfection corrected multiple processing pathways in *ldlB* cells. To determine whether the abnormal processing of other glycoproteins (5a) was also corrected, *ldlB*, LET-B, and CHO cells were infected with vesicular stomatitis virus (VSV) for 5 h, labeled with [^{35}S]methionine (20 μCi/ml) for 10 min, chased for 1 h, lysed, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography (5a, 9, 10). Although the original *ldlB* mutant synthesized an abnormal form of the VSV-encoded G glycoprotein, the transfected cells synthesized VSV G proteins with wild-type mobility (Fig. 3, lower panel, and data not shown). In addition, the transfected cells showed essentially wild-type sensitivities to a panel of toxic lectins which bind different carbohydrate structures (Table 1 and data not shown). This indicates that the processing of many different glycoconjugates was corrected in the LET-B cells. Since the glycosylation-defective phenotype of *ldlB* cells was always corrected after transfection-mediated reversion of their LDL receptor-deficient phenotype (11 of 11 cases), both the structural and functional phenotypes of *ldlB* mutants are almost certainly due to defects in a single gene.

Although many glycosylation mutants have previously been isolated, only a few of these mutants have defects which affect multiple processing pathways (16). In addition, detailed genetic characterization of many previously isolated glycosylation mutants has been difficult because of the lack of specific reversion selections (17). In the current studies, we used DNA transfection techniques and efficient selection for LDL receptor expression to isolate phenotypic revertants of an LDL receptor-deficient mutant with multiple glycosylation defects. The transfected cells express normal levels of LDL receptor activity and show normal processing of both LDL receptors and other glycoconjugates. These results indicate that a single gene is responsible for the complex phenotypes of the *ldlB* mutant. The secondary transfectants of *ldlB* cells contain a small number of common human DNA sequences that probably represent the human analog of the *ldlB* gene. Isolation and characterization of these sequences may help to identify the biochemical defect in *ldlB* cells and to explain the apparent interdependence of different Golgi processing functions in these cells.

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LITERATURE CITED

1. **Beisiegel, U., W. J. Schneider, J. L. Goldstein, R. G. W. Anderson, and M. S. Brown.** 1981. Monoclonal antibodies to the low density lipoprotein receptor as probes for study of receptor-mediated endocytosis and the genetics of familial hypercholesterolemia. J. Biol. Chem. **256**:11923–11931.
2. **Feinberg, A. P., and B. Vogelstein.** 1984. A technique for radiolabeling DNA restriction fragments to high specific activity. Addendum. Anal. Biochem. **137**:266–267.
3. **Goldstein, J. L., and M. S. Brown.** 1983. Familial hypercholesterolemia, p. 672–713. *In* J. B. Stanbury, J. B. Wyngaarden, J. L. Goldstein, and M. S. Brown (ed.), The metabolic basis of inherited disease. McGraw-Hill Book Co., New York.
4. **Goldstein, J. L., J. A. S. Helgeson, and M. S. Brown.** 1979. Inhibition of cholesterol synthesis with compactin renders growth of cultured cells dependent on the low density lipoprotein receptor. J. Biol. Chem. **254**:5403–5409.
5. **Kingsley, D. M., K. F. Kozarsky, L. Hobbie, and M. Krieger.** 1986. Reversible defects in O-linked glycosylation and LDL receptor expression in an UDP-Gal/UDP-GalNAc 4-epimerase deficient mutant. Cell **44**:749–759.

5a. **Kingsley, D. M., K. F. Kozarsky, M. Segal, and M. Krieger.** 1986. Three types of LDL receptor-deficient mutant have pleiotropic defects in the synthesis of N-linked, O-linked, and lipid-linked carbohydrate chains. J. Cell Biol. **102**:1567–1575.

6. **Kingsley, D. M., and M. Krieger.** 1984. Receptor-mediated endocytosis of low density lipoprotein: somatic cell mutants define multiple genes required for expression of surface receptor activity. Proc. Natl. Acad. Sci. USA **81**:5454–5458.

6a. **Kozarsky, K. F., H. A. Brush, and M. Krieger.** 1986. Unusual forms of LDL receptors in hamster cell mutants with defects in the receptor structural gene. J. Cell Biol. **102**:1576–1585.

7. **Krieger, M.** 1983. Complementation of mutations in the LDL pathway of endocytosis by cocultivation of LDL receptor-defective hamster cell mutants. Cell **33**:413–422.
8. **Krieger, M., D. M. Kingsley, R. D. Sege, L. Hobbie, and K. F. Kozarsky.** 1985. Genetic analysis of receptor-mediated endocytosis. Trends Biochem. Sci. **10**:447–452.
9. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
10. **Laskey, R. A.** 1980. The use of intensifying screens or organic scintillators for visualizing radioactive molecules resolved by gel electrophoresis. Methods Enzymol. **65**:363–371.
11. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity in vitro by nick-translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
12. **Sege, R. D., K. Kozarsky, D. L. Nelson, and M. Krieger.** 1984. Expression and regulation of human low-density lipoprotein receptors in Chinese hamster ovary cells. Nature (London) **307**:742–745.

13. **Shih, C., and R. A. Weinberg.** 1982. Isolation of a transforming sequence from a human bladder carcinoma cell line. Cell **29**:161–169.
14. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
15. **Stanley, P.** 1981. Selection of specific wheat germ agglutinin-resistant (Wga^R) phenotypes from Chinese hamster ovary cell populations containing numerous lec^R genotypes. Mol. Cell. Biol. **1**:687–696.
16. **Stanley, P.** 1985. Lectin-resistant glycosylation mutants, p. 745–772. *In* M. M. Gottesman (ed.), Molecular cell genetics: the Chinese hamster cell. John Wiley & Sons, Inc., New York.
17. **Stanley, P., V. Caillibot, and L. Siminovitch.** 1975. Selection and characterization of eight phenotypically distinct lines of lectin-resistant Chinese hamster ovary cells. Cell **6**:121–128.
18. **Wahl, G. M., M. Stern, and G. R. Stark.** 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxy-methyl-paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. USA **76**:3683–3687.
19. **Yamamoto, T., C. G. Davis, M. S. Brown, W. J. Schneider, M. L. Casey, J. L. Goldstein, and D. W. Russell.** 1984. The human LDL receptor: a cysteine rich protein with multiple Alu sequences in its mRNA. Cell **39**:27–38.