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Assignment of the human gene for the low density lipoprotein receptor to chromosome 19: Synteny of a receptor, a ligand, and a genetic disease

(gene mapping/somatic cell hybrids/monoclonal antibodies/familial hypercholesterolemia)

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ABSTRACT **The availability of a species-specific monoclonal antibody that recognizes the low density lipoprotein (LDL) receptor of human but not hamster origin permitted assignment of the structural gene for the human receptor to chromosome 19. The antibody was used to detect the human LDL receptor in a series of hamster–human somatic cell hybrids by two assays: (*i*) a structural assay that measured cellular incorporation of [^{35}S]methionine into immunoprecipitable receptor and (*ii*) a functional assay that measured the rate of receptor-dependent uptake and degradation of the ^{125}I-labeled anti-receptor monoclonal antibody. Both assays showed that the human LDL receptor was expressed in 15 out of 20 hybrid cell lines. Expression of the human LDL receptor was 100% concordant with the presence of human chromosome 19; all other human chromosomes showed at least 25% discordance. As expected, the gene for the LDL receptor (*LDLR*) is located on the same chromosome as the gene for the disease familial hypercholesterolemia, which has been previously mapped to chromosome 19 by pedigree studies and is caused by allelic mutations at the LDL receptor locus. The gene for apolipoprotein E, a ligand for the LDL receptor, is also known to be located on chromosome 19, raising the possibility of an evolutionary link between a protein ligand and its receptor.**

The low density lipoprotein (LDL) receptor plays a major role in controlling the level of cholesterol in plasma of humans and animals (1). Located on the surface of hepatocytes and other cells, the receptor binds LDL, a cholesterol transport protein, and facilitates its entry into cells by receptor-mediated endocytosis. Inside the cell, LDL is degraded in lysosomes and its cholesterol is released for metabolic use. By controlling the rate of degradation of plasma LDL, the LDL receptor determines the plasma level of the lipoprotein.

A common autosomal dominant disease, familial hypercholesterolemia, has been traced to a series of mutant alleles at the LDL receptor locus (2). About 1 in 500 individuals is heterozygous for one of these defects. Heterozygotes express half the normal number of LDL receptors and maintain plasma LDL–cholesterol levels about 2-fold above normal. They suffer myocardial infarctions as early as age 35. Individuals who inherit two mutant LDL receptor genes (i.e., either homozygotes or compound heterozygotes) express few, if any, LDL receptors. They have extremely high LDL levels and usually develop myocardial infarctions before age 20 (2).

The chromosomal site of the LDL receptor gene in man, or in any other animal species, is unknown. However, the gene responsible for the clinical phenotype of familial hypercholesterolemia has been assigned to chromosome 19 on the basis of pedigree analyses that have shown linkage with the third component of complement (C3) (3, 4). The latter has been shown by somatic cell genetic techniques to reside on chromosome 19 (5). If familial hypercholesterolemia is caused by mutations at the LDL receptor locus, then the gene for the LDL receptor should also map to chromosome 19.

Mapping the LDL receptor locus has become feasible as a result of purification of the receptor from bovine adrenal cortex (6) and the preparation of mouse monoclonal antibodies directed against the receptor (7). One of the monoclonal antibodies, designated IgG-C7, recognizes the human and the bovine LDL receptor but does not recognize the LDL receptor of hamsters, mice, or other rodent species (7). The human LDL receptor binds ^{125}I-labeled IgG-C7 (^{125}I-IgG-C7) in amounts stoichiometric to the binding of LDL; i.e., there is one molecule of IgG-C7 bound for each molecule of LDL bound. The receptor-bound ^{125}I-IgG-C7 is taken up by cells and degraded in lysosomes in a similar fashion to LDL. Thus, measurements of the uptake and degradation of ^{125}I-IgG-C7 by cells can be related to the number of human LDL receptors on the surface of a cell (7). Inasmuch as monoclonal IgG-C7 does not recognize the hamster LDL receptor, it should be useful for detection of human receptors on the surfaces of hamster–human hybrid cells. This, in turn, should permit assignment of the human LDL receptor gene to a specific human chromosome.

In the current studies, we have used the IgG-C7 monoclonal antibody to map the human gene for the LDL receptor in a series of hamster–human hybrids. The results indicate that chromosome 19 is both necessary and sufficient for expression of the human LDL receptor in these hybrids, and we postulate that this chromosome carries the structural gene for the LDL receptor, which we designate *LDLR*.

MATERIALS AND METHODS

Somatic Cell Hybrids. Chinese hamster × human hybrid cell lines were derived from seven independent series. The Chinese hamster established parental cell lines were V79/380-6, hypoxanthine phosphoribosyltransferase (EC 2.4.2.8)-deficient (hybrid series XII, XIII, XV, XVII, and XVIII) and Don/a23, thymidine kinase (EC 2.7.1.75)-deficient (series XXI and XXV). Human cells fused to hamster cells were skin fibroblasts (series XV, XVII, XVIII, XXI and XXV) and peripheral blood leukocytes (series XII and XIII) (8). All human donors were heterozygous for a defined chromosomal rearrangement. References to the derivation, subcloning, and initial characterization of the hamster–human hybrids are described in ref. 9.

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Abbreviations: LDL, low density lipoprotein; *LDLR*, genetic locus specifying the LDL receptor; ^{125}I-IgG-C7 and ^{125}I-LDL, ^{125}I-labeled monoclonal antibody IgG-C7 and LDL, respectively.

Chromosome Analysis. Chromosomes of hybrid clones were reanalyzed with trypsin/Giemsa banding (8) and in some cases with Giemsa 11 staining (10) immediately before, and in five hybrids immediately after, the LDL receptor assays. Human chromosome content was determined quantitatively on photographs of 15–25 banded metaphase spreads.

Assays of Enzyme Markers. Enzyme markers for human chromosomes 11 (lactate dehydrogenase A, EC 1.1.1.27), 12 (lactate dehydrogenase B, EC 1.1.1.27), 14 (nucleoside phosphorylase, EC 2.4.2.1), 15 (mannosephosphate isomerase, EC 5.3.1.8 and pyruvate kinase 2, EC 2.7.1.40), 19 (glucosephosphate isomerase, EC 5.3.1.9), 20 (adenosine deaminase, EC 3.5.4.4), and 22 (mitochondrial aconitase, EC 4.2.1.3) were studied by cellogel electrophoresis (11, 12).

Monoclonal Antibody Against Human LDL Receptor. A species-specific mouse monoclonal antibody (IgG-C7) that binds to the human LDL receptor was prepared with the lymphocyte hybridoma technique (13). IgG-C7 binds to the human and bovine LDL receptor, but it does not react with the LDL receptor of hamster, mouse, rat, rabbit, or canine cells (7). IgG-2001, a mouse monoclonal antibody directed against an irrelevant antigen (Hemophilus influenza, type B) was prepared as described (13). IgG-C7 was radiolabeled with ^{125}I to specific activities of 5–17 × 10^3 cpm/ng of protein by the chloramine-T method (13).

Cultures. Human, Chinese hamster, and hybrid cells were grown in monolayer culture at 37°C with 5% CO_2/95% air. On day 0 of growth, 8 × 10^4 cells were seeded into Petri dishes (60 × 15 mm) containing (each) 3 ml of medium A (Ham's F-12 medium supplemented with penicillin at 100 units/ml and streptomycin at 100 μg/ml) and 10% (vol/vol) fetal calf serum. After 2 or 3 days of growth, cells were switched to lipoprotein-deficient serum for 24 or 48 hr to induce synthesis of LDL receptors (14).

Immunoprecipitation of LDL Receptors. After 24 hr in lipoprotein-deficient serum, each monolayer was switched to 1.2 ml of methionine-free Dulbecco's modified Eagle medium containing [^{35}S]methionine at 100 μCi/ml (1 Ci = 37 GBq) and 10% lipoprotein-deficient serum. After 3 hr at 37°C, monolayers were washed, and detergent extracts were prepared (15). The extracts were incubated with immunocomplexes containing IgG-C7 or IgG-2001, and the precipitates were washed (15). Pellets were dissolved in 8 M urea/0.2 M dithiothreitol and subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis and fluorography (15).

Functional Assay for LDL Receptors. After 48 hr in lipoprotein-deficient serum, monolayers were incubated with 2 ml of Ham's F-12 medium containing 10% human lipoprotein-deficient serum and either ^{125}I-IgG-C7 (1 μg/ml, 5–17 × 10^3 cpm/ng of protein) in the absence or presence of unlabeled IgG-C7 at 250 μg/ml or ^{125}I-labeled LDL (^{125}I-LDL; 10 μg/ml, 160 cpm/ng protein) (14) in the absence or presence of unlabeled LDL at 400 μg/ml. After 1–5 hr at 37°C, total cellular uptake (surface-bound plus internalized) of ^{125}I-IgG-C7 or ^{125}I-LDL and total amount of ^{125}I-IgG-C7 or ^{125}I-LDL degraded by the cells and excreted into the culture medium were measured (14). High affinity uptake plus degradation was calculated by subtracting the ^{125}I-labeled ligand values obtained in the presence of unlabeled ligand from those obtained in its absence. LDL receptor studies were carried out on coded hybrid cell samples without knowledge of the human chromosome content.

RESULTS

Two types of assays were used to detect the human LDL receptor in hamster–human hybrids. In the immunoprecipitation assay, the cells were incubated with [^{35}S]methionine under conditions of cholesterol deprivation, which elicits maximal synthesis of LDL receptors. Detergent-solubilized extracts were incubated with IgG-C7, and the immunoprecipitates were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. The spectrum of results obtained is shown in Fig. 1. Human fibroblasts synthesized an immunoprecipitable receptor of 160,000 daltons (lane HF) (15). Chinese hamster ovary (CHO-K1) cells did not synthesize any pro-

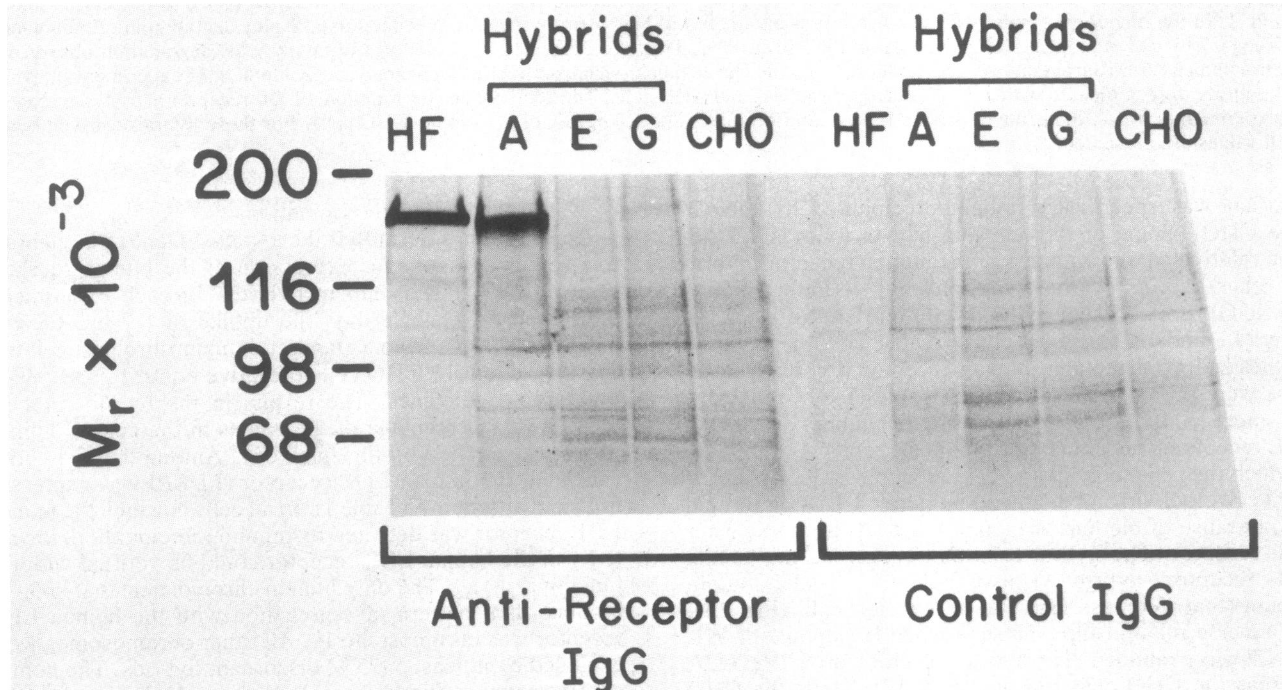

FIG. 1. $NaDodSO_4$ gel electrophoresis of ^{35}S-labeled LDL receptors synthesized by human fibroblasts (HF), CHO cells, and three hamster–human hybrids (A, E, and G). Cells were pulse-labeled for 3 hr with [^{35}S]methionine. Cell extracts were incubated with immunocomplexes containing either mouse monoclonal IgG-C7 (anti-receptor IgG) or IgG-2001 (control IgG). The washed immunoprecipitates were subjected to $NaDodSO_4$ electrophoresis in a 7% slab gel. The gel was exposed to x-ray film for 4 days. Hybrids were scored on a 0 to 4+ scale as follows: hybrid A, 4+; hybrid E, 0; hybrid G, 1+.

Table 1. Expression of the human LDL receptor locus (*LDLR*) in human–hamster hybrid clones containing different human chromosomes

Hybrid		LDL receptor*				Presence or absence of human chromosomes†					
Series	Code	Immunoprecipitation assay	Functional assay, % of control	GPI‡	Frequency of chromosome 19§	1	2	3	4	5	6
XV	A	4+	14, 13	+	0.9	–	+	P[a]	+	+	–
XV	B	4+	11, 8	+	1.0	P[b]	+	+	–	+	+
XV	R	2+	8	+	1.1	+	+	+	+	+	+
XV	T	3+	3, 5	+	0.2	–	+	+	+	–	+
XVII	J	4+	23	+	0.8	–	+	+	–	–	+
XVII	N	0	<1	–	0	–	+	+	–	–	+
XVII	S	1+	2, 3	+	0.5	–	+	P[a]	–	–	+
XVIII	L	2+	5	+	0.7	–	–	–	+	–	+
XVIII	M	3+	15	+	1.0	–	+	+	+	+	+
XVIII	O	2+	6, 5	+	0.8	–	–	–	–	–	–
XXI	C	3+	4	+	0.8	–	–	–	–	+	+
XXI	D	1+	13	+	0.7	–	–	–	–	–	–
XXI	E	0	<1	–	0	–	–	+	–	+	–
XXI	G	1+	2, 3	+	0.7	+	–	–	–	+	–
XXI	K	3+	19	+	0.7	+	–	–	–	–	–
XXI	P	0	<1, <1	–	0	–	–	+	–	+	+
XXV	I	0	<1	–	0	–	–	–	–	–	–
XII	U	2+	3	+	0.8	+	+	+	+	–	+
XII	W	3+	9	+	0.8	+	–	+	+	–	+
XIII	H	0	<1	–	0	–	–	–	–	–	–
LDLR/chromosome											
Concordant +/+						5	8	7	7	6	10
–/–						5	4	2	5	3	3
Discordant +/–						9	7	6	8	9	5
–/+						0	1	3	0	2	2
Ratio of discordant/total hybrids						9/19	8/20	9/18	8/20	11/20	7/20
Percent discordancy						47	40	47	40	55	35

*LDL receptor activity was determined by two assays. In the immunoprecipitation assay, the receptor was scored on a 0 to 4+ scale as shown in Fig. 1. In the functional assay, values shown represent amount of high affinity receptor-mediated uptake plus degradation of monoclonal ^{125}I-IgG-C7 by cell monolayers after incubation for 5 hr at 37°C. Data are expressed as percentage of uptake plus degradation observed in control human fibroblasts assayed in the same experiment. The 20 human–hamster hybrids were analyzed in a total of 11 experiments in which 1–4 hybrids were compared with control human fibroblasts and CHO cells. The 100% values for functional LDL receptor activity (average of 11 experiments) were 226 ng/mg of protein for human fibroblasts and 1.0 ng/mg of protein for CHO cells. For those hybrids assayed twice, both values are presented.

tein that was specifically immunoprecipitated by IgG-C7 (lane CHO). Some of the hamster–human hybrids synthesized relatively large amounts of the human receptor (hybrid A); other hybrids synthesized small but definite amounts (hybrid G). Both of these hybrids were scored positive in the assay (4+ and 1+, respectively, in Table 1). Other hybrids synthesized no detectable human receptor (hybrid E), and these were scored as negative (0 in Table 1).

A more quantitative estimate of the number of human LDL receptors was obtained from measurements of the rate at which the cells took up and degraded ^{125}I-IgG-C7 (Fig. 2). CHO cells took up and degraded human ^{125}I-LDL at a rapid rate, because of binding of human ^{125}I-LDL to the hamster LDL receptor. The hybrid cells that expressed the human LDL receptor (hybrid A) also took up ^{125}I-LDL but in amounts that were less than that of the CHO cells (Fig. 2*A*). The inverse relationship was seen when the uptake of ^{125}I-IgG-C7 was examined. The hybrid cells took up ^{125}I-IgG-C7, whereas the CHO cells did not (Fig. 2*B*). Thus, the CHO cells were synthesizing the hamster LDL receptor, which binds ^{125}I-LDL but not ^{125}I-IgG-C7, whereas the hybrid cells were synthesizing the human receptor, which binds both ^{125}I-LDL and ^{125}I-IgG-C7. Presumably, these hybrid cells were also synthesizing hamster LDL receptor, but we have no way to make this determination.

These findings permitted the establishment of a quantitative screening assay for expression of the human receptor gene in the hamster–human hybrids. In each experiment, performed on a single day, the uptake of ^{125}I-IgG-C7 was measured in cells from a standard human fibroblast cell line (positive control), CHO cells (negative control), and one to four hybrid cell lines. The results in the hybrids are expressed as a percentage of the values in the control human fibroblasts studied on the same day. Among the 20 hybrids analyzed, the human LDL receptor (*LDLR*) was expressed in 15 and absent in 5 (Table 1). In all cells in which the human LDL receptor was detected by immunoprecipitation, the activity of the human LDL receptor could be verified with the functional assay. The only human chromosome that showed a concordant pattern of segregation with the human LDL receptor was chromosome 19. All other chromosomes were excluded by at least 5 (25%) discordant hybrids. The human chromosomes retained in each of the hybrids were usually present in 50–90% of the metaphases analyzed and sometimes in more than one copy per cell. For chromosome 19, the frequencies (average copy number per cell) are presented in Table 1. There was complete concordance between the presence of chromosome 19 as determined cytologically and

Presence or absence of human chromosomes†																
7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
−	+	−	−	+	+	−	+	+	+	+	−	+	+	+	+	+
−	+	−	−	−	−	+	+	−	+	−	+	+	+	+	+	−
+	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
+	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+
−	−	−	−	+	+	+	+	P[c]	−	−	+	+	−	+	P[d]	+
−	−	−	−	+	+	−	+	−	−	−	+	−	−	+	−	+
−	+	−	−	+	+	+	+	P[c]	+	−	+	+	+	+	P[d]	+
−	P[e]	−	−	−	−	+	+	−	+	−	−	+	−	+	+	+
−	−	−	−	+	+	+	+	−	+	−	+	+	+	+	+	−
−	−	−	−	+	−	−	+	−	−	−	−	+	+	−	−	+
−	+	−	−	+	−	+	−	+	−	+	+	+	+	+	+	−
−	+	−	−	P[f]	+	−	−	+	−	+	−	+	+	+	+	−
+	+	−	+	+	+	+	+	−	−	+	−	−	−	+	+	+
−	−	−	−	P[f]	+	−	−	+	+	+	+	+	−	−	−	+
−	+	−	−	P[f]	−	−	+	−	−	+	−	+	−	R	+	+
−	+	−	+	+	+	−	+	−	−	−	−	−	−	+	+	+
−	−	−	+	−	+	−	+	−	−	+	−	−	−	−	−	−
−	+	+	−	−	+	+	+	+	+	−	+	+	+	+	+	P[g]
−	+	−	−	−	+	−	+	+	+	−	+	+	−	+	+	P[g]
−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	+	P[g]
2	8	2	2	8	10	9	12	8	9	5	10	15	10	12	11	9
4	3	5	2	2	1	4	0	5	4	3	4	5	5	2	2	1
13	6	13	13	4	5	6	3	5	6	10	5	0	5	2	2	4
1	2	0	3	3	4	1	5	0	1	2	1	0	0	3	3	3
14/20	8/19	13/20	16/20	7/17	9/20	7/20	8/20	5/18	7/20	12/20	6/20	0/20	5/20	5/19	5/18	7/17
70	42	65	80	41	45	35	40	28	35	60	30	0	25	26	28	44

†+, Presence of chromosome in at least 10% of cells; −, absence of chromosome; P, presence of part of chromosome as specified by a–g: a, 3qter → cen → qter; b, 1pter → p3200; c, 15q14 → qter; d, 22pter → q13.31; e, 8pter → q22; f, 11p11 → qter; g, Xp22.2 → qter; R, rearranged chromosome. P and R were excluded from calculating the ratio of discordant hybrids.

‡GPI, glucose phosphate isomerase, human enzyme present (+) or absent (−).

§Expressed as average copy number per cell.

expression of human glucose phosphate isomerase (*GPI*), an enzyme marker for 19. No other human chromosome was consistently present, in addition to 19, in the hybrids that expressed human *LDLR*.

DISCUSSION

We have localized the human gene for the LDL receptor (*LDLR*) to chromosome 19 on the basis of expression studies in hamster–human somatic cell hybrids. A mouse monoclonal antibody with specificity for the LDL receptor of human but not hamster origin was used to detect the expression of the human *LDLR* gene in a panel of hybrids. Species-specific monoclonal antibodies have been used previously in conjunction with interspecies somatic cell hybrids for the chromosomal localization of genes for other cell surface antigens of known or unknown function (16–18) and of genes for intracellular enzymes (19, 20).

The LDL receptor undergoes complex posttranslational processing en route from its site of synthesis in the endoplasmic reticulum to its site of function on the cell surface (15, 21). The receptor gains both N-linked and O-linked carbohydrates, which causes its apparent molecular weight to increase from 120,000 to 160,000 as estimated from $NaDodSO_4$ gels (15, 21). It is therefore possible that human chromosome 19 does not contain the structural gene for the receptor but rather some enzyme that allows the hamster receptor to be processed into a form that reaches the surface and is recognized by IgG-C7. We consider this unlikely because hamster cells contain all of the factors necessary to process their own receptors to a fully active form. For example, experiments with a polyclonal antibody that recognizes the hamster receptor have shown that CHO-K1 cells process their own receptors by adding N-linked and O-linked sugars in a fashion similar to that of human cells (21). Moreover, monoclonal antibody IgG-C7 recognizes the unprocessed 120,000-dalton precursor of the human receptor (15). If the hamster–human hybrid cells were accumulating this precursor in the absence of human chromosome 19, the immunoprecipitation reactions should have revealed it. These data strongly suggest that the hamster enzymes are able to process the human LDL receptor precursor and that human chromosome 19 contains the structural gene for the receptor itself.

The current results agree with family linkage data that place the familial hypercholesterolemia locus (*FHC*) on chromosome 19 (3, 4). *FHC* is linked to the gene for complement component 3 (*C3*) at a distance of 20 centimorgans, with a lod score of 4.75 (22). *C3* has previously been assigned to chromosome 19 by somatic cell hybrid studies (5) and indirectly by linkage to the polymorphic enzyme locus for peptidase D (*PEPD*) (23). With this assignment, genes previously linked to *C3*—*Lu* (Lutheran blood group antigen), *Le* (Lewis blood group antigen), H (H antigen), *Se* (ABH secre-

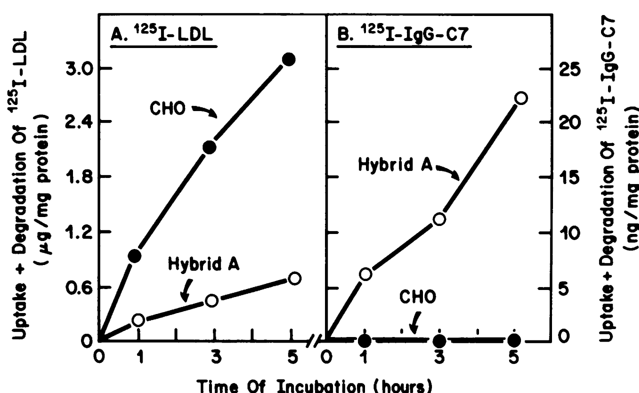

FIG. 2. Receptor-mediated uptake and degradation of ^{125}I-LDL and monoclonal anti-receptor ^{125}I-IgG-C7 in CHO cells and hamster–human hybrid A. Cells were incubated for the indicated times at 37°C with either ^{125}I-LDL (*A*) or ^{125}I-IgG-C7 (*B*), and the high affinity receptor-mediated uptake plus degradation of the two ^{125}I-labeled ligands was measured. Each value is the average of triplicate incubations.

tion), and *DM* (myotonic dystrophy, Steinert disease)—are also mapped to chromosome 19.

The LDL receptor binds two different apoproteins: apoprotein B, which is found on LDL, and apoprotein E, which is a constituent of very low density and intermediate density lipoproteins (1, 24). It is striking that the gene for apoprotein E (*APOE*) has recently been assigned to chromosome 19 on the basis of pedigree studies showing linkage with *C3* (25). Thus, the genes for the LDL receptor and for one of its two ligands are located on the same chromosome. *APOE* and *C3* are linked at a distance of 15 centimorgans with a lod score of 5.94 in males (22). *FHC* is not closely linked to *APOE*, as suggested by family studies (26). This suggests that *APOE* and *LDLR* (which is equivalent to *FHC*) are on opposite sides of *C3*. The chromosomal location of the gene for apoprotein B is not yet known.

The possibility of a syntenic localization of plasma proteins and their cell surface receptors is intriguing. The chromosomal loci for only two other cell surface receptors that undergo receptor-mediated endocytosis are known—the human receptor for transferrin (located on chromosome 3) (17) and the human receptor for epidermal growth factor (located on chromosome 7) (27, 28). The gene for human transferrin, the ligand for the transferrin receptor, has been provisionally mapped to the same chromosome as the gene for its receptor—i.e., chromosome 3 (29). The location of the gene for epidermal growth factor is not known. Thus, in the only two cases in which the chromosomal locus of a cell surface receptor and its ligand are known, the receptors and their ligands appear to be on the same chromosome. If this turns out to be a general finding, it would suggest an evolutionary link between protein ligands and their receptors.

Since familial hypercholesterolemia is caused by allelic mutations in the *LDLR* gene (2, 15), there is only one locus involved on chromosome 19. According to current policies of the International Workshops on Human Gene Mapping, a genetic locus should be called by the name of the wild-type gene product, if known, rather than by the name of a mutation in that locus. Therefore, we propose that the locus symbol be *LDLR*, for LDL receptor, and that *FHC*, for familial hypercholesterolemia, be reserved as a secondary synonym.

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