Progress in understanding the LDL receptor and HMG-CoA reductase, two membrane proteins that regulate the plasma cholesterol

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The discovery of the low density lipoprotein (LDL) receptor 11 years ago and the subsequent elucidation of its mode of action in the cell and in the body have provided a conceptual framework for understanding the mechanisms that control the concentration of the most abundant cholesterol-carrying lipoprotein in human blood. Study of the LDL receptor has taught us that human and animal cells possess at least two mechanisms for obtaining the cholesterol required for synthesis of membranes, steroid hormones, and bile acids: *1*) they can synthesize cholesterol de novo through the classic cholesterol synthetic pathway, of which the rate-determining step is the reaction catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase); or *2*) they can supply themselves with cholesterol through the receptor-mediated endocytosis of LDL, an event that is mediated by the LDL receptor (reviewed in ref. 1). Under the usual circumstances of tissue culture, most cells, such as human skin fibroblasts, rely primarily on LDL receptors as a source of cholesterol. They maintain a low rate of cholesterol synthesis by expressing a small amount of HMG-CoA reductase activity. Cells from individuals with genetic defects in the LDL receptor, such as those with homozygous familial hypercholesterolemia (FH), cannot obtain cholesterol from LDL, and thus they must express increased amounts of HMG-CoA reductase to supply their cholesterol needs (1).

In tissue culture cells, the LDL receptor and HMG-CoA reductase are both subject to end-product feedback regulation by cholesterol. When cellular cholesterol levels rise, the synthesis of HMG-CoA reductase and the synthesis of LDL receptors are suppressed. On the other hand, when cells have an increased demand for cholesterol, the production of LDL receptors and HMG-CoA reductase increase (1).

A similar type of feedback regulation of these two proteins has been observed in the livers of several animal species. Under certain conditions, the level of LDL in plasma is dictated by the balance between the activities of HMG-CoA reductase and LDL receptors in the liver. If this balance is not preserved, hypercholesterolemia and atherosclerosis can result. If plasma LDL levels are to be kept low, the activities of the LDL receptor and HMG-CoA reductase must be regulated in a coordinate manner in the body as well as in tissue culture. Until quite recently, little was known about the mechanism of regulation of either protein. For this reason, we turned our attention several years ago to studies of the protein structures of these two crucial membrane molecules and the genes that encode them. Over the past few years, our group has made considerable progress in understanding the structure, biosynthesis, and molecular genetics of both of these molecules. In this article, we briefly review recent progress in these areas and relate the newer information on the genetic regulation of the LDL receptor and HMG-CoA reductase to the control of the plasma LDL concentration in the general population.

THE LDL RECEPTOR: STRUCTURE

The LDL receptor was purified from bovine adrenal cortex by Wolfgang Schneider (2), a partial amino acid sequence was obtained, and this sequence was used by David Russell and Tokuo Yamamoto to isolate a full-length cDNA for the human LDL receptor (3, 4). Studies of the receptor protein, coupled with the amino acid sequence that was deduced from the nucleotide sequence of the cDNA, have provided insight into the structure of the LDL receptor (4). The mature human

Abbreviations: EGF, epidermal growth factor; ER, endoplasmic reticulum; FH, familial hypercholesterolemia; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; SDS, sodium dodecyl sulfate; VLDL, IDL, and LDL, very low, intermediate, and low density lipoproteins, respectively.

LDL receptor consists of 839 amino acids. The sequence can be divided into five domains. The *first domain* consists of the NH_2-terminal 322 amino acids, which is composed of a sequence of 40 amino acids that is repeated, with some variation, eight times. Each 40-residue repeat contains six cysteine residues (save the last repeat, which contains only five) for a total of 47 cysteine residues in the 322-amino acid segment. All of these cysteine residues are bound up in disulfide bonds. This finding indicates that the NH_2-terminal domain of the LDL receptor is an extremely rigid structure with multiple loops that confer extreme stability. Thus, the LDL receptor can be boiled in sodium dodecyl sulfate (SDS) or guanidine and retain binding activity so long as the disulfide bonds are not reduced (5).

Each of the eight 40-amino acid repeats in the first domain of the receptor contains a cluster of negatively charged amino acids (4). The two ligands for the LDL receptor, apoprotein B and apoprotein E, are known to contain positively charged lysine and arginine residues that are crucial for receptor binding (6). In apoE the crucial positive residues are believed to be clustered in a single α-helix (6). It is thus likely that the negatively charged, cysteine-rich domain of the receptor constitutes its binding site. In view of the data of Mahley and Innerarity (6), which suggests that four apoprotein E molecules can bind to a single LDL receptor, we suggest that two of the eight 40-amino acid repeats may constitute each ligand binding site.

The *second domain* of the LDL receptor consists of ~350 amino acids that bear a strong homology with the polyprotein precursor of epidermal growth factor (EGF), a 6000-dalton peptide that stimulates the growth of epidermal cells. EGF is synthesized as a 133,000-dalton precursor (7, 8) that contains a membrane-spanning region near its COOH-terminal end (9, 10). EGF is liberated from this precursor by proteolysis prior to secretion. The second domain of the LDL receptor exhibits a strong homology to a segment of the EGF precursor that does not include EGF itself, but does include several other extracellular domains of the EGF precursor molecule (4, 10). The strength of this homology (33% identity over a segment of 350 amino acids) (4) strongly suggests that the LDL receptor, a cell surface protein that supplies a nutrient (i.e., cholesterol), must have evolved from the same gene that gave rise to EGF, a hormone that stimulates growth. This implies that growth control and nutrient supply originally may have been primordial functions of an ancestral gene for a membrane molecule that has subsequently duplicated and given rise to several of the cell surface receptors and growth-stimulating factors that we recognize today (10).

The *third domain* of the human LDL receptor consists of a stretch of 48 amino acids, 18 of which are serine and threonine residues (4). These serines and threonines contain carbohydrate molecules attached in O-glycosidic linkage (10, 11). This region is located immediately external to the plasma membrane and may serve to extend the LDL receptor so that the binding sites in the first domain stick out from the membrane.

The *fourth domain* consists of a membrane-spanning region of 22 amino acids (4). This stretch is devoid of charged residues and has been shown by proteolysis studies to lie within the membrane (10).

The *fifth domain* consists of a sequence of 50 amino acids at the COOH-terminal end of the receptor that projects into the cytoplasm. These sequences are strongly conserved in the human and bovine LDL receptors (4, 10). We postulate that these sequences bind to clathrin or some clathrin-associated protein, a reaction that allows the receptor to be incorporated into clathrin-coated pits so that it can be internalized and recycled during receptor-mediated endocytosis (12).

In addition to its 18 O-linked sugar chains, the LDL receptor also contains at least two asparagine-linked (N-linked) carbohydrate chains of the classic complex type (11). Their precise location on the receptor protein is unknown.

A summary of the structural properties of the LDL receptor is presented in **Table 1.**

THE LDL RECEPTOR: BIOSYNTHESIS

The receptor is synthesized in the rough endoplasmic reticulum (ER) as a precursor that migrates with an apparent molecular weight of 120,000 on SDS polyacrylamide gels. About 30 min after its synthesis, the receptor is modified in such a way that it migrates on SDS gels with an apparent molecular weight of 160,000 (13, 14). This change in electrophoretic mobility occurs at the same time that the carbohydrates are undergoing modification. The precursor contains at least two high-mannose N-linked oligosaccharide chains that are converted in the Golgi to the complex N-linked chains found on the mature receptor (11). The precursor also contains up to 18 N-acetylgalactosamine molecules attached in O-linkage to serine and threonine residues (11). The decrease in migration on SDS gels (or apparent increase in molecular weight from 120,000 to 160,000) coincides in time with the processing of the high-mannose N-linked chains to the complex form and the elongation of the O-linked core chains by the addition of one galactose and two sialic acid residues to each N-acetylgalactosamine. The mass of sugar that is added

TABLE 1. Structural properties of the LDL receptor and HMG-CoA reductase, two membrane glycoproteins

Property	Molecule	
	LDL Receptor (Human)	HMG-CoA Reductase (Hamster)
Cellular location	Coated pits on cell surface	ER
Protein structure	1 Chain of 839 amino acids after cleavage of signal peptide of 21 amino acids	1 chain of 887 amino acids; no signal peptide
Carbohydrate structure	2 N-linked chains (complex); 18 O-linked chains	1 N-linked chain (high mannose)
Protein mass	93,102 daltons	97,092 daltons
Carbohydrate mass	~22,000 daltons	~2,000 daltons
Apparent molecular weight on SDS gels		
Precursor	120,000	97,000
Mature	160,000	97,000
Number of membrane-spanning regions	1	7
Orientation in membrane	NH_2-terminus is outside of cell; COOH-terminus is in the cytoplasm	NH_2-terminus is inside lumen of ER; COOH-terminus is in the cytoplasm

does not amount to 40,000 daltons. Thus, the decrease in electrophoretic mobility is due in large part to an apparent conformational change that slows the migration of the receptor on SDS gels (11). This change seems to be dependent upon the lengthening of the O-linked sugars, rather than upon the modification of the N-linked sugars, since it is not abolished by tunicamycin, which prevents the addition of N-linked sugars (11, 13, 14).

THE LDL RECEPTOR: mRNA AND GENE

The sequence of the full-length cDNA for the human LDL receptor shows that the protein contains a signal sequence of 21 amino acids that is cleaved prior to the movement of the protein to the cell surface (4). Presumably, this hydrophobic signal sequence directs the receptor mRNA to the rough ER so that the receptor can be inserted into the membrane co-translationally in the fashion of the classic signal sequences described for secretory and cell surface proteins (15).

Hybridization studies of the receptor mRNA in cultured cells have shown that this mRNA is markedly reduced in amount when sterols are added to the culture medium (4, 10), an observation that explains the previously observed feedback regulation of LDL receptor protein (1). Moreover, the receptor mRNA is 9-fold more abundant in bovine adrenal glands than in bovine liver (10), an observation that is consistent with the relative amounts of LDL receptor in these tissues as estimated previously by measurements of ^{125}I-labeled LDL binding to isolated membranes (16).

The mRNA for the human LDL receptor contains a 5′ untranslated region of at least 13 nucleotides, which has not yet been fully characterized (4). This mRNA also contains an unusually long 3′ untranslated region of 2,500 nucleotides. This 3′ untranslated region is quite unusual in that it contains multiple copies of a repetitive sequence (4). Such repetitive sequences, which are designated as *Alu* sequences, are usually found in the introns that separate the coding regions of genes and in the flanking regions between genes (17). However, the mRNA for the human LDL receptor contains three *Alu* sequences within the 3′ untranslated region (4). Whether this has any significance for the function of the LDL receptor mRNA, or for the relatively large number of mutations that are known to occur in this gene, is unknown. Unlike the human mRNA, the 3′ untranslated region of the mRNA for the bovine receptor does not contain any repetitive sequences (3).

The structural gene for the human LDL receptor has recently been mapped to chromosome 19 (18), a finding that agrees with family linkage data that place the locus responsible for FH on chromosome 19. The gene for apoprotein E is also known to be on chromosome 19 (19), raising the possibility of an evolutionary link between a protein ligand and its receptor. Thomas Sudhof and David Russell are in the process of isolating the human LDL receptor gene from a bacteriophage λ

genomic library. Preliminary studies suggest that there is only a single copy of the receptor gene per haploid genome and that no pseudogenes are detectable. The receptor gene appears to be extremely long, at least 50,000 nucleotides, and its coding region is split by many introns.

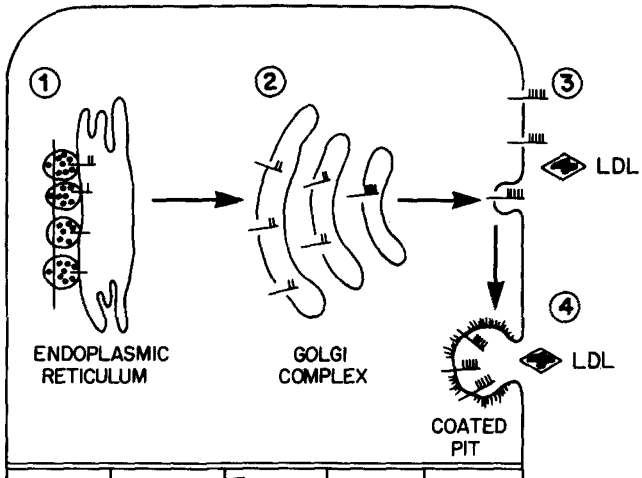
THE LDL RECEPTOR: MUTATIONS

At least ten different mutations in the LDL receptor gene have been identified in patients with the clinical syndromes of homozygous and heterozygous FH (13, 14, 20). These allelic mutations can be divided into four broad classes (**Fig. 1** and **Table 2**). In Class 1, the most frequent class of mutations, the mutant gene fails to specify synthesis of a receptor protein that can be recognized by any of our available antibodies. These Class 1 mutations thus represent "null" alleles. These mutations may well differ from patient to patient in a fashion that has not yet been determined. Class 2 mutations produce receptors that are synthesized in the rough ER, but are not transported to the Golgi apparatus and thus do not undergo the carbohydrate processing reactions. As a result, these receptors do not appear on the cell surface. Some of the proteins produced by the Class 2 alleles have a molecular weight of 120,000 on SDS gels, which is the same as the normal receptor precursor. Others have abnormal molecular weights of 100,000 and 135,000. None of these proteins undergoes the normal 40,000-dalton increase in apparent molecular weight. It seems likely that these mutant proteins have been affected in such a way that they cannot be recognized by the cellular transport machinery that is necessary to carry the proteins from the ER to the Golgi apparatus.

We have observed an interesting variant of the Class 2 mutations in which the mutant allele specifies a 120,000-dalton receptor protein that is eventually converted to the 160,000-dalton mature form, but at an abnormally slow rate. Such kinetic mutations have been identified in several individuals with homozygous FH (21), including three South African FH homozygotes (unpublished observations and ref. 22). A similar kinetic mutation appears to be responsible for the FH syndrome in Watanabe heritable hyperlipidemic (WHHL) rabbits (21).

Class 3 mutations encode receptors that are synthesized, processed, and transported to the cell surface normally. However, the receptors specified by these abnormal genes fail to bind LDL normally, although they can bind certain monoclonal antibodies directed against the LDL receptor (14, 23). Some of these mutant proteins are synthesized with a normal molecular weight of 120,000 that increases to 160,000 in the mature form. Other members of this class are synthesized as precursors with abnormal molecular weights of 100,000 and 170,000 that increase by an apparent 40,000 daltons (i.e., to 140,000 and 210,000 daltons, respectively) prior to their arrival at the cell surface (13, 14). Since these mutations do not affect carbohydrate structure (11), it seems likely that they involve duplications or deletions of part of the receptor protein. The LDL receptor may be unusually prone to such mutations because of the highly repeated structure at the NH_2-terminal end of the protein (4). Such a repeat structure creates the genetic potential for unequal crossing-over due to homologous recombination, which would delete or add segments to the receptor protein.

Class 4 mutations are those in which the receptors are synthesized, processed, and transported to the cell surface where they bind LDL normally. However, these mutant receptors fail to cluster in coated pits and hence they do not internalize receptor-bound LDL. We postulate that the molecular lesion disrupts the sequence of the receptor in its cytoplasmic domain in such a way that the receptor cannot interact with clathrin or a clathrin-associated protein that coats the cytoplasmic surface of a coated pit.



Class of Mutation	Synthesis	Transport from ER to Golgi	Binding of LDL	Clustering in Coated Pits
①	X			
②	→	X		
③	→	→	X	
④	→	→	→	X

Fig. 1. Four classes of mutations in the structural gene for the LDL receptor have been identified. Each mutation affects a different region in the gene and thus interferes with a different step in the normal process by which the receptor is synthesized, processed in the Golgi complex, and transported to coated pits where it is available to bind LDL or IDL. Each class of mutations can be further subdivided into different mutant alleles that are summarized in Table 2.

TABLE 2. Mutant alleles at the LDL receptor locus that produce familial hypercholesterolemia

Class of Mutation	Allele Designation	Apparent Molecular Weight of Receptor on SDS Gels		Frequency Among FH Patients
		Precursor	Mature	
Class 1 (no detectable precursor)	*R–0*	none detected	none detected	Common
Class 2 (precursor not processed)	*R–100*	100,000	100,000	Rare; found in Lebanese
	R–120	120,000	120,000	Common
	R–135	135,000	135,000	Rare
Class 2 Variant (precursor processed at abnormally slow rate)	*R–120 → 160 (slow)*	120,000	160,000	Rare; found in South Africans and in WHHL rabbits
Class 3 (precursor processed normally, but does not bind LDL normally)	R^{b-} *100 → 140*	100,000	140,000	Rare
	R^{b-} *120 → 160*	120,000	160,000	Common
	R^{b-} *170 → 210*	170,000	210,000	Rare
Class 4 (precursor processed normally and binds LDL normally, but does not cluster in coated pits)	R^{b+,i^*} *110 → 150*	110,000	150,000	Rare
	R^{b+,i^*} *120 → 160*	120,000	160,000	Rare

The genetic analysis that produced this classification is based on studies of cultured skin fibroblasts from 98 FH homozygotes and many of their heterozygous parents. The three common alleles (*R–0, R–120,* and R^{b-} *120 → 160*) are each genetically heterogeneous and each will require further subdivision into additional alleles when DNA sequence data become available.

The high incidence of mutations at the LDL receptor locus may also be related in some way to the repetitive *Alu* sequence in the 3′ untranslated region of the mRNA. It is possible that the presence of this sequence in long-lived cytoplasmic mRNA might lead to abnormal homologous recombination events that would occur through an RNA intermediate.

HMG-CoA REDUCTASE: STRUCTURE

HMG-CoA reductase is found in the ER of the liver and other cells. Structural studies of the protein and cloning of its cDNA were made possible through the development of UT-1 cells, a line of Chinese hamster ovary cells that was adapted to growth in the presence of compactin, an inhibitor of reductase (24). In response to compactin, the UT-1 cells have undergone a 15-fold amplification of the reductase gene, and they also transcribe each reductase mRNA at a 20-fold higher rate (25). As a result, at least 2% of the mRNA and 2% of the total cell protein in these cells corresponds to HMG-CoA reductase (24, 25). UT-1 cells house the HMG-CoA reductase in an elaborate system of ER membranes that is packed together in a tubular arrangement, designated crystalloid ER (24, 26).

A full-length cDNA for HMG-CoA reductase was isolated from UT-1 cells by Daniel Chin and Kenneth Luskey, and its nucleotide sequence was determined by Gregorio Gil and David Russell (27). Protein chemistry studies carried out by Laura Liscum, together with the nucleotide sequence data, have revealed that the hamster enzyme has a protein molecular weight of 97,092 and consists of 887 amino acid residues (27, 28). The protein is divided into two domains (28, 29). The *first domain,* the NH_2-terminal 35,000 daltons, is extremely hydrophobic and is believed to criss-cross the ER membrane seven times (28, 29). The NH_2-terminal end of this segment is located in the lumen of the ER. The COOH-terminal end of the first domain is contiguous with a 62,000-dalton water-soluble stretch of amino acids that projects into the cytoplasm and comprises the *second domain* of the reductase. This cytoplasmic domain contains the catalytic site of the enzyme (28, 29). Secondary structure predictions, based on various computer-modeling techniques, suggest that this domain consists of two elongated β-barrel structures surrounded by amphipathic helices. The hydrophilic catalytic domain can be separated from the hydrophobic membrane domain by treatment with any of several proteases (28, 29). The function of the membrane domain is not yet known. One hypothesis is that this region serves as a "receptor" for LDL-derived cholesterol (29). When such cholesterol enters cells, the rate of degradation of HMG-CoA reductase is known to be enhanced about 3-fold, and this acceleration might be produced by the binding of cholesterol to the membrane domain of the reductase protein (30).

The structural properties of HMG-CoA reductase are summarized in Table 1.

HMG-CoA REDUCTASE: BIOSYNTHESIS

HMG-CoA reductase is believed to be synthesized on membrane-bound ribosomes (31). This implies that it contains a signal sequence that allows the nascent chain

to bind to the ER. However, cDNA sequencing studies (27) and cell-free translation studies (31) show that the reductase does not contain a hydrophobic sequence at its NH_2-terminus. Rather, the NH_2-terminal nine amino acids are quite hydrophilic, and they are not preceded by a cleaved hydrophobic sequence (27). Somehow, this hydrophilic NH_2-terminus must be inserted into the lumen of the ER. It seems likely that this insertion may be mediated by a hydrophobic sequence within the protein that binds to signal recognition particle (15) and mediates the insertion of the protein into the membranes of the ER. However, such an internal signal sequence has not yet been identified.

The reductase contains at least one N-linked carbohydrate chain that is bound to an asparagine in the membrane domain (28). This chain never undergoes modification to the complex type. Rather, it remains in the high-mannose precursor form, although some of the mannose residues are trimmed so that the majority of reductase molecules contain six mannose residues rather than the initial nine. This finding suggests that the HMG-CoA reductase never goes to the Golgi apparatus, the site at which high-mannose chains of secretory and plasma membrane proteins are processed to the complex type.

HMG-CoA REDUCTASE: mRNA AND GENE

Studies by Gary Reynolds, Sandip Basu, Tim Osborne, and Kenneth Luskey revealed that the mRNA for HMG-CoA reductase is unusual in that it contains an extremely long 5′ untranslated region that can be up to 729 nucleotides long in hamster liver and up to 670 nucleotides long in UT-1 cells (32). Heterogeneity in the length of reductase mRNA in UT-1 cells arises because at least four different sites can be used to initiate transcription (32). Moreover, there is an intron in the 5′ untranslated region that is processed out of the mRNA by cleavage at a single 3′ splice site, but at variable 5′ splice sites. This creates a situation in which several families of reductase mRNAs exist, each with the same coding region, but each containing a different 5′ untranslated region (32). Whether this heterogeneity has any functional significance is unknown.

Some of the reductase mRNAs contain as many as eight methionine codons upstream of the AUG that is used to initiate translation of the reductase protein (32). Some of these codons might initiate abortive protein synthesis, and this might affect the overall rate of translation of reductase mRNA. It appears that the heterogeneity observed in the UT-1 cell mRNA is also found in reductase mRNAs from the liver of hamsters that have been induced by treatment with cholestyramine and compactin (32). It will be important to determine whether different transcripts with different 5′ untranslated regions are produced in various tissues, or under varying conditions of metabolic regulation.

The 3′ untranslated region of the reductase mRNA is also quite long (1,942 nucleotides) (27). It contains several potential sites that could be used for polyadenylation and hence termination of the mRNA (32). It does not contain repetitive sequences of the type found in the 3′ untranslated region of the human LDL receptor.

The gene for hamster HMG-CoA reductase has recently been isolated and characterized in some detail (32). It spans 25,000 nucleotides and contains 20 exons that are separated by 19 introns. Interestingly, each of the seven hydrophobic segments in the NH_2-terminal domain of the reductase protein is specified by a separate exon (29, 32). This finding lends support to the notion that the seven postulated hydrophobic segments are in fact membrane-spanning regions. It also suggests that the multiple membrane-spanning regions have been assembled in the reductase gene in an orderly fashion through evolutionary time. None of the seven membrane-spanning regions is homologous to the others in terms of amino acid sequence, suggesting that each hydrophobic stretch arose as an independent exon and not by repeated duplication of the gene for a single exon.

Sterols, such as cholesterol derived from the receptor-mediated endocytosis of LDL or 25-hydroxycholesterol added to culture media in solvents, suppress the activity of HMG-CoA reductase by several mechanisms. The most important mechanism involves an inhibition of the transcription of the reductase gene (25). A second mechanism involves a stimulation of the degradation of preexisting reductase protein (30, 33). It is not yet known whether sterols also affect the post-transcriptional processing of the reductase mRNA or alter the stability of this mRNA in the nucleus or cytoplasm.

The promoter in the 5′ flanking region that is responsible for transcription of the reductase mRNA is highly unusual (32). Unlike that of almost all other cellular genes, the reductase promoter does not contain a characteristic TATA box or CCAAT box. Rather, it contains a long sequence that is rich in guanosine (G) and cytosine (C) residues. This region contains three repeats of the hexanucleotide sequence CCGCCC, a sequence that was initially identified in the early promoter of the SV40 virus and which is known to promote transcription of the SV40 DNA (34).

The lack of a TATA box appears to explain why the reductase mRNA has multiple transcription initiation sites. In other genes, deletion of the TATA box has been shown to alter transcription in such a way that multiple initiation sites are used, rather than the single

site that is used when the TATA box is present (34). The part of the reductase gene that is responsible for inhibition of transcription when cholesterol is available has not yet been identified. However, it is known that the CCGCCC-rich region of the reductase promoter functions as a strong positive promoter when it is placed in front of another gene such as the gene for chloramphenicol acetyltransferase (Osborne, T., et al., unpublished observations).

Fig. 2 shows a schematic diagram illustrating the structural features of hamster HMG-CoA reductase at the level of the gene, the mRNA, and the protein.

COORDINATE REGULATION OF THE LDL RECEPTOR AND HMG-CoA REDUCTASE IN THE BODY

By varying the activities of the LDL receptor and HMG-CoA reductase, cells can obtain cholesterol either from exogenous lipoproteins or from endogenous synthesis (1). The exact proportion of cholesterol contributed by each pathway in specific body cells cannot be determined directly. However, indirect measurements in rats suggest that most cells that have low requirements for cholesterol satisfy these requirements by synthesizing a small amount of cholesterol through the HMG-CoA reductase pathway and do not express large numbers of LDL receptors (35).

There are two well-studied tissues that have a large requirement for cholesterol and that express large numbers of LDL receptors. These tissues are the adrenal gland and the liver, both of which express a much larger number of LDL receptors than do other body tissues (16, 35–38). The adrenal gland uses LDL receptors to supply much of the cholesterol that is needed for synthesis of steroid hormones. The liver uses the LDL receptor to supply cholesterol for excretion into bile, conversion into bile acids, and for synthesis of lipoproteins. Although the adrenal gland has the highest concentration of receptors per mass of tissue (16), the liver expresses the largest total number of receptors per organ (36–38). Studies in several species, including rats, hamsters, guinea pigs, and rabbits, suggest that the liver removes at least two-thirds of the LDL from the circulation daily and that the majority of this LDL is removed by LDL receptors (reviewed in ref. 38).

In livers of rabbits and dogs, LDL receptors are subject to regulation in a fashion that resembles the regulation in cultured cells (39). Thus, hepatic receptors are reduced when animals are fed cholesterol, causing cholesterol to accumulate in liver cells (6, 38–40). This

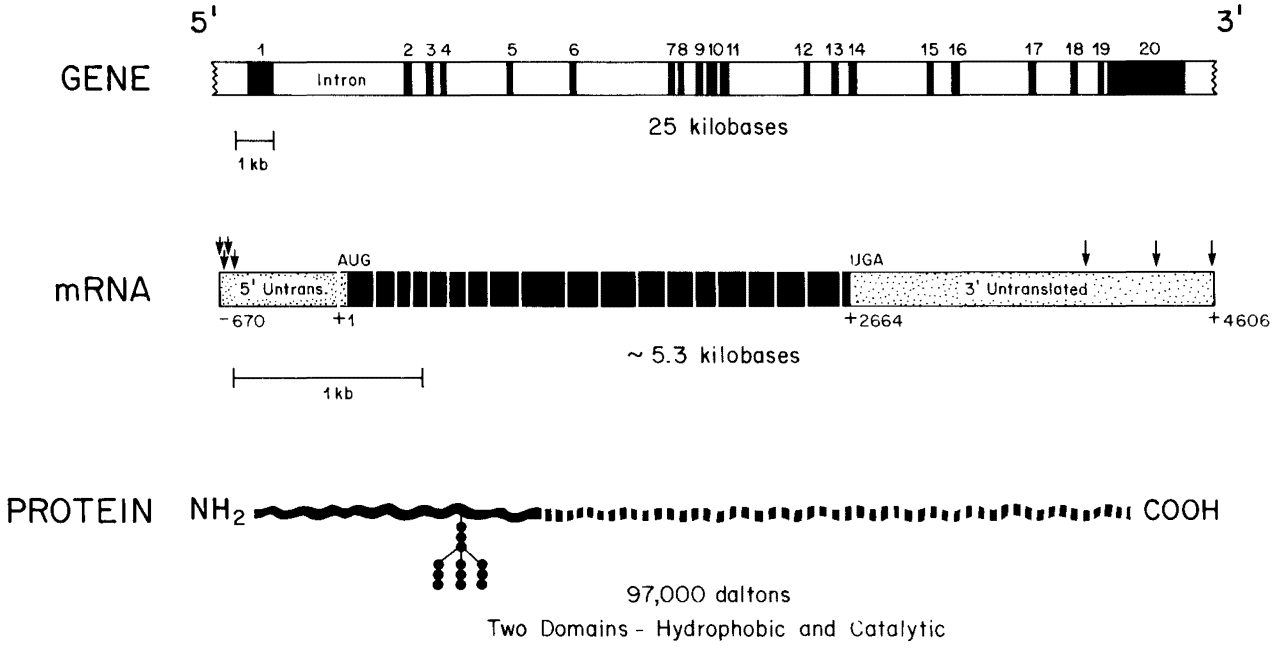


Fig. 2. Structural organization of the gene, mRNA, and protein for hamster HMG-CoA reductase. The 25-kilobase gene is encoded by 20 exons (closed squares) that are interrupted by 19 introns (open squares). The arrows at the 5′ untranslated region of the mRNA denote the multiple sites at which transcription initiates in UT-1 cells; the arrows in the 3′ untranslated region denote the multiple sites at which transcription terminates. The coding region in the mRNA begins at the AUG initiator codon and ends with the UGA terminator codon. The NH_2-terminal one-third of the protein contains the 35,000-dalton hydrophobic membrane domain with its one N-linked oligosaccharide chain (solid line); the COOH-terminal two-thirds of the protein contains the 62,000-dalton hydrophilic catalytic domain (stippled line).

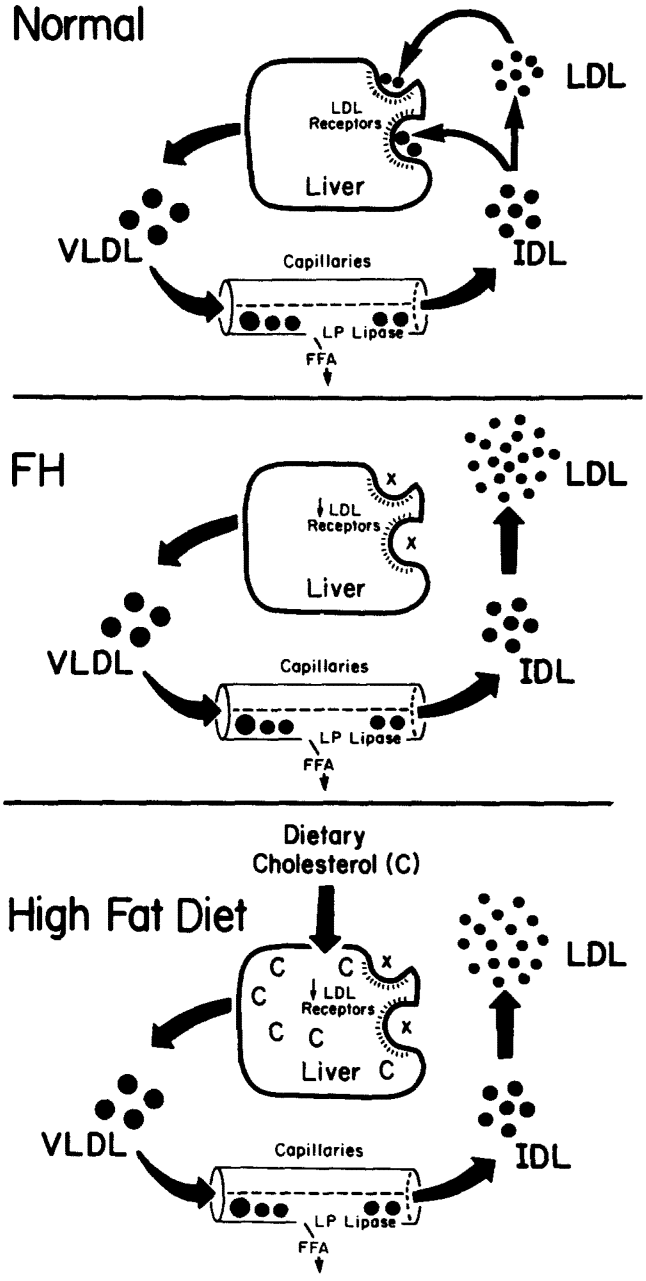


Fig. 3. Schematic diagram showing the mechanism by which LDL receptors in the liver control both the production and catabolism of plasma LDL. Panel A: In normal human subjects, VLDL is secreted by the liver and converted to IDL. About half of the plasma IDL particles are taken up rapidly by binding to LDL receptors in the liver; the remainder of the IDL particles escape uptake in the liver and are converted to LDL. Panel B: In individuals with familial hypercholesterolemia (FH), the number of LDL receptors in the liver is diminished owing to a defect in the gene encoding the receptors. Panel C: An analogous (although less complete) deficiency of receptors can be produced in normal animals by the ingestion of diets rich in cholesterol and saturated fats. By filling the liver with cholesterol, these diets cause the liver to diminish its production of LDL receptors. The deficiency of receptors, whether genetic or acquired, has the same consequences for LDL metabolism: IDL particles can no longer enter the liver at a normal rate, and so they remain in the circulation where they are converted to LDL in increased amounts. The LDL, in turn, is removed slowly from the plasma. Thus, a receptor deficiency, either genetic or acquired, elevates the LDL level by two mechanisms: an increased rate of LDL production owing to increased conversion from IDL and a decreased rate of LDL catabolism owing to the slow removal of LDL from the circulation.

reduction in receptor number contributes to the buildup of LDL in the plasma of these animals. Conversely, LDL receptors are induced when the liver's demand for cholesterol is enhanced, as occurs with the administration of bile acid-binding resins such as cholestyramine (41, 42). This effect is amplified if the liver is prevented from synthesizing increased amounts of cholesterol by the simultaneous administration of an inhibitor of HMG-CoA reductase such as compactin or mevinolin (42). When a bile acid-binding resin is given together with a reductase inhibitor, livers of normal dogs can produce up to three times the normal number of LDL receptors, and the plasma LDL-cholesterol level falls by as much as 75% (42).

Recent studies in rabbits have indicated that the LDL receptor plays an additional role in cholesterol metabolism that may be even more important than its role in removing LDL from the circulation: the LDL receptor appears to be the main route for the removal of intermediate density lipoproteins (IDL) from the circulation (43, 44).

IDL and LDL are both generated from very low density lipoproteins (VLDL) after the triglycerides of VLDL have been hydrolyzed by lipoprotein lipase. Initially, IDL are formed, and if these particles are not removed from the plasma rapidly they undergo a further conversion to LDL (**Fig. 3**). Inasmuch as IDL particles are rich in apoE, a high affinity ligand for the LDL receptor (6, 39), their uptake into the liver is ordinarily quite rapid. However, when hepatic LDL receptors are reduced, either as a result of genetic defects or as a result of metabolic suppression by high cholesterol diets, IDL particles remain in the circulation where they undergo further lipolysis and are converted in LDL (43, 44) (Fig. 3). Thus, a deficiency in LDL receptors leads to an increase in LDL production via enhanced conversion from IDL; at the same time there is a diminished LDL catabolism. The combination of enhanced production and diminished catabolism of LDL leads to a marked increase in the plasma LDL level (43, 44).

Because of their dual effects on LDL production and degradation, hepatic LDL receptors play a dominant role in dictating the plasma LDL level. For this reason, it is important to understand the factors that regulate the production of LDL receptors in the liver. The liver has three main sources of cholesterol that are subject to regulation: *1*) the liver can synthesize cholesterol in a

reaction that is regulated by the activity of HMG-CoA reductase; *2*) the liver can take up cholesterol from LDL in a reaction that is regulated by the number of LDL receptors; and *3*) the liver can take up dietary cholesterol in a reaction that is regulated predominantly by the eating habits of the individual. The interplay between these three regulated processes determines in large part the plasma LDL level.

A dramatic illustration of the importance of this interplay is shown by a comparison of the responses of two rodent species, rabbits and rats, to a high intake of dietary cholesterol (**Table 3**). In rabbits, a high cholesterol diet leads to an accumulation of cholesterol in the liver and this suppresses the activity of HMG-CoA reductase, blocking cholesterol synthesis (45). The accumulation of cholesterol also leads to a profound suppression of the production of LDL receptors (40). Because of this suppression, IDL and LDL circulate for a prolonged period, and this contributes to the severe hypercholesterolemia observed in cholesterol-fed rabbits.

A qualitatively different response is seen in the rat (Table 3). In these animals a high cholesterol diet causes cholesterol to accumulate in the liver, just as in the rabbit. As a result, HMG-CoA reductase is suppressed and cholesterol synthesis is inhibited (36). However, in rats hepatic cholesterol accumulation does not suppress receptor-mediated uptake of LDL (36). As a result, the liver continues to clear IDL and LDL efficiently, and hypercholesterolemia does not occur. Hypercholesterolemia can be produced in rats only when cholesterol feeding is coupled with another maneuver that is known to decrease hepatic LDL receptors, such as the creation of a state of thyroid hormone deficiency (39).

The reason for the failure of the rat liver to suppress hepatic LDL receptors in the absence of hypothyroidism is unknown, but the consequence is clear: as long as hepatic LDL receptors remain high, hypercholesterolemia does not occur. Other animal species seem to have responses that are intermediate between the extremes of the rat and the rabbit. In dogs, for example, cholesterol feeding suppresses LDL receptors partially and leads to moderate hypercholesterolemia (6, 46). However, in dogs, as in rats, further hypercholesterolemia results if the animals are also rendered hypothyroid (46).

TABLE 3. Different responses of hepatic LDL receptors and plasma LDL levels in rats and rabbits on high cholesterol diets

Species	Cholesterol Content of Diet	Source of Hepatic Cholesterol: Diet	HMG-CoA Reductase	LDL Receptors	Plasma LDL Level
Rabbit	Low	−	+	+	Low
	High	+	−	−	High
Rat	Low	−	+	+	Low
	High	+	−	+	Low

In many animal species the degree of hypercholesterolemia that develops in response to a high cholesterol diet is extremely variable among individuals. Is it possible that the so-called "hyper-responders" (i.e., animals that develop profound hypercholesterolemia on a cholesterol-rich diet) are like animals in which LDL receptors can be readily suppressed? Do the "hypo-responders" resemble rats in that they fail to suppress LDL receptors in response to a high cholesterol diet? These questions should be open to examination with the monoclonal antibodies and molecular cDNA probes that are now available to measure the LDL receptor protein and its mRNA.

FUTURE DIRECTIONS IN RESEARCH ON THE LDL RECEPTOR AND HMG-CoA REDUCTASE

The recent advances in the understanding of the LDL receptor and HMG-CoA reductase have raised many questions. Many of these questions can be addressed by existing techniques, and it is hoped that answers will be forthcoming shortly. Some of these questions are:

1. How does the LDL receptor navigate through the various membrane compartments of a cell, and how do mutations in the receptor disrupt this process? This question can be answered through analysis of the naturally occurring LDL receptor mutations that disrupt the movement of the receptor from one compartment to another. The answer will not only clarify specific defects in FH, but it will also provide important new information for cell biology in general. At present, the signals that direct membrane proteins to move from one place to another in a cell are largely unknown. The LDL receptor system offers a superb system for uncovering many of these signals.

2. Why does HMG-CoA reductase have such an elaborate membrane domain consisting of seven membrane-spanning segments? Is this membrane domain the structural signal that retains the reductase in the ER after synthesis there, preventing the enzyme from moving to the plasma membrane or some other membrane compartment? Answers to this question can be obtained by site-directed mutagenesis studies in which the nucleotide sequence of the reductase cDNA can be altered at will. Various mutated cDNAs can then be introduced

by standard DNA transfection into cultured cells that lack HMG-CoA reductase (47), and the functional properties of the altered proteins expressed by the mutated cDNA can be analyzed. By this means, it should be possible to determine whether a truncated form of HMG-CoA reductase protein lacking the entire 35,000-dalton membrane domain can synthesize sufficient amounts of mevalonate for normal cell function.

3. Do some individuals in the population have subtle defects in the *regulation* of the genes for the LDL receptor and/or HMG-CoA reductase that make them susceptible to environmental agents, such as dietary fat, that raise plasma LDL levels? It seems reasonable to speculate that some individuals in the population who have high plasma LDL levels and who seem to be sensitive to dietary cholesterol owe their dietary sensitivity to genetic polymorphisms in the structure of the promoters of one of these two genes. For example, if an individual were to suppress his or her LDL receptors too efficiently when fed cholesterol, he or she would then develop an abnormal elevation in plasma LDL levels. By the same token, if an individual did not suppress his or her HMG-CoA reductase gene when consuming cholesterol, the resulting increase in hepatic cholesterol would lead indirectly to enhanced suppression of LDL receptors and elevated plasma levels of LDL. It may be possible to detect such genetic polymorphisms by examining the promoter regions of the HMG-CoA reductase gene and the LDL receptor gene in individuals who have high plasma LDL levels, but who do not have FH.

4. Is dietary and environmental suppression of LDL receptor activity an important cause of the high blood cholesterol levels observed in most individuals in industrialized societies of the world? Abundant epidemiologic studies over the past three decades have shown that plasma LDL levels in certain industrialized nations are much higher than those in primitive societies. These differences have been attributed to the high fat diet that is prevalent in industrialized societies. However, no one has been able to demonstrate experimentally the mechanism by which a high fat diet leads to an elevated level of an endogenous lipoprotein such as LDL, which originates in the liver and not in the intestine. The tissue culture and whole animal experiments on the LDL receptor suggest that a high fat diet leads to suppression of LDL receptors, which in turn may lead to an accumulation of LDL in plasma. Bombardment of the liver with dietary cholesterol over a long period of time could conceivably lead to resetting of the "thermostat" (i.e., the promoter of the gene) that controls LDL receptor production, so that LDL receptors are regulated about a lower setting. In addition, saturated fats might modify the composition of LDL so that it binds to the receptor with lower affinity. It should be possible to test these hypotheses by measuring LDL receptor activity in individuals from a variety of populations with different LDL levels throughout the world.

LDL receptors are currently measured in vivo by determining the rate of disappearance of ^{125}I-labeled LDL from the circulation (44, 48, 49). These studies do not give an exact measurement of the number of LDL receptors because investigators remove the LDL from a given individual and inject it back into the same individual. As suggested by the recent studies of Witztum et al. (50), this procedure obscures the measurement of LDL receptor number. If an animal or individual expresses a high number of LDL receptors, then the LDL that remains in the circulation is the LDL that binds the poorest to the receptors. When this "sluggish" LDL is removed, radiolabeled, and injected back into the circulation, one will find a relatively low catabolic rate for a given number of receptors.

It would be ideal to use one standardized LDL preparation and to inject that preparation into multiple individuals, thus assuring a uniform test ligand. In order for such studies to be performed, it will be necessary to overcome the possible objections that nonautologous LDL might carry viruses such as hepatitis virus into the recipients. However, the amounts of injected LDL are extremely small, and a limited panel of reliable, hepatitis-free donors could be used, essentially obviating the possibility of transmitting hepatitis.

Alternatively, it may be possible to use monoclonal antibodies against the LDL receptor (23) to measure receptor levels in vivo (51). These antibodies bind to the receptor and are internalized and degraded by receptor-bearing cells. The rate of their disappearance from the circulation is proportional to the number of receptors in the whole body (51). Monoclonal antibodies are uniform reagents and they have the additional advantage that they are not subject to competition by the LDL that is present in the circulation. Thus, LDL receptors can be measured in a fashion that is independent of the size of the circulating pool of plasma LDL (51). However, in order for these studies to be carried out, it will be necessary to show that monoclonal antibodies do not carry any toxic agents with them and do not stimulate any immune response. It seems likely that as experience with monoclonal antibodies grows, such objections will be overcome, and the monoclonal antibody technique might provide an extremely reliable method by which to measure the total number of LDL receptors in the body in vivo.

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