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A Receptor-Mediated Pathway for Cholesterol Homeostasis

MICHAEL S. BROWN AND JOSEPH L. GOLDSTEIN

In 1901, after studying a patient with black urine, a physician named Archibald Garrod suggested that a single mutant gene can produce a discrete block in a biochemical pathway, which he called an “inborn error of metabolism.” Garrod’s brilliant insight anticipated by 40 years the one gene–one enzyme concept of Beadle and Tatum. Similarly, the chemist Linus Pauling and the physician Vernon Ingram, through study of patients with sickle cell anemia, showed that mutant genes alter the amino acid sequences of proteins. Clearly, many fundamental advances in biology were spawned by perceptive studies of human genetic diseases (1).

We began our work in 1972 in an attempt to understand a human genetic disease, familial hypercholesterolemia (FH). In patients with this disease, the concentration of cholesterol in the blood is elevated many times above normal and heart attacks occur early in life. We postulated that this dominantly inherited disease results from a failure of end-product repression of cholesterol synthesis. The possibility fascinated us because genetic defects in feedback regulation had not been observed previously in humans or animals, and we hoped that study of this disease might throw light on fundamental regulatory mechanisms.

Our approach was to apply the techniques of cell culture to unravel the postulated regulatory defect in FH. These studies led to the discovery of a cell surface receptor for a plasma cholesterol transport protein called low density lipoprotein (LDL) and to the elucidation of the mechanism by which this receptor mediates feedback control of cholesterol synthesis (2, 3). FH was shown to be caused by inherited defects in the gene encoding the LDL receptor; these defects disrupt the normal control of cholesterol metabolism. Study of the LDL receptor in turn led to an understanding of receptor-mediated endocytosis, a general process by which cells communicate with each other through internalization of regulatory and nutritional molecules (4). Receptor-mediated endocytosis differs from previously described biochemical pathways because it depends on the continuous and highly controlled movement of membrane-embedded proteins from one cell organelle to another in a process termed receptor recycling (4). Many of the mutations in the LDL receptor that occur in FH patients disrupt the movement of the receptor between organelles. These mutations define a new type of cellular defect that has broad implications for normal and deranged human physiology.

The Problem of Cholesterol Transport

Cholesterol is the most highly decorated small molecule in biology. Thirteen Nobel Prizes have been awarded to scientists who devoted major parts of their careers to cholesterol (5). Ever since it was first isolated from gallstones in 1784, cholesterol has exerted an almost hypnotic fascination for scientists from the most diverse areas of science and medicine. Its complex four-ring structure and its
synthesis from a simple two-carbon substrate (acetate) through the action of at least 30 enzymes has attracted the attention of organic chemists and biochemists. Physiologists and cell biologists have been fascinated with cholesterol because of its essential function in membranes of animal cells, where it modulates fluidity and maintains the barrier between cell and environment, and because it is the raw material for the manufacture of steroid hormones and bile acids. And finally, the observation that elevated levels of blood cholesterol accelerate the formation of atherosclerotic plaques, leading to heart attacks and strokes, has been of great interest to physicians.

Cholesterol is a Janus-faced molecule. The very property that makes it useful in cell membranes, namely its absolute insolubility in water, also makes it lethal. When cholesterol accumulates in the wrong place, for example within the wall of an artery, it cannot be readily mobilized and its presence eventually leads to the development of an atherosclerotic plaque. The potential for errant cholesterol deposition is aggravated by its dangerous tendency to exchange passively between blood lipoproteins and cell membranes. If cholesterol is to be transported safely in blood, its concentration must be kept low and its tendency to escape from the bloodstream must be controlled.

Multicellular organisms solve the problem of cholesterol transport by esterifying the sterol with long-chain fatty acids and packaging these esters within the hydrophobic cores of plasma lipoproteins (Fig. 1). With its polar hydroxyl group esterified, cholesterol remains sequestered within this core, which is essentially an oil droplet composed of cholesteryl esters and triglycerides, solubilized by a surface monolayer of phospholipid and unesterified cholesterol and stabilized by protein. The small amounts of unesterified cholesterol on the surface of the particle are maintained in equilibrium exchange with the cholesterol of cell membranes, but the larger amounts of cholesteryl esters remain firmly trapped in the core of the particle and leave the particle only as the result of highly controlled processes.

The major classes of plasma lipoproteins were delineated in the 1950's and 1960's through work in many laboratories, most notably those of Oncley (6), Gofman (7), and Fredrickson (8). The four major classes are LDL, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and high density lipoprotein (HDL). A schematic representation of LDL, the most abundant cholesterol-carrying lipoprotein in human plasma, is shown in Fig. 1.

Fig. 1. Structure of plasma LDL and its cholesteryl and cholesteryl ester components. LDL is a spherical particle with a mass of $3 \times 10^6$ daltons and a diameter of 22 nm. Each LDL particle contains about 1500 molecules of cholesteryl ester in an oily core that is shielded from the aqueous plasma by a hydrophobic coat composed of 800 molecules of phospholipid, 500 molecules of unesterified cholesterol, and 1 molecule of a 400,000-dalton protein called apoprotein B-100 (128). Elevations in blood cholesterol are usually attributable to an increase in the number of LDL particles.

Packaging of cholesteryl esters in lipoproteins solves the problem of nonspecific partitioning of cholesterol into cell membranes, but it creates another problem, namely one of delivery. Cholesteryl esters are too hydrophobic to pass through membranes. How then can esterified cholesterol be delivered to cells? The delivery problem is solved by lipoprotein receptors, of which the prototype is the LDL receptor (9). Strategically located on the surfaces of cells, these receptors bind LDL and carry it into the cell by receptor-mediated endocytosis. The internalized lipoprotein is delivered to lysosomes where its cholesteryl esters are hydrolyzed. The liberated cholesterol is used by the cell for the synthesis of plasma membranes, bile acids, and steroid hormones, or stored in the form of cytoplasmic cholesteryl ester droplets. Two properties of the receptor—its high affinity for LDL and its ability to cycle multiple times in and out of the cell—allow large amounts of cholesterol to be delivered to body tissues, while at the same time keeping the concentration of LDL in blood low enough to avoid the buildup of atherosclerotic plaques. When LDL receptor function is inappropriately diminished as a result of genetic defects or in response to regulatory signals, the protective mechanism is lost, cholesterol builds up in plasma, and atherosclerosis ensues (10).

Familial Hypercholesterolemia: Origin of the LDL Receptor Concept

As a disease, FH has a rich clinical history. It was first described in 1938 by Carl Müller as an "inborn error of metabolism" that produced high blood-cholesterol levels and myocardial infarctions in young people (11). Müller concluded that FH is transmitted as a single gene-determined autosomal dominant trait. In the mid-1960's and early 1970's, Khachadurian (12) and Fredrickson and Levy (13) showed that FH exists clinically in two forms: the less severe heterozygous form and the more severe homozygous form.

FH heterozygotes, who carry a single copy of a mutant LDL-receptor gene, are quite common, accounting for one out of every 500 persons among most ethnic groups throughout the world (14). These individuals have a twofold increase in the number of LDL particles in plasma from the time of birth. They begin to have heart attacks at 30 to 40 years of age. Among people under age 60 who suffer myocardial infarctions, about 5 percent have the heterozygous form of FH, a 25-fold enrichment over the incidence in the general population (15–17).

The attractiveness of FH as an experimental model stems from the existence of homozygotes. These rare individuals, who number about one in 1 million persons, inherit two mutant genes at the LDL receptor locus, one from each parent. Their disease is much more severe than that of heterozygotes. They have six to ten times the normal concentrations of plasma LDL from the time of birth, and they often have heart attacks in childhood (12–14). The severe atherosclerosis that develops in these patients in the absence of any other risk factors is formal proof that high levels of plasma cholesterol can produce atherosclerosis in humans. Experimentally, the availability of FH homozygotes permits study of the manifestations of the mutant gene without any confounding effects from the normal gene.

At the time that our studies began in 1972, it was generally thought that all important events in cholesterol metabolism take place in the liver or intestine (18). It was obviously impossible to perform meaningful studies in livers of humans with FH. Our only chance to explain its mysteries depended on the mutant phenotype being faithfully manifested in long-term cultured cells such as skin fibroblasts. Inherited enzyme defects were known to be expressed in cultured fibroblasts from patients with rare recessive diseases such as
galactosemia, and Lesch-Nyhan syndrome, and Refsum's syndrome. By 1970, Neufeld's classic studies of the mucopolysaccharidoses (a form of lysosomal storage disease) were beginning to establish the value of cultured skin fibroblasts in elucidating complex cellular pathways (19).

There was some reason to believe that the defect associated with FH might be studied in cultured skin fibroblasts. Studies in the 1960's by Bailey (20) and Rothblat (21) had demonstrated that several types of cultured animal cells synthesize cholesterol and that this synthesis is subject to negative feedback regulation. When serum was absent from the medium, cultured cells produced little cholesterol from radioactive acetate. When serum lipoproteins were removed from the culture medium, cholesterol synthesis increased.

Regulation of HMG CoA reductase by LDL in fibroblasts. We began our work by setting up a microassay for 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase), the rate-determining enzyme of cholesterol biosynthesis. This assay was used to measure HMG CoA reductase activity in extracts of cultured fibroblasts (2, 22). Studies in rat livers by Bucher and Lynen (23) and by Siperstein (24) had shown that the activity of this enzyme was reduced when rats ingested cholesterol and that this reduction limited the rate of cholesterol synthesis. We soon found that the activity of HMG CoA reductase was subject to negative regulation in fibroblasts (2, 22). When normal human fibroblasts were grown in the presence of serum, HMG CoA reductase activity was low (Fig. 2A). When the lipoproteins were removed from the culture medium, the activity of HMG CoA reductase rose by at least 50-fold over a 24-hour period. The induced enzyme was rapidly suppressed when lipoproteins were added back to the medium (Fig. 2B).

Not all lipoproteins could suppress HMG CoA reductase activity. Of the two major cholesterol-carrying lipoproteins in human plasma, LDL and HDL, only LDL was effective (22, 25). This specificity was the first clue that a receptor might be involved. The second clue was the concentration of LDL that was required. The lipoprotein was active at concentrations as low as 5 µg of protein per milliliter, which is less than 10^{-6}M (22, 25). A high-affinity receptor mechanism must be responsible for enzyme suppression.

The key to this mechanism emerged from studies of cells from patients with homozygous FH (2, 25). When grown in serum that contained lipoproteins, the homozygous FH cells had HMG CoA reductase activities that were 50- to 100-fold above normal (Fig. 2A). This activity did not increase significantly when the lipoproteins were removed from the serum, and there was no suppression when LDL was returned to the medium. Clearly, the genetic defect was expressed in cell culture (Fig. 2, A and B).

The simplest interpretation of these results was that FH-homozygotes had a defect in the gene encoding HMG CoA reductase that rendered the enzyme resistant to feedback regulation by LDL-derived cholesterol. This working hypothesis was immediately disproved by the next experiment. Cholesterol, dissolved in ethanol, was added to normal and FH-homozygous cells. When mixed with albumin-containing solutions, cholesterol forms a quasi-soluble emulsion that enters cells passively, apparently by incorporation into the plasma membrane. When cholesterol was added in this form, the HMG CoA reductase activities of normal and FH-homozygous fibroblasts were suppressed at the same rate and to the same extent (25).

Clearly, the defect in the FH-homozygous cells must reside in their ability to extract cholesterol from the lipoprotein, and not in the ability of the cholesterol, once extracted by the cells, to act. But how do normal cells extract the cholesterol of LDL? The high affinity of the process suggested that a cell surface receptor was involved. The existence of cell surface receptors for protein hormones and other chemical messengers had been known for many years. It was generally thought that these receptors acted by binding the ligand at the surface and then generating a "second messenger" on the intracellular side of the plasma membrane. The classic second messenger was adenosine 3',5'-monophosphate (cyclic AMP) (26). Perhaps LDL was binding to a receptor and generating some second messenger that suppressed HMG CoA reductase.

Delineation of the LDL receptor pathway. The existence of an LDL receptor was confirmed when LDL was radiolabeled with 125I (125I-LDL) and incubated with normal and FH-homozygous fibroblasts. These studies showed that normal cells had high-affinity binding sites for 125I-LDL, whereas FH-homozygous cells lacked high-affinity receptors (3, 27). This seemed to explain the genetic defect in FH, but it did not reveal how LDL generated the signal that suppressed HMG CoA reductase. The answer came from studies of the fate of the surface-bound 125I-LDL. Techniques were developed to distinguish surface-bound from intracellular 125I-LDL (28), and these revealed that the receptor-bound LDL remained on the surface for less than 10 minutes on average (Fig. 3A). Within this time, most of the surface-bound LDL particles entered the cell; within another 60 minutes, the protein component of 125I-LDL was digested completely to amino acids and the 125I-I, which had been attached to tyrosine residues on LDL, was released into the culture medium as 125I-monoiodotyrosine (27, 28). Meanwhile, the cholesterol esters of LDL were hydrolyzed, generating unesterified cholesterol that remained within the cell (29).

The only cellular organelle in which LDL could have been degraded so completely and rapidly was the lysosome. Originally described by de Duve (30), lysosomes were known to contain a large number of acid hydrolases that could easily digest all of the components of LDL. The hypothesis of lysosomal digestion of LDL was confirmed through the use of inhibitors such as chloroquine (31), which raises the pH of lysosomes and inhibits lysosomal enzymes (32), and through studies of cultured fibroblasts from
patients with a genetic deficiency of lysosomal acid lipase (29). Cells from such patients bound and internalized LDL but failed to hydrolyze its cholesteryl esters, even though they were able to degrade its protein component.

The cholesterol that was generated from LDL within the lysosome proved to be the second messenger responsible for suppressing HMG CoA reductase activity. We now know that cholesterol (or an oxygenated derivative that is formed within the cell) acts at several levels, including suppression of the synthesis of the HMG CoA reductase gene (33) and acceleration of the degradation of the enzyme protein (34). The LDL-derived cholesterol also regulates two other cellular processes in a coordinated action that stabilizes the cell’s cholesterol content. It activates a cholesteryl-esterifying enzyme, acyl CoA: cholesterol acyltransferase (ACAT), so that excess cholesterol can be stored in the cytoplasm as cholesteryl ester droplets (35). It also suppresses synthesis of LDL receptors by lowering the concentration of receptor messenger RNA (mRNA) (36, 37). The latter action allows cells to adjust the number of LDL receptors to provide sufficient cholesterol for metabolic needs without causing cholesterol overaccumulation (9). Through these regulatory mechanisms, cells keep their level of unesterified cholesterol remarkably constant despite wide fluctuations in cholesterol requirements and exogenous supply.

Receptor-mediated endocytosis of LDL: binding coupled to internalization in coated pits. The rapidity of internalization of receptor-bound LDL and the completeness with which the protein of LDL was hydrolyzed implied that fibroblasts have a special mechanism for transport of the lipoprotein from the cell surface to the lysosome. The likely mechanism was endocytosis, the process by which surface membranes pouch inward and pinch off to form vesicles that eventually fuse with lysosomes. Endocytosis was first demonstrated by cinematography of phagocytic cells in the 1930’s, and its widespread occurrence was established in the 1950’s by the electron microscopic studies of Palade (38). Endocytosis was felt to be a nonspecific process that transported bulk fluid and its contents into cells. There was no precedent for entry of specific receptors into cells by this route.

In collaboration with R. G. W. Anderson, we coupled LDL to electron-dense ferritin and found that receptor-bound LDL was internalized by endocytosis. More important, however, these morphological studies explained the efficiency of internalization—efficiency was contingent upon the clustering of the LDL receptors in small pockets on the surface called coated pits (39). Coated pits had been described in detail by Roth and Porter (40) in electron microscopic studies of the uptake of yolk proteins by mosquito oocytes. These investigators showed that coated pits pinch off from the surface to form coated endocytic vesicles that carry extracellular fluid and its contents into the cell.

The finding that LDL receptors were clustered in coated pits raised the possibility that these structures serve as gathering places for cell surface receptors that are destined for endocytosis (4). Other cell surface proteins, being excluded from coated pits, could not rapidly enter the cell.

This interpretation of the function of coated pits was strengthened by study of fibroblasts from a unique FH homozygote. Cells from most of these subjects simply failed to bind LDL, but cells from one FH patient (J.D.) bound LDL but failed to internalize it (Fig. 3B) (41, 42). In collaboration with Anderson, we showed that the receptors in these mutant cells were excluded from coated pits (43). This was an important finding, for it established the essential role of coated pits in the high-efficiency uptake of receptor-bound molecules (4).

The sequential steps in the LDL receptor pathway, as deduced from the biochemical, genetic, and ultrastructural studies performed between 1972 and 1976, are summarized in Fig. 4. The striking “all or none” biochemical differences in the metabolism of LDL and its regulatory actions in fibroblasts derived from a normal subject and from an FH homozygote with a complete deficiency of LDL receptors are shown in Fig. 5.

Soon after the initial studies of the LDL receptor pathway, Pearse (44) purified coated vesicles and found that the cytoplasmic coat was composed predominantly of a single protein, clathrin. At the same time, Cohen and his collaborators were studying the action of epidermal growth factor (EGF) on cultured fibroblasts (45). They found that this peptide hormone was internalized by cells in a manner that was indistinguishable from that of LDL. Similar observations were made by Terris and Steiner (46) with insulin in hepatocytes; by Neufeld and co-workers (47) and by Sly and co-workers (48) with lysosomal enzymes in fibroblasts; and by Ashwell, Morell, and co-workers (49) with asialoglycoproteins in hepatocytes. Moreover, Helenius, Simons, and their co-workers (50) showed that several lipid-enveloped viruses enter cells by this route.
The LDL Receptor: Structure Adapted to Function

The LDL receptor is a cell surface glycoprotein that contains approximately two asparagine-linked (N-linked) oligosaccharide chains of the complex type and approximately 18 serine/threonine-linked (O-linked) oligosaccharide chains (55, 56). About two-thirds of the O-linked sugars are clustered in one region of the molecule (57). The LDL receptor binds two proteins: (i) apo B-100, the 400,000-dalton glycoprotein that is the sole protein of LDL (27); and (ii) apo E, a 34,000-dalton protein that is found in multiple copies in IDL and a subclass of HDL (58, 59). Innerarity and Mahley (59) demonstrated that lipoproteins that contain multiple copies of apo E bind to LDL receptors with up to 20-fold higher affinity than LDL, which contains only one copy of apo B.

The LDL receptor (Fig. 6) is synthesized in the rough endoplasmic reticulum (ER) as a precursor (60) that contains high-mannose N-linked carbohydrate chains and the core sugar (N-acetylglucosamine) of the O-linked chains (56). The O-linked core sugars are added before the mannose residues of the N-linked chains are trimmed (while the receptor is still in the endoglycosidase H-sensitive stage). Thus, the O-linked sugars must be added either in the ER or in a transitional zone between the ER and the Golgi apparatus. The receptor precursor migrates during sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis as a single band corresponding to an apparent molecular weight of 120,000 (60).

Within 30 minutes after its synthesis, the LDL receptor decreases in mobility on SDS gels. The apparent molecular weight increases from 120,000 to 160,000 (60). This change is coincident with the conversion of the high-mannose N-linked oligosaccharide chains to the complex endoglycosidase H–resistant form (56). At the same time, each O-linked chain is elongated by the addition of one galactose and one or two sialic acid residues (56). The amount of carbohydrate is not sufficient to account for an increase in molecular mass of 40,000 daltons. Rather, the decrease in electrophoretic mobility is primarily caused by a change in conformation of the protein that results from the elongation of the clustered O-linked sugars (56, 57).

About 45 minutes after synthesis, LDL receptors appear on the cell surface, where they gather in coated pits. Within 3 to 5 minutes of their formation, the coated pits invaginate to form coated endocytic vesicles. Very quickly, the clathrin coat dissociates. Multiple endocytic vesicles then fuse to create larger sacs of irregular contour, called endosomes or receptosomes (4, 61). The pH of the endosomes falls below 6.5, because of the operation of adenosine triphosphate–driven proton pumps in the membrane (53, 54, 61). At this acid pH, the LDL dissociates from the receptor. The latter returns to the surface, apparently by clustering with other receptors in a segment of the endosomal membrane that pinches off to form a recycling vesicle. Once it reaches the surface, the receptor binds another lipoprotein particle and initiates another cycle of endocytosis (4). Each LDL receptor makes one round trip every 10 minutes in continuous fashion whether or not it is occupied with LDL (4, 62). The LDL that dissociates from the receptor is delivered to a lysosome when the membranes of the endosome and lysosome fuse. There the protein component of LDL is hydrolyzed to amino acids and the cholesterol esters are hydrolyzed by an acid lipase, liberating cholesterol.

The striking feature of this pathway is that it requires the continuous movement of a membrane-embedded protein from one organelle to another in a highly ordered fashion. Each time it moves, the receptor must be segregated from neighboring membrane.
proteins that do not follow the same route. This raises a crucial question: What are the signals that direct the highly selective movement of receptors from one membrane organelle to another? Clearly, the signals must lie in the structures of the receptors.

The LDL receptor: a multi-domain protein. The LDL receptor was purified from bovine adrenal cortex by W. J. Schneider in our laboratory (55). A partial amino acid sequence was obtained, and this sequence was used by D. W. Russell and T. Yamamoto to isolate a full-length complementary DNA (cDNA) for the human LDL receptor (37, 63). Biochemical 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 structural domains of the LDL receptor (Fig. 7) (63–65).

At the extreme NH₂-terminus of the LDL receptor, there is a hydrophobic sequence of 21 amino acids that is cleaved from the receptor immediately after it is translated. This segment functions as a classic signal sequence to direct the receptor-synthesizing ribosomes to the ER membrane. Because it does not appear in the mature receptor, the signal sequence is omitted from the structural domains. The mature receptor (without the signal sequence) consists of 839 amino acids (63).

The first domain of the LDL receptor consists of the NH₂-terminal 292 amino acids, which is composed of a sequence of 40 amino acids that is repeated with some variation seven times (65, 66). Antibody-binding studies on intact cells revealed that this domain is located on the external surface of the plasma membrane (67). Each of the seven 40-amino-acid repeats contains six cysteine residues, which are in register for all of the repeats. The receptor cannot be labeled with [³H]iodoacetamide without prior reduction, suggesting that all of these cysteines are disulfide-bonded (65). This region of the receptor must therefore exist in a tightly cross-linked, convoluted state. This explains the extreme stability of the binding domain of the receptor; the receptor can be boiled in strong denaturants and still retain its binding activity as long as the disulfide bonds are intact (65).

A striking feature of each cysteine-rich repeat sequence is a cluster of negatively charged amino acids near the COOH-terminus of each repeat (65, 66). The changes on these sequences are complementary to a cluster of positively charged residues that are believed to occupy one face of a single α-helix in apo E, the best studied ligand for the LDL receptor (68). Elegant studies by Mahley and Innerarity (68) with mutant and proteolyzed forms of apo E and with monoclonal antibodies against different regions of apo E showed that the positively charged region contains the site whereby this protein binds to the LDL receptor. It is therefore tempting to speculate that the negatively charged clusters of amino acids within the cysteine-rich repeat sequence of the LDL receptor constitute multiple binding sites, each of which binds a single apo E molecule by attaching to its positively charged α-helix (65).

The second domain of the LDL receptor, consisting of approximately 400 amino acids, is 35 percent homologous to a portion of the extracellular domain of the precursor for EGF (63, 64, 69). The EGF precursor is a molecule of 1217 amino acids that, like the LDL receptor, appears to span the plasma membrane once (69–72).

Analysis of the amino acid sequence of the EGF precursor, as revealed from the sequence of the cloned cDNA (70, 71), suggests that EGF, a peptide of 53 amino acids, is liberated from the EGF precursor by proteolysis. The sequence of EGF is not homologous to the LDL receptor. Rather, the homology involves a part of the EGF precursor that is on the NH₂-terminal side of EGF itself. The function of this region in either the LDL receptor or the EGF precursor is unknown.

The third domain of the LDL receptor lies immediately external to the membrane-spanning domain and consists of a stretch of 58 amino acids that contains 18 serine or threonine residues (63, 66). This domain is encoded within a single exon. Proteolysis studies have revealed that this region contains the clustered O-linked sugar chains (64).

The fourth domain consists of a stretch of 22 hydrophobic amino acids that span the plasma membrane, as demonstrated by proteolysis experiments (63, 64). Comparison of the amino acid sequences of the human and bovine LDL receptors reveals that the membrane-spanning region is relatively poorly conserved (65). Of the 22 amino acids in this region, seven differ between human and cow, but all of the substitutions retain a hydrophobic character.

The fifth domain is the cytoplasmic tail. The human and bovine LDL receptors each contain a COOH-terminal segment of 50 amino acids that projects into the cytoplasm (63, 64). Localization of this domain to the cytoplasmic side of the membrane was determined by means of an antibody directed against the COOH-terminal sequence (64). When inside-out membrane vesicles containing receptor were digested with pronase, the antibody-reactive material was removed, and the molecular weight of the receptor was reduced by approximately 5000. The cytoplasmic sequence is strongly conserved among species. Of the 50 amino acids in this
region, only four differ between human and cow, and each of these substitutions is conservative with respect to charge (65).

The cytoplasmic domain of the LDL receptor plays an important role in clustering in coated pits, either through interaction with clathrin itself or with some protein that is associated with clathrin on the cytoplasmic side of the membrane (4). This conclusion is based on a molecular analysis of three naturally occurring mutations at the LDL receptor locus that produce receptors that bind LDL normally but fail to cluster in clathrin-coated pits. All three of these mutations produce defects in the cytoplasmic tail as discussed below (65, 73).

The LDL receptor: a mosaic gene. The haploid human genome contains a single copy of the LDL receptor gene (66) on chromosome 19 (74). Sequences representing almost the entire gene have been isolated from bacteriophage λ and cosmid libraries (66). The position of each intron within the gene was mapped, and the sequence of each exon-intron junction was determined.

The LDL receptor gene spans approximately 45 kilobases and is made up of 18 exons separated by 17 introns (66). There is a striking correlation between the exons in the gene and the functional domains of the protein (Fig. 8). The first intron is located just at the end of the DNA encoding the cleaved signal sequence. The binding domain is encoded by exons 2 to 6. Within this domain (which contains the seven cysteine-rich repeats), introns occur precisely at the ends of repeats I, II, V, VI, and VII (Fig. 8). Repeats III, IV, and V are included in one exon. The binding domain is terminated by an intron at amino acid 292, the last residue in the seventh repeat. Thus, the binding domain is composed of a single exon that has been duplicated multiple times to produce seven repeats of a single 40-amino-acid sequence. The repeat sequence is strongly homologous to a stretch of 40 amino acids that occurs in the middle of the C9 component of complement, a plasma protein of 537 amino acids that participates in the complement cascade (66, 75).

The next eight exons in the LDL receptor gene (exons 7 to 14) encode the region that is homologous with the EGF precursor (Fig. 8). The gene for the EGF precursor contains the same eight exons (69). These exons form a cassette that has been lifted out of some ancestral gene during evolution and placed in the middle of the EGF precursor gene and the LDL receptor gene. Three of these exons have also been used by another class of genes. These exons encode a cysteine-rich sequence of 40 amino acids (labeled A, B, and C in Fig. 8) that is repeated three times in the LDL receptor and occurs once in several proteins of the blood clotting system, including factor IX, factor X, and protein C (69, 76). Thus, these exons have been used by members of at least three different gene families.

The O-linked sugar domain is also encoded by a single exon (exon 15). However, not all domains of the protein are encoded by single exons. Thus, the membrane-spanning region is encoded by parts of two exons (exons 16 and 17). The cytoplasmic tail is also encoded by two exons (exons 17 and 18) (Fig. 8).

The sharing of exons between the LDL receptor gene and other genes provides strong evidence to support Gilbert's hypothesis concerning the nature and function of introns (77). As originally proposed, introns permit functional domains encoded by discrete exons to shuffle between different proteins, thus allowing proteins to evolve as mosaic combinations of preexisting functional units. The LDL receptor is a vivid example of such a mosaic protein (66, 78). It seems likely that other cell surface receptors will also be found to be mosaic structures assembled from exons shared with other genes.

Genetic Defects in the LDL Receptor

The mutations in the LDL receptor gene in FH patients have helped to delineate the crucial steps of receptor-mediated endocytosis. We have studied fibroblasts from 110 patients with the clinical phenotype of homозygous FH. All show evidence of defects in the LDL receptor, but not all defects are the same. At least ten different mutations can be distinguished by structural criteria (65), and these can be separated into four classes (Fig. 9). Many of the apparent FH homozygotes are actually compound heterozygotes who inherited different mutant alleles from each parent.

Class 1 mutations: no receptors synthesized. This is the most common class of mutant alleles, accounting for approximately half of the mutations so far analyzed. These genes produce either no LDL receptor protein or only trace amounts as determined by reaction with polyclonal or monoclonal antibodies. One of these alleles has been analyzed by molecular cloning; the gene contains a large deletion that extends from exon 18 to an Alu repetitive element in intron 15 (79). This deletion is easily recognized by DNA hybridization of genomic DNA. We have not found evidence of a similar deletion in any other individual with the receptor-negative phenotype, so this particular deletion must be rare.

Class 2 mutations: receptor synthesized, but transported slowly from ER to Golgi. This is the second most common class of mutations. These alleles produce receptors that are synthesized as precursors whose apparent molecular weights vary from 100,000 to 135,000. Most have an apparent molecular weight similar to that of the normal precursor (120,000). These receptors contain high-mannose N-linked sugars and the core N-acetylgalactosamine of the O-linked
sugars (56, 80). However, the N-linked sugars are not converted to the complex endoglycosidase H–resistant form nor are the O-linked sugar chains elongated. These mutant receptors do not appear on the surface of the cell; rather, they seem to remain in the ER until they are eventually degraded. Some mutations in this class are complete (there is no detectable processing of carbohydrate), while others are partial (some of the receptors are processed and move to the surface at a rate that is one-tenth of normal) (80, 81). The molecular defect in this class of mutations has not been determined.

Class 3 mutations: receptors are processed and reach cell surface, but fail to bind LDL normally. In the mature form, these mutant receptors can have a normal apparent molecular weight of 160,000 or aberrant apparent molecular weights of 140,000 or 210,000 (65). They are all synthesized as precursors that appear to be 40,000 daltons smaller than the mature form. They all undergo normal carbohydrate processing and reach the cell surface, and they bind a variety of antibodies directed against the LDL receptor. However, they have a markedly reduced ability to bind LDL. We suspect that these mutations may involve amino acid substitutions, deletions, or duplications in the cysteine-rich LDL binding domain or the EGF precursor region, but none has yet been fully elucidated at the molecular level.

Class 4 mutations: receptors reach cell surface and bind LDL, but fail to cluster in coated pits. Study of these internalization-defective mutants at the cellular level originally revealed the importance of coated pits in receptor-mediated endocytosis (42, 43). Three of the mutations have now been elucidated in molecular detail. All involve alterations in the cytoplasmic tail of the receptor (Fig. 10). The mutations have been unraveled through the preparation of genomic DNA libraries and the subsequent isolation and sequencing of exons 17 and 18, which encode the cytoplasmic domain. In the most drastic case, a cryptophan codon has been converted to a nonsense (stop) codon at a position that is two residues distal to the membrane-spanning region (73). This produces a receptor with only two amino acids in the cytoplasmic tail. Another mutation involves a duplication of four nucleotides following the codon for the sixth amino acid of the cytoplasmic tail (73). The duplication alters the reading frame and leads to a sequence of eight random amino acids followed by a stop codon. This receptor has only six of the normal amino acids in the cytoplasmic domain. Protein-chemistry studies have confirmed that these two proteins lack the normal COOH-terminus (73).

The third mutation is the most informative. In this patient, who was the original internalization-defective subject to be described (J.D., Fig. 3), a single base change leads to the substitution of a cysteine for a tyrosine residue at position 807, which is in the middle of the cytoplasmic tail domain (Fig. 10). We have recently reproduced this amino acid substitution in the normal LDL receptor cDNA by oligonucleotide-directed mutagenesis. When the altered cDNA was introduced into Chinese hamster ovary cells by gene transfer techniques, it produced a receptor that bound LDL but did not cluster in coated pits, confirming that the single base change is responsible for the internalization defect in J.D.’s cells (82).

Inasmuch as all three internalization-defective mutations involve the cytoplasmic tail, this region must normally play a crucial role in the clustering of LDL receptors in coated pits. It is likely that the cytoplasmic tail binds to clathrin or some other protein that is itself linked to clathrin. The puzzling feature at the moment is that other cell surface receptors that cluster in coated pits do not show obvious homology with the LDL receptor in the amino acid sequences of their cytoplasmic tails (65). Thus, the precise structure that links receptors to coated pits remains a mystery.

We have identified several interesting variants of the class 4 mutations in which the mutant genes produce LDL receptors that are secreted into the culture medium. In two mutants of this class (each from an unrelated family), the responsible mutation is a large deletion that results from a recombination between two repetitive Alu sequences, one in intron 15 and the other in the 3’ untranslated region of exon 18. The deletion joints in the two mutants are similar but not identical, indicating that the two mutations arose by independent events (83). In each mutant, the deletion removes the exons encoding the membrane-spanning region as well as the cytoplasmic tail. Presumably these prematurely terminated proteins have a short random sequence of amino acids at the COOH-terminus, because of read-through of an unspliced mRNA. The receptors are transported to the surface, where some of them remain bound to the membrane. The vast majority, however, are released.
into the culture medium (83). The few receptors that remain on the surface bind LDL, but do not migrate to coated pits, thus giving rise to an internalization-defective phenotype. These findings emphasize the importance of the membrane-spanning region in anchoring the LDL receptor to the plasma membrane.

Figure 11 shows the location of nine mutations in the LDL receptor gene that have been analyzed at the molecular level. Each FH family examined to date has had a different mutation, and multiple types of mutational events have occurred. Of the nine mutations, two involve single base-substitutions, two involve insertions (one small and one large), and five involve large deletions. Many of the deletion joints occur in Alu repetitive elements.

**Functions of the LDL Receptor in the Body**

The LDL receptor was elucidated by an investigative route that is opposite to the one usually used to uncover metabolic pathways in animals. These pathways are usually observed first in intact animals or tissues and then they are studied in isolated cells. The LDL receptor was first observed in a totally artificial environment—namely, tissue culture. The question immediately arose: What tissues express LDL receptors in the body, and how do they work? We knew at the outset that the receptor must play some role in the body as evidenced by the devastating consequences of LDL receptor deficiency in FH homozygotes and the proportionately less severe abnormalities in FH heterozygotes. Clearly, the receptor must be functioning somewhere. But where?

**Detection of LDL receptor expression in vivo.** The first cells that were demonstrated to have LDL receptor activity in vivo were circulating blood lymphocytes. In the initial studies, in collaboration with Y. K. Ho, lymphocytes were isolated from the bloodstream and incubated for 67 hours in vitro in the absence of exogenous cholesterol so as to “derepress” receptor synthesis (84). Under these conditions the lymphocytes expressed abundant LDL receptors as determined by measurements of the high-affinity uptake and degradation of $^{125}$I-LDL (Fig. 12A). Lymphocytes from FH homozygotes did not express detectable LDL receptor activity, and lymphocytes from FH heterozygotes had an intermediate level of expression consistent with the presence of only a single functional gene (85). LDL receptors were also detectable on lymphocytes immediately after their isolation from the bloodstream, although the level of activity was lower than it was after derepression for 67 hours (85). Thus, LDL receptors were expressed in at least one cell type in vivo.

Another early clue to the function of LDL receptors in vivo came from studies of the rate of disappearance of intravenously injected $^{125}$I-LDL from plasma (Fig. 12B). Such LDL is removed from the circulation more slowly in FH heterozygotes than it is in normal people (86, 87). The removal defect is even more profound in FH homozygotes (87–89). The sluggishness of LDL catabolism in vivo correlates with the relative deficiency of LDL receptors as determined in isolated lymphocytes.

More detailed demonstrations of LDL receptor function in vivo have been obtained in experimental animals. Together with S. K. Basu, an assay was established for the binding of $^{125}$I-LDL to membranes from homogenates of cultured cells and various tissues of the cow and other animals (90). By means of this assay, P. T. Kovanen found that most tissues of the cow had detectable high-affinity LDL-binding; the adrenal gland and ovarian corpus luteum had the highest activity on a per gram basis (91). When the weight of the organ was taken into consideration, the liver was found to produce by far the largest number of LDL receptors. Similar results were obtained in studies of human fetal tissues (91). In collaboration with workers in Havel’s laboratory, we showed that $^{125}$I-LDL was taken up by perfused rat livers by a high-affinity receptor-mediated process that could be markedly accelerated by administration of the estrogenic hormone, 17α-ethinyl estradiol (92).

High levels of hepatic LDL receptors were also observed when radiolabeled LDL was injected into the circulation of experimental animals and its uptake into various tissues was compared. Steinberg and co-workers (93) and Dietzschy and co-workers (94) showed that approximately 70 percent of the total-body uptake of radiolabeled LDL took place in the liver by LDL receptor-dependent pathways, but that the highest rates of uptake on a weight basis were seen in the adrenal gland. Various other tissues also showed receptor-mediated uptake of LDL in excess of that seen with nonspecific bulk-phase markers such as radiolabeled albumin.

Measurements of receptor-mediated LDL uptake by tissues of animals were made more practical as a result of two developments: (i) Steinberg and co-workers developed a method to label LDL with radioactive sucrose and later with tyramine-cellobiose (95). In contrast to $^{125}$I-labeling of tyrosines, the latter method produced a radioactive marker that remained trapped in lysosomes after uptake and degradation, thus allowing slow rates of uptake to be quantified cumulatively over long periods. (ii) Shepherd and Packard (96) showed that LDL whose arginine residues were modified by reaction with cyclohexanedione was cleared from the human circulation much more slowly than was native LDL. The rationale for these latter studies lay in previous work from our laboratory (97) and from Mahley’s laboratory (98), which showed that modification of arginine or lysine residues on LDL abolished its ability to bind to the LDL receptor. These observations provided a crude estimate of the fraction of LDL clearance that was attributable to LDL receptors.

We had earlier estimated the fraction of total LDL clearance that was receptor-dependent by comparing the rate of catabolism of intravenously injected $^{125}$I-LDL in normal individuals and in FH homozygotes (99). The fractional catabolic rate for LDL (the fraction of the total plasma pool of LDL removed per unit time) was threefold higher in normal subjects than in FH homozygotes (87). From this observation, we reasoned that approximately two-thirds of LDL clearance is normally mediated through the LDL receptor (99). This conclusion has generally been borne out by a number of studies in which degradation rates for native versus lysine-modified or arginine-modified LDL were compared both in normal human subjects and in a wide variety of experimental animals (100).

The Watanabe Heritable-Hyperlipidemic (WHHL) rabbit and the role of the LDL receptor in clearance of LDL. One of the most important functions of LDL receptors in vivo was appreciated only in the past few years as a result of studies performed in WHHL rabbits (101). These rabbits have a mutation in the LDL receptor gene that is similar to the class 2 mutations in human FH (81, 102).
When present in the homozygous form, this mutation gives rise to extremely high LDL-cholesterol levels; the rabbits develop atherosclerosis early in life (101, 102).

The WHHL rabbits proved invaluable in explaining a previously puzzling feature of homozygous FH. Kinetic studies of $^{125}$I-LDL metabolism by Myant and co-workers (88) and by Bilheimer and Grundy (87, 89) indicated that FH subjects have a dual defect. In addition to degrading LDL more slowly, FH homozygotes and heterozygotes also appeared to overproduce LDL. How does a genetic defect in the LDL receptor lead simultaneously to overproduction and reduced degradation of LDL? The answer lies in the complex biosynthetic pathway for LDL.

Early studies by Gitlin (103) and later those of Bilheimer, Levy, and Eisenberg (104) suggested that LDL is not secreted directly from the liver, but is produced in the circulation from a blood-borne precursor, VLDL (Fig. 13A). VLDL is a large, triglyceride-rich lipoprotein that is secreted by the liver; it transports triglyceride to adipose tissue and muscle. The triglycerides in VLDL are removed in capillaries by the enzyme lipoprotein lipase, and the VLDL returns to the circulation as a smaller particle intermediate-density lipoprotein (IDL). The IDL particles have lost most of their triglyceride, but they retain cholesteryl esters. Some of the IDL particles are rapidly taken up by the liver; others remain in the circulation where they undergo further triglyceride hydrolysis and are converted to LDL. A distinguishing feature of the IDL particles is their content of multiple copies of apo E in addition to a single copy of apo B-100. The multiple copies of apo E allow IDL to bind to the LDL receptor with very high affinity. When IDL is converted to LDL, the apo E leaves the particle and only apo B-100 remains.
Thereafter, the affinity for the LDL receptor is much reduced (102).

With T. Kita, we showed that the apparent overproduction of LDL in WHHL rabbits is due to the failure of IDL to be removed from the plasma (102, 105) (Fig. 13B). Thus, when \(^{125}\text{I}-\text{VLDL}\) was administered to WHHL rabbits, the resultant IDL was not taken up by the liver, as it was in normal rabbits (105). Rather it remained in the circulation and was converted in increased amounts to LDL. These findings strongly suggest that IDL is normally cleared from plasma by binding to LDL receptors in the liver. Although experiments of similar detail cannot be carried out in humans, the observations of Soutar, Myant, and Thompson (106) are consistent with the idea that enhanced conversion of IDL to LDL also occurs in FH homozygotes, thus accounting for much of the apparent overproduction of LDL.

Fig. 13A illustrates the dual role of the LDL receptor in LDL metabolism as determined from the studies of WHHL rabbits. First, the receptor limits LDL production by enhancing the removal of the precursor, IDL, from the circulation. Second, it enhances LDL degradation by mediating cellular uptake of LDL. A deficiency of LDL receptors causes LDL to accumulate as a result both of overproduction and of delayed removal (Fig. 13B). By this quirk of dual functionality, LDL receptors become crucially important modulators of plasma LDL levels in humans and animals.

**Perspectives**

Receptor regulation: therapeutic implications. The therapeutic implications of the LDL receptor studies center on strategies for increasing the production of LDL receptors in the liver, thereby lowering plasma LDL-cholesterol levels. In FH heterozygotes this goal can be attained by stimulating the normal gene to produce more than its usual number of LDL receptors, thus compensating for the defective allele (107). The rationale for such therapy emerged from studies of cultured fibroblasts, which showed that the production of LDL receptors is driven by the cell’s demand for cholesterol (9, 36).

When demands for cholesterol are high, the cells have high levels of mRNA for the LDL receptor. Conversely, when demands for cholesterol are reduced, excess cholesterol accumulates in cells, and the amount of receptor mRNA falls (36, 37).

Inasmuch as the liver is the major site of expression of LDL receptors, the therapeutic problem is reduced to the development of methods to increase hepatic demands for cholesterol. This can be achieved by two techniques: (i) inhibition of the intestinal reabsorption of bile acids and (ii) inhibition of cholesterol synthesis. These techniques can be used alone or in combination (Fig. 14).

The liver converts cholesterol into bile acids, this being the major route by which cholesterol is excreted from the body (18). However, only a fraction of the bile acids secreted by the liver actually leaves the body. The vast bulk of bile acids are reabsorbed in the terminal ileum and returned to the liver for reutilization. As a result, the liver converts only a minimal amount of cholesterol into bile acids (Fig. 14, left). The liver’s demand for cholesterol can be enhanced by the ingestion of resins that bind bile acids in the intestine and prevent their reabsorption. Since the liver can no longer reuse old bile acids, it must continually make new bile acids and the liver’s demand for cholesterol increases. In order to obtain this cholesterol, the liver makes a dual response: (i) it synthesizes increased amounts of cholesterol through an increase in the activity of HMG CoA reductase; and (ii) it attempts to take up additional plasma cholesterol by increasing the production of LDL receptors. The increased LDL receptor activity causes plasma LDL levels to decrease (Fig. 14, center). The problem with bile acid resin therapy (and the physiologically equivalent procedure of ileal bypass surgery) is that the effects are not profound. The increase in cholesterol production partially offsets the hepatic demand for cholesterol and so there is only a 15 to 20 percent increase in the synthesis of LDL receptors and only a 15 to 20 percent drop in plasma LDL-cholesterol levels.

The second method for increasing LDL receptor production, namely, inhibition of hepatic cholesterol synthesis, is much more powerful than bile acid depletion. The technique emerged from the discovery in 1976 of a class of fungal metabolites that inhibit HMG CoA reductase. The original compound, discovered by A. Endo in Japan, is compactin (108), while a more recent version, developed in the United States by A. W. Alberts, is called mevinolin (109). These two agents are potent competitive inhibitors of HMG CoA reductase; the inhibitory constant is approximately $10^{-7}$M (108).

When given to experimental animals, compactin or mevinolin initially inhibit cholesterol synthesis in the liver, and this triggers a complex regulatory mechanism that lowers the plasma LDL-cholesterol level. With Kovanen and Kita, we showed that the inhibition of cholesterol synthesis elicits a dual compensatory response: (i) hepatocytes synthesize increased amounts of HMG CoA reductase; and (ii) they synthesize increased numbers of LDL receptors (110). When a new steady state is attained, the increase in HMG CoA reductase is almost sufficient to overcome the inhibitory effects of compactin. Total body cholesterol synthesis is only slightly reduced (111). Meanwhile, the plasma LDL level has decreased as a result of the increase in LDL receptors. The fall in plasma LDL levels is balanced by the increase in LDL receptors, and so the absolute amount of cholesterol entering the liver through the receptor pathway is the same as it was earlier. The difference, however, is that this delivery is now occurring at a lower plasma LDL level (107).
When given as a single agent to FH heterozygotes, mevinolin routinely produces a 30 percent decrease in plasma LDL–cholesterol levels. When given together with cholestyramine, mevinolin blocks the compensatory increase in cholesterol synthesis, and the increase in LDL receptors is even greater (Fig. 14, right). Plasma LDL cholesterol levels decrease by 50 to 60 percent (112).

The important principle to emerge from these studies is that stimulation of LDL receptor activity lowers the plasma LDL–cholesterol level without grossly altering cholesterol delivery (107, 111). At present, mevinolin and related compounds are in the early stages of clinical testing. Their efficacy in lowering plasma LDL–cholesterol levels has been well established, but there is no information regarding long-term toxicity in patients. If these drugs are shown to be nontoxic, they will have an important role in the therapy of FH heterozygotes and probably of other hypercholesterolemic individuals as well.

The principles applied to treatment of FH heterozygotes cannot be applied to homozygotes, especially those who have totally defective LDL receptor genes. These individuals do not respond to the above-mentioned drugs because they cannot synthesize LDL receptors (113). Current therapy for these individuals involves removal of LDL from plasma extracorporeally through repeated plasmapheresis (114). Such procedures, which must be repeated every 2 to 3 weeks, are technically difficult and are very demanding of patient and physician.

Recently, a more direct therapeutic approach was taken in an FH homozygote (S.J.) who has two mutant genes at the LDL receptor locus. This 6-year-old girl, who is a patient of D. Bilheimer, had a total plasma cholesterol level over 1000 mg/dl (greater than six times above normal limits), and she sustained repeated episodes of myocardial infarction. After she failed to respond to coronary bypass procedures plus a mitral valve replacement, she was subjected to combined heart-liver transplantation by a team of surgeons led by T. E. Starzl (115). The liver transplant was designed to provide a source of LDL receptors. The heart transplantation was necessitated by the poor condition of her own heart as a result of the atherosclerotic process.

Immediately after the operation, S.J.’s total plasma cholesterol level fell from 1100 mg/dl to the range of 200 to 300 mg/dl, and it remained in that range for the next 13 months (Fig. 15A). She was then treated with the HMG CoA reductase inhibitor mevinolin, and her cholesterol level fell further to the range of 150 to 200 mg/dl (Fig. 15A). Liver transplantation not only lowered the plasma cholesterol level but it also restored responsiveness to mevinolin, which requires a normal LDL receptor gene in order to act. Lipoprotein turnover studies performed 6 months after surgery confirmed that the new LDL receptors furnished by the transplanted liver were responsible for the dramatic drop in plasma cholesterol level (Fig. 15B). S.J. remains asymptomatic at the time of this writing, and her cutaneous xanthomas have disappeared. However, she requires continuous therapy with cyclosporin to prevent rejection of the transplanted organs, and her long-term prognosis is uncertain. The response to liver transplantation in S.J. underscores the importance of hepatic LDL receptors in vivo and raises the possibility that other FH homozygotes may respond to similar transplantation procedures.

**Speculations: LDL receptors and the general problem of atherosclerosis.**

We now leave the realm of solidly established scientific fact and enter the much more controversial realm of speculation about the relation between cholesterol levels, LDL receptors, and atherosclerosis in the general population. After all, FH heterozygotes account for only 5 percent of myocardial infarctions in patients under the age of 60. What causes the other 95 percent of heart attacks?

Extensive epidemiologic studies performed in many populations in many countries over the past three decades have pointed strongly to a general association of high blood-cholesterol levels with heart attacks. Among the most striking examples is the seven-country study of coronary artery disease directed by Keys (116). A similar correlation has been observed within a single population in the extensive studies in Framingham, Massachusetts (117).

These studies have shown that the incidence of myocardial infarction rises in proportion to the plasma cholesterol level, more specifically the plasma level of LDL-cholesterol. When LDL-cholesterol levels are below 100 mg/dl (equivalent to a total plasma cholesterol level of approximately 170 mg/dl), heart attacks are rare; when they are above 200 mg/dl (equivalent to a total plasma cholesterol level of approximately 280 mg/dl), heart attacks are frequent. Controversy arises over the middle ground—individuals with plasma LDL-cholesterol levels between 100 and 200 mg/dl (total plasma cholesterol of 170 to 280 mg/dl). This is the range in which most heart attacks occur. Somewhere within this range there is an inflection point in which heart attacks begin to become more frequent. How much of the heart attack burden is attributable to plasma cholesterol in this middle ground? There is no definite answer. In addition to cholesterol, heart attacks in this group are aggravated by smoking, hypertension, stress, diabetes mellitus, and poorly understood genetic factors. However, it seems reasonable to propose that plasma cholesterol has something to do with heart attacks in these subjects and that the incidence of heart attacks would be reduced if plasma cholesterol could be lowered (10).

The LDL-receptor studies lend experimental support to the epidemiologists’ suggestion that the levels of plasma cholesterol usually seen in Western industrialized societies are inappropriately high (9). This support derives from knowledge of the affinity of the LDL receptor for LDL. The receptor binds LDL optimally when the lipoprotein is present at a cholesterol concentration of 2.5 mg/dl (28). In view of the 10- to 1 gradient between concentrations of LDL in plasma and interstitial fluid, a level of LDL-cholesterol in plasma of 25 mg/dl would be sufficient to nourish body cells with cholesterol (118). This is roughly one-fifth of the level usually seen in Western societies (Fig. 16) (119). Several lines of evidence suggest that plasma levels of LDL-cholesterol in the range of 25 to 60 mg/dl (total plasma cholesterol of 110 to 150 mg/dl) might
Indeed be physiologic for human beings. First, in other mammalian species that do not develop atherosclerosis, the plasma LDL-cholesterol level is generally less than 80 mg/dl (Fig. 16) (120). In these animals, the affinity of the LDL receptor for their own LDL is roughly the same as the affinity of the human LDL receptor for human LDL, implying that these species are designed by evolution to have similar plasma LDL levels (9, 119). Second, the LDL level in newborn humans is approximately 30 mg/dl (121), well within the range that seems to be appropriate for receptor binding (Fig. 16). Third, when humans are raised on a low fat diet, the plasma LDL–cholesterol tends to stay in the range of 50 to 80 mg/dl. It only reaches levels above 100 mg/dl in individuals who consume a diet rich in saturated animal fats and cholesterol that is customarily ingested in Western societies (116, 122).

What is the mechanism for the high levels of plasma LDL that are so frequent in Western industrialized societies? Extensive evidence implicates two major factors—diet and heredity. When people habitually consume diets low in animal fats, their plasma LDL–cholesterol levels tend to remain low. When even moderate amounts of animal fat are introduced into the diet, the plasma cholesterol level rises (116, 122). However, the level does not rise equally in every person. Clearly, genetic as well as dietary factors play a role.

How might a diet rich in animal fats and cholesterol elevate the plasma LDL–cholesterol level? Here we believe that two properties of the LDL receptor play a role—saturation and suppression. As the plasma LDL level rises, the receptors become saturated. This saturation of receptors sets an upper limit on the rate at which LDL can be removed efficiently from plasma (123). Each receptor can handle only one particle of LDL at a time. Once the receptors become saturated, the rate of removal of LDL can be accelerated only by an increase in clearance by non-receptor pathways that operate at low efficiency. In order to drive these alternate pathways, the LDL level must be quite high (99). At ordinary levels of LDL, the major factor that limits the removal of LDL from plasma is saturation of the LDL receptor (123).

Once LDL receptors become saturated, the removal rate of LDL is proportional to the number of receptors. Whenever the number of receptors is reduced, plasma LDL levels must rise. Experiments in animals indicate that the consumption of a high fat diet decreases the number of LDL receptors in the liver (123, 124). We believe that this mechanism operates through feedback suppression as described above. That is, when excess dietary cholesterol accumulates in the liver, the liver responds by decreasing the production of LDL receptors (Fig. 13C). The entry of dietary cholesterol into the liver is mediated by a receptor, termed the chylomicron remnant receptor, whose activity is genetically distinct from the LDL receptor (125). The chylomicron remnant receptor is unaffected by cholesterol accumulation (126), and it causes cholesterol to accumulate to high levels in liver when the diet contains excess fat.

The combination of saturation and suppression of hepatic LDL receptors contributes in a major way to the buildup of LDL in plasma when a diet rich in saturated fats and cholesterol is ingested. Insofar as such a diet also may increase production of LDL in the face of a fixed or declining removal capacity, the LDL level would rise even higher.

If the LDL receptor does limit the removal of LDL from plasma, then maneuvers that increase LDL receptor activity might be effective in individuals who have high plasma LDL–cholesterol levels, but who do not have defective LDL receptor genes. Such therapy seems feasible with the development of HMG CoA reductase inhibitors. However, it is still too early to tell whether such therapy would decrease the incidence of myocardial infarctions in individuals with moderately elevated plasma LDL-cholesterol levels in the range of 100 to 200 mg/dl. There is much circumstantial evidence to expect such improvement (127), but unequivocal data are not yet available.

In considering the role of diet and drugs in treatment of high cholesterol levels, it is necessary to bear in mind the genetic variability between individuals. This variability exists at three levels: (i) The degree of increase in plasma cholesterol upon ingestion of a high cholesterol diet is variable. Not all people develop hypercholesterolemia. Some people, such as the Pima Indians, maintain low plasma cholesterol levels despite ingestion of a high fat diet (10). (ii) Even when the plasma cholesterol level becomes elevated, the propensity for atherosclerosis varies. For example, a substantial proportion of FH heterozygotes (10 to 20 percent) escape myocardial infarction until the eighth or ninth decade despite pronounced hypercholesterolemia from birth (14). (iii) Genetic susceptibility to contributory risk factors is variable. Some people can withstand hypertension and cigarette smoking for decades without developing atherosclerotic complications; others are highly sensitive. An important goal will be to identify the genes that determine such predispositions and to analyze them in each individual.

Receptor recycling: a novel cellular pathway. The studies of the LDL receptor focused attention on the process by which membrane-embedded receptors cycle continuously into and out of cells. The receptors move from one organelle to another as a result of two sequential events: (i) segregation from other proteins by lateral movement in the plane of the membrane, and (ii) pinching off of receptor-enriched membranes to form vesicles that eventually fuse with a different organelle. These receptors have been designated as "migratory" membrane proteins to distinguish them from "resident" membrane proteins that do not move in this manner (4). One purpose of such intracellular traffic is to integrate the behavior of multiple organelles to form coherent biochemical pathways. Thus, the movement of the LDL receptor links the cell surface to the endosome and to the lysosome. The cholesterol liberated from LDL in lysosomes exerts regulatory effects in two other organelles, the endoplasmic reticulum and the nucleus. Selective movement of membrane proteins from one organelle to another allows such multi-organelle regulation to occur.

What are the signals that dictate the path that each migrant membrane protein must follow? We are beginning to obtain some insight into the signals necessary for LDL receptors to be incorporated into one sorting structure, the coated pit. However, there is still no information with regard to signals that cause proteins to leave other organelles such as the endoplasmic reticulum and move to different organelles such as the Golgi complex. Delineation of these sorting signals is a major challenge facing the field of cell biology.

REFERENCES AND NOTES
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