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CHAPTER 21

Lipoprotein receptors

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1. Introduction

From a systemic lipoprotein metabolism point of view, the main task of lipoprotein receptors is the clearance of lipoproteins from the circulation, body fluids, and interstitial spaces. One can envision several reasons why lipoproteins need to be cleared from extracellular fluids into cellular compartments, for instance: (1) they are transport vehicles for components that are vital to the target cells; (2) their uptake serves signalling and/or regulatory roles in cellular metabolism; (3) they have done their job and have become dispensable; and (4) they might have deleterious effects if allowed to remain extracellular for prolonged time. Chapters 18, 19, and 20 have described the structures, syntheses, and interconversion pathways of lipoprotein particles in the circulation. Here, the mechanisms of lipoprotein transport from the plasma compartment to various types of cells of the body, which is one of the best understood aspects of receptor biology, is described. In addition, newly discovered functions of receptors thus far thought to be specialized exclusively for lipoprotein transport are described.

In the context of lipoprotein transport via cell surface receptors, it helps to recall that the two major transported lipid components of lipoproteins, triacylglycerols and cholesterol, have quite different fates. Triacylglycerols are delivered primarily to adipose tissue and muscle where their fatty acids are stored or oxidized for production of energy, respectively. Cholesterol, in contrast, is continuously shuttled among the liver, intestine, and other extra-hepatic tissues. Actually, the major transport form of cholesterol is its esterified form; within cells the cholesteryl esters are hydrolyzed and the unesterified sterol has multiple uses. Among their many functions, sterols serve as structural components of cellular membranes, as substrates for the synthesis of steroid hormones and bile acids, and they perform several regulatory functions (a classical example is the low density lipoprotein (LDL) receptor pathway, see Section 2.2). For correct targeting of lipoproteins to sites of metabolism and removal, the lipoproteins rely heavily on the apolipoproteins (apos) associated with their surface coat. Apos mediate the interaction of lipoprotein particles with enzymes, transfer proteins, and with cell surface receptors, the main topic of this chapter.

Key features of human lipoprotein metabolic pathways are schematically summarized in Fig. 1; this outline necessarily omits many of the details which are less significant for aspects of receptor-mediated removal of lipoproteins. The interwoven complex pathways can be divided into exogenous and endogenous branches, concerned with the transport of dietary and liver-derived lipids, respectively. Both metabolic sequences start with the

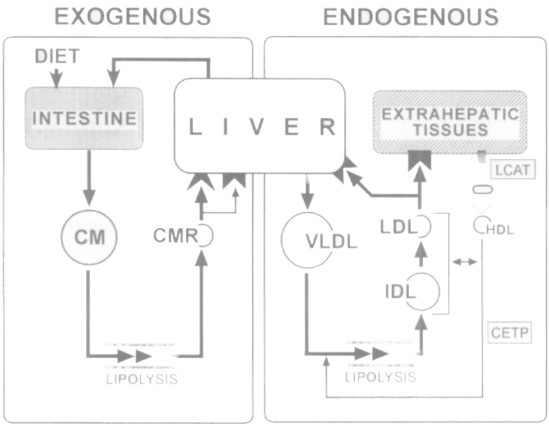

Fig. 1. Highly schematized summary of the pathways for receptor-mediated lipoprotein metabolism in humans. The liver is the crossing point between the exogenous pathway (left-hand side) dealing with dietary lipids, and the endogenous pathway (right-hand side) that begins with hepatically synthesized lipoproteins. The exogenous metabolic branch starts with the production of chylomicrons (CM) in the intestine, whereas the liver synthesizes very low density lipoprotein particles (VLDL). Abbreviations: CMR, chylomicron remnants; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; LCAT, lecithin : cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; ⮝, LDL receptor-related protein (LRP); and ⮜, LDL receptor; 'Lipolysis' denotes lipoprotein lipase-catalyzed triacylglycerol lipolysis in the capillary bed.

production and secretion of triacylglycerol-rich lipoproteins (Chapter 19). Intestinally derived chylomicrons (CM in Fig. 1) are secreted into the lymph and from there enter the bloodstream, where they function as energy carriers by providing triacylglycerol-derived fatty acids to peripheral tissues. This lipolytic extraction of fatty acids from the triacylglycerol core of the lipoprotein particles ('Lipolysis' in Fig. 1) is achieved mainly by the enzyme lipoprotein lipase, which is bound to the lumenal surface of the endothelial cells lining the capillary bed. Removal of triacylglycerol in extrahepatic tissues results in decreased size of the chylomicrons and produces cholesteryl ester-rich lipoprotein particles termed chylomicron remnants. During this conversion, apoCs are lost from the surface of the particles; the remnants, having finished their task, are destined for catabolism by the liver, which occurs almost exclusively by receptor-mediated processes. Both the so-called LDL receptor-related protein (⮝ in Fig. 1, and see Section 3.1) and the LDL receptor (⮜ in Fig. 1, and see Section 2) mediate their removal via recognition of apoE. The apoB48, which resides on chylomicrons throughout their life span, is not recognized by these receptors.

In analogy to the exogenous lipid transport branch, the endogenous pathway begins with the production and secretion of triacylglycerol-rich lipoproteins by the liver, here termed very low density lipoprotein (VLDL). In significant contrast to chylomicrons, VLDL contains apoB100, in addition to the apoCs and apoE. Lipoprotein lipase in the capillary bed hydrolyzes triacylglycerol of secreted VLDL, but less efficiently than from chylomicrons, which is likely one of the reasons for slower plasma clearance of VLDL ($t_{1/2}$, days) compared to chylomicrons ($t_{1/2}$, minutes to a few hours). Lipolysis

during the prolonged residency of VLDL particles in the plasma compartment generates intermediate density lipoproteins (IDL) (Fig. 1) and finally, LDL. In parallel, apos and surface components (mostly phospholipids and unesterified cholesterol), but also cholesteryl esters and triacylglycerol, are subject to transfer and exchange between particles in the VLDL lipolysis pathway and certain species of high density lipoproteins (HDL). In addition, it appears that another receptor akin to the LDL receptor, the so-called VLDL receptor (see Section 3.2), might act in concert with lipoprotein lipase in delivering fatty acids to a limited set of peripheral tissues. Enzymes involved in cholesterol loading and esterification, and in interparticle-transfer reactions are, e.g., lecithin–cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) (see Chapter 20). IDL particles (which still harbor some apoE) to a variable degree, and LDL as the end product of VLDL catabolism in the plasma (and at least in man, free of apoE), are catabolized via the LDL receptor. This receptor is found in the liver (which harbors 60–70% of all the LDL receptors in the body) as well as in extrahepatic tissues, and is a key regulatory element of systemic cholesterol homeostasis (see Section 2.2).

Thus, steady-state plasma LDL levels are not only the result of lipoprotein receptor numbers, but also are influenced by the rate of VLDL synthesis, the activity of lipoprotein lipase and other lipases, the VLDL receptor, and by other metabolic processes. As far as HDL levels are concerned, one result of the LCAT- and CETP-catalyzed reactions is the production of a dynamic spectrum of particles with a wide range of sizes and lipid compositions. The further metabolic fates of these HDL fractions are described in Chapter 20.

In addition to receptor-mediated metabolism of lipoproteins, which clearly is the predominant mechanism for removal from the plasma of intact lipoproteins, individual components of lipoproteins, in particular unesterified cholesterol, might diffuse into cells across the plasma membrane. Other minor uptake processes may include so-called fluid-phase endocytosis, which does not involve binding of lipoproteins to specific cell surface proteins, and phagocytosis, in which lipoproteins are thought to attach to the cell surface via more or less specific forces, and are engulfed by the plasma membrane.

2. *Removal of LDL from the circulation*

The supply of cells with cholesterol via receptor-mediated endocytosis of LDL is one of the best characterized processes of macromolecular transport across the plasma membrane of eukaryotic cells. The following sections describe this process, provide an overview of the biochemical and physiological properties of the LDL receptor, and discuss the molecular basis for the genetic disease, familial hypercholesterolemia.

2.1. Receptor-mediated endocytosis

This multi-step process, originally defined as a distinct mechanism for the cellular uptake of macromolecules, emerged from studies by M.S. Brown, J.L. Goldstein and their colleagues which they performed in the mid-1970s in order to elucidate the

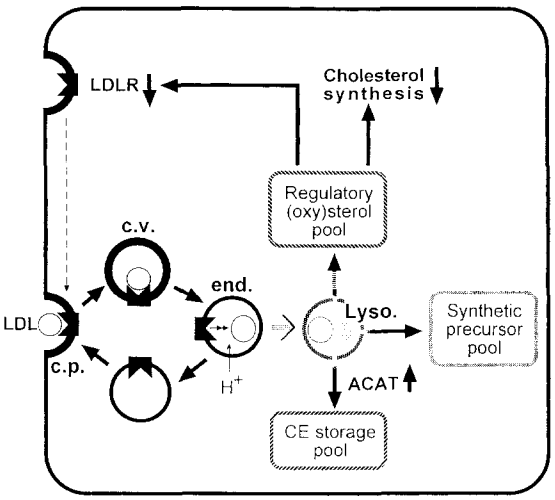

Fig. 2. The LDL receptor pathway, regulation of cellular cholesterol homeostasis. LDL receptors are synthesized in the endoplasmic reticulum and undergo post-translational modification in the Golgi compartment; from there they travel to the cell surface and collect in coated pits (c.p.). LDL particles bound to LDL receptors (⚑) are internalized in coated vesicles (c.v.) which become uncoated and acidified by protons (H^+) being pumped into their lumen, resulting in endosomes (end.), in which the LDL particles dissociate from the receptors due to the low pH. From there, the LDL are delivered to lysosomes (Lyso.), but almost all of the receptors travel back to the cell surface (where they become incorporated into c.p. again) within a recycling vesicle. Lysosomal degradation of LDL results in complete breakdown of apoB100 and liberation of cholesterol via hydrolysis of cholesteryl esters. The LDL-derived cholesterol has three main fates: (a) it is reconverted to cholesteryl esters via stimulation of acyl-CoA cholesterol acyltransferase (ACAT) for storage in droplets (CE storage pool; bottom); (b) it is used as biosynthetic precursor for bile acids, steroid hormones, membranes, etc. (synthetic precursor pool; right-hand side); and (c) it serves, especially if converted to oxysterols (top), several regulatory functions. The most important of these are suppression of cholesterol synthetic enzymes, and decreasing the production of LDL receptors.

normal function of LDL. The salient features of the itinerary of a LDL particle (mean diameter, ~22 nm) from the plasma into a normal human fibroblast are summarized in Fig. 2. First, the lipoprotein particle binds to one of the approximately 15,000 LDL receptors on the surface of the cell. LDL receptors are not evenly distributed on the cell surface; rather, up to 80% are localized to specialized regions of the plasma membrane comprising only 2% of the cell surface. These regions form pits and are lined on their cytoplasmic side with material that in electron micrographs has the appearance of a fuzzy coat. Each of these so-called 'coated pits' contains several kinds of endocytic receptors in addition to LDL receptors, but LDL particles bind only to 'their' receptors, due to their extremely high affinity and specificity. Next, the receptor/LDL complex undergoes rapid invagination of the coated pit, which eventually culminates in the release of the coated pit into the interior of the cell. At this point, the coated pit has been transformed into an endocytic 'coated vesicle', a membrane-enclosed organelle that is coated on its exterior (cytoplasmic) surface with a polygonal network of fibrous protein(s), the main structural component of which is a fascinating protein called clathrin [1]. Subsequently, the coat is rapidly removed, in concert with acidification

of the vesicles' interior and fusion with other uncoated endocytic vesicles. Transiently, LDL and the receptor are found in smooth vesicles in which the lipoprotein particle dissociates from the receptor due to the acidic environment. LDL is then delivered to lysosomes, where it is degraded, while the receptor escapes this fate and recycles back to the cell surface, homes in on a coated pit and is ready to bind and internalize new ligand molecules [1].

There are variations to the theme; not in all systems of receptor-mediated endocytosis are ligand degradation and receptor recycling coupled; however, all have in common the initial steps leading to the formation of endosomes. Then, the receptors are either degraded, recycled back to the cell surface, or are transported (for example, across polarized cells); their respective ligands can follow the same or divergent routes [1]. The reutilization of the LDL receptor via recycling constitutes an economical way to ensure efficient removal of LDL from the extracellular space.

2.2. The LDL receptor pathway

The LDL receptor is the key component in the feedback-regulated maintenance of cholesterol homeostasis in the body [1]. In fact, as an active interface between extra- and intra-cellular cholesterol pools, it is itself subject to regulation at the cellular level (cf. Fig. 2). LDL-derived cholesterol (generated by hydrolysis of LDL-borne cholesteryl esters) and its intracellularly generated oxidated derivatives mediate a complex series of feedback control mechanisms that protect the cell from over-accumulation of cholesterol. First, (oxy)sterols suppress the activities of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase, and HMG-CoA reductase, two key enzymes in cellular cholesterol biosynthesis. Second, the cholesterol activates the cytoplasmic enzyme acyl-CoA : cholesterol acyltransferase (ACAT; E.C. 2.3.1.26) which allows the cells to store excess cholesterol in re-esterified form. Third, the synthesis of new LDL receptors is suppressed, preventing further cellular entry of LDL and thus cholesterol overloading. The coordinated regulation of LDL receptors and cholesterol synthetic enzymes relies on the sterol-modulated proteolysis of a membrane-bound transcription factor, SREBP, as described in Chapter 15.

The overall benefits from, and consequences of, this LDL receptor-mediated regulatory system are the coordination of the utilization of intra- and extra-cellular sources of cholesterol at the systemic level. Mammalian cells are able to subsist in the absence of lipoproteins because they can synthesize cholesterol from acetyl-CoA. When LDL is available, however, most cells primarily use the LDL receptor to import LDL cholesterol and keep their own synthetic activity suppressed. Thus, a constant level of cholesterol is maintained within the cell while the external supply in the form of lipoproteins can undergo large fluctuation.

Most of these concepts have arisen from detailed studies on cultured fibroblasts from normal subjects and from patients with the disease, familial hypercholesterolemia (FH). Lack of the above described regulatory features in FH fibroblasts led to the conclusion that the abnormal phenotype is caused by lack of LDL receptor function, and thus, disruption of the LDL receptor pathway. In particular, the balance between extracellular and intracellular cholesterol pools is disturbed. Clinically, the most important effect of

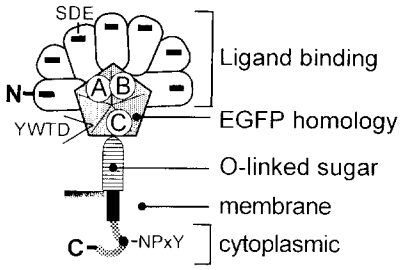

Fig. 3. Domain model of the LDL receptor. The five domains of the mature protein, from the N-terminus (bold N) to the carboxy-terminus (bold C) are as follows. (1) The ligand binding domain, characterized by seven cysteine-rich repeats, each with a cluster of negatively charged amino acids whose core consists of Ser–Asp–Glu ('SDE'); repeats 2–7 cooperatively bind apoB100 and apoE. (2) The EGF-precursor (EGFP) homology region, consisting of 400 amino acid residues (central pentagon); adjacent to the ligand binding domain and at the carboxy-terminus of this region, respectively, are located three repeats with high homology to repeat motifs found in the precursor to epidermal growth factor (encircled letters A, B, and C). The remaining portion of this domain consists of five internally homologous stretches of approximately 50 amino acid residues each of which contains the sequence Tyr–Trp–Thr–Asp (YWTD). (3) The *O*-linked sugar region, consisting of 58 amino acids with 18 serine and threonine residues containing *O*-linked carbohydrate chains. (4) A single membrane-spanning domain. (5) The cytoplasmic tail with 50 amino acid residues containing the internalization sequence Asn–Pro–Val–Tyr (NPxY; the Val is not absolutely conserved in all species).

LDL receptor deficiency is hypercholesterolemia with ensuing accelerated development of atherosclerosis and its complications (Chapter 22). In the following sections, a detailed description of the LDL receptor is provided, with emphasis on the impact of mutations on its structure and function.

2.3. Relationships between structure and function of the LDL receptor

Studies at the levels of protein chemistry, molecular biology, and cell biology have led to a detailed understanding of the biology of the LDL receptor. The mature receptor is a highly conserved integral membrane glycoprotein consisting of five domains (Fig. 3). In order of appearance from the amino terminus these domains are: (1) the ligand binding domain; (2) a domain that has a high degree of homology with the epidermal growth factor precursor (EGFP); (3) a domain that contains a cluster of *O*-linked carbohydrate chains; (4) a transmembrane domain; and (5) a short cytoplasmic region. Until direct information on the three-dimensional structure of the 839-residue receptor becomes available, an arrangement of these domains as presented in Fig. 3 may serve as a useful model.

2.3.1. The ligand binding domain

This domain mediates the interaction between the receptor and lipoproteins containing apoB100 and/or apoE [2]. The function is localized to a region at the amino terminus of the receptor, comprised of seven repeats of approximately 40 residues each. These seven repeats have six cysteines each, which presumably mediate the folding of the domain into a rigid structure with clusters of negatively charged residues on its surface (with

the signature tripeptide Ser–Asp–Glu). These clusters are thought to participate in the binding of lipoprotein(s) via positively charged residues on apoB100 or apoE.

2.3.2. The epidermal growth factor precursor homology domain

This region of the LDL receptor lies adjacent to the ligand binding site and is comprised of approximately 400 amino acids; the outstanding feature is the sequence similarity of this region to parts of the EGFP, i.e., three regions termed 'growth factor repeats'. Two of these repeats (A, B in Fig. 3) are located in tandem at the amino terminus, and the other (C21) is at the carboxy-terminus of the precursor homology region of the LDL receptor. The remainder consists of five ~50-residue stretches that contain tetrapeptide sequences with a consensus of Tyr–Trp–Thr–Asp. Experimental evidence suggests an involvement of this region in the receptor's acid-dependent dissociation from LDL and its subsequent recycling (cf. Fig. 2).

2.3.3. The O-linked sugar domain

The *O*-linked sugar domain of the human LDL receptor is a 58-amino acid stretch highly enriched in serine and threonine residues, located just outside the plasma membrane. Most, if not all, of the 18 hydroxylated amino acid side chains are glycosylated. The *O*-linked oligosaccharides undergo elongation in the course of receptor synthesis and maturation: when leaving the endoplasmic reticulum, *N*-acetylgalactosamine is the sole *O*-linked sugar present, and upon processing in the Golgi, galactosyl and sialyl residues are added. Despite the detailed knowledge about the structure of this region, its functional importance remains unclear.

2.3.4. The membrane anchoring domain

The membrane anchoring domain lies carboxy-terminally to the *O*-linked carbohydrate cluster. It consists of 22–25 hydrophobic amino acids; as expected, the deletion of this domain in certain naturally occurring mutations, or by site-directed mutagenesis, leads to secretion of truncated receptors from the cells.

2.3.5. The cytoplasmic tail

The cytoplasmic tail of the LDL receptor constitutes a short stretch of 50 amino acid residues involved in the targeting of LDL receptors to coated pits. Naturally occurring mutations and site-specific mutagenesis [3] have identified an 'internalization signal', Asn–Pro–Xxx–Tyr (NPxY; where x denotes any amino acid). Recently, the cytoplasmic domains of the LDL receptor and structural relatives have come into new focus, since they hold the key to the involvement of these receptors in signal transduction as indicated below (Section 5).

2.4. The human LDL receptor gene — organization and naturally occurring mutations

The ~48 kb human LDL receptor gene contains 18 exons and is localized on the distal short arm of chromosome 19. There is a strong correlation between the functional domains of the protein and the exon organization in the gene. For instance, the seven cysteine-rich repeats of the ligand binding domain are encoded by exons 2 (repeat 1), 3

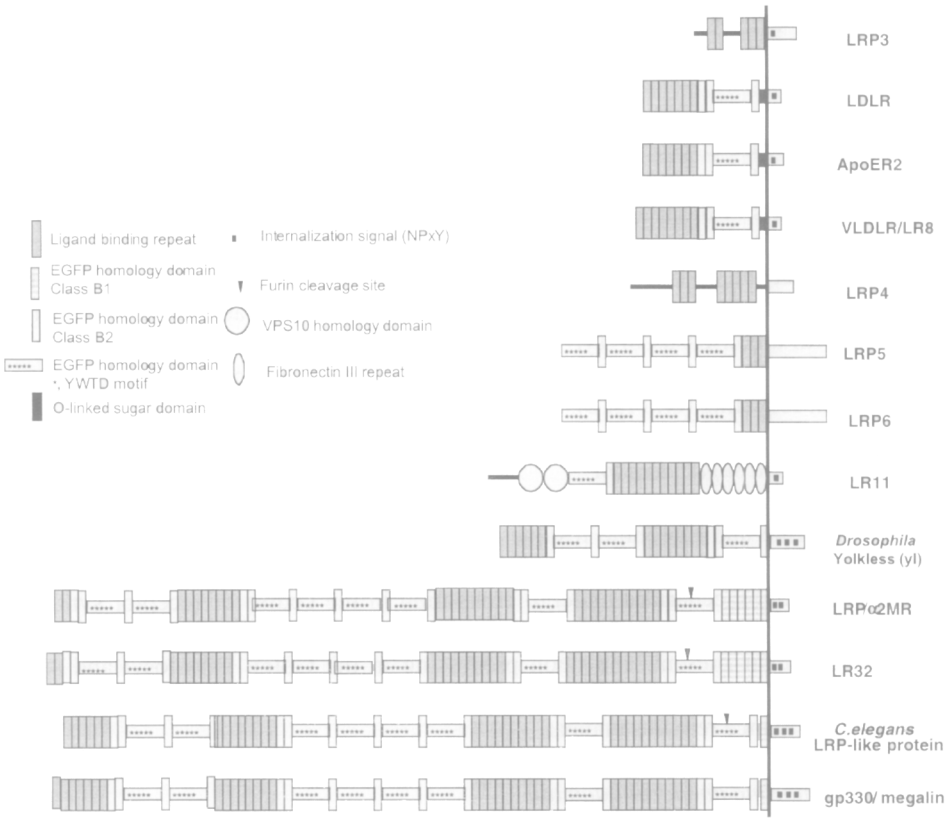

Fig. 4. The LDL receptor gene family. The structural building blocks making up these proteins are listed in the left-hand top part (for more details, see Fig. 3); presumed extracellular domains are depicted to the left of the plasma membrane (black vertical line). The standard modules are: negatively charged ligand binding repeats with six cysteines each; EGFP homology repeats (in the entire family, two subclasses with slightly different consensus sequences are distinguished, termed B1 and B2; these repeats also contain six cysteines each); the five 'YWTD' motifs within the 5-times-50 residues structure of EGFP homology domains; the *O*-linked sugar domains, just outside the plasma membrane, typical for LDL receptor, apoER2 and VLDL receptor/LR8; and the consensus or presumed internalization signals, NPxY. Several large members of the gene family harbor consensus furin cleavage sites; LR11 (Section 6.2) contains domains not found in other relatives, i.e., two VPS10 domains and six fibronectin type III repeats. Except for the yolk protein transport receptor of *Drosophila melanogaster* (yl), all receptors are discussed in the text.

(repeat 2), 4 (repeats 3, 4, and 5), 5 (repeat 6) and 6 (repeat 7). The EGFP homology domain is encoded by eight exons, organized in a manner very similar to the gene for the EGFP itself. The third domain is translated from a single exon between introns 14 and 15. Thus, the LDL receptor gene is a compound of shared coding sequences; in fact, many more molecules containing all or some of these elements have been discovered and likely will continue to be found. Membrane proteins with clusters of ligand binding repeats in their extracellular domains are now recognized as relatives of the so-called LDL receptor gene family (Fig. 4).

Molecular genetic studies in FH patients have identified over 600 different mutations in the LDL receptor gene. A listing of all mutations with their original literature citations can be found at http://www.ucl.ac.uk/fh/. In order to gain some insight into the nature of these mutations, they are grouped into five classes according to their effects on the protein [4] as follows.

2.4.1. Class 1: null alleles — no detectable receptor

These mutant alleles fail to produce receptor proteins as determined by immunoprecipitation with many anti-receptor antibodies, and thus, cells carrying these mutations do not bind any LDL. The spectrum of these mutations includes point mutations causing premature termination codons early in the protein coding region, mutations in the promoter region that block transcription, mutations that lead to abnormal splicing and/or instability of the mRNA, and large deletions.

2.4.2. Class 2: slow or absent processing of the precursor

These alleles, probably accounting for at least half of all mutant LDL receptor alleles, specify transport-deficient receptor precursors which fail to move with normal rates from the endoplasmic reticulum to and through the Golgi compartment(s) and on to the cell surface. As a consequence, the typical sudden increase in apparent M_r observed during biosynthesis of the normal receptor is lacking. While some mutations only slow down processing, most of these mutations are complete in that there is total absence of transport from the endoplasmic reticulum, and the mutant receptors never reach the cell surface.

2.4.3. Class 3: defective ligand binding

These receptors in general reach the cell surface at normal rates, but are unable to bind LDL efficiently due to subtle structural changes near the ligand binding domain. By definition, these mutant receptors undergo the normal maturation process.

2.4.4. Class 4: internalization-defective

Here, one of the prerequisites for effective ligand internalization — localization of LDL receptors to coated pits — is not met. The failure of these 'internalization-defective' receptors to localize to coated structures results from mutations that directly or indirectly disrupt the carboxy-terminal domain of the receptor. Variants of class 4 mutations have been identified in which large deletions lead to a lack of both the cytoplasmic and transmembrane domains; the majority of these mutant truncated proteins are, as expected, secreted.

2.4.5. Class 5: recycling-defective

The classification of these mutations into a separate class [4] is based on the observation that upon deletion of the first two EGFP domains of the human LDLR, the truncated receptor binds and internalizes ligand, but fails to release it in the acidic environment of the endosome [5]. The mutant receptor is rapidly degraded without returning to the surface in an unoccupied state. Class 5 mutants all affect the EGFP homology domain, and most often the YWTD regions thereof.

In summary, to a large extent through the delineation of natural mutations in the LDL receptor gene, structural as well as regulatory features of receptor-mediated metabolism of the major cholesterol-carrying lipoprotein in human plasma are now well understood. In the following sections, our still somewhat limited knowledge about the events involved in receptor-mediated plasma clearance of triacylglycerol-rich lipoproteins is outlined.

3. Removal of triacylglycerol-rich lipoproteins from the plasma

3.1. Catabolism of chylomicrons

Chylomicrons are too large to cross the endothelial barrier; thus, their prior lipolysis to remnants serves a dual function: transport of energy to tissues, and decrease in size to facilitate terminal catabolism. Classical experiments in hepatocytes, perfused rat livers, and transgenic and knockout mice studies have shown that chylomicron remnant transport into the liver is mediated by cell surface receptors. Although apoE, which is recognized by the LDL receptor, is the surface component that targets chylomicron remnants to their site of uptake, studies in LDL receptor-deficient model systems predicted that chylomicron remnant removal would be LDL receptor independent. Individuals with homozygous FH, who lack functional LDL receptors, show no signs of delayed clearance of chylomicron remnants. Furthermore, evidence for a separate hepatic chylomicron remnant removal mechanism came from studies in which dietary, drug, and hormonal factors were shown to regulate the number of hepatic LDL receptors without greatly affecting the clearance rate for chylomicron remnants.

Since the LDL receptor and the proposed chylomicron remnant receptor share at least one property, namely apoE binding, attempts to isolate this receptor were based on the presumed similarity of its ligand binding region to that of the LDL receptor. Indeed, homology cloning resulted in the characterization of an unusually large membrane protein, composed exclusively of structural elements found in the LDL receptor molecule; it has therefore been termed LDL receptor-related protein, or LRP [6]. As shown in Fig. 4, LRP, a 4526-amino acid integral membrane glycoprotein, contains (among other structural elements found in the LDL receptor) 31 repeats of the type forming the ligand binding domain in the LDL receptor and 22 repeats of the growth factor type. The unusually large membrane protein binds lipoproteins in apoE-dependent fashion.

Soon after its cloning, LRP was shown to be identical to the receptor for α_2-macroglobulin (α_2MR), a major plasma protein that functions in 'trapping', and thereby inactivating, cellular proteinases that have entered the plasma compartment. Since then, many more plasma proteins and protein complexes have been identified, which at least in vitro bind to LRP [7]. Importantly, α_2-macroglobulin–proteinase complexes are cleared rapidly by the liver (with the same kinetics as chylomicron remnants), indicating that LRP may indeed perform multiple functions in the removal of spent vehicles of intestinal lipid transport and of potentially harmful proteinases. Another LDL receptor

relative with an even broader range of functions is introduced in the following section, and further properties of this protein are described in detail in Sections 4 and 5.

3.2. The so-called VLDL receptor: a role in catabolism of VLDL?

The name VLDL receptor was coined for a protein discovered in 1992 by Takahashi et al. [8]. The overall modular structure of the VLDL receptor is virtually superimposable with that of the LDL receptor, except that the ligand recognition domain contains an additional binding repeat located at the amino terminus (Fig. 4). The VLDL receptor shows an amazing degree of conservation among different species; within mammals, there is 95% identity between the corresponding proteins. Even the VLDL receptor homologs of more distant species such as the chicken and frog share 84% and 73%, respectively, of identical residues with the human VLDL receptor. In addition, VLDL receptors exist in variant forms, arising from differential splicing of exon 16 which specifies an *O*-linked sugar domain.

However, uptake of VLDL as such into tissues in vivo has not been conclusively shown to involve the eight ligand binding repeat receptor to a significant extent. This is despite the fact that its tissue distribution is highly suggestive of a role in triacylglycerol transport into metabolically active tissues, such as heart, skeletal muscle, and adipose tissue. In contrast to the LDL receptor, and as expected from a receptor implicated in triacylglycerol transport, the VLDL receptor is not regulated by cellular sterols, but its level appears to be influenced by hormones such as estrogen and thyroid hormone. On the other hand, its expression pattern is not congruent with that of lipoprotein lipase with which the VLDL receptor would be expected to act in concert. Nevertheless, numerous studies suggest that the VLDL receptor is, at least in part, involved in the delivery of fatty acids derived from VLDL-triacylglycerols to peripheral tissues, such as adipose tissue [9].

Subsequent to the revelation that despite the proposed and/or intuitive physiological function, the VLDL receptor likely has only a limited role in lipoprotein metabolism, our view of VLDL receptor function has dramatically changed. In 1999, it was discovered by elegant experimentation that it clearly plays a role in neuronal migration in the developing brain, via binding of a ligand quite distinct from lipoproteins. These important results are described in Section 5. However, there is a homologue of the VLDL receptor which indeed does deserve this name, since it has a very well defined function in lipoprotein metabolism, as described in the following section.

4. Multifunctional receptors in the chicken

A particularly interesting VLDL receptor homolog is that of the chicken (termed LDL receptor relative with eight binding repeats, or LR8; Fig. 4), as its functions are documented by both biochemical and genetic evidence: it mediates a key step in the reproductive effort of the hen, i.e., oocyte growth via deposition of yolk lipoproteins [10,11]. This conclusion is based on studies of a non-laying chicken strain carrying a single mutation at the *lr8* locus that disrupts LR8 function (the 'restricted ovulator',

R/O, strain) [12]. As a consequence of the mutation, the hens fail to deposit into their oocytes VLDL and the lipophosphoglycoprotein vitellogenin, which are produced at normal levels in the liver, and the mutant females develop severe hyperlipidemia and features of atherosclerosis. The phenotypic consequences of the single-gene mutation in R/O hens revealed the extraordinary multifunctionality of LR8, i.e., that the receptor recognizes in essence over 98% of all the yolk precursors that eventually constitute the mass of the fully grown oocyte [11]. Obviously, R/O hens, which represent a unique animal model for an oocyte-specific receptor defect leading to familial hypercholesterolemia (Section 2.2), are sterile due to non-laying.

Those tissues which express the VLDL receptor in mammals, i.e., heart, skeletal muscle, brain, and adipose tissue, but not the liver, also express this receptor in chicken, albeit at very low levels compared to the oocytes [13]. One difference between the structures of the major VLDL receptors in mammals and chicken LR8 is the presence (in mammalian tissues) and absence (in chicken oocytes) of the *O*-linked sugar domain, respectively [13]. Here, the larger form is termed $LR8^+$, and the smaller one, $LR8^-$. It was found that in chicken, the somatic cells and tissues, in particular the granulosa cells surrounding the oocytes, heart, and skeletal muscle express predominantly $LR8^+$ (at very low levels, as indicated above), while the oocyte is by far the major site of LR8-expression. In the male gonad, the same expression dichotomy exists in that somatic cells express the larger, and spermatocytes the shorter, form of LR8 [14].

The properties of LR8 and its central role in reproduction strengthen the hypothesis that the avian receptor is the product of an ancient gene with the ability to interact with many, if not all, ligands of more recent additions to the LDL receptor gene family (Fig. 4). In this context, vitellogenin, absent from mammals, and apoE, not found in birds, possess certain common biochemical properties and regions of sequence similarities, and have been suggested to be functional analogues [15]. Even high density lipophorin, an abundant lipoprotein in the circulatory compartment of insects, is likely endocytosed in a variety of tissues via an LR8 homologue with very high similarity to the VLDLR/LR8 group [16]. Presumably, binding of lipophorin to this receptor is mediated by apolipophorins I and II, which share sequence homology with mammalian apoB, and thus may behave similarly to the major yolk precursor proteins.

Furthermore, studies in the chicken have revealed that members of the LDL receptor gene family from different animal kingdoms have common structures, and share a growing list of physiological roles, including the most recently discovered function(s) in signal transduction, as described in the following section.

5. VLDL receptor and apoE receptor type 2 (apoER2) as signal transducers

5.1. ApoER2 — a close relative of the VLDL receptor

The structure of apoER2, which was discovered by homology cloning, is highly reminiscent of that of the VLDL receptor (Fig. 4). However, the produced proteins are now known to harbor a cluster of either three, four, five, seven, or eight binding

repeats, dependent on the species and organ expressing apoER2 [17]. Murine apoER2 mRNA may contain an additional small exon following that encoding repeat 8, giving rise to a variant containing a furin consensus cleavage site at the carboxy-terminal end of the ligand binding domain. This may lead to the secretion of a soluble receptor fragment constituting the ligand binding domain, which could act as a dominant negative ligand-trapping receptor. Most interestingly, this variant is the only one detectable in the placenta, showing that some of the splice events are tissue-specific.

ApoER2 is predominantly found in brain, placenta, and testis. This is in contrast to other members of the LDL receptor family, which are all expressed to a small extent in the brain, but most prominently in a variety of other organs and cells. Besides the liver, the brain is also the most prevalent site of apoE expression in mammals, and it is widely believed that apoE serves a role in local lipid transport in the central nervous system [18]. Ligand binding studies with the human apoER2 demonstrated high affinity of the receptor for β-VLDL, indicating that the receptor might be involved in apoE-mediated transport processes in the brain. In addition, at least the apoER2 splice variant containing eight binding repeats can act as receptor for α_2-macroglobulin (also a ligand of LRP, see Section 3.1) in brain, which suggests a role in the clearance of α_2-macroglobulin–proteinase complexes from the cerebrospinal fluid and from the surface of neurons. In turn, proteinases may play a role in synaptic plasticity [19], and the balance between proteolytic activity and its inhibition might be controlled by proteinase inhibitors and their receptors. Despite these intriguing possibilities, an even more important, recently delineated, function of apoER2 — and of the VLDL receptor — is described below.

5.2. Genetic models reveal new roles for apoER2 and VLDL receptor

In addition to their potential to bind and endocytose ligands in a variety of metabolic and cellular contexts, these receptors are now known to be involved in signal transduction [20], which likely is independent of ligand internalization. Surprisingly, targeted disruption of both the VLDL receptor and the apoER2 genes in mice (so-called double-knockout mice) display a dramatic phenotype, essentially identical to that of mice lacking the extracellular matrix glycoprotein reelin [20]; single-knockout mice of either receptor gene show only very subtle phenotypes. Reelin is secreted by Cajal–Retzius cells in the outermost layer of the developing cerebral cortex and orchestrates the migration of neurons along radial fibers, thus forming distinct cortical layers in the cerebrum.

The reason for the grossly abnormal phenotype of the double-knockout mice, i.e., disturbed foliation of the cortical layers, is that reelin normally interacts with the extracellular domains of both the VLDL receptor and apoER2 [21,22], but the ensuing vital signal cascade remains inactivated when the receptors are missing. The current model suggests that reelin binding to the VLDL receptor and apoER2 leads to phosphorylation of the cytoplasmic adapter protein Disabled-1 (mDab-1), which is associated with the NPxY-motifs present in the receptors' tails. Reelin-triggered tyrosine-phosphorylation of mDab-1 may then start kinase cascade(s) controlling cellular motility and shape by acting on the neuronal cytoskeleton. The specificity of the reelin signalling via

apoER2 and VLDL receptor seems to be achieved by selective binding of reelin to these receptors and not to other members of the LDL receptor family. This is an important aspect, since mDab-1 not only binds to the VLDL receptor and apoER2, but also to LRP and the LDL receptor [23].

6. *Other relatives of the LDL receptor family*

In the past few years, several additional LDL receptor gene family members have been identified at the molecular level. Since all of these, by definition, contain LDL receptor ligand binding repeat clusters and may therefore play roles in lipid-related metabolism, they are also described in this chapter. For simplified schematized structures of these membrane proteins, please refer to Fig. 4.

6.1. Small and mid-sized LDL receptor relatives: LRP 3, 4, 5, and 6

These rather new additions to the LR gene family were discovered more or less serendipitously. Degenerative probes corresponding to the highly conserved amino acid sequence WRCDGD, found in LDL receptor ligand repeats, were used to screen a rat liver cDNA library, resulting first in the cloning of LRP3, and then of its human homologue from a HepG2 cDNA library [24]. The same approach, using a murine heart cDNA library, resulted in cloning of LRP4. LRP3 is a 770 residue membrane protein with clusters of two and three binding repeats, respectively. Murine LRP4 contains two clusters of LDL receptor binding repeats with three and five modules, respectively. Future studies will help to clarify whether these proteins are capable of binding ligands that have been shown to interact with other LDL receptor relatives, whether they are endocytotically competent, and of course, what their relevant in-vivo functions are.

Two other new members of the LDLR family, LRP5 [25] and LRP6 [26] have been discovered (Fig. 4), quite surprisingly, in the course of attempts to identify the nature of the insulin-dependent diabetes mellitus locus IDDM4 on chromosome 11q13. Human and mouse LRP5 and LRP6 are type I membrane proteins, approximately 1600 residues long (about twice as large as the LDL receptor), and their extracellular domains are organized exactly as a portion of LRP (Fig. 4). The cytoplasmic domains of LRP5 and LRP6 do contain motifs (dileucine, and aromatic-X-X-aromatic/large hydrophobic) similar to those known to be functional in endocytosis of other receptors.

Probably more importantly, they harbor serine- and proline-rich stretches that may serve as ligands for Src homology 3 (SH3) and WW (a variant of SH3) domains, properties that relate these receptors to signal transduction pathways, albeit different ones from those of apoER2 and the VLDL receptor described above. Indeed, LRP6 has been shown to be a co-receptor for proteins of the Wnt family, which trigger signalling pathways important for correct development of anterior structures. Most recently, LRP6 has been demonstrated to interact with proteins called Dickkopf and Axin, respectively [27]. Dickkopf inhibits Wnt signalling by releasing receptor-bound Wnt. Axin is one of the components in the cascade that regulates the activation of gene expression in the nucleus of target cells. The interplay of Wnt-, Dickkopf- and Axin-binding to LRP5/6

may hold the key to important developmental signals, similar to the role of VLDL receptor and apoER2 in neuronal migration (Section 5.2).

6.2. The unusual one: LR11

Also by homology cloning, LR11, a novel and unusually complex member of the LDL receptor gene family (Fig. 4) has been discovered first in rabbit, and subsequently in man, mouse and chicken [28]. Significantly, overall sequence identities between the ~250 kDa proteins range from 80% (man vs. chicken) to 94% (man vs. rabbit). The predicted amino acid sequence of LR11 suggests that the polypeptide is made up of seven distinct domains (Fig. 4), among which is a cluster of eleven LDL receptor ligand repeats. Unusual features are a large domain (~400 residues) highly homologous to a yeast receptor for vacuolar protein sorting, VPS10p, and modules found in cellular adhesion molecules (six tandem fibronectin type III repeats). The membrane spanning and cytoplasmic domains are extremely highly conserved; e.g., the presumed internalization signal is FANSHY in all LR11s known to date.

LR11 is found mainly in the nervous system, and depending on the species, also in testis, ovary, adrenal glands, and kidney. It appears that LR11 is developmentally regulated, and several studies have demonstrated induced expression during morphogenetic processes. For instance, LR11 levels are increased during proliferation, but become downregulated following differentiation of neuroblastoma cells. LR11 is also markedly increased in arterial intimal smooth muscle cells during atherogenesis and in the proliferative phase of smooth muscle cells in culture. Thus, to date, the limited information available suggests that LR11 is involved in cellular proliferation during development and possibly, in pathological processes.

However, knowledge about the cell biology of LR11 is still highly rudimentary. Only 10% of the receptors are found on the cell surface in transfected cells, and thus far identified ligands of LR11, such as apoE-containing lipoproteins, have not offered significant insights into LR11 functions [29]; finally, endocytic competence of the receptor has not been demonstrated unambiguously to date.

6.3. Large LDL receptor relatives: megalin and LR32

6.3.1. Megalin, a true lipid transport receptor

Encoded by a different gene from that for LRP, this 600-kDa protein is another large member of the LDL receptor gene family containing four LDL receptor ligand binding repeat clusters. Although many proteins which bind to LRP are also ligands for megalin (with the exception of apoJ, also known as clusterin), its expression pattern and specificity for certain ligands account for physiological roles distinct from those of LRP. Megalin is essential for development of the forebrain by taking up apoB-containing lipoproteins into the embryonic neuroepithelium (reviewed in Willnow et al. [7]). Another important function is its involvement in the metabolism of certain lipophilic vitamins. For instance, in the kidney, vitamin B12/transcobalamin complexes are recaptured from the ultrafiltrate directly by binding to megalin expressed on proximal tubule cells [30]. Furthermore, megalin mediates the reabsorption from the proximal

tubules of 25-(OH) vitamin D3/vitamin D binding protein complexes, which constitutes a key step in converting the precursor into active vitamin D3 in the kidney [7].

6.3.2. LR32 (LRP1B)

This 4599-residue type I membrane protein contains 32 LDL receptor ligand binding repeats in its extracellular portion (Fig. 4). Among all of its relatives, LR32 shows the highest homology to LRP, containing one additional ligand binding repeat and an insertion of 33 amino acids in the cytoplasmic domain compared to the sequence of LRP (cf. Fig. 4). This newly discovered receptor molecule may thus have a role in lipoprotein metabolism.

However, homology searches revealed that LR32 is identical to the product of a candidate tumor suppressor gene, *lrp1b* [31]. The human LR32 gene locus was mapped to chromosome 2q21 by fluorescence in-situ hybridization. The receptor is expressed mainly in brain and skeletal muscle, and its regulation has been studied in smooth muscle cells derived from rabbit arteries and in an established smooth muscle cell-line. In both systems, LR32 expression is induced during the exponential phases of cellular proliferation. Peaks of expression seem to occur at later time points than those observed for LR11 induction (see Section 6.2), consistent with different roles of LR11 and LR32. The future will tell us more about the true physiological role(s) of this close relative of LRP.

7. Scavenger receptors

In addition to the type of scavenger receptors (SRs) described in Chapter 20, there is also a growing list of hepatic and extrahepatic SRs with potential disease-related functions. For many of these, the criterion to be considered as SR is their broad spectrum of ligands which includes diverse polyanionic compounds and, importantly, modified lipoproteins, such as oxidized LDL. The more than a dozen currently known SRs are classified according to their primary structure, tissue distribution, and proposed function(s) into six groups (SR-A to -F). The most prominent and probably best understood SRs in the context of lipoprotein metabolism are the SR class A (SR-A) and a class E SR, LOX-1 (for a concise review of SRs, see Terpstra et al. [32]).

7.1. Class A SRs

SR-As are trimeric membrane proteins characterized structurally by the presence of a small amino terminal intracellular region, an extracellular coiled-coil collagen-like stalk, and a cysteine-rich carboxy-terminal domain [33]. Three isoforms of this receptor are produced from the same gene by differential splicing. SR-A isoforms are expressed at different levels in tissue macrophages, Kupffer cells, and various extrahepatic endothelial cells. SR-A expression is induced by some of its ligands, which include, in addition to modified lipoproteins and polyanions, Gram-positive bacteria, heparin, lipoteichoic acid, and a precursor of lipid A from LPS of Gram-negative bacteria.

The potential role of SR-A in atherosclerotic plaque development was demonstrated in a study on apoE- and SR-A double-knockout mice. Mice deficient in apoE develop

severe plaques, but simultaneous absence of SR-A leads to a reduction in plaque size by 58%. This reduction may be related to the greatly reduced uptake of acetylated LDL and oxidized LDL that can be observed in in-vitro uptake studies using macrophages and liver cells of SR-A knockout mice.

7.2. Lectin-like oxidized LDL receptor (LOX)-1

This 50-kDa transmembrane protein shows no structural similarity to other SRs. It belongs to the C-type lectin family of molecules, and its carboxy-terminal cytoplasmic tail contains several potential phosphorylation sites [34]. It can act as endocytic receptor for atherogenic oxidized LDL, but in contrast to SR-A, it interacts only weakly, if at all, with acetylated LDL. Thus, LOX-1 differs from other SRs in that binding of oxidized LDL is inhibited by polyinosinic acid and delipidated oxidized LDL, but not by acetylated LDL, maleylated bovine serum albumin, or fucoidin. LOX-1 is found in thoracic and carotid vessels, and highly vascularized tissues such as placenta, lungs, brain, and liver. Its expression is apparently not constitutive, but can be induced by proinflammatory stimuli; it is then detectable in cultured macrophages and in activated smooth muscle cells. LOX-1 also mediates the recognition of aged red blood cells and apoptotic cells. However, little is known about whether LOX-1 has similar functions in clearance of damaged cell in vivo.

In summary, SRs are a widely expressed and highly diverse group of proteins that are appropriately named for their recognition of a broad array of ligands. At least some of this intriguing group of receptors, as indicated here, may play roles in the metabolism of modified lipoproteins, and thus may well be related to lipid anomaly disorders.

8. Future directions

It is exciting to realize that multifunctionality of LDL receptor gene family members can no longer be viewed as being limited to their extracellular moiety. The originally established concepts regarding their functions in lipoprotein metabolism remain valid, but have been extended significantly by the discovery that these membrane proteins also play a role in important signalling pathways. Given the wealth of signalling pathways, future efforts will be directed towards developing concepts that define the contributions of the individual intracellular domains of LDL receptor relatives. These efforts can also be expected to add alternative aspects to the ongoing quest to delineate the evolutionary history of the LDL receptor family. In fact, based on the most recently gained knowledge, an evolutionary theory will have to consider the combinatorial events arising from the multitude of both intra- and extra-cellular domains of these proteins in the generation of new signalling and/or transport units.

Another point to consider is the functional redundancy of receptors involved in a multitude of metabolic pathways and events. This aspect has been addressed here not only for the LDL receptor gene family, but also for the growing group of scavenger receptors, which show broad overlapping ligand specifities (Section 7). In the case of the well understood LDL receptor gene family, when need arises (e.g., when one

or more receptors are unfunctional), certain members can substitute for others, or are at least sufficiently active for preserving life. For example, VLDLR$^{-/-}$ mice, and apoER2$^{-/-}$ mice, have phenotypes that are at first sight indistinguishable from normal; but double-knockout mice are grossly abnormal, as described in Section 5.2. Moreover, (1) LRP can physiologically substitute for the LDL receptor in hepatic clearance of chylomicron remnants (which also bind to the LDL receptor in vitro), (2) VLDLR, not expressed in the liver and normally not significantly involved in lipoprotein metabolism, can, when expressed in the liver, substitute for the LDL receptor, and (3) in chickens, an oocyte-specific LRP mediates growth of oocytes, although not sufficient for egg laying, in the LR8-deficient R/O hens (see Section 4). Thus, functional redundancy of LDL receptor relatives can be due to their simultaneous expression on the same cells in a given organ. In turn, different functions despite overlapping ligand spectra may arise from their expression in different cell types within a tissue or organism. LDL receptor family members presumably have in-vivo access to different ligands in different environments, and in addition, their cytoplasmic domains interact with cell-type-specific adaptor proteins in order to mediate a spectrum of signal transduction pathways.

As a consequence, specific functions of the gene family must be defined at two levels: the cellular level, in order to delineate molecular events, and in physiological studies including state-of-the-art genetic manipulations, which should reveal the functional relevance of receptor redundancy.

Abbreviations

α_2MR	α_2-macroglobulin receptor
ACAT	acyl-CoA : cholesterol acyltransferase
Apo	apolipoprotein
CETP	cholesteryl ester transfer protein
CM(R)	chylomicron (remnants)
EGFP	epidermal growth factor precursor
FH	familial hypercholesterolemia
HDL	high density lipoprotein
HMG-	3-hydroxy-3-methylglutaryl-
LCAT	lecithin–cholesterol acyltransferase
LOX-1	lectin-like oxidized LDL receptor
LR	LDL receptor relative
LRP	LDL receptor-related protein
(V)LDL	(very) low density lipoprotein

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