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The life cycle of the low-density lipoprotein receptor: insights from cellular and in-vivo studies

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Abstract

Purpose of review Long-term exposure to elevated concentrations of low-density lipoprotein (LDL) cholesterol increases the risk of cardiovascular events. The main player in clearing LDL cholesterol is the LDL receptor (LDLR) trafficking pathway, however, our fundamental knowledge about the mechanisms regulating this pathway is still incomplete.

Recent findings The LDLR pathway is very complex and involves multiple proteins. Endocytosis is regulated by two different adaptor proteins, i.e. autosomal recessive hypercholesterolemia (ARH) and disabled-2 (Dab2). The proteolysis of LDLR is regulated by inducible degrader of the LDL receptor (IDOL) and proprotein convertase subtilisin/kexin type 9 (PSCK9). However, only a few proteins have been identified that provide insights into endosomal sorting and recycling of LDLR.

Summary Since the discovery of LDLR, knowledge about its function has greatly expanded. Because of its importance in maintaining homeostatic LDL levels, the LDLR pathway has emerged as a key therapeutic target to reduce circulating cholesterol. In order to be able to treat and diagnose individuals with hypercholesterolemia in the future, it is important to learn more about the LDLR trafficking pathway, as we still lack a full mechanistic understanding of how LDLR trafficking is controlled.

Keywords: low-density lipoprotein, low-density lipoprotein receptor, intracellular trafficking, cardiovascular disease

Keypoints:

- The LDLR trafficking pathway is orchestrated by numerous proteins and is cell-type specific.
- In the liver, which is responsible for the main part of LDL clearance, ARH and PCSK9 are responsible for endocytosis and degradation respectively, whereas in other cell types Dab2 and IDOL are also involved.
- Proteins that are involved in LDLR degradation, such as IDOL and PCSK9, are interesting pharmaceutical targets to lower LDL cholesterol.
Introduction

A high level of circulating low-density lipoprotein-cholesterol (LDL) is a major risk factor for coronary heart disease (CHD). A central player in regulating the levels of plasma cholesterol and overall cholesterol homeostasis is the low-density lipoprotein receptor (LDLR) (1). LDLR belongs to the extended family of LDL receptors, which comprises seven cell surface endocytic receptors that internalize extracellular ligands for lysosomal degradation (reviewed in (2, 3)). Ligands that can be internalized by LDLR are the very low-density lipoprotein (VLDL), VLDL remnants, and the low-density lipoprotein (LDL). VLDL and its remnants bind to the receptor via apolipoprotein (apo) E, whereas LDL-LDLR binding is facilitated by apoB100. Mutations in LDLR are associated with familial hypercholesterolemia (FH), and more than 1,200 genetic variants have been described that are associated with high plasma LDL cholesterol levels, dyslipidemia and early CHD (4). Apart from LDLR mutations, FH can be caused by mutations in genes encoding for proteins involved in the formation of the LDL-LDLR complex, endocytosis, or degradation of LDLR, such as apoB100, the autosomal recessive hypercholesterolemia (ARH) protein, and proprotein convertase subtilisin/kexin type 9 (PCSK9), respectively. These mutations directly affect LDL clearance via LDLR and have been essential to understanding the LDLR trafficking pathway. Despite these discoveries, our fundamental knowledge about the mechanisms by which trafficking of LDLR is coordinated remains incomplete. In this review we will describe our current understanding of the LDLR “life cycle”.

Endocytosis of LDLR

LDLR is synthesized at the endoplasmic reticulum (ER), and is processed to its mature form by glycosylation in the Golgi apparatus before it is transported to the plasma membrane. Upon binding of the lipoproteins to the receptor at the cell surface, the LDLR-lipoprotein complex is internalized through clathrin-coated pits into clathrin-coated vesicles (5) (Fig. 1). These vesicles fuse with early endosomes, where the acidic environment leads to release of the lipoprotein from LDLR (6). Subsequently, the ligand and receptor are sorted in two different directions. LDLR is either sorted back to the plasma membrane for reuse, or directed to the lysosomes for degradation (see below). During its lifespan, LDLR can make approximately 100 of these cycles (7). The ligand is sorted to the late endosomes and eventually to the lysosomes, from which cholesterol is taken up by the cell. Endolysosomal trafficking of the LDLR ligand is likely to depend on the two-pore channel TPC2, while TPC1 is involved in Ca²⁺ signaling, which seems to be needed for the endolysosomal fusion processes (8). The transfer of cholesterol into the cytosol is mediated by the Niemann-Pick disease proteins,
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Internalization of LDLR depends mainly on the NPxY motif within its cytoplasmic tail. The NPxY motif is a universal signal for mediating endocytosis or signal transduction events in different protein families, including the LDLR family, the amyloid precursor protein family, beta integrins and receptor tyrosine kinases (10, 11). The motif serves as a binding site for many adaptor proteins such as Disabled (Dab) 1, Dab2, ARH, and β-arrestin, but only ARH and Dab2 appear to play a significant role in targeting LDLR for endocytosis (reviewed in (10)) (Fig. 1). The importance of the NPxY motif was proven in a patient with the Y807C mutation. In both fibroblasts of the patient, as well as in Chinese hamster ovary cells in which LDLR with this cysteine substitution was introduced, disturbance of the NPxY motif led to an internalization defective receptor (12). Although the NPxY motif within the cytoplasmic tail of LDLR seems to be indispensable for the binding of many adaptor proteins, ARH interacts with a wider part of the LDLR cytoplasmic tail than only the NPxY motif, as it binds to INFDNPVYQKT sequence (13).

When ARH binds to the NPxY motif, LDLR internalization is promoted by ARH binding to clathrin and adaptin 2 (AP-2), two components of clathrin-coated pits (14). Subsequently, AP-2 recruits accessory proteins and enzymes that help with the incorporation of LDLR. The significance of ARH in LDLR endocytosis has been demonstrated in patients with autosomal recessive hypercholesterolemia (15). Interestingly, despite defective LDL clearance, loss of ARH translates into a milder disease phenotype than loss of LDLR. This is presumably because ARH deficiency does not affect VLDL clearance by LDLR, and thus the circulating precursor pool of LDL will still be cleared (16, 17).

The pathway involved in LDLR internalization is cell-type-specific. ARH is indispensable in hepatocytes and lymphocytes to internalize LDLR (18). In fibroblasts and the cervical cancer cell line HeLa however, ARH is redundant. This redundancy was shown by using a single or a double knockout cell line of ARH and Dab2, where endocytosis was only perturbed in a major way when both genes were absent (19). Despite the fact that ARH and Dab2 functionally overlap in various cell types, the mechanisms by which ARH and Dab2 sort LDLR into clathrin-coated pits are slightly different (20). ARH and Dab2 both interact with LDLR via the NPxY motif and couple the receptor to clathrin-coated pits (21). Furthermore, both adaptor proteins require simultaneous binding to clathrin and phosphoinositides (PtdIns(4,5)P2), and to LDLR (22). However, ARH couples the receptor to the coated pits by binding to AP-2, whereas Dab2-mediated endocytosis is independent of AP-2 (19). Also, in
contrast to Dab2, ARH activity is regulated by nitrosylation via nitric oxide (23). ARH can be nitrosylated at two cysteines, and this is necessary for ARH to associate with AP2, and thus for internalizing LDLR (23). This modification of ARH is likely to be involved in additional fine-tuning of LDLR-mediated LDL uptake in hepatocytes.

In contrast to ARH, no mutations in Dab2 have been reported in patients with hypercholesterolemia, which could be explained because Dab2 is vital for embryogenesis, as
previously demonstrated in mice (24). Dab2-deficient mouse embryos die in an early stage of development due to disorganization of the extra-embryonic endoderm (25). However, depletion of Dab2 specifically in the embryonic part results in normal development, and adult mice show elevated levels of plasma LDL cholesterol (24).

Although these *in vivo* data support the notion that both Dab2 and ARH regulate clearance of circulating LDL, the elevated LDL levels in Dab2-deficient mice are remarkable as Dab2 is not expressed in hepatocytes, but is mainly expressed in kidney and placenta. Further research is therefore needed to understand the cell-type-specific role of these two adaptor proteins in LDLR trafficking.

**LDLR trafficking**

LDLR can be reused multiple times, but the mechanisms by which internalized LDLR is sorted from the endosomes and directed back to the plasma membrane has not been completely resolved yet. To our knowledge, only sorting nexin 17 (SNX17) is shown to be involved in recycling of LDLR (Fig. 1) (26). So far, 25 members have been identified in the human sorting nexin family. These proteins are membrane-associated and play a role in various aspects of endocytosis and protein trafficking. SNX17 binds to LDLR via the NPxY motif, and is also associated with several other LDLR family members, including the VLDL receptor, ApoER2 and LDLR-related protein (26, 27). Binding of SNX17 to LDLR increases the endocytosis rate by a factor two, without changing the number of receptors on the cell surface (27, 28). This suggests that interaction of SNX17 with LDLR does not influence degradation of the receptor by directing it to the lysosome, but rather accelerates its movement through the early endocytic compartment (27).

Also the Rab family is likely involved in vesicle transport of LDLR. The Rab family consists of over 60 members that are associated with intracellular membranes and are regulating (polarized) membrane traffic (reviewed in (29)). LDLR is transported by Rab3B and Rab13 to the basolateral membrane (Fig. 1) (30, 31). The specific function of Rab13 in LDLR trafficking was further elucidated by a study making use of two LDLR variants (31). One variant travels to the membrane directly, while the other variant first passes the recycling endosomes. In the case of perturbing Rab13 function, only the LDLR variant that travels via the recycling endosomes is prevented from reaching the cell membrane, suggesting that Rab13 mediates LDLR trafficking between the trans-Golgi network and the recycling endosomes (31).

Interestingly, Megalin, another member of the LDLR family, is accompanied by ARH during
recycling from the plasma membrane through the early endosomes and recycling endosomes back to the plasma membrane, and ARH is specifically required for trafficking from the early endosome to the recycling endosome (32). This role of ARH has not yet been investigated in LDLR (Fig. 1), but it is possible that ARH has a dual role in endocytosis and trafficking.

**Degradation of LDLR**

Degradation of LDLR is regulated by two independent pathways, which are orchestrated by PCSK9 and E3 ubiquitin ligase IDOL (inducible degrader of the LDL receptor) (Fig. 1) (33, 34). PCSK9 was discovered in families suffering from familial hypercholesterolemia. These FH patients were heterozygous for a PCSK9 gain-of-function mutation that resulted in autosomal dominant hypercholesterolemia (35). In addition, PCSK9 null mutations have been described that correlate with very low levels of LDL cholesterol, but without an apparent clinical phenotype (36). PCSK9 can mediate the proteolysis of LDLR intra- or extracellularly (Fig. 1). Extracellularly, PCSK9 binds to the epidermal growth factor like-A domain (EGFA) of LDLR, which induces ARH-dependent internalization (37). When acting intracellularly, PCSK9 binds to LDLR at the trans-Golgi network after synthesis and directly targets the receptor to the lysosomes for degradation (38). In both cases, LDLR will be chaperoned to the endosomes where normal recycling of the receptor is disrupted by preventing the acid-dependent conformational switch from open to closed (39). The inhibition of this switch marks LDLR for degradation by the endolysosomal compartment, where both LDLR and PCSK9 are broken down (40, 41). **PCSK9** expression, like **LDLR** expression, is positively regulated by sterol regulatory element binding protein 2 (SREBP2). SREBP2 is activated in the case of low levels of cellular cholesterol and turns on genes for cholesterol uptake such as **LDLR** (42). This co-regulation of PCSK9 and LDLR seems contradictory, but immediate breakdown of LDLR is prevented by the presence of LDL in the plasma. LDL binds to PCSK9, and prevents PCSK9-mediated LDLR breakdown (43). PCSK9 activity is probably influenced by various other factors, in addition to protein levels. This has been endorsed by the fact that PCSK9 levels alone cannot predict the amount of PCSK9-LDLR complexes (44). Furthermore, PCSK9 is more prone to dissociate from LDLR when reaching the endosome in PSCK9-resistant fibroblasts (45), even though the affinity of PCSK9 for LDLR increases dramatically at acidic pH (41).

Similar to PCSK9, IDOL drives the degradation of LDLR, but IDOL’s mechanism of action differs from PCSK9. Where PCSK9 is upregulated by SREBP2, IDOL expression is regulated by liver X receptor (LXR), which is activated in the case of intracellular cholesterol excess (34). Expression of IDOL causes raised plasma LDL cholesterol by sending LDLR for proteolysis.
This is effectuated by two functional domains. The FERM (Band 4.1, ezrin-radixin-moesin) domain interacts with the cytoplasmic domain of LDLR, and after binding, the RING (really interesting new gene) domain ubiquitinates LDLR as well as IDOL itself, which targets both proteins for lysosomal destruction (34, 46).

The role of IDOL in LDLR degradation was confirmed in mice. Overexpressing IDOL in the liver led to hypercholesterolemia and exacerbated atherosclerosis (47). In humans, the association of IDOL with cholesterol homeostasis was first shown in genome-wide association studies, where a strong statistical association between LDL levels and IDOL was found (48, 49). One of the SNPs found, rs9370867, encodes for variant N342S and is associated with cholesterol levels. This variant is located in the FERM domain and S342 results in decreased ability of IDOL to degrade LDLR, whereas N342 causes reduced LDLR levels (50). Another mutation that has been associated with low circulating LDL levels is R266X (51). This nonsense mutation leads to a truncated IDOL protein, which lacks the complete RING domain, rendering it unable to degrade LDLR (51).

When LDLR is targeted by IDOL, LDLR is endocytosed and needs to be sorted before it is directed to the lysosomes (Fig. 1). A recent study by Scotti et al. showed that the IDOL-mediated internalization is independent of clathrin, ARH, caveolin and dynamin (46). They suggested that IDOL ubiquitinates LDLR present in lipid rafts at the plasma membrane, and is subsequently internalized in an Epsin-dependent manner. Epsins form a class of ubiquitin adaptor proteins involved in the internalization of numerous receptors such as Notch, EGFR, VEGFR2 (52, 53), and Epsin-dependent internalization of LDLR has been demonstrated in C. elegans (54). After LDLR is internalized, the endosomal-sorting complex required for transport machinery (ESCRT) mediates the sorting of LDLR to the multivesicular body pathway and finally into the lysosomes (46, 55). It was also demonstrated that the ubiquitin-specific protease 8 (USP8), a deubiquitinating enzyme (DUB), prevents IDOL-mediated degradation (46). Altogether, IDOL directs LDLR to the lysosome via different pathways than those shown for PCSK9 and for LDL uptake.

Why two different pathways regulate LDLR degradation is still not clear. As with endocytosis, the pathways that mediate LDLR proteolysis are partially determined by the cell type. The highest levels of PCSK9 activity are detected in the liver, with lower quantities in the kidney, intestine and brain (56). On the other hand, IDOL exerts only a small effect in the liver, and LXR-mediated IDOL activation is mainly observed in the intestine and peritoneal macrophages (34). Because PCSK9 is mainly responsible for degradation of LDLR in the liver,
it appears to have a dominant role in regulating plasma LDL levels.

**Conclusion**

Since the discovery in the ‘80s, by Goldstein and Brown, that LDLR is related to familial hypercholesterolemia (57-60), our knowledge on the LDLR pathway in cholesterol homeostasis has expanded significantly. The identification of the LDLR-PCSK9-LDL axis has emerged as one of the most potent drug targets for lowering cholesterol (reviewed in (61)). Several PCSK9 clinical trials are currently in phase III and have demonstrated that pharmacological inhibition of PCSK9 can decrease circulating LDL levels by as much as 40-72% (62). Despite these valuable discoveries, our understanding of the molecular mechanisms that regulate intracellular trafficking of LDLR remains poor. In particular, we need to learn more about the mechanisms by which internalized LDLR is sorted at the endosomes to direct the receptor back to the cell surface or to the lysosomes. A better understanding of these pathways could provide additional therapeutic targets to treat hypercholesterolemia, since improved recycling of LDLR increases the cellular uptake of LDL (26). In addition, uncovering these pathways will likely reveal novel candidate genes that might help to explain the etiology in unresolved cases of hypercholesterolemia: approximately 40% of patients with hypercholesterolemia did not have mutations in the known FH genes (63). More research to broaden our fundamental knowledge on the LDLR trafficking pathway is required to treat and genetically diagnose individuals with hypercholesterolemia in the future.

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**Conflicts of interest**

The authors report no conflicts of interest.
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