Scratching the surface: Regulation of cell surface receptors in cholesterol metabolism

Nelson, J.K.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 11
General Discussion
Elevated plasma levels of LDL-cholesterol are an established risk factor for the development of atherosclerosis and CVDs. The LDLR is a key determinant in regulating LDL levels in plasma, and current lipid-lowering strategies aim to increase its cell surface expression. Thus far, three major pathways are known to regulate plasma membrane LDLR expression: SREBP, PCSK9 and IDOL. These are discussed in detail in Chapter 1. The SREBP transcription factors have an established role in cholesterol homeostasis by regulating the expression of the LDLR as well as all the genes necessary for de novo cholesterol biosynthesis (e.g. HMGCR and SQLE). In fact, their ability to regulate LDLR expression is the main reason current statin therapies are effective at lowering plasma LDL. Next to transcriptional control, the half-life of the LDLR and its function are governed by post-translational mechanisms. In recent years, the SREBP-regulated PCSK9 and the LXR-induced E3 ligase IDOL have been identified as potent regulators of LDLR abundance and function. In humans, the PCSK9 pathway has been thoroughly investigated and strategies for its inhibition are being currently introduced into clinical practice. Yet, the physiological relevance of IDOL-mediated LDLR degradation is less understood.

**New dimensions to the LXR-IDOL-LDLR axis**

In 2009, the E3 ubiquitin ligase IDOL was identified as a direct LXR target gene and a new post-translational regulator of the LDLR. Unlike PCSK9, this sterol-dependent regulation of the LDLR occurs independently of SREBPs. Given that LDL is the primary source of cholesterol in the plasma, containing ~1600 molecules of cholesterol per particle, this pathway illustrates a potent feedback loop which allows cells to rapidly control uptake of LDL in response to intracellular sterol fluctuations. Acting as an E3 ligase, IDOL promotes ubiquitylation of the LDLR and thereby marks the receptor for subsequent lysosomal degradation. Ubiquitylation is a dynamic post-translational modification implicated in all aspects of cellular biology. This process occurs through a three-step enzymatic cascade, described in detail in Chapter 1. As an E3 ubiquitin ligase, IDOL’s RING domain mediates the formation of both Lys63 and Lys48 poly-ubiquitin chains on its targets and on itself, respectively. This diversity of chain topographies is dependent on IDOL’s association with members of the UBE2D1-4 E2 ligase family. However, IDOL’s RING domain alone cannot promote ubiquitylation of the LDLR. Rather, the ability of IDOL to specifically target and degrade the LDLR requires its N-terminal FERM domain. Different lines of evidence demonstrate that this domain targets IDOL to the plasma membrane, where it preferably ubiquitylates the pool of LDLR residing in lipid rafts (Chapter 3). Upon binding, IDOL’s RING domain catalyzes the conjugation of ubiquitin to the conserved lysine residue present immediately after the NPxY endocytosis motif, which is located in the intracellular tail of its targets. Collectively, these molecular and biochemical studies laid the groundwork underlying the studies presented in this thesis.
**Trafficking of ubiquitylated-LDLR: from the plasma membrane to the lysosome**

Since its discovery, the LDLR pathway for lipoprotein uptake has served as a paradigm for receptor mediated endocytosis (Chapter 1 and 13). The key feature of this pathway is its dependency on clathrin-associated adaptor proteins that anchor the LDLR to the clathrin machinery 14. This endocytic route is essential for facilitating LDL uptake (Chapter 2) as well as for PCSK9-mediated degradation 15. However, unlike PCSK9, this machinery is not required for IDOL-induced internalization and degradation of the LDLR. Nor is it dependent on caveolin-, dynamin- or macroautophagy-related pathways. Instead, IDOL-mediated internalization of the LDLR, and subsequent sorting, requires Epsins and the ESCRT machinery (Chapter 3). Epsins are a class of ubiquitin adaptors that are implicated in internalization and sorting of many ubiquitylated plasma membrane receptors, including EGFR 16-18 and Notch 19. Accordingly, a dominant negative Epsin1 construct blocks IDOL-mediated degradation of the LDLR and leads to accumulation of ubiquitylated receptor on the plasma membrane (Chapter 3 and Chapter 6). These findings suggest that IDOL ubiquitylation occurs independently and upstream of Epsin-mediated internalization, and strengthens our observations that IDOL targets the LDLR residing on the plasma membrane. Once internalized, the ESCRT system is required for proper sorting of the ubiquitylated receptor through the early endosomes and MVBs, and together with NDRG1, directs the LDLR to lysosomes for degradation (Chapter 3 and Chapter 4). In line with this cellular routing, functional inhibition of NDRG1 or the ESCRT components VPS4, TSG101 (ESCRT-1) and USP8 attenuates IDOL-mediated degradation (Chapter 3 and Chapter 4). The involvement of NDRG1 is particularly intriguing as loss-of-function mutations in humans are linked to the demyelinating neuropathy Charcot-Maria-Tooth disease type 4D (CMT4D) 20-22. Therefore, the studies in Chapter 4 contribute to our understanding of the underlying molecular mechanisms involved in the pathology of CMT4D, and potentially implicate the dysregulation of LDLR trafficking and cholesterol metabolism in general therein. Moreover, the identification of the ESCRT-associated DUB USP8 represents the first identified DUB to regulate the LXR-IDOL-LDLR pathway (Chapter 3 and 23). Previous studies have shown that USP8 can hydrolyze both Lys48- and Lys63-ubiquitin chains on ubiquitylated cargo as well as modify the ubiquitylation status of ESCRT components 24. In the presence of IDOL, over-expression of USP8 attenuates LDLR degradation. This USP8-dependent control of LDLR ubiquitylation could possibly be an indirect consequence of perturbing USP8 expression, akin to the dominant negative effect of Epsin1. In support of this, Scotti et al. demonstrated that silencing USP8 does not prevent IDOL-mediated ubiquitylation of the LDLR, but rather prevents it from entering MVBs and therefore prevents its degradation 23. These findings suggest that USP8 activity resides downstream of IDOL ubiquitylation at the plasma membrane, and functions to salvage ubiquitin prior to lysosomal destruction of the receptor. Therefore, as USP8 can modify the ubiquitylation state of ESCRT components 24, we propose that perturbing USP8 levels in the cell could result in ESCRT machinery functional impairment and blockage of ubiquitylated cargo transport to the lysosome (Chapter 3). In this scenario, ubiquitylated LDLR would accumulate in the early endosomal compartment and total LDLR protein levels would increase, similar to the results observed in silencing NDRG1 (Chapter 4).
**DUB’ing IDOL: the role of USP2**

Similar to other post-translational modifications, ubiquitylation is effectively reversed through the actions of DUBs. The human genome encodes approximately 100 DUBs, with the Cysteine USP proteases being the largest family (~55 members) (Chapter 1 and 25). The substrate specificity of DUBs is determined by their sub-cellular localization, specific binding interactions, and preference for ubiquitin chain topology. The ESCRT-associated DUB USP8 was the first identified DUB to regulate the LXR-IDOL-LDLR axis (Chapter 3 and 23). However, USP8 does not interact with IDOL and its range of action is determined by its association with the ESCRT system, therefore placing its activity downstream of IDOL-mediated ubiquitylation of the LDLR. Given the reversible nature of ubiquitylation, we hypothesized that DUBs could directly counteract IDOL activity. Through a genetic screening approach, the DUB USP2 was identified as a novel IDOL binding partner (Chapter 6). Molecular and functional characterization of this interaction revealed that USP2 binds and increases IDOL protein stability (Chapter 6), in line with the emerging concept of regulation of E3 ligase activity by DUBs 6,25,26. Several studies highlight stabilization of the E3 by its interaction with a DUB, largely explained by removal of an auto-ubiquitylation signal. This results in increased degradation of the E3 targets 6,25-28. In line with this, USP2 has been shown to deubiquitylate and stabilize MDM2 and ITCH, leading to increased ubiquitylation and degradation of their cognate targets 29,30. However, in the context of IDOL, its stabilization by USP2-mediated deubiquitylation results in reduced ubiquitylation and degradation of its targets (i.e. LDLR and VLDLR). This represents a paradoxical scenario in which there is more IDOL protein yet reduced degradation of the LDLR, which functionally leads to enhanced cellular uptake of LDL. Mechanistically, IDOL, USP2 and the LDLR form a tri-partite complex at the plasma membrane, which leads to lower levels of ubiquitylated LDLR. Several *in vitro*-based findings suggest that USP2 can hydrolyze diverse chain topographies, including both Lys48- and Lys63-linked ubiquitin chains 31-33. Therefore, two plausible mechanisms could account for this outcome (Chapter 6):

1) **Sequential:** USP2 counteracts IDOL-mediated degradation of the LDLR by subsequently removing Lys63-linked chains on the receptor required for lysosomal sorting and degradation. In this scenario, USP2 would be recruited by IDOL to act directly on the receptor, reminiscent of its reported effect on the EGFR after ligand binding 34.

2) **Trans-ubiquitylation:** USP2 deubiquitylates IDOL rendering it inactive. In this scenario, IDOL ubiquitylation serves to both regulate its stability (auto-ubiquitylation) and activity (i.e. LDLR ubiquitylation). While speculative, both *cis* and *trans* ubiquitylation of E3 ligases have been reported 35-37. In preliminary studies, USP2 was able to remove ubiquitin chains from an IDOL mutant lacking auto-ubiquitylation activity (*cis* ubiquitylation) (unpublished data). Therefore, this could suggest that IDOL is ubiquitylated in *trans* by another E3 ligase, and that USP2 would remove this activating ubiquitin signal. In essence, this unidentified trans-ubiquitylation event would activate IDOL activity, which would effectively be attenuated by USP2.
Our current experiments cannot distinguish these two potential modes of action of USP2 on IDOL. One method to address this would be to reconstitute the enzymatic reactions in vitro. However, this is not trivial, as USP2 may only recognize the intracellular tail of the LDLR when ubiquitylated and only in the context of a biological membrane. Proteomics-based studies could be an alternative approach, and could provide insight into the Lysine residue(s) and chain type(s) on IDOL sensitive to USP2 deubiquitylation. Moreover, several in vitro and in vivo studies have suggested that USP2 expression is sensitive to nutritional and circadian input. Given that cholesterol metabolism is subject to these cues as well, it will be important to investigate how USP2 is integrated with the IDOL-LDLR axis in vivo. We observe that USP2 expression is not regulated by sterol content or LXR activation (Chapter 6). However, it could be that the functional interaction between USP2 and IDOL, and the formation of a tri-partite complex with the LDLR, is regulated through additional post-translational modifications (e.g. phosphorylation), nutritional cues, or to the dynamic level of these proteins.

**DUB’ing IDOL through transcription**

The identification of USP8 and USP2 introduced a new layer of post-translational modulation into the LDLR pathway. To further elucidate the role of DUBs, we used pharmacological inhibition of cellular DUBs. These pan-DUB inhibitors were previously shown to block the deubiquitylase activity of a broad range of Cysteine protease DUBs in vitro. In agreement with these studies, we also observed broad DUB inhibition in treated cells (Chapter 7). Interestingly, short-term DUB inhibition (2 hours) led to a dramatic decrease in LDLR protein abundance and associated LDL uptake in hepatic cells, which we could attribute to LXR-independent induction of IDOL (Chapter 7). The rapid induction of IDOL mRNA and activity occurred in both human and mouse cell culture lines as well as in human primary cells. To date, this is the first example of LXR-independent regulation of IDOL. In response to elevated sterol content, LXRα induce the expression of IDOL to degrade LDLR from the plasma membrane to prevent the uptake LDL particles. The identified LXRE-containing genomic region in intron 2 of the IDOL/Idol locus in human and mice mediates this response by LXR (Chapter 7). However, induction of IDOL expression by pharmacological DUB inhibition occurs independently of this region, and is not dependent on LXRα and LXRβ (Chapter 7). Instead, this response occurs through a 70-base pair region mapped to the proximal promoter of IDOL. The underlying transcriptional mechanism that mediates this response is currently under investigation. However, we postulate that an unidentified transcription factor induces IDOL expression in response to DUB activity inhibition. This concept is not without precedent, as the abundance and activity of several transcription factors are known to be regulated by ubiquitin, such as p53 and members of the Forkhead box O-class (FOXO) family. Previous studies have shown that depletion of the DUB USP7 leads to increased monoubiquitylation of FOXO4 and subsequent increased transcription activity. Interestingly, the HBX41-108 pan-DUB inhibitor used in our assays was originally described as a selective USP7 inhibitor. Therefore, we speculated that if USP7 was involved in the induction of IDOL, silencing its expression would mimic the effects observed with pharmacological DUB inhibition. However, effective silencing of
USP7 in hepatic cells did not perturb IDOL mRNA, nor inhibit the induction of IDOL by DUB inhibition (data not shown). Identification of a single DUB activity responsible for this induction may be difficult, as multiple DUBs are sensitive to the pharmacological inhibitors we used. Therefore, it will be more interesting to elucidate the physiological significance of non-sterol (i.e. LXR-independent) regulation of IDOL. Next to promoting degradation of LDLR, IDOL also regulates the degradation of the VLDLR and ApoER2. We also demonstrated that IDOL degrades the VLDLR in response to pan-DUB inhibition (Chapter 7). These two receptors are particularly important in the Reelin signaling pathway during the development of the central nervous system (Chapter 1 and 49). IDOL is also highly expressed in the brain and is functionally involved in the regulation of lipid uptake in oligodendrocytes, which are the myelinating cells of the central nervous system (Chapter 4). However, unlike other LXR targets (e.g. ABCA1), IDOL is not subject to LXR-dependent regulation in neurons. Currently it is unknown what regulates IDOL expression in the brain, and its significance to the development of the central nervous system. Potentially, Chapter 7 uncovers a transcriptional process important for controlling IDOL expression and activity in neurons. However, this transcriptional event may still have implications for cholesterol metabolism. Genome-wide association studies have linked polymorphisms at the IDOL locus to plasma LDL cholesterol levels in humans. These studies have largely focused on functional coding variants, even though variation in promoter elements can also have a large impact on expression of the studied gene. Chapter 7 demonstrates that a limited region in the IDOL promoter can have a large effect on its expression and activity. Therefore, future investigations into the contribution of genetic variation in this region to LDL cholesterol levels in humans will be needed.

Physiological roles of IDOL and potential therapeutics implications

Our understanding of the LXR-IDOL-LDLR axis in humans and in vivo is just beginning to emerge. Recent genome-wide association studies suggest that IDOL contributes to variation in circulating levels of LDL cholesterol in humans. In Chapter 5 we identify the first IDOL loss-of-function rare variant in individuals with low circulating LDL cholesterol. These findings suggest that IDOL inhibition would be beneficial for developing new lipid lowering strategies. IDOL’s ability to regulate the LDLR is dependent on its ubiquitylation activity. This is a desirable feature as a small molecule inhibitor could be used to target its activity, which would be more affordable than the expensive monoclonal antibodies currently used to target PCSK9. Nevertheless, characterizing novel IDOL inhibitors may present some challenges when using these compounds in murine models. The fact that mice carry most of their cholesterol on HDL makes it difficult to assess LDL metabolism in this model without dietary or genetic manipulations, which complicates the development of human therapeutics. In this regard, the LXR-IDOL pathway has additional challenges as several in vivo studies have reported tissue- and species-specific regulation of LDLR. Characterization of Idol-null mice revealed increased LDLR protein and LDL uptake in peripheral tissues and primary peripheral cells (i.e. macrophages), but these mice lacked a hepatic phenotype. Similarly, pharmacological dosing with an LXR ligand strongly induces Idol expression and decreases LDLR abundance in a
number of peripheral tissues (e.g. intestine, heart, spleen), embryonic stem cells and macrophages, but only marginally in the liver. These results are surprising since the liver is the primary site for cholesterol homeostasis; as it regulates the de novo synthesis, the formation and uptake of lipoproteins, and the biliary excretion. Recently, it was shown that, unlike mice, pharmacological activation of the LXR pathway in primates induces hepatic IDOL expression and activity. In line with our reported human data, this study demonstrated that silencing IDOL expression in primates leads to elevated plasma LDL levels, which is a phenotype not observed in mice. Although the pathway for IDOL-mediated degradation of the LDLR is intact in the rodent liver, as forced overexpression of Idol leads to decreased LDLR abundance and increased LDL levels in plasma, the underlying molecular mechanisms for the species-dependent differences in hepatic regulation of the LXR-IDOL remains to be explored. While unexpected, this observation has been reported for several other cholesterol-related genes. For example, Cyp7A1 is an LXR target in mice but not humans, and the CETP (Cholesteryl Ester Transfer Protein) gene is an LXR target in humans that is not present in mice. Collectively, these studies indicate that the LXR-IDOL axis is not a strong determinant of the LDLR pathway in mouse liver. Whether its activity in the peripheral tissues (i.e. intestine) can influence cellular or systemic cholesterol metabolism remains to be addressed, and answering this question will rely heavily on the availability of Idol-deficient mice.

Post-HMGCR pathways that regulate the LDLR

Statin-based therapies are currently the mainstay treatment for hypercholesterolemia and for preventing CVDs, as they promote LDL clearance and effectively reduce circulating LDL levels in humans. Mechanistically, statins inhibit the first rate-limiting step in the cholesterol synthesis pathway (HMGCR), which elicits a positive feedback response to induce the SREBP transcriptional pathway (i.e. LDLR expression). However, HMGCR functions very early in the cholesterol synthesis pathway, which is before a branch point directing flux to either sterols or isoprenoids. As a consequence, statins not only inhibit the synthesis of cholesterol, but also the production of essential isoprenoids needed for protein prenylation (a post-translational modification important for cell cycle progression, proliferation and cell signaling). Hence, there is a growing interest to elucidate post-HMGCR pathways that regulate cholesterol biosynthesis and potentially LDLR expression, which could serve as an alternative to statins. One approach to this would be to target the second rate-limiting enzyme in cholesterol biosynthesis, SQLE, which converts squalene to squalene-2,3-epoxide in the first oxygenation step of cholesterol synthesis. As an SREBP target, SQLE expression is modulated by sterol depletion, and is post-translationally regulated by cholesterol-dependent proteasomal degradation.

Recently, we identified MARCH6 as the E3 ubiquitin ligase regulating the cholesterol-dependent proteasomal degradation of SQLE. By regulating this second rate-limiting enzyme in the biosynthesis pathway, we observed that MARCH6 influences both the SREBP and LXR signaling pathways. In hepatic cells, depletion of MARCH6 leads to a stabilization of SLQE protein. However, through mechanisms not fully elucidated yet, we
observed a simultaneous up-regulation of SREBP target genes (i.e. *LDLR*) and LXR-induced *IDOL* expression and IDOL-mediated degradation of the LDLR (*Chapter 9*). Previous studies have shown that over-expression of SQLE leads to formation of 24(S),25-epoxycholesterol, a potent LXR ligand. We have not directly determined whether this ligand is formed in hepatic cells upon MARCH6 depletion, but we have shown that the metabolic flux across the SQLE point, which also converts MOS to DOS, is increased. Since this is the limiting step for synthesis of 24(S),25-epoxycholesterol we think it is reasonable to assume that the level of this metabolite increases as well in response to MARCH6 silencing. How 24(S),25-epoxycholesterol activates LXR without attenuating SREBP processing is unclear. Unraveling these regulatory mechanisms will be key in understanding the role MARCH6 plays in the cholesterol biosynthesis pathway. While still speculative, inhibiting SQLE activity through increased MARCH6-mediated proteasomal degradation could be an attractive lipid lowering strategy, as this mode of action would produce similar effects observed with statin therapy but with the added benefit of not inhibiting isoprenoid production.

**The identification of EEPD1 as a novel LXR target gene in macrophages**

Cholesterol homeostasis is achieved through a balance of tightly regulated transcriptional and post-transcriptional mechanisms. Under low sterol conditions, the SREBP pathway is activated to induce *de novo* synthesis and lipoprotein uptake, as described above. Once activated, these processes will increase the cellular sterol levels and subsequently lead to the formation of oxysterols and accumulation of intermediate cholesterol metabolites. These molecules act as endogenous activators of LXRs and potently induce its transcriptional activity to reduce the cholesterol burden, as described in *Chapter 1* and *Chapter 10*. The LXR transcriptional pathway occurs in all cell types, but its role in macrophages is particularly important. Macrophages play a key role in clearing apoptotic cells and modified-LDL (*e.g.* oxidized-LDL) in the vessel wall. In response to this cellular lipid loading, LXRs regulate their homeostatic response by inducing the reverse cholesterol transport pathway. This system exports excess cholesterol to HDL particles by transporting the cholesterol to the liver for biliary excretion (*Chapter 1*). This efflux pathway is mediated through several LXR target genes, primarily ABCA1, which is a cell surface transporter highly expressed in cholesterol-loaded macrophages. In a transcriptional profiling analysis of human macrophage cells treated with endogenous and synthetic LXR ligands, we identified a novel LXR target gene EEPD1 (*Chapter 10*). In macrophages, EEPD1 is highly induced upon LXR activation and localizes to the cytosolic surface of the plasma membrane. Our preliminary data indicate that EEPD1 regulates ABCA1 protein abundance and activity in response to LXR signaling. The underlying mechanism for this regulation is currently being investigated, but we speculate that EEPD1 could function as a phosphatase to attenuate ABCA1 protein degradation. The physiologic significance of EEPD1 *in vivo*, in the context of cholesterol homeostasis, has yet to be investigated. Based on the *in vitro* findings presented in *Chapter 10*, we speculate that EEPD1 could regulate the reverse cholesterol transport pathway and therefore act to limit atherogenesis.
Concluding Remarks

This thesis elaborates on the post-translational pathways that govern LDLR and the ABCA1 protein abundance. Both LDLR and ABCA1 play an essential role in regulating cellular and systemic cholesterol metabolism. These receptors and transporters are regulated at multiple levels and their transcriptional regulation is already well described. Mechanisms that regulate LDLR and ABCA1 protein stability and activity, on the other hand, have only recently been described. The LXR-induced E3 ubiquitin ligase IDOL is one of those recently described regulators of LDLR stability, and through genetic, molecular and biochemical characterization, this thesis presents novel mechanisms and components that regulate IDOL-mediated degradation of the LDLR (Figure 1). These new players in the LXR-IDOL-LDLR axis offer new avenues for research. Future studies aimed at elucidating the roles of these new targets in vivo will be particularly valuable, as the physiological impact of IDOL in mice and man is gradually emerging. Moreover, LXRs ability to regulate cell surface receptors expands beyond the regulation of IDOL, highlighted by the discovery of EEPD1 as a novel regulator of ABCA1 stability and activity. As a previously undescribed protein, we open the door for future studies directed at elucidating the molecular and physiological roles of EEPD1 in cholesterol homeostasis. In summary, this thesis “scratches the surface” of cholesterol metabolism by describing novel mechanisms that regulate cell surface expression of key receptors and transporters, and establishes the foundation for future studies.

Figure 1 | Schematic representation of novel regulators of cell surface LDLR expression. (1) Upon binding with LDL-cholesterol, the LDLR is internalized through the clathrin-mediated endocytosis pathway. In the endosomal compartments the ligand and receptor dissociate. This allows for the receptor to be recycled back to the plasma membrane, whereas the LDL particle is sorted to the MVBs and lysosomes for hydrolysis. (2) The E3 ubiquitin ligase IDOL binds the LDLR on the plasma membrane, and with its E2 partner (UBE2D), conjugates a Lys63 poly-ubiquitin chain to the intracellular tail of the receptor. This signals the receptor to be internalized through a clathrin-independent, Epsin-dependent mechanism. (3) The ubiquitylated-receptor is sorted through the endosomal compartments via the ESCRT machinery and the ESCRT-associated DUB USP8 is required for the LDLR to enter the MVBs. (4) NDRG1 is required for MV8 formation and sorting the ubiquitylated-LDLR towards lysosomal degradation. (5) The DUB USP2 forms a tri-partite complex with IDOL and the LDLR, and attenuates IDOL-mediated degradation of the LDLR. (6) Pan-DUB inhibition leads to an LXR-independent transcriptional activation of IDOL and subsequent increased IDOL activity. (7) The E3 ubiquitin ligase MARCH6 regulates the metabolic flux in the cholesterol biosynthesis pathway and consequently regulates the transcriptional activation of both SREBP-2 (e.g. LDLR expression) and LXR/RXR (e.g. IDOL induction). (8) PRR and SORT1 bind and mediate the trafficking of nascent LDLR to the plasma membrane.
Reference: