

The UPS and downs of cholesterol homeostasis

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An emerging theme in the regulation of cholesterol homeostasis is the role of the ubiquitin proteasome system (UPS), through which proteins are ubiquitylated and then degraded in response to specific signals. The UPS controls all aspects of cholesterol metabolism including its synthesis, uptake, and efflux. We review here recent work uncovering the ubiquitylation and degradation of key players in cholesterol homeostasis. This includes the low-density lipoprotein (LDL) receptor, transcription factors (sterol regulatory element binding proteins and liver X receptors), flux-controlling enzymes in cholesterol synthesis (3-hydroxy-3-methylglutaryl-CoA reductase and squalene monooxygenase), and cholesterol exporters (ATP-binding cassette transporters ABCA1 and ABCG1). We explore which E3 ligases are involved, and identify areas deserving of further research.

The UPS: a rapid mode of regulation

Cholesterol homeostasis is governed by intricate regulatory networks that permit exquisite control over the levels of this essential but toxic lipid. Transcriptional programs operate to mediate longer-term control, but deactivation and/or destruction of any excess protein acts as an additional layer of control that allows more acute regulation of cholesterol homeostasis when required. Indeed, post-translational events are emerging as important determinants of cholesterol metabolism. In particular, the conjugation of ubiquitin to target proteins (ubiquitylation; see [Glossary](#)), is a common post-translational modification, second only to phosphorylation in the curated database PhosphoSitePlus [1]. Ubiquitylation typically results in degradation of the marked protein, and recent work indicates that the UPS plays a crucial role in controlling key nodes of cholesterol metabolism, including proteins involved in cholesterol synthesis, uptake, and efflux ([Figure 1](#)). As such, the UPS is an important determinant of cellular cholesterol levels.

The UPS involves the conjugation of ubiquitin to substrates through an E1 activating enzyme, E2 conjugating

enzyme, and an E3 ubiquitin ligase. The mammalian genome encodes two E1s and ~40 E2s, but more than 600 E3s [2,3]. Thus, the E3s provide specificity towards particular target proteins, and are classified into one of three families: the majority comprise the RING ligases, and the remainder are either HECT or U-box ligases [2] ([Table 1](#)). Following ubiquitylation of target proteins, degradation generally occurs at the 26S proteasome. Although ubiquitylation can have other consequences for the protein, in this review we focus on ubiquitylation that leads to protein degradation because this is the primary ubiquitin-dependent mechanism that has been linked to mammalian cholesterol homeostasis.

UPS and the master transcriptional regulators of cholesterol metabolism

Cellular cholesterol levels reflect the net balance of biosynthesis, efflux, and uptake of cholesterol. This balance is largely governed by the coordinated actions of two transcription factor families: the liver X receptors (LXRs) α and β [4], and the sterol regulatory element binding proteins 1 and 2 (SREBPs) [5,6]. LXRs are ligand-dependent transcription factors that are activated by their endogenous ligands when cellular cholesterol levels rise. These ligands include oxidized cholesterol derivatives (oxysterols), and intermediates and byproducts of the cholesterol synthesis pathway, the most potent being desmosterol and 24,25 epoxycholesterol [7]. On activation, LXRs induce the expression of a set of genes whose main function is to reduce

Glossary

Degradation: the process by which a protein is destroyed.

Deubiquitylase: enzyme that removes ubiquitin from substrates.

E3 ligase: enzyme that catalyzes the addition of ubiquitin to target substrates.

F-box domain: structural domain that binds the ubiquitylated substrate and then associates with the SCF complexes.

HECT (homologous to the E6AP carboxyl terminus) domain: found in E3 ligases that directly conjugate ubiquitin to their substrates.

Lysosome: membrane-bound organelle that contains hydrolytic enzymes to destroy proteins.

Proteasome: a complex of proteins that destroys ubiquitylated substrates.

RING (really interesting new gene) domain: found in E3 ligases that facilitate the conjugation of ubiquitin to its substrate from its E2.

SUMOylation: addition of a small ubiquitin-like modifier (SUMO) to target substrates.

Ubiquitin proteasome system (UPS): encompasses a set of components required for degradation including E1, E2, E3, DUBs, and proteasomes.

Ubiquitylation: conjugation of the small protein ubiquitin to target substrates.

U-box domain: closely related to the RING domain, but differing in Zn²⁺-binding.

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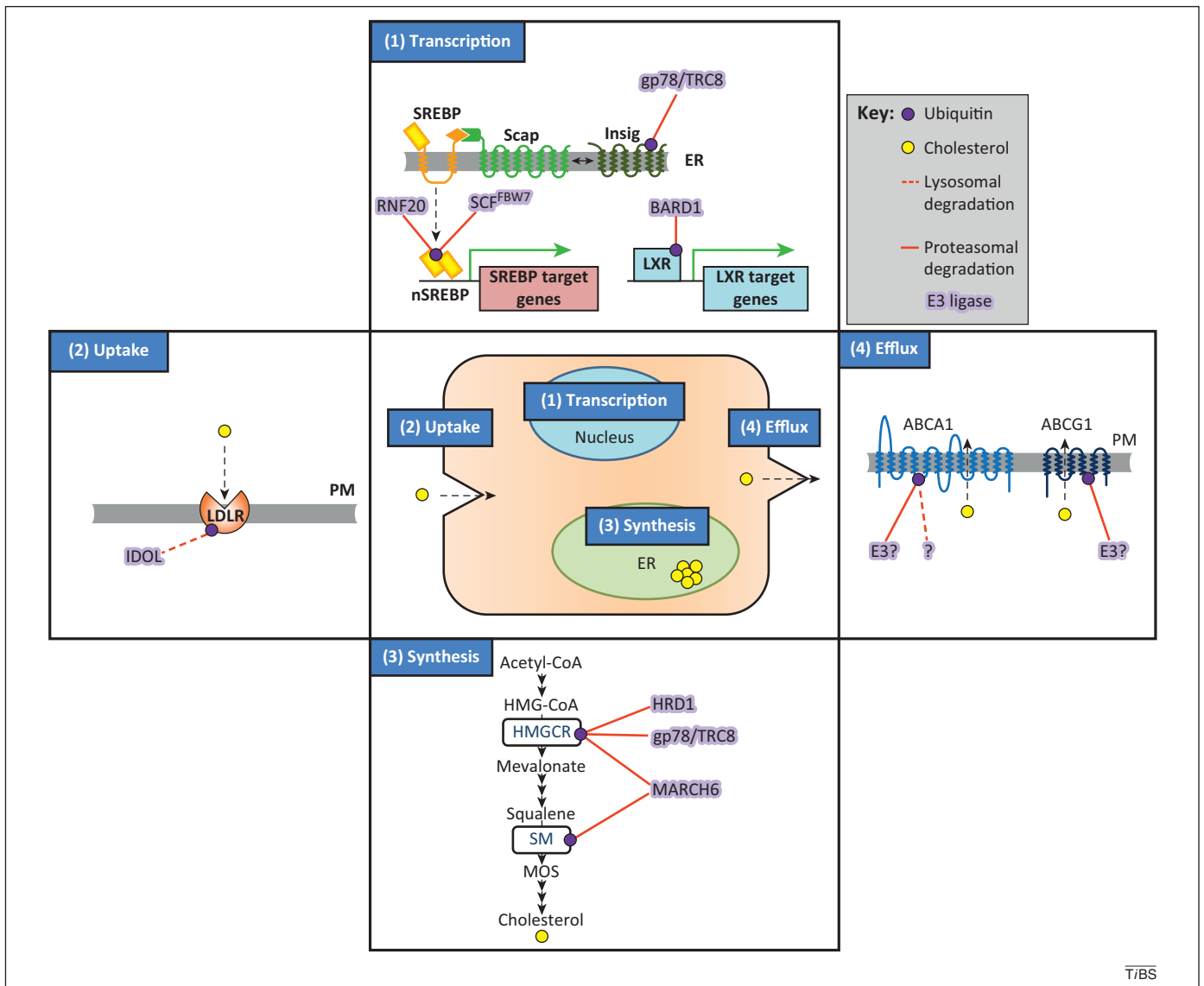


Figure 1. The ubiquitin proteasome system (UPS) in cholesterol homeostasis. E3 ligases and ubiquitylation (shown in purple) play a role in the degradation of proteins involved in each area of cholesterol homeostasis. The central panel indicates the cellular location of each process involved in cholesterol homeostasis. (1) In transcription, gp78/TRC8 ubiquitylate and promote degradation of Insig, BARD1 stimulates degradation of LXR, and active nSREBP is ubiquitylated by RNF20 and SCF^{FBW7}. (2) IDOL mediates lysosomal degradation of LDLR. (3) The key cholesterol synthesis enzymes, HMGCR and SM, are proteasomally degraded. HMGCR has four known E3 ligases: HRD1, gp78, TRC8, and MARCH6, whereas SM is only known to be ubiquitylated by MARCH6. (4) ABCA1 and ABCG1 are both ubiquitylated and degraded by as yet unknown E3 ligases. Abbreviations: ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; BARD1, breast and ovarian cancer susceptibility 1 (BRCA1)-associated RING domain 1; ER, endoplasmic reticulum; HMGCR, HMG CoA reductase; IDOL, inducible-degrader of LDLR; LDLR, low-density lipoprotein receptor; LXR, liver X receptor; MARCH6, membrane-associated ring finger (C3HC4) 6; MOS, monooxidosqualene; nSREBP, nuclear SREBP; PM, plasma membrane; Scap, SREBP cleavage activating protein; SCF^{FBW7}, Skp1-Cul1-FBW7 E3 ubiquitin ligase complex; SM, squalene monoxygenase; SREBP, sterol regulatory element binding protein; TRC8, translocation in renal cancer from chromosome 8.

Table 1. E3 ligases involved in cholesterol homeostasis

E3 ligase	Other names	Type	Target(s)	Process	Location
BARD1	–	RING	LXR	Transcription	Nucleus
FBW7	–	F-box and WD repeat	SREBP		
RNF20	BRE1A	RING	SREBP-1c		
gp78	AMFR, RNF45	RING	HMGCR, Insigs, ApoB	Synthesis	ER membrane
MARCH6	Doa10 (yeast), RNF176, TEB4	RING	HMGCR, SM		
TRC8	RNF139	RING	Insigs, HMGCR		
HRD1	SYVN1	RING	HMGCR, ABCG5, ABCG8		
CHIP	STUB1	U-box	NPC1	Transport	Cytosol
IDOL	MYLIP	RING	LDLR, ApoER2, VLDLR	Uptake	Plasma membrane
RNF5	–	RING	ABCG5, ABCG8	Efflux	

Table 2. Degradation of key cholesterol homeostasis components

Component	Function in cholesterol homeostasis	Ubiquitylation sites ^a	E3 ligase(s)	Location	Refs
ABCA1	Efflux	Unknown	Unknown	Proteasome and lysosome	[72,73,75]
ABCG1	Efflux	Unknown	Unknown	Proteasome	[75]
ABCG5	Efflux	Unknown	RNF5, HRD1	Proteasome	[74]
ABCG8	Efflux	Unknown	RNF5, HRD1	Proteasome	[74]
ApoB	Secretion	K2911, K2915	gp78	Proteasome	[80]
ApoER2	Uptake	K868	IDOL	Lysosome	[58]
HMGCR	Synthesis	K142, K248, K288, K299, K351, K362, K381, K396, K401, K460, K474, K480, K502, K619, K633, K662, K666, K692, K704,	gp78, TRC8, HRD1, MARCH6	Proteasome	[33,39–41]
Insig-1	Interacts with Scap and HMGCR	K156, K158, K273	gp78	Proteasome	[37,38]
Insig-2	Interacts with Scap and HMGCR	K100, K102	gp78	Proteasome	[37,38]
LDLR	Uptake	K830 and C839 required ^b	IDOL	Lysosome	[56]
LXR	Transcription	Unknown	BARD1	Proteasome	[28,31]
NPC1	Intracellular transport	K84, K392, K585, K822, K1180	CHIP	Proteasome	[79]
SM	Synthesis	K288, K290, K426	MARCH6	Proteasome	[41,43]
SREBP-1	Transcription	K256, K313, K347, K356, K470, K587, K675, K924, K1070, K1121	SCF ^{FBW7} and RNF20 (SREBP-1c)	Proteasome	[11,16]
SREBP-2	Transcription	K354, K405, K464, K579, K660, K817, K907, K989, K1067, K1115	SCF ^{FBW7}	Proteasome	[11]
VLDLR	Uptake	K828	IDOL	Lysosome	[58]

^aPublished human ubiquitylation sites from PhosphoSitePlus [1].

^bAs indicated in [56].

the cellular cholesterol burden, including the cholesterol efflux pumps ABCA1 and ABCG1, and the E3 ligase IDOL (inducible degrader of the LDL receptor, LDLR) [4]. By contrast, when cellular sterol levels decline SREBPs are activated (see below) and induce the full set of genes required for *de novo* cholesterol biosynthesis [6,8]. In addition, SREBPs induce expression of LDLR, leading to increased uptake of LDL-cholesterol. Components of the LXR and SREBP pathway, as well as the transcription factors themselves, are subject to ubiquitylation-dependent control (Figure 1, Table 2).

SREBP

Three isoforms of SREBP coordinate the transcriptional program of lipid metabolism; despite some overlap in their function, SREBP-1c and SREBP-2 control mostly fatty acid and cholesterol metabolism, respectively, whereas SREBP-1a controls both processes [8].

To induce their transcriptional program, SREBPs need to be processed from their precursor, transcriptionally-inactive form to release the active N-terminal basic helix-loop-helix domain [5]. In mammalian cells, this process is largely governed by the cholesterol content in the endoplasmic reticulum (ER), sensed by Scap [9]. When cholesterol levels are low, Scap escorts SREBP to the Golgi apparatus for subsequent processing and activation. When there is sufficient cholesterol, the SREBP–Scap complex associates with Insig proteins and is retained in the ER, preventing SREBP maturation. This coordinated process

includes several steps in which the UPS contributes to regulation of SREBP-mediated signaling.

Focusing on the nuclear forms of SREBPs (nSREBPs), Hirano *et al.* [10] were the first to point out that both SREBP-1 and SREBP-2 were stabilized, and transcription of their target genes was increased, by blocking the proteasome. Central to this process is the Skp1–Cul1–FBW7 E3 ubiquitin ligase complex (SCF^{FBW7}) [11]. The signal promoting ubiquitylation and subsequent proteasomal degradation of nSREBP is a hierarchical cascade of phosphorylation of three adjacent SREBP threonine and serine residues by the kinase GSK-3 β [11,12]. This cascade seems to be specific for SREBP-1 because the corresponding serine in SREBP-2 is not phosphorylated [12]. Once phosphorylated, this ‘phosphodegron’ recruits FBW7, the substrate recognition component of the SCF^{FBW7} complex, to nSREBPs resulting in their ubiquitylation and degradation. Importantly, DNA binding promotes phosphorylation and subsequent SCF^{FBW7}-mediated ubiquitylation of nSREBP [13], a finding that likely explains why degradation of nSREBPs is coupled to their transcriptional activity (‘destruction by activation’) [14]. This system may also underlie, at least in part, the mechanism behind the ability of hepatic insulin signaling to promote SREBP transcriptional activity because engagement of the insulin receptor promotes Akt-dependent phosphorylation of GSK-3 β , a modification that inhibits its kinase activity [11,12]. Insulin receptor signaling was recently shown to also reduce the protein level of CDK8 and its regulatory cofactor cyclin

C, that together form a kinase complex implicated in phosphorylation-dependent ubiquitylation of nSREBP-1 [15]. The identity of the ubiquitin ligase acting downstream of CDK8-dependent phosphorylation of nSREBP-1 has not yet been determined. While SCF^{F^{EW}7} represents a potential candidate for this activity, a recent study identified an additional E3 ligase, RNF20, that promotes hepatic degradation of SREBP-1c [16], further emphasizing the complex regulation of nSREBPs by the UPS.

Next to phosphorylation, acetylation of nSREBP also controls its ubiquitylation. In contrast to phosphorylation, acetylation stabilizes nSREBPs and enhances their transcriptional activity, presumably by directly competing with ubiquitylation for the same lysine residues [17]. The deacetylase SIRT1 can directly remove acetyl groups from all nSREBPs, thereby promoting their ubiquitylation and subsequent degradation [18,19]. Similarly, SUMOylation of SREBPs also negatively regulates the transcriptional activity of SREBP-1 and -2 [20,21]. However, whether this modification is coupled to subsequent ubiquitylation and degradation of SREBPs is not fully clear. Combined, SUMOylation of SREBPs and the SIRT1-dependent acetylation switch could explain, at least in part, how SREBP signaling is shut down in the fasted state when SIRT1 activity and PKA-stimulated SUMOylation of SREBP-1c increase.

The UPS has also been implicated in controlling maturation of SREBP in the ER in a process involving the E3 ligase TRC8 (translocation in renal cancer from chromosome 8). Originally identified as a tumor-suppressor gene in renal cell carcinoma [22], TRC8 is a multimembrane-spanning E3 ligase located in the ER [23]. Overexpression of TRC8 inhibits cell growth, an effect attributed to inhibition of protein translation and cholesterol and lipid synthesis [24]. Indeed, TRC8 has been shown to promote ubiquitylation of Insig, the proteins responsible for ER retention of SREBPs, and HMGCR (3-hydroxy-3-methylglutaryl-CoA reductase, see next section), a key cholesterol synthesis enzyme. In addition, two recent reports indicate that TRC8 also limits the maturation of SREBPs, although the underlying mechanism is still unclear [25,26]. Whereas one study proposes that TRC8 associates with SREBP and prevents its trafficking to the Golgi [25], the second study suggests that TRC8 reduces the level of precursor SREBP in a process sensitive to proteasomal degradation [26]. Despite the mechanistic ambiguity, these experiments implicate the E3 ligase TRC8 in regulation of cholesterol homeostasis through multiple, yet unresolved, mechanisms.

LXR

Despite their important roles in cholesterol homeostasis and the potential therapeutic benefits of their activation [27], there is limited information regarding the ubiquitylation of LXRs. However, it is unclear whether this indicates a lack of research in this area, or that ubiquitylation of LXRs is not a major avenue for their regulation. Only two studies specifically address ubiquitylation of LXR, and these present conflicting scenarios. The first study [28] showed that SIRT1 binds to LXR, promoting its deacetylation, which in turn promotes its ubiquitylation and release from DNA. This then allows subsequent recruitment of

new LXR to further enhance expression of LXR target genes. This mechanism suggests that increased proteasomal degradation (and thus higher turnover of LXR) leads to higher LXR activity, akin to what has been proposed for the estrogen and thyroid receptors [29,30]. By contrast, the second study found that decreased proteasomal degradation increased LXR activity [31]. BARD1 [breast and ovarian cancer susceptibility 1 (BRCA1)-associated RING domain 1] promoted basal ubiquitylation of LXR, but binding of ligand displaced BARD1 and prevented LXR ubiquitylation. Thus, the ligand-dependent decrease in ubiquitylation and degradation was proposed to increase LXR signaling. More work is clearly necessary to resolve these conflicting studies and clarify how ubiquitylation and proteasomal degradation regulate LXR signaling.

Although much is known about the ubiquitylation and degradation of SREBPs, this is not the case for LXR. It will be interesting to discover whether ubiquitylation plays a similarly important role in regulation of LXR, or whether this is specific to SREBP, the transcription factor responsible for upregulating the entire cholesterol synthesis pathway.

UPS and the flux-controlling enzymes in cholesterol synthesis

The cholesterol synthesis pathway involves over 20 enzymes. The two key flux controlling enzymes are HMGCR and squalene monooxygenase (SM, also known as squalene epoxidase, SQLE). Both enzymes are ubiquitylated, which leads to their degradation [32,33] (Figure 1). In addition, proteomic screens have identified ubiquitylation sites on many other cholesterol synthesis enzymes [7] (Table 2), representing a possible avenue for future research in this area. However, not all of these are robustly regulated by the UPS. For example, DHCR24 has seven known ubiquitylation sites [1], but appears to be very stable [34].

HMGCR

One of the original examples of ER-associated degradation (ERAD), HMGCR has been the most extensively studied cholesterol-related substrate of the UPS. As the target of the statins, HMGCR is of major interest clinically and is generally considered the key rate-controlling step in cholesterol synthesis. In mammalian cells, the E3 ligase HRD1 appears to be involved in the basal degradation of HMGCR but not in sterol-regulated degradation [33]. In yeast, regulated degradation of HMGCR seems to occur through a 'controlled structural transition' of the HMGCR transmembrane domain that allows the HRD quality-control machinery to recognize it as a substrate [35].

The first E3 ligase implicated in the sterol-dependent degradation of mammalian HMGCR was gp78 [36]. Embedded in the ER membrane, gp78 associates with and degrades the ER resident proteins, Insig-1 and Insig-2, in sterol-depleted cells [37,38]. The Insig proteins are required both for the sterol-dependent degradation of HMGCR and for preventing the proteolytic activation of SREBP. Insig provides a scaffold to recruit gp78 which then promotes HMGCR degradation.

Further studies have identified additional E3 ligases involved in HMGCR regulation. Knockdown experiments

have suggested that HMGCR degradation is mediated primarily through gp78 and another membrane-bound E3 ligase, TRC8 [39]. Studies in gp78-knockout mice, including in isolated hepatocytes, also supported a role for gp78 in HMGCR degradation [40]. Puzzlingly, Tsai and colleagues [37] used similar experimental settings and approaches but found no evidence to support roles for either gp78 or TRC8 in sterol-mediated HMGCR degradation in three model cell lines. This controversy opens up the possibility that other E3 ligase(s) may be involved. Indeed, a fourth membrane-associated E3 ligase, MARCH6 [membrane-associated ring finger (C3HC4) 6], has recently been implicated in controlling basal, and perhaps to some extent sterol-mediated, degradation of HMGCR [41]. MARCH6 (also known as TEB4) is the mammalian homolog of Doa10, the other yeast E3 ligase besides Hrd1 that is involved in ERAD [42]. As discussed in the next section, MARCH6 also targets SM [41,43]. Further work will be necessary to ascertain the exact contribution of each E3 ligase in determining HMGCR levels, which is likely to differ between cell types and conditions.

Squalene monoxygenase

The enzyme SM converts squalene to squalene-2,3-epoxide and represents an underappreciated rate-limiting step in the biosynthesis of cholesterol. Unlike the well-appreciated rate-limiting enzyme HMGCR, the post-transcriptional regulation of SM has only recently received attention [7].

An intriguing difference between sterol-stimulated degradation of HMGCR and SM is that degradation of the former is stimulated by oxysterols and metabolic intermediates of the mevalonate pathway, whereas that of SM is accelerated by cholesterol itself [32]. The identity of the E3 ligase mediating SM ubiquitylation was recently identified as MARCH6 [41,43]. MARCH6 is a multispanning ER membrane protein implicated as a component of the ERAD system, but DIO2 is its only previously described mammalian target [44]. Importantly, loss of MARCH6 activity in mammalian cells increases abundance of SM protein, as expected from reduced proteasomal degradation, and this corresponds with altered metabolic flux through the mevalonate pathway [41]. This regulatory system is potentially conserved in plants [45] and partially conserved in yeast. Similarly to MARCH6, the yeast homolog Doa10 promotes degradation of ERG1, the SM homolog [43]; however, the first 100 amino acids of SM that are necessary and sufficient for cholesterol-dependent degradation are lacking in lower organisms such as yeast, suggesting that the mechanism through which Doa10 regulates degradation of ERG1 is likely distinct [32].

MARCH6 does not seem to be transcriptionally regulated by cellular cholesterol levels [41]. Instead, cholesterol acts to promote MARCH6 activity (observed as degradation of SM), while unsaturated fatty acids attenuate this by inhibiting the interaction between SM and MARCH6 [32,46]. At present, precisely how cholesterol induces degradation of SM is unclear. However, it probably involves both altered membrane properties and specific cholesterol interactions, considering that cholesterol directly binds to SM [47], and the enantiomer of cholesterol can also induce SM degradation by affecting membrane properties alone [48].

An additional difference between sterol-stimulated degradation of HMGCR and SM is that, unlike the former, degradation of SM does not require Insig or Scap, nor any SREBP-2-dependent genes [32]. In fact, the structural and accessory proteins playing a role in SM degradation by MARCH6 remain to be elucidated. As indicated above, MARCH6 is able to control the two key rate-limiting steps of the mevalonate pathway, HMGCR and SM. By using distinct sterol triggers, this may enable MARCH6 to control the flux through the mevalonate pathway depending on demand. For example, cholesterol synthesis could be selectively shut down while sparing the production of important isoprenoid products [41].

Both HMGCR and SM are regulated by ubiquitin-dependent degradation, and research is ongoing into both of these enzymes. Other enzymes in the cholesterol synthesis pathway may be similarly degraded, and this work will undoubtedly be explored in future studies. Cholesterol synthesis is an important but energetically expensive process for obtaining cholesterol, and we therefore next discuss the alternative, cholesterol uptake.

UPS in uptake of cholesterol

LDLR is the major endocytic route for uptake of exogenous cholesterol by cells, and is transcriptionally regulated by SREBP. As such, mutations in the LDLR are the leading cause for development of familial hypercholesterolemia [49]. Next to transcriptional regulation, two additional post-translational mechanisms that control LDLR abundance have been established, involving IDOL and PCSK9 (proprotein convertase subtilisin-kexin isotype 9).

PCSK9 and LDLR are transcriptionally coregulated by SREBPs in hepatocytes [50]. This seems counterintuitive because PCSK9 then degrades LDLR, but may prevent hepatic uptake of secreted VLDL particles, ensuring a supply of lipoprotein-derived cholesterol to peripheral tissues. In hepatocytes, PCSK9 is autocatalytically cleaved and subsequently secreted, where it can bind an extracellular domain of LDLR [51]. PCSK9-bound LDLR is then internalized via clathrin-mediated endocytosis, and directed to the lysosome where it is degraded [52]. This mode of LDLR degradation is ubiquitin-independent [52], does not require the intracellular tail of the receptor [53,54], and also applies to two receptors closely related to LDLR, very low density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2) [55].

The ubiquitin-dependent degradation of LDLR occurs through an IDOL-mediated mechanism. Unlike PCSK9, IDOL binds to the cytoplasmic tail of LDLR and acts as an E3 ligase to promote ubiquitylation of specific residues in this domain, leading to lysosomal degradation of the receptor. IDOL was first named and its function characterized in 2009, when it was found to be under the transcriptional control of LXR [56].

Our understanding of the LXR–IDOL–LDLR axis is rapidly developing [57]. Similarly to PCSK9, IDOL also promotes the degradation of the VLDLR and ApoER2 [58]. Degradation of these receptors requires interaction of their cytoplasmic tails with the N-terminal FERM domain of IDOL [59,60]. The C terminus of IDOL contains a RING domain, typical of the eponymous family of E3

ligases, which is essential for its function [59,61]. The residues in the cytoplasmic tail of LDLR that are required for binding to IDOL are in close proximity to the membrane and to the residues required for ubiquitylation [59,60] (Table 2).

Although IDOL can interact with LDLR at multiple steps in its cellular itinerary, plasma membrane-localized LDLR seems to be particularly sensitive to IDOL-mediated ubiquitylation [59,62]. Once ubiquitylated, LDLR is rapidly removed from the plasma membrane and is internalized via a clathrin-, caveolae-, and dynamin-independent pathway that requires sorting by the ESCRT (endosomal sorting complexes required for transport) system [61,62]. This cellular route for LDLR degradation is distinct from that used by PCSK9 [52] and that used for LDL uptake. Therefore, even though both PCSK9 and IDOL ultimately degrade LDLR, they achieve this by independent mechanisms.

There is considerable interest in therapeutically targeting the degradation of LDLR to lower cholesterol levels because statins, which represent the mainstay cholesterol-lowering treatment, are not free from limitations [63]. These approaches mostly target PCSK9 (e.g., [51]), but also IDOL (e.g., [64]), or both (e.g., [65]). The recent finding that individuals carrying a loss-of-function IDOL allele have lower circulating levels of LDL further supports targeting IDOL in hypercholesterolemia [66].

Uptake of cholesterol through the LDLR is one way in which cells interact with circulating cholesterol, and this is exquisitely controlled by degradation. To return excess cholesterol to the circulation, reverse cholesterol transport involving efflux is employed.

UPS in cholesterol efflux

To decrease cellular cholesterol levels, ApoA-I is loaded with cholesterol and phospholipids via ABCA1, ABCG1, and in some tissues ABCG4, to form high-density lipoprotein (HDL) particles [67]. HDL is then transported to the liver in a process termed 'reverse cholesterol transport', and HDL-cholesterol is taken up for subsequent biliary efflux by the ABCG5/ABCG8 heterodimeric transporters [4]. The ability of cells to promote cholesterol efflux has been particularly well studied in the context of macrophages given their crucial function in development of atherosclerotic disease. Activation of the LXR genetic program (discussed earlier) in response to elevated cellular cholesterol levels promotes cholesterol efflux, primarily as a result of transcriptional induction of the cholesterol transporters ABCA1, ABCG1, ABCG5, ABCG8, and ApoE [4]. However, there is accumulating evidence that, in addition to transcriptional regulation, levels of the aforementioned transporters, and thus cholesterol efflux, are also subject to post-translational regulation.

ABCA1 is the primary transporter that mediates efflux of cholesterol and phospholipids to nascent HDL particles. Several distinct processes have been implicated in controlling degradation of ABCA1. The presence of a PEST-sequence in the C terminus of ABCA1 was recognized early on as a factor promoting degradation of the transporter [68,69]. This pathway seems to be specifically inhibited by the presence of exogenous ApoA-I [70,71]. Ubiquitylation

has not been implicated in this PEST-dependent degradation pathway, but is nevertheless important in ABCA1 turnover. Somewhat confusingly, ubiquitylation can lead to both lysosomal [72] and proteasomal [73] degradation of ABCA1, and the signals that determine which degradative pathway operates are presently unclear.

Two other cholesterol efflux transporters, ABCG5 and ABCG8, were shown to be degraded via the action of the E3 ligases RNF5 and HRD1 [74]. Although direct ubiquitylation of these two transporters was not demonstrated, these ligases may contribute to regulation of cholesterol efflux in the intestine and liver, the major sites of ABCG5 and ABCG8 expression [4].

The UPS also plays an important role in the degradation of ABCG1 [75]. The degradation of both ABCG1 and ABCA1 is intrinsically coupled to their physiological functions. In response to cholesterol loading, both transporters are stabilized due to reduced ubiquitylation and proteasomal degradation [75]. This in turn increases cellular cholesterol efflux capacity. Blocking the proteasome promoted cellular cholesterol efflux *in vitro* [75], and remarkably also enhanced *in vivo* cholesterol efflux [73]. Many of the molecular determinants controlling the degradation of ABCA1 and ABCG1 remain elusive, including the E3 ligases responsible for their ubiquitylation. Although a major challenge, identification of these ligases may allow development of degradation inhibitors as a therapeutic strategy to increase reverse cholesterol transport (removal of excess peripheral cholesterol for excretion) and combat coronary artery disease.

Deubiquitylases in cholesterol homeostasis

Substrate ubiquitylation, promoted by E3 ubiquitin ligases, can be effectively reversed through the activity of the approximately 100 deubiquitylases (DUBs) encoded in the human genome [76]. As such, the E3-DUB balance determines the ubiquitylation state of the substrate protein. In contrast to E3 ligases, whose role in cholesterol metabolism has gained attention in recent years, the role of DUBs in this process is largely unexplored. With the exception of LDLR, no DUBs have been reported to directly affect the ubiquitylation status of the proteins discussed in this review thus far. Two studies [61,62] identified ubiquitin-specific protease 8 (USP8) as a regulator of LDLR degradation. USP8 is an ESCRT-associated DUB and acts to salvage ubiquitin from ubiquitylated cargo before it enters multivesicular bodies (MVBs) for subsequent lysosomal degradation [77]. Removal of ubiquitin from polyubiquitylated LDLR by USP8 – but not by another ESCRT-associated DUB, AMSH (associated molecule with the SH3 domain of STAM) – is a necessary step for receptor entry into MVBs, and is essential for promoting lysosomal degradation of the LDLR downstream of IDOL-mediated ubiquitylation [61,62]. However, it is likely that the role of USP8 in controlling LDLR degradation is not specific, but rather is related to its function in sorting ubiquitylated cargo through the ESCRT system. Given that regulation of E3 ligase activity by DUBs is an emerging concept [78], it is likely that additional DUBs influencing cholesterol metabolism will be discovered.

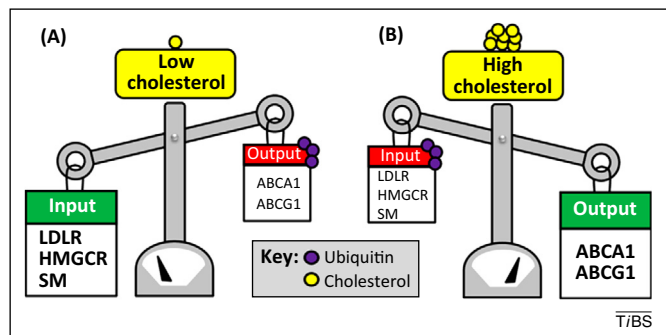


Figure 2. The UPS and downs of cholesterol homeostasis. **(A)** Under conditions of low cholesterol, LDLR, HMGCR, and SM are stabilized to increase cholesterol synthesis and uptake, whereas ABCA1 and ABCG1 are degraded. **(B)** Under conditions of high cholesterol, ABCA1 and ABCG1 are stabilized to increase cholesterol efflux, whereas LDLR, HMGCR, and SM are degraded.

Concluding remarks

In summary, ubiquitylation and degradation is a recurring theme in most if not all aspects of cholesterol homeostasis, including transcriptional programming (SREBP, LXR), cholesterol synthesis (HMGCR, SM), uptake (LDLR), and efflux (ABCA1, ABCG1). As a result, cells are able to rapidly toggle between low and high cholesterol conditions through increased stabilization or turnover of these key proteins (Figure 2). Ubiquitylation is a common factor in these processes, although degradation does not always occur via the proteasome (e.g., LDLR). The UPS also plays roles in other cholesterol-related processes, including intracellular transport via Niemann–Pick disease type C (NPC1) [79] and ApoB-containing lipoprotein secretion [80].

Many unanswered questions remain (Box 1). Multiple E3 ligases may target an individual substrate, as is apparently the case for HMGCR. Which E3 operates, or at least predominates, probably depends on the cellular and/or (patho)physiological context, providing versatile and exquisite regulation. It seems that the quality-control machinery has been co-opted for meeting the cellular demands for cholesterol such that hydrophobic substrates such as HMGCR are recognized by various E3 ligases, possibly by evaluating the folding state rather than specific sequence motifs within the target [35]. Considering the hydrophobic nature of cholesterol, the protein machinery that has evolved to deal with cholesterol metabolism is also hydrophobic and predominantly membrane-bound, often anchored in the ER (Table 1). One of the major challenges

Box 1. Outstanding questions

- What other players in cholesterol homeostasis are subjected to regulated degradation through ubiquitylation?
- Why does HMGCR appear to have numerous E3 ligases?
- Do cholesterol-related ERAD substrates have specific degrons or less-specific regions (e.g., exposed hydrophobic stretches) that are recognized by the quality-control machinery, as proposed for yeast HMGCR [35]?
- Why do both PCSK9 and IDOL exist to degrade LDLR?
- Which deubiquitylases are involved in cholesterol homeostasis?
- How do other post-translational modifications such as phosphorylation or acetylation affect ubiquitylation and impact degradation?
- Can components of the UPS be targeted to treat cholesterol-related diseases?

in studying these proteins is navigating this membranous world, with the inherent technical difficulties that often entail. However, this area is attracting increased attention [81,82], suggesting that there are potentially rich rewards on offer for better understanding how cholesterol homeostasis is acutely controlled through the UPS and protein degradation.

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