# Mechanisms and regulation of cholesterol homeostasis

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Abstract | Cholesterol homeostasis is vital for proper cellular and systemic functions. Disturbed cholesterol balance underlies not only cardiovascular disease but also an increasing number of other diseases such as neurodegenerative diseases and cancers. The cellular cholesterol level reflects the dynamic balance between biosynthesis, uptake, export and esterification — a process in which cholesterol is converted to neutral cholesteryl esters either for storage in lipid droplets or for secretion as constituents of lipoproteins. In this Review, we discuss the latest advances regarding how each of the four parts of cholesterol metabolism is executed and regulated. The key factors governing these pathways and the major mechanisms by which they respond to varying sterol levels are described. Finally, we discuss how these pathways function in a concerted manner to maintain cholesterol homeostasis.

Sterols
A subgroup of steroids with a hydroxyl group at the C-3 position of the A-ring. A steroid is a biologically active organic compound with four rings (A–D) arranged in a specific molecular configuration.

Sphingolipids
A class of lipids with a polar head group and two non-polar tails. The core of a sphingolipid is an amino alcohol called sphingosine.

Glycosylphosphatidylinositol-anchored proteins
Proteins with glycosylphosphatidylinositol (GPI) attached at the C-termini. The GPI anchor is a unique mode of protein binding to the plasma membrane.

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Since its initial isolation from human gallstones more than two centuries ago, the lipid cholesterol ($C_{27}H_{46}O$) has continued to occupy scientists and clinicians, and its physiological and pathological importance cannot be denied. In particular, levels of cholesterol have been strongly associated with the risk of atherosclerosis and cardiovascular disease. Like other sterols, cholesterol is largely hydrophobic. It is biosynthesized by all mammalian cells and predominantly localizes to cell membranes, where it interacts with the adjacent lipids to regulate rigidity, fluidity and permeability of the bilayer. In addition, cholesterol can bind numerous transmembrane proteins, helping to maintain or alter their conformations. Cholesterol also interacts with numerous sterol transport proteins that facilitate cholesterol trafficking and regulate its subcellular distribution[1,2].

On the plasma membrane, where the majority of total cellular cholesterol resides[3], cholesterol is often packed with sphingolipids and glycosylphosphatidylinositol-anchored proteins, forming dynamic, nanoscale microdomains that can coalesce to form relatively ordered structures with established roles in the modulation of membrane trafficking, signal transduction and host–pathogen interactions[4]. Beyond its role in membrane structure and function, cholesterol via enzymatic and non-enzymatic routes gives rise to various oxysterols[5], some of which are further metabolized into bile acids. Oxidative cleavage of the side chain of cholesterol generates pregnenolone, the common precursor to all other steroid hormones. These cholesterol derivatives are actively engaged in a diverse array of biological processes. Further, cholesterol can covalently modify Hedgehog and Smoothened proteins, ensuring proper Hedgehog signalling and embryonic development[6,7]. Given such crucial functions in diverse physiological contexts, disrupted metabolism of cholesterol can cause several congenital human diseases (TABLE 1). There is also increasing evidence of a close relationship between cholesterol metabolism and acquired diseases that include, as discussed above, cardiovascular disorders but also Alzheimer disease and many types of cancer[8–10].

A series of landmark discoveries led to the current understanding of cholesterol metabolism. It is now known that cellular cholesterol levels are determined by the interplay between de novo biosynthesis, uptake, export and storage (FIG. 1). In brief, cholesterol synthesis starts from acetyl-CoA and involves concerted actions of more than 20 enzymes, most of which localize in the membrane of the endoplasmic reticulum (ER). Cholesterol can also be derived from dietary sources. In this case, cholesterol is absorbed by Niemann–Pick type C1 (NPC1)-like 1 (NPC1L1) protein on the apical surface of enterocytes in the intestine[11], which then releases this dietary cholesterol as chylomicrons, from which cholesterol is taken up by the liver. The liver — the main site of cholesterol biosynthesis — delivers both endogenously synthesized and exogenously acquired cholesterol to the bloodstream as very-low-density lipoproteins (VLDLs). After processing in the bloodstream, the VLDLs generate circulating low-density lipoproteins (LDLs), which can be taken up by peripheral cells via receptor-mediated endocytosis[12]. Within the cell, cholesterol is dynamically transported between various organelles by vesicular and non-vesicular mechanisms to fulfil its multifaceted functions[13]. Surplus cholesterol can be exported to lipid-free

Table 1 | **Genetic diseases caused by disturbed cholesterol homeostasis**

| Disease | Underlying mechanism | Featured symptoms | Mutant genes | Refs |
|---|---|---|---|---|
| Niemann–Pick type C disease | Cholesterol accumulation within lysosomes | Neurodegeneration; enlarged liver and spleen | *NPC1*, *NPC2* | 297 |
| Schnyder corneal dystrophy | Enhanced cholesterol production due to HMGCR stabilization | Cholesterol accumulation in the cornea; corneal opacification | *UBIAD1* | 298 |
| Smith–Lemli–Opitz syndrome | 7-Dehydrocholesterol accumulation and cholesterol deficiency | Mental and growth retardation; cleft palate; malformations of heart, kidney and genitals (males); polydactyly or syndactyly | *DHCR7* | 299 |
| Familial hypercholesterolaemia | Impaired LDLR-mediated LDL uptake | Markedly elevated plasma levels of cholesterol containing LDLs; premature coronary heart disease | *LDLR*, *APOB*[a], *PCSK9*[b], *ARH* | 300,301 |
| Tangier disease | Impaired ABCA1-mediated cholesterol efflux | Extremely low HDL and apoA-I; massive deposition of cholesteryl esters in macrophage-rich tissues; increased risk of coronary artery disease | *ABCA1* | 302 |
| Sitosterolaemia | Impaired ABCG5 and ABCG8-mediated cholesterol efflux | Elevated plasma and tissue levels of plant sterols and cholesterol; xanthomas; premature cardiovascular disease | *ABCG5*, *ABCG8* | 303 |

ABCA1, ATP-binding cassette subfamily A member 1; ABCG, ATP-binding cassette subfamily G member; apo, apolipoprotein; ARH, autosomal recessive hypercholesterolaemia; DHCR7, 7-dehydrocholesterol reductase; HDL, high-density lipoprotein; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LDL, low-density lipoprotein; LDLR, LDL receptor; NPC, Niemann–Pick type C; PCSK9, proprotein convertase subtilisin/kexin type 9; UBIAD1, UbiA prenyltransferase domain-containing protein 1. [a]The mutations impair LDL–LDLR association. [b]Gain-of-function mutations that promote LDLR degradation.

or lipid-poor apolipoprotein A-I (apoA-I) produced by the liver, intestine and pancreas via passive or active mechanisms to generate high-density lipoproteins (HDLs)[14]. Excess cholesterol is esterified by acyl coenzyme A:cholesterol acyltransferase (ACAT; also known as SOAT) to cholesteryl esters[15], which are either stored as a cholesterol reservoir in cytosolic lipid droplets or released as a major constituent of plasma lipoproteins, including the aforementioned chylomicrons, VLDLs, LDLs and HDLs. HDLs are finally transported from peripheral tissues back to the liver and intestine, where cholesterol is recycled or eliminated, as well as to steroidogenic organs, where cholesterol is used to generate steroid hormones. These processes are tightly governed by multiple transcriptional and post-translational regulatory circuits that function as an integrative system capable of responding to varying intracellular and physiological cues to ensure cholesterol homeostasis.

In this Review, we summarize the current knowledge of key molecular pathways involved in each major aspect of cellular cholesterol metabolism. We also discuss how these pathways are coordinated to maintain cholesterol homeostasis under perturbations, prominently in cholesterol-rich conditions, which occur commonly as a result of a western-style diet. The mechanisms underlying vesicular and non-vesicular cholesterol trafficking in cells will only be briefly mentioned and the reader is referred to other recent reviews on this topic[1,2,16,17].

## Regulation of cholesterol biosynthesis

Almost all cells can synthesize cholesterol, and about 50% of total synthesis in humans occurs in the liver[18]. Cholesterol biosynthesis is an energetically expensive process requiring significant inputs from acetyl-CoA, ATP, oxygen and the reducing factors NADPH and NADH. Accordingly, it must be tightly regulated. In this section, we briefly review three crucial players of the cholesterol biosynthetic pathway, namely, sterol regulatory element-binding protein 2 (SREBP2), which functions as a master transcriptional regulator of cholesterol biosynthesis, and two rate-limiting enzymes of the biosynthetic pathway: 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) and squalene monooxygenase (FIG. 2).

### *Regulation of SREBP2*

Three closely related isoforms of SREBPs (SREBP1a, SREBP1c and SREBP2) exist in mammals, among which SREBP2 selectively regulates the genes encoding cholesterologenic enzymes[19,20]. SREBP2 is indispensable for embryonic development, and *Srebp2*-deficient mice die in utero with limb bud malformations[21], probably as a result of impaired Hedgehog signalling. The regulation of SREBP2 is discussed below.

***Regulation of SREBP2 protein egress from the ER and its proteolytic activation.*** SREBP2 is synthesized as an ER-anchored precursor consisting of an N-terminal transcription factor domain containing a basic-helix–loop–helix–leucine zipper motif; two transmembrane segments separated by a short, lumen-facing loop; and a C-terminal regulatory domain that interacts with the WD-repeat domain of SREBP-cleavage activating protein (SCAP) in a stoichiometric ratio of 4:4 in yeast[22]. To become active, SREBP2 must translocate from the ER to the Golgi apparatus, where site 1 protease (S1P) and S2P act sequentially to liberate the N-terminal fragment from the membrane. The processed SREBP2, designated nuclear SREBP2 (nSREBP2), then enters the nucleus as a homodimer, binds to the sterol regulatory element (SRE) sequence in the promoters of target genes, including *HMGCR* and *SQLE* (encoding squalene monooxygenase), and upregulates their transcription[23].

The exit of SREBP2 precursor from the ER is regulated by cholesterol via SCAP, which binds SREBP2 via its C-terminal domain. SCAP senses and responds to ER cholesterol fluctuations by switching between open and closed conformations to modulate its binding to COPII-coated vesicles[23]. When ER membrane cholesterol is depleted, the SCAP–SREBP2 complex is sorted into COPII vesicles and moves from the ER to the Golgi for proteolytic activation of SREBP2 (FIG. 2a). As shown in

Oxysterols
The oxidized derivatives of cholesterol.

Bile acids
The hydroxylated steroids which are amphipathic and synthesized from cholesterol in the liver. Bile acids are secreted into the intestine where they play an important role in emulsifying dietary lipids to facilitate their absorption.

Niemann–Pick type C1
(NPC1). A large (1278 amino acids in humans), 13-pass transmembrane protein that binds cholesterol with the 3β-hydroxyl group and the tetracyclic ring of cholesterol buried and the iso-octyl side chain exposed via the N-terminal domain. NPC1 is ubiquitously expressed and localizes on lysosomal membrane. Mutations in *NPC1* cause 95% of NPC cases.

Chylomicrons
The triglyceride-rich lipid particles in the blood and lymph that are solely produced by the intestine. Chylomicrons deliver lipids to the liver and extrahepatic tissues. After depletion of their triglycerides by the extrahepatic tissues, chylomicrons become chylomicron remnants that are cleared by the liver.

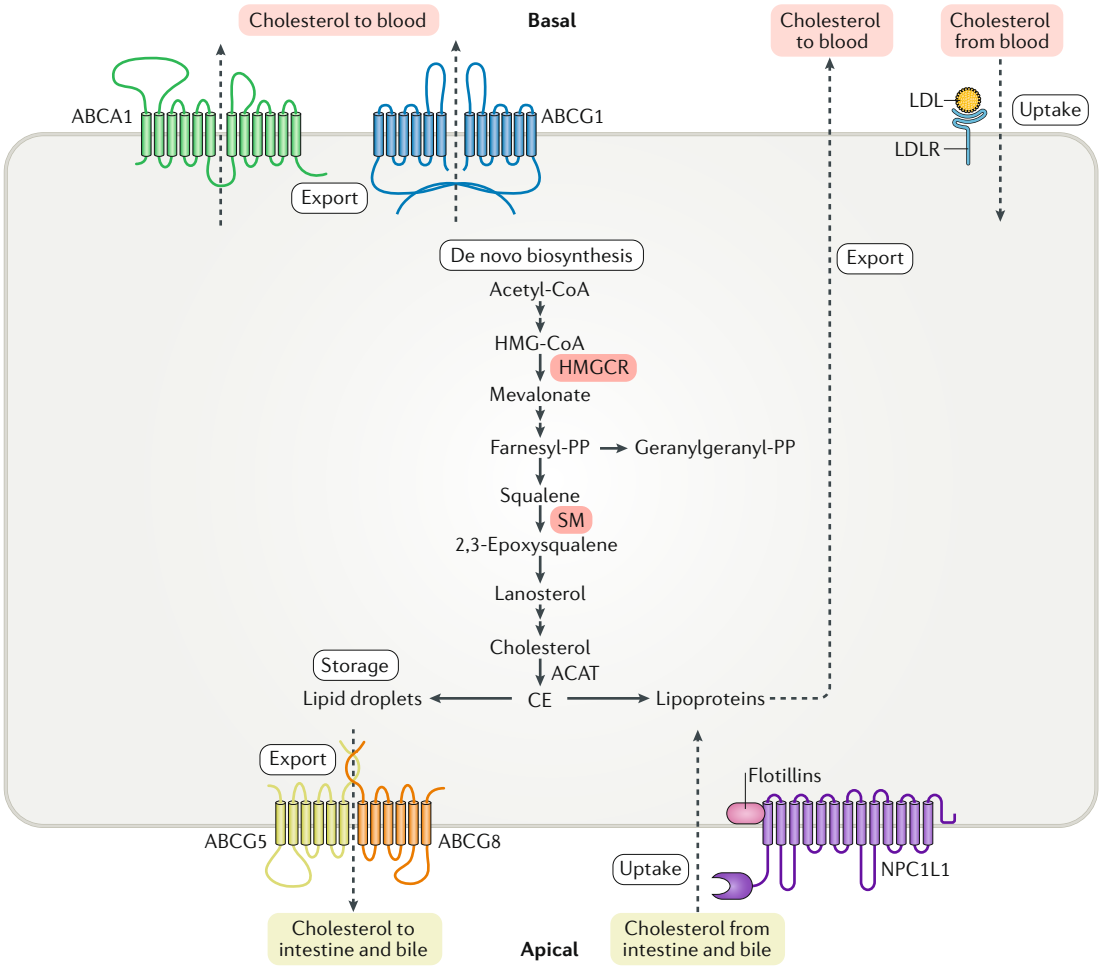



Fig. 1 | **Major pathways of cholesterol metabolism in a polarized cell.** Cholesterol is synthesized from acetyl-CoA through a series of ~30 reactions using 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) and squalene monooxygenase (SM) as the rate-limiting enzymes (highlighted in red). In addition to de novo biosynthesis, cholesterol carried by low-density lipoprotein (LDL) particles in the blood can be taken up by LDL receptor (LDLR) at the basal surface of polarized cells (such as enterocytes or hepatocytes). Free cholesterol can also be absorbed from dietary sources by enterocytes in the intestine and from bile in the biliary ducts by hepatocytes in the liver. This absorption is mediated by Niemann–Pick type C1-like 1 (NPC1L1) and the associated flotillins present on the apical surface of these cells. Excess cholesterol is exported to the blood by ATP-binding cassette subfamily A member 1 (ABCA1) or the homodimer of ATP-binding cassette subfamily G member 1 (ABCG1), or to the intestinal lumen and bile ducts by the ABCG5 and ABCG8 heterodimer. Cholesterol can also be converted to cholesteryl ester (CE) by acyl coenzyme A:cholesterol acyltransferase (ACAT; also known as SOAT) for storage in lipid droplets or for secretion as lipoproteins. CoA, coenzyme A; PP, pyrophosphate.

Chinese hamster ovary cells, when ER membrane cholesterol is above 5 mol% of total ER lipids[24], it binds to loop 1 of SCAP and triggers the SCAP sterol-sensing domain (SSD, comprising transmembrane domains 2–6) to interact with insulin-induced gene (INSIG) proteins (BOX 1; FIG. 2b). This blocks COPII binding to SCAP and causes the SCAP–SREBP2 complex to stay in the ER. Oxysterols such as 25-hydroxycholesterol are much more potent than cholesterol in triggering ER retention of the SCAP–SREBP2 complex by binding directly to INSIGs and promoting INSIG binding to SCAP[25]. In line with this, mutants of INSIG2, one of the two INSIG isoforms in mammals, that are unable to bind 25-hydroxycholesterol fail to suppress proteolytic SREBP processing[25]. The central roles of SCAP and INSIGs in sterol-regulated SREBP2 activation are highlighted by the findings that mutants with disrupted SCAP–INSIG interaction (hamster SCAP Y298C, L315F or D443N mutations) display constitutive SREBP2 cleavage and activation regardless of the sterol status[26,27], whereas those with impaired SCAP–INSIG dissociation (D428A) have attenuated SREBP2 cleavage even in the absence of sterols[28].

Sterol-induced binding of SCAP to INSIGs also stabilizes INSIG1, which otherwise undergoes degradation by the ubiquitin–proteasome pathway[29,30] (FIG. 2a). Under sterol depletion conditions, this degradation of INSIG1 contributes to rapid dissociation of the SCAP–INSIG complex and activation of the SREBP2 pathway, allowing transcription of downstream genes including *Insig1* itself[31]. The newly synthesized INSIG1 continues to be targeted for degradation unless a sufficient

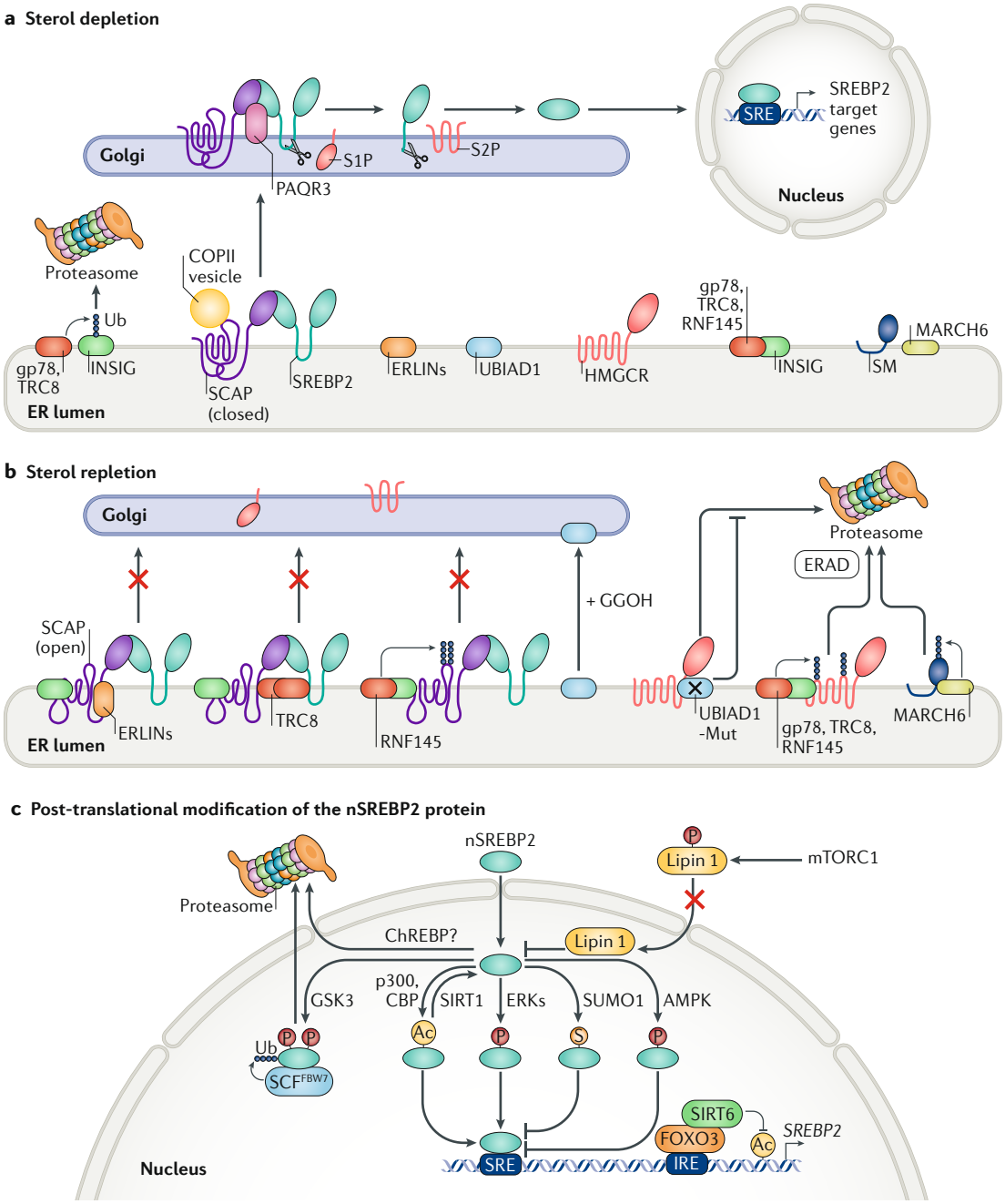
**Very-low-density lipoproteins**
(VLDLs). The triglyceride-rich lipid particles in the blood that are produced by the liver. VLDLs enable fats and cholesterol to move within the water-based bloodstream. VLDLs are converted to intermediate-density lipoproteins and low-density lipoproteins in the bloodstream.

**Low-density lipoproteins**
(LDLs). The lipid particles enriched in cholesteryl esters. Each LDL particle contains a single apolipoprotein B-100 molecule and delivers lipids, mainly cholesterol, and vitamins to extrahepatic tissues, where it is taken up by an LDL receptor.

**Apolipoprotein**
(apo). A protein that binds lipids to form lipoproteins, which then transport lipids and fat-soluble vitamins in circulation.

**COPII-coated vesicles**
The membrane vesicles coated by coatomer II (COP II), which is a type of vesicle coat protein that facilitates the formation of transport vesicles from the endoplasmic reticulum (ER). COPII-coated vesicles exit from specialized regions of the ER membrane devoid of bound ribosomes, known as 'ER exit sites', and deliver their content to the Golgi.

**Insulin-induced gene (INSIG) proteins**
INSIG proteins, including INSIG1 and INSIG2, are integral membrane proteins of the endoplasmic reticulum that mediate sterol regulation of sterol regulatory element-binding protein cleavage-activating protein (SCAP) and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase).




amount of cholesterol is produced to induce a conformational change of SCAP to which INSIG1 can bind. The dual requirement of cholesterol and INSIG1 protein for switching off the SREBP pathway constitutes a convergent feedback mechanism regulating cholesterol biosynthesis and uptake[29]. INSIG2 functions similarly to INSIG1 but does not rely on SREBP activation for production[32].

Additional negative regulation of SREBP2 egress from the ER is provided by the multimeric ER proteins ERLINs and two RING-finger ubiquitin ligases, TRC8 (also known as RNF139) and RNF145 (REFS[33–35]). ERLINs interact with the SCAP–SREBP2–INSIG complex tightly under cholesterol repletion conditions (FIG. 2b), and silencing of ERLINs stimulates SREBP2 processing as potently as cholesterol depletion does[33]. TRC8 is able to bind SREBP2 and SCAP directly to retain the complex in the ER independently of its E3 ligase activity[35], whereas RNF145 can ubiquitylate SCAP within a cytoplasmic loop essential for COPII binding[34]; these E3 ligase-mediated events impair COPII binding to the SCAP–SREBP2 complex, leading to attenuated SREBP2 processing[34,35] (FIG. 2b).

In addition to ER exit, proper ER-to-Golgi targeting and Golgi anchoring of the SCAP–SREBP2 complex are also critical for SREBP2 activation. Serine/threonine protein kinase AKT (also known as PKB) promotes anterograde trafficking of the complex towards the Golgi in the COPII vesicles[36]. At the Golgi, the transmembrane protein progestin and adipoQ receptor 3 (PAQR3) — which is transcriptionally induced under cholesterol-depleting conditions — interacts with SCAP and

RING-finger ubiquitin ligases
The largest type of E3 ubiquitin ligases with the RING (really interesting new gene) finger domains that bind two zinc ions in a unique 'cross-brace' arrangement through a defined motif of cysteine and histidine residues.

◀ Fig. 2 | **Mechanisms regulating cholesterol biosynthesis.** The key mediator of cholesterol biosynthesis is sterol regulatory element-binding protein 2 (SREBP2), which is extensively regulated on several levels. **a** | SREBP2 is synthesized on the endoplasmic reticulum (ER) but requires transfer to the Golgi for activation. In the ER, SREBP2 interacts with SREBP-cleavage activating protein (SCAP). When ER cholesterol is depleted, loop 1 and loop 7 of SCAP interact, allowing coatomer II (COPII) to bind SCAP. The SCAP–SREBP complex exits the ER and anchors to the Golgi through progestin (not shown) and adipoQ receptor 3 (PAQR3). SREBP2 then undergoes proteolytic cleavage by site 1 protease (S1P) and S2P, thereby releasing the N-terminal domain that enters the nucleus and activates transcription of target genes by binding the sterol regulatory element (SRE) in the promoter. The insulin-induced gene (INSIG) proteins in the ER, due to their dissociation from SCAP, are ubiquitylated by gp78 (also known as AMFR) and TRC8 (also known as RNF139) and degraded by the proteasome. Sterol depletion also prevents 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) from binding to INSIGs and the associated E3 ubiquitin ligases (gp78, TRC8 and RNF145), as well as preventing squalene monooxygenase (SM) from binding to another E3 ubiquitin ligase, MARCH6, thereby stabilizing HMGCR and SM to support cholesterol biosynthesis (FIG. 1). **b** | When ER cholesterol levels are high, INSIGs are recruited by SCAP, causing loop 1 and loop 7 separation and COPII detachment from SCAP. The SCAP–SREBP2–INSIG complex is further retained in the ER by ERLINs and TRC8, which directly bind to the complex, and by ubiquitylation of SCAP by RNF145. Sterols also induce INSIGs–E3 ligase complex binding to HMGCR and MARCH6 binding to SM. The ubiquitylated HMGCR and SM are eventually degraded in proteasomes through ER-associated degradation (ERAD). In addition, in the presence of sterols, UbiA prenyltransferase domain-containing protein 1 (UBIAD1) binds to HMGCR, preventing its removal through ERAD. However, when sufficient geranylgeraniol (GGOH) is generated, UBIAD1 translocates from the ER to the Golgi and no longer interacts with HMGCR, thereby accelerating HMGCR degradation. By contrast, Schnyder corneal dystrophy-associated UBIAD1 mutants (UBIAD1-Mut) are constitutively retained in the ER and delay sterol-induced ERAD of HMGCR. **c** | In the nucleus, the protein level of nuclear SREBP2 (nSREBP2) is downregulated by lipin 1, a phosphatidic acid phosphatase whose phosphorylation by mTOR complex 1 (mTORC1) prevents its entry into the nucleus. nSREBP2 can be phosphorylated by the serine/threonine protein kinase GSK3 and targeted for proteasomal degradation by the Skp, Cullin, F-box (SCF)–FBW7 ubiquitin ligase complex. Ubiquitylation of nSREBP2 is also mediated by carbohydrate response element-binding protein (ChREBP), but the exact mechanism is unknown. In addition, nSREBP2 can be acetylated by the histone acetyltransferase p300 and its related protein CBP, or phosphorylated by ERK proteins, for increased transcriptional activity. Sirtuin 1 (SIRT 1) can deacetylate nSREBP2 and counteract the stimulatory effects of p300 and CBP. SUMO1-mediated sumoylation and AMP-activated protein kinase (AMPK)-mediated phosphorylation repress the transcriptional activity of nSREBP2. Forkhead box O3 (FOXO3) represses SREBP2 expression by binding a conserved insulin response element (IRE) and recruiting SIRT6 for deacetylation of histone H3 at the SREBP2 promoter. Ac, acetyl group; P, phosphate group; S, small ubiquitin-like modifier (SUMO); Ub, ubiquitin.

SREBP2 and retains the complex in the Golgi[37] (FIG. 2a). Notably, the interactions of SCAP–SREBP2 with PAQR3 or with INSIG1 are mutually exclusive and regulated by cholesterol levels[37].

Finally, the regulation of proteolytic activation of SREBP2 is supported by heat shock protein 90 (HSP90)[38]. HSP90 binds and stabilizes the SCAP–SREBP2 complex both in the ER, thereby facilitating its transit to the Golgi, and in the Golgi (even after the N terminus of SREBP2 is cleaved), thereby preventing premature degradation of SCAP and SREBP2 in the proteasomes and promoting SREBP2 activity.

***Regulation of nSREBP2 protein.*** The protein level and transcriptional activity of nSREBP2 add another layer of regulation to the SREBP2 pathway (FIG. 2c). mTOR complex 1 (mTORC1), the master regulator of anabolic reactions, increases nSREBP2 proteins by phosphorylating and preventing nuclear entry of lipin 1 (REF.[39]), and by suppressing cholesterol trafficking from lysosomes to the ER[40] (see also Intracellular routes of LDLR and cholesterol after endocytosis below). On the contrary, the lipogenic transcription factor carbohydrate response element-binding protein (ChREBP) promotes ubiquitylation and proteasomal degradation of nSREBP2 in an as yet unexplored mechanism[41]. The nSREBP2 phosphorylated by the serine/threonine protein kinase GSK3 can be targeted for degradation by FBW7, a substrate receptor of the SCF ubiquitin ligase complex[42].

The transcriptional activity of nSREBP2 is also subject to modulation by post-translational modifications. The histone acetyltransferase p300 and its related protein CBP can bind and acetylate the N terminus of SREBP2, enhancing its expression and transcriptional activity[43]. Accordingly, ablation or inhibition of sirtuin 1 (SIRT1), which deacetylates SREBP2, increases SREBP2 abundance in the nucleus[44]. As SIRT1 is activated by fasting[45], SIRT1-mediated deacetylation of SREBP2 halts the energy-consuming biosynthetic process of cholesterol under nutrient deprivation conditions. Aside from acetylation, nSREBP2 can be phosphorylated by ERKs and AMPK for increased and decreased transcriptional activity, respectively[46,47], as well as modified by sumoylation for decreased transcriptional activity[48].

***Transcription regulation of the* SREBP2 *gene.*** Like other SREBP2 targets, the *SREBP2* gene itself is upregulated by nSREBP2 owing to the presence of a 10-base-pair SRE upstream of the transcription initiation site[49]. *SREBP2* also harbours the binding sites for transcription factors NF-Y and SP1, both of which act synergistically with nSREBP2 to upregulate SREBP2-dependent gene expression[49]. Between the SRE and the transcription initiation site there is a conserved insulin response element, to which the transcription factor forkhead box O3 (FOXO3) can bind[50]. FOXO3 then recruits SIRT6, which deacetylates histone H3 and downregulates the expression of *Srebp2* in mouse liver[50] (FIG. 2c). Of note, the FOXO3–SIRT6 complex also represses the expression of the proprotein convertase subtilisin/kexin type 9 (*Pcsk9*) gene[51], which is an SREBP2 target and a negative regulator of the LDL receptor (LDLR) pathway (see also PCSK9-induced degradation of LDLR and its regulation below). Under starvation conditions, when FOXO3 and SIRT6 are both active, decreased cholesterol biosynthesis together with increased LDLR-mediated cholesterol uptake from blood ensures maximal utilization of the cholesterol pool in the body.

### *Regulation of HMGCR*

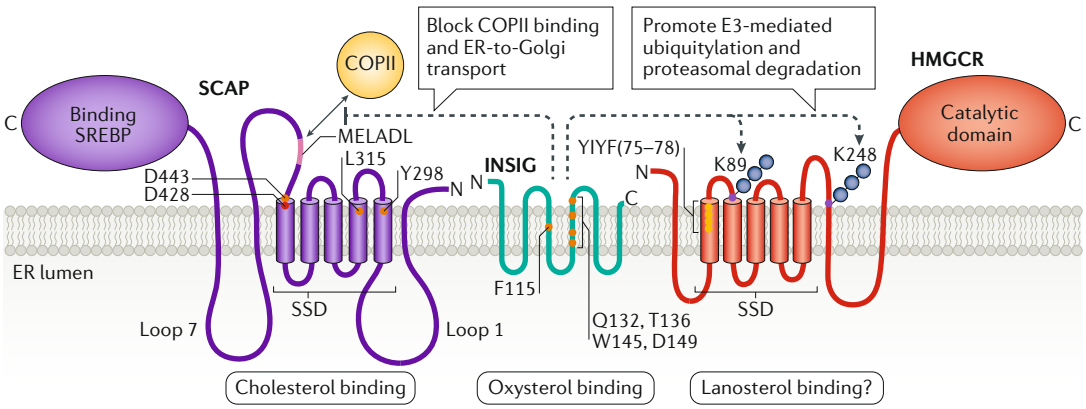
Mammalian HMGCR is an ER-localized glycoprotein comprising a hydrophobic N-terminal domain that spans the membrane eight times and a large soluble C-terminal domain that projects into the cytosol[52]. Akin to SCAP, transmembrane domains 2–6 serve as the SSD that confers HMGCR sensitivity to sterol levels in the ER membrane (BOX 1). The cytosolic C-terminal domain is responsible for converting HMG-CoA to mevalonate, using two molecules of NADPH as the reducing reagent.

As the rate-limiting enzyme for cholesterol biosynthesis, HMGCR is highly regulated at transcriptional, translational and post-translational levels[53]. The *HMGCR* gene is activated by nSREBP2 when sterol

SCF ubiquitin ligase complex
The complex that catalyses the ubiquitylation of proteins for degradation. The core components of the Skp, Cullin, F-box (SCF) complex include the scaffold protein Cul1, the RING-finger protein RBX1/ROC1 and the adaptor protein Skp1. The F-box protein (FBP) is the variable component determining substrate specificity. In most cases, FBPs recognize phosphorylated proteins.

**Box 1 | Comparison of sterol-induced binding of INSIGs to SCAP and to HMGCR**

Sterol regulatory element-binding protein (SREBP)-cleavage activating protein (SCAP) and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) are both bound and regulated by insulin-induced gene (INSIG) proteins (see the figure). The key residues involved in formation of the SCAP–INSIG complex include Y298, L315 and D443 on SCAP and F115, Q132, T136, W145 and D149 on INSIG2 (orange dots); the D428 position in SCAP (red dot) is responsible for regulating the dissociation of INSIGs from SCAP. Binding of INSIGs to HMGCR depends on the presence of the YIYF sequence in the 75–78 position of HMGCR (yellow dots). Although SCAP and HMGCR display little sequence similarity, they do share some common key features. First, both proteins are composed of eight transmembrane segments (with the sterol-sensing domain (SSD) comprising transmembrane domains 2–6) and a large C-terminal domain that projects into the cytosol. Second, both are induced to interact with INSIGs by sterols. Cholesterol can bind loop 1 of SCAP directly, whereas oxysterols bind first to INSIG2 to recruit SCAP. The formation of the HMGCR–INSIG complex is simulated by lanosterol and oxysterols. Lanosterol is hypothesized to bind HMGCR directly. Whether oxysterol binding to INSIGs is required for the INSIG–HMGCR interaction is unclear. Third, INSIG binding to both proteins reduces cholesterol biosynthesis (FIG. 2). For SCAP, INSIG binding changes the conformation of SCAP so that the MELADL motif responsible for coatomer II (COPII) binding is no longer exposed and the trafficking of SCAP in complex with SREBP2 towards the Golgi is blocked. For HMGCR, INSIGs in complex with E3 ligases drive HMGCR ubiquitylation, targeting it for proteasomal degradation. ER, endoplasmic reticulum.




Sirtuin 1
(SIRT1). Member of a family of proteins that act predominantly as NAD-dependent deacetylases. There are seven sirtuins in mammals, SIRT1–SIRT7. Some sirtuins can remove various acyl lysine modifications from proteins.

ERKs
Widely expressed protein-serine/threonine kinases that are activated via the phosphorylation of tyrosine. Activation of ERK can affect cell proliferation, survival, apoptosis, motility, metabolism and differentiation.

AMPK
(AMP-activated protein kinase). A central regulator of energy homeostasis that is activated when the cellular ATP level is low. AMPK activation inhibits cholesterol and fatty acid synthesis.

Mevalonate
A product generated from 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) by the action of HMG-CoA reductase. The mevalonate pathway in mammals leads to the synthesis of sterols, isoprenoids, dolichol, haeme, ubiquinone and so forth.

Isoprenoids
A class of naturally occurring organic compounds that are composed of two or more units of isoprene. They are synthesized in the mevalonate pathway in mammals.

Lanosterol
The first sterol intermediate in the mevalonate pathway consisting of 30 carbons. Lanosterol is synthesized by cyclization of squalene and can potently stimulate degradation of hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) without inhibiting the processing of sterol regulatory element-binding protein (SREBP).

ER-associated degradation
(ERAD). A surveillance system that clears misfolded proteins in the endoplasmic reticulum (ER) via ubiquitylation and proteasomal degradation.

concentrations are low. Translation of HMGCR mRNA can be blocked by unknown non-sterol isoprenoids through a poorly characterized mechanism[54]. In this section, we review regulation of HMGCR stability and activity through post-translational modifications.

***INSIG-mediated ubiquitylation and degradation of HMGCR.*** HMGCR is relatively stable under sterol depletion conditions (half-life of about 12 h in cultured human fibroblasts)[55]. Its degradation can be induced by sterols, mostly oxysterols such as 25-hydroxycholesterol and 27-hydroxycholesterol, and methylated sterols such as lanosterol and 24,25-dihydrolanosterol (REFS[56,57]). Two members of the vitamin E family, δ-tocotrienol and γ-tocotrienol, can also induce HMGCR degradation[58]. By contrast, cholesterol is a relatively weak cue for HMGCR degradation[56].

INSIGs are absolutely required for the sterol-induced degradation of HMGCR. INSIG1 associates with ubiquitin ligases gp78 (also known as AMFR)[59], TRC8 (REF.[60]) and RNF145 (REF.[61]). When oxysterols and lanosterol accumulate in the cell, INSIG1 is induced to bind the membrane domain of HMGCR[56,62], triggering HMGCR ubiquitylation and its subsequent extraction from the membrane and proteasomal degradation via ER-associated degradation (ERAD)[55,63,64] (BOX 1; FIG. 2b). INSIG1-mediated ubiquitylation and degradation of HMGCR is enhanced by UFD1, a component of the ERAD machinery that directly binds gp78 (REF.[65]). These processes are further regulated by the prenyltransferase UBIAD1 (UbiA prenyltransferase domain-containing protein 1), which competes with INSIG1 to bind HMGCR and protects it from membrane extraction during ERAD[66,67]. Geranylgeraniol accelerates the ERAD of HMGCR by facilitating ER-to-Golgi transport of UBIAD1 (REF.[68]). By contrast, specific mutations in UBIAD1 associated with Schnyder corneal dystrophy (TABLE 1) stabilize and sequester UBIAD1 in the ER, thereby also stabilizing HMGCR protein, which was shown to increase cholesterol biosynthesis in the cornea[66,69] (FIG. 2b).

INSIG2 is also required for sterol-stimulated HMGCR degradation and, like INSIG1, recruits E3 ubiquitin ligases to HMGCR[55]. Ectopic expression of INSIG2 in cells deficient in INSIG1 and INSIG2 fully restores sterol-accelerated ubiquitylation and degradation of HMGCR[70]. Recently, in the liver, the *Insig2* gene was found to be a direct target of hypoxia inducible factor 1α, a major transcription regulator of hypoxia[71]. Accordingly, in mice exposed to hypoxia, upregulation of INSIG2 and accumulation of lanosterol and 24, 25-dihydrolanosterol contribute to reduced levels of hepatic HMGCR[71,72]. Fasting elevates the expression of *Insig2*, particularly *Insig2a*, in mouse livers as well[73]. The consequent increase of INSIG2 protein suppresses the SREBP pathway[74], which, together with INSIG2-mediated HMGCR degradation as discussed above, blocks cholesterol biosynthesis during food deprivation.

A complex interplay exists between INSIGs and ubiquitin ligases. In the absence of sterols, INSIG1 is

ubiquitylated, independently by gp78 (REFS[29,30]) and TRC8 (REF.[75]), and degraded by proteasomes. INSIG2 is relatively stable in cultured cells[30,76]. However, in mouse livers, depletion of gp78 elevates the protein levels of both INSIGs, with a more noticeable effect on INSIG2 (REF.[77]). These liver-specific *gp78*-knockout mice exhibit reduced biosynthesis of cholesterol and fatty acids, as inhibition of the SREBP pathway by INSIGs outweighs the stabilization of HMGCR by gp78 deficiency[77]. It is unclear why gp78 targets INSIG2 for degradation specifically in hepatocytes. gp78, TRC8 and RNF145 are predicted to share similar structures[78], and TRC8 and RNF145 display sterol-dependent protein stability owing to the presence of the SSD[35,79]. However, TRC8 has a much longer half-life than RNF145 (REFS[35,79]). Knockdown of gp78 markedly slows TRC8 degradation without affecting RNF145 stability[60,79]. The presence of multiple, dynamically regulated ubiquitin ligases is likely to provide a fail-safe mechanism for efficient ERAD of HMGCR, as each E3 ligase may have different sensitivity to sterol levels and express in a tissue-specific manner.

***Regulation of HMGCR activity by phosphorylation.*** HMGCR can be interconverted between phosphorylated and dephosphorylated forms. A single serine residue close to the very end of the catalytic domain (Ser871 in rodents and Ser872 in humans) is the key phosphorylation site[80]. Phosphorylation abolishes HMGCR activity without affecting sterol-induced degradation of the protein[81]. In the liver, HMGCR is primarily phosphorylated by AMPK[82], which blocks cholesterol biosynthesis when intracellular ATP levels are low[81]. Inhibition of AMPK activity activates HMGCR and increases cholesterol production[83,84]. miR-34a, a microRNA overexpressed in non-alcoholic fatty liver disease, suppresses SIRT1, causing dephosphorylation of AMPK and, consequently, activation of HMGCR, which contributes to cholesterol accumulation in non-alcoholic fatty liver disease[85]. Despite these insights, much work is still needed to elucidate the physiological role of HMGCR phosphorylation in cholesterol biosynthesis.

### *Regulation of squalene monooxygenase*

Squalene monooxygenase has been recently recognized as another rate-limiting enzyme beyond HMGCR in the cholesterol biosynthetic pathway[86]. The mammalian squalene monooxygenase contains an extended N-terminal domain that is absent in lower organisms[86,87], with the first 100 amino acids of the N terminus anchoring the enzyme to the ER via a loop that partially transverses the membrane[88]. However, a recent structural analysis shows two membrane-binding helical domains at the C terminus[89]. The exact membrane topology of squalene monooxygenase is still unknown.

Like HMGCR, squalene monooxygenase is controlled at both transcriptional and post-translational levels. The *SQLE* gene has SREs and NF-Y and SP1 binding sites, and responds to sterols via SREBP2 (REFS[90,91]). The squalene monooxygenase protein can be potently degraded in the presence of cholesterol in a process requiring an amphipathic helix in the first 100 amino acids of the N terminus, the E2 enzyme UBE2J2 and the E3 ubiquitin ligase MARCH6, but not INSIGs[86,92–94] (FIG. 2b). The amphipathic helix is proposed to attach transiently to the membrane at low cholesterol levels but to dissociate and unravel in response to excess cholesterol, thereby allowing MARCH6 to bind[92]. This causes squalene monooxygenase ubiquitylation at the serine residues flanking the amphipathic helix[95]. Cholesterol also stabilizes MARCH6 by blocking its autoubiquitylation[96]. Beyond functioning as a ubiquitin ligase for squalene monooxygenase, MARCH6 also negatively regulates the SREBP2 transcriptional programme and thus expression of the *SQLE* gene[97]. Notably, although *HMGCR* and *SQLE* are transcriptionally co-regulated by nSREBP2, degradation pathways of HMGCR and squalene monooxygenase are triggered by different metabolic cues and rely on different molecular machineries. These different means of regulation have a physiological importance, whereby modulation of squalene monooxygenase independently of HMGCR permits cholesterol biosynthesis to be shut down while maintaining the biosynthesis of essential isoprenoids.

## Regulation of cholesterol uptake

Besides biosynthesis, the diet and subsequent cholesterol uptake from the blood have key roles in maintaining cholesterol homeostasis. In this section, we overview mechanisms and regulators of NPC1L1-mediated cholesterol absorption from the intestinal lumen and LDLR-mediated uptake of cholesterol containing LDL particles (LDL-c) from the blood (BOX 2; FIG. 3).

### *NPC1L1-mediated cholesterol uptake*

NPC1L1 is a key mediator of cholesterol absorption, governing cholesterol uptake in enterocytes via clathrin-mediated endocytosis. NPC1L1 was first identified by virtue of its high sequence homology (42% identity and 51% similarity) to NPC1 (REF.[98]). It is an extensively glycosylated, multi-spanning membrane protein expressed on the apical surface of enterocytes and the membrane of bile canaliculi of human hepatocytes[11]. NPC1L1 includes three large extracellular domains, 13 transmembrane segments and a short cytoplasmic C-terminal tail[99]. The N-terminal domain of NPC1L1 selectively binds cholesterol and oxysterols in vitro[100,101]. One of the extracellular loops (loop 2) has been determined as a binding site for the cholesterol absorption inhibitor ezetimibe[102], thereby supporting pharmacological modulation of cholesterol absorption. Transmembrane segments 3–7 comprise a predicted SSD as seen in SCAP, HMGCR, NPC1 and several other cholesterol regulators[23]. Finally, the C-terminal domain of NPC1L1 bears an endocytic signal sequence YVNxxF (where x is any amino acid) that binds to the endocytic adaptor NUMB to regulate NPC1L1 internalization, as well as the QKR sequence that recruits the LIM domain and actin-binding protein 1 (LIMA1) to modulate NPC1L1 trafficking back to the cell surface[103,104] (see below).

***Mechanisms of NPC1L1-mediated intestinal cholesterol uptake.*** The molecular mechanisms of NPC1L1-mediated cholesterol uptake have been well delineated using cultured rat hepatoma cells. NPC1L1 resides

Prenyltransferase
A class of enzymes that transfer prenyl moieties to acceptor molecules. They are responsible for menaquinone and ubiquinone biosynthesis, or protein modification called prenylation that is the covalent linkage of a lipid consisting of three or four isoprene units to a thiol of a cysteine side chain.

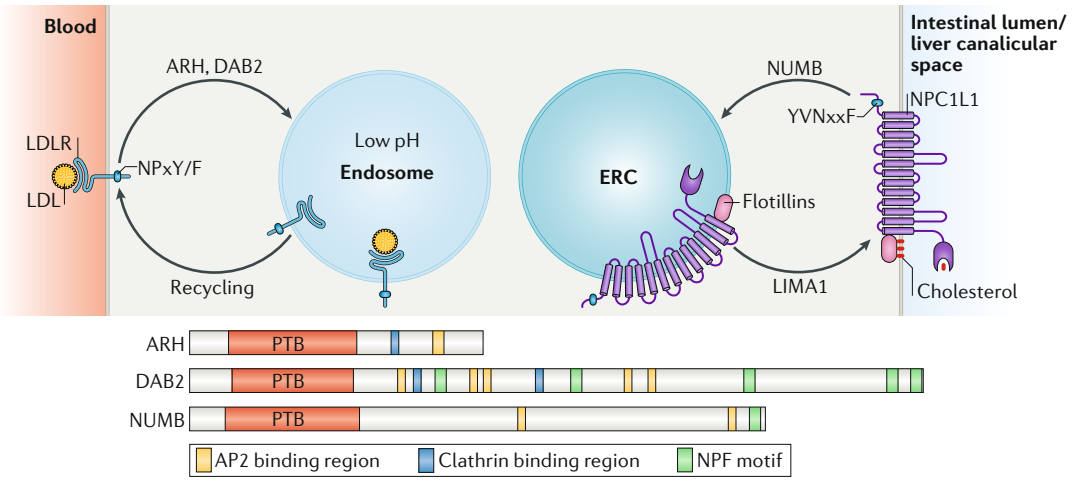
Geranylgeraniol
A diterpene alcohol containing 20 carbons that is synthesized in the mevalonate pathway. Geranylgeraniol can be used to synthesize vitamins E and K, and to modify proteins in a process known as geranylgeranylation.

E2 enzyme
Also known as ubiquitin-conjugating enzyme. E2 enzymes perform the second step in the ubiquitylation reaction. Through the series of reactions of E1, E2 and E3, cellular proteins are linked to ubiquitin.

Bile canaliculi
Thin tubes formed by intercellular space between hepatocytes. They carry biles towards the interlobar bile ducts.

Box 2 | **Comparison of LDLR-mediated and NPC1L1-mediated cholesterol uptake**

Low-density lipoprotein (LDL) receptor (LDLR) and Niemann–Pick type C1-like 1 protein (NPC1L1) both mediate cholesterol uptake from the extracellular sources through the clathrin-dependent pathway. However, several obvious differences exist between these two processes (see the figure). Within a polarized cell such as enterocytes and hepatocytes, LDLR is localized at the basolateral membrane and is responsible for acquiring cholesterol in the form of LDLs from the blood, whereas NPC1L1 is on the opposite side to receive unesterified cholesterol from the intestine lumen and liver canalicular space. LDLR-mediated LDL endocytosis employs autosomal recessive hypercholesterolaemia (ARH) and DAB2 as the clathrin adaptor proteins, whereas NPC1L1-mediated cholesterol uptake requires NUMB. Notably, ARH, DAB2 and NUMB are all clathrin-associated sorting proteins sharing a phosphotyrosine-binding (PTB) domain that recognizes the canonical (F/Y)xNPx(F/Y) endocytic sorting signal, to which the NPxY/F motif of LDLR and YVNxxF of NPC1L1 conform (where x is any amino acid). Upon internalization and shedding the clathrin coats, the LDLR–LDL complex undergoes a pH-induced dissociation, which releases LDLR for recycling and LDL for further hydrolysis in the late endosomes and lysosomes. The NPC1L1–cholesterol complex is instead delivered to a designated endocytic recycling compartment (ERC), from which it can be recycled back to the plasma membrane through actin-mediated trafficking regulated by LIM domain and actin-binding protein 1 (LIMA1). Regardless of sources and pathways, the absorbed cholesterol eventually arrives at the endoplasmic reticulum for sensing, transport or esterification (also FIG. 3).




primarily in the endocytic recycling compartment (ERC) under normal growth conditions (the steady state) and translocates rapidly to the plasma membrane upon cholesterol depletion[105,106]. Replenishment of cholesterol triggers the inward transport of NPC1L1 together with cholesterol from the plasma membrane to the ERC[105]. Mechanistically, NPC1L1 interacts with cholesterol on the extracellular side and flotillins at the inner leaflet of the plasma membrane[100,107]. Cholesterol binding to NPC1L1 promotes the formation of specialized membrane microdomains enriched in cholesterol, flotillins and gangliosides[100,107,108], and causes the dissociation of the NPC1L1 C-terminal tail from the plasma membrane so that the YVNxxF sequence is available for NUMB recognition[103]. As a clathrin adaptor protein, NUMB further recruits clathrin and clathrin adaptor AP2 to the invaginated microdomains, generating coated vesicles and then endocytic vesicles that migrate along actin filaments to the ERC[105] (FIG. 3, bottom).

From the ERC, NPC1L1 can be recycled back to the plasma membrane for reuse. This process requires LIMA1, the small GTPase CDC42, the motor protein myosin Vb and actin filaments[104,105,109,110]. At steady state, CDC42 remains in the GDP-bound, inactive form and weakly interacts with NPC1L1, which in these conditions predominantly associates with another small GTPase RAB11a and RAB11 family-interacting protein 2 (REFS[109,110]). Cholesterol depletion promotes the formation of GTP-bound, active CDC42 with increased avidity for NPC1L1, resulting in the dissociation of RAB11a from NPC1L1 (REF.[109]) and the association of NPC1L1 with myosin Vb and actin mediated by LIMA1 (REF.[104]). The downstream effectors of activated CDC42, N-WASP and Arp2/3 complex, further contribute to NPC1L1 translocation to the plasma membrane by promoting actin polymerization[109].

This NPC1L1-mediated cholesterol uptake model is supported by multiple lines of evidence from in vivo studies. Both endogenous mouse NPC1L1 protein and transgenic human NPC1L1 protein are localized on the brush border membrane of mouse small intestine[111,112]. Oral gavage of cholesterol induces the internalization of NPC1L1 and cholesterol from the brush border membrane to the subapical region, and this process is blocked by ezetimibe[111]. Intestine-specific *Numb* or *Lima1*-knockout mice show impaired NPC1L1 recycling and significantly reduced cholesterol absorption, and are resistant to diet-induced hypercholesterolaemia[103,104]. Similarly, individuals carrying a G595D mutation in NUMB that decreases the interaction between NUMB and AP2, or a K306 frameshift mutation in LIMA1 that impairs NPC1L1 recycling back to the surface, display significantly lower cholesterol absorption and plasma LDL-c, high levels of which are strongly

**Endocytic recycling compartment**
(ERC). An intracellular cholesterol-rich site composed of a mixture of individual and interconnected vesicles and tubules near the microtubule-organizing centre. The ERC is RAB11a positive and regulates vesicular recycling to the plasma membrane.

**Flotillins**
A family of two ubiquitously expressed, membrane-associated proteins, namely, flotillin 1 and flotillin 2. They play a role in forming cholesterol-rich membrane microdomains, endocytosis and signal transduction.

**Gangliosides**
A species of plasma membrane-concentrated lipids. Each ganglioside molecule is composed of a glycosphingolipid linked to one or more sialic acid.

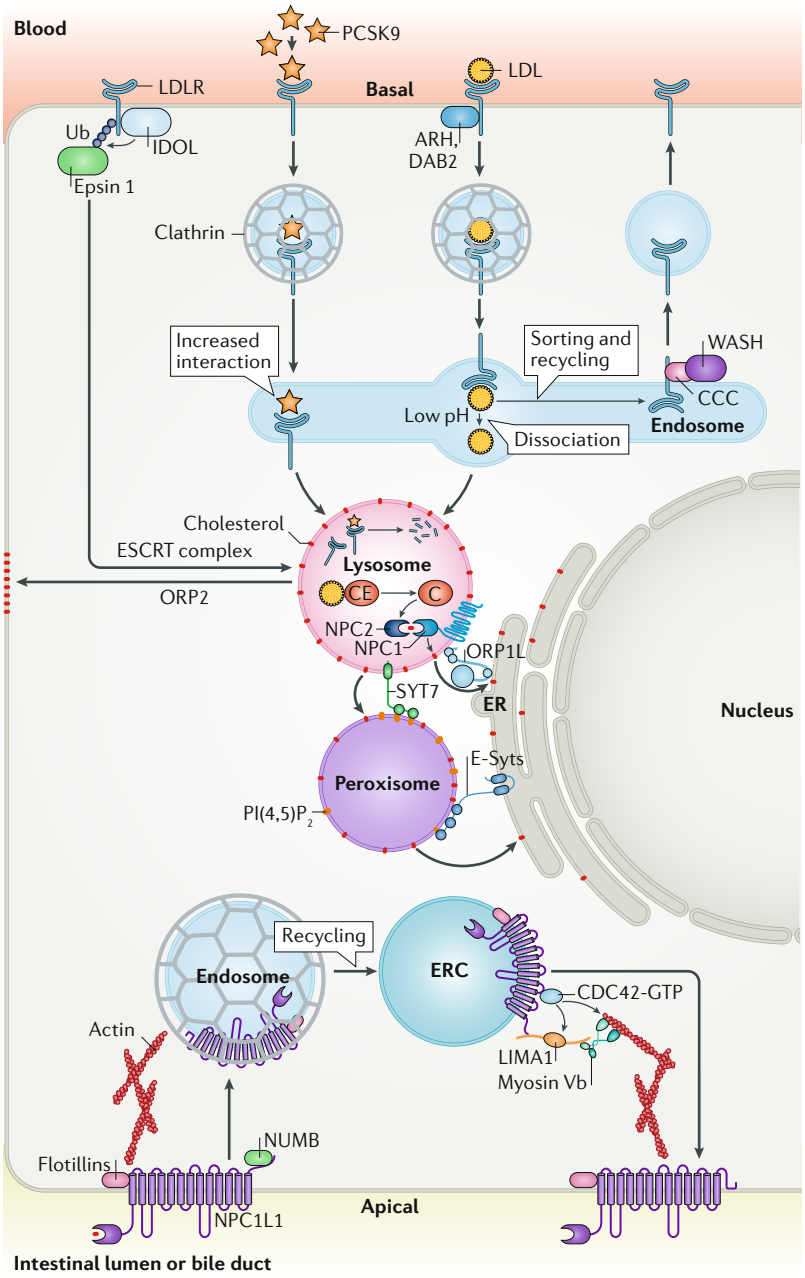



Fig. 3 | **Mechanisms regulating cholesterol uptake.** Low-density lipoprotein (LDL) receptor (LDLR) is expressed on the plasma membrane of most cells, including the basolateral surface of enterocytes and hepatocytes. Niemann–Pick type C1 (NPC1)-like 1 (NPC1L1) is specifically expressed on the apical surface of enterocytes and human hepatocytes. Top: LDL in the blood is captured by LDLR on the cell surface and the LDL–LDLR complex is internalized in clathrin-coated pits involving the adaptor proteins autosomal recessive hypercholesterolemia (ARH) or DAB2. As the endosomal pH decreases, LDLR dissociates from LDL and is recycled by the CCC and WASH protein complex back to the surface for additional uptake. LDL is further delivered towards lysosomes, and the carried cholesteryl esters (CEs) are hydrolysed to cholesterol (C). Cholesterol is inserted into the limiting membrane of lysosome by coordinated actions of NPC2 and NPC1, followed by trafficking to downstream membranes including the plasma membrane and endoplasmic reticulum (ER). The oxysterol-binding protein-related protein 2 (ORP2) may deliver cholesterol from lysosomes to the plasma membrane. Transfer to the ER occurs at ER–lysosome contact sites and is mediated by oxysterol-binding protein-related protein 1L (ORP1L). Peroxisomes are also engaged in cholesterol transport from lysosomes to the ER by forming membrane contact sites with both organelles using lysosomal synaptotagmin VII (SYT7), peroxisomal phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) and ER-resident extended synaptotagmins (E-Syts). Proprotein convertase subtilisin/kexin type 9 (PCSK9) binds LDLR on the plasma membrane and is internalized with the LDLR protein in clathrin-coated vesicles. The PCSK9–LDLR interaction increases as the endosomal pH decreases, preventing LDLR from recycling back to the surface. The PCSK9–LDLR complex is eventually degraded in lysosomes. The intracellular ubiquitin ligase inducible degrader of the LDL receptor (IDOL; also known as MYLIP) ubiquitylates LDLR at the cytoplasmic C-terminal tail. The ubiquitylated LDLR is internalized by epsin 1 and sorted by ESCRT complexes (endosomal sorting complexes required for transport) to multivesicular bodies (not shown) and eventually to lysosomes for degradation. Bottom: cholesterol in the intestinal lumen or bile binds the N-terminal domain of NPC1L1. This causes a conformational change of NPC1L1 that exposes the C-terminal endocytic motif, which is recognized by NUMB. This promotes NPC1L1 endocytosis. The resulting clathrin-coated vesicles migrate along actin filaments to the endocytic recycling compartment (ERC). Exactly how cholesterol is delivered from the ERC to the ER is unknown. Upon cholesterol depletion, NPC1L1 establishes an interaction with myosin Vb through LIM domain and actin-binding protein 1 (LIMA1), and translocates on actin filaments from the ERC back to the plasma membrane. These processes are promoted by the GTP-bound, active form of small GTPase CDC42. Ub, ubiquitin.

correlated with the risk of cardiovascular disease[104,113]. In line with this, the R1325X mutation produces a truncated NPC1L1 protein lacking the NUMB-recognizing endocytic motif[103], and is associated with lower plasma LDL-c and lower cardiovascular disease incidence in humans[114].

***Regulation of NPC1L1 expression.*** The human *NPC1L1* gene contains two SRE sites and is activated by SREBP2 (REFS[115,116]). Multiple SREs are also present in the mouse promoter[117], and animals on a high-cholesterol diet have markedly reduced expression of *Npc1l1* in the intestine[118,119], indicating a negative feedback loop between the cholesterol abundance and the pathway of its absorption.

In addition to SREBP2, other mechanisms contribute to regulating NPC1L1 expression as well. In human liver and intestinal cells, SREBP2-mediated activation of *NPC1L1* is increased by hepatocyte nuclear receptor 4α (HNF4α)[120]. In human liver-derived HepG2 cells, the PPARα–RXRα nuclear receptor complex can upregulate *NPC1L1* transcription[121]. Negative regulation of *Npc1l1* expression is driven by the transcription factor CREBH, which binds and represses the activity of mouse *Npc1l1* promoter[122]. Moreover, the transcriptional co-repressor SHP can inhibit SREBP2-mediated transactivation of *Npc1l1* in mouse intestine, acting downstream

Brush border
The apical plasma membrane consisting of an array of densely packed microvilli, which are tiny projections intended to increase the surface area for absorption.

Liver X receptors
(LXRs). The sterol-sensitive transcription factors that belong to the nuclear receptor family and are activated by oxysterols and desmosterol. LXRs promote cholesterol efflux mainly by upregulating ATP-binding cassette (ABC) subfamily A member 1 (ABCA1) and ABC subfamily G member 1 (ABCG1), ABCG5 and ABCG8. They also increase fatty acid synthesis by elevating sterol regulatory element-binding protein 1c (SREBP1c) expression.

Thyroid hormones
Two tyrosine-based, iodine-containing hormones produced by the thyroid gland. They participate in the regulation of metabolism and growth.

ARH
(Autosomal recessive hypercholesterolaemia). An adaptor protein that binds low-density lipoprotein receptor and mediates its endocytosis in hepatocytes and lymphocytes. Mutations in *ARH* cause an autosomal recessive form of hypercholesterolaemia.

NPC2
(Niemann–Pick type C2). A small (132 amino acids in humans), luminal protein that resides in late endosomes and lysosomes, and binds cholesterol with the iso-octyl side chain of cholesterol buried and the 3β-hydroxyl group exposed. Mutations in *NPC2* cause 5% of NPC cases.

ESCRT complexes
(Endosomal sorting complexes required for transport). These protein complexes comprise multiple cytosolic subunits. They transport ubiquitylated cargo to cellular vesicles by promoting membrane budding into the endosomes to form multivesicular bodies, which eventually fuse with lysosome and cause degradation of the cargo.

of a postprandial increase in FGF19 signalling[117]. Surprisingly, there is evidence that fasted mice and glucose-deprived Caco-2 cells have reduced *NPC1L1* mRNA levels[123]. Activation of liver X receptors (LXRs) or ablation of sortilin, a sorting receptor closely implicated in cardiovascular disease, negatively regulates *NPC1L1* expression[124,125], with the underlying mechanisms yet to be determined.

Expression of NPC1L1 is also dependent on the regulation of protein degradation. The mechanisms of NPC1L1 degradation are poorly understood, but both lysosomal and ubiquitin–proteasome pathways of degradation seem to contribute[126].

### *LDLR-mediated LDL endocytosis*

LDLR is a main player responsible for the uptake of cholesterol (as a component of LDLs) by peripheral cells from the circulation. LDLR is a cell surface glycoprotein consisting of five structurally and functionally distinct modules: an N-terminal apoB and apoE binding domain, a large epidermal growth factor (EGF) precursor homology domain (encompassing two EGF-like repeats (EGF-A, EGF-B), a six-bladed β-propeller and a third EGF-like repeat (EGF-C)), an O-linked oligosaccharide-rich domain, a single transmembrane domain and a relatively short C-terminal tail containing a highly conserved NPxY internalization motif[127].

***Intracellular routes of LDLR and cholesterol after endocytosis.*** *LDLR* is the transcriptional target of SREBP2. Thyroid hormones also induce *LDLR* expression by binding directly to its promoter[128]. Once synthesized, the 120-kDa LDLR precursor is glycosylated as it transits along the secretory pathway, converting to a 160-kDa mature protein that is found in the plasma membrane of most cell types[129]. In polarized cells, such as hepatocytes and enterocytes, LDLR is localized to the basolateral membranes in contrast to NPC1L1, which is found apically (BOX 2; FIG. 3). Surface LDLR captures the circulating LDL via the extracellular ligand binding domain, and recruits endocytic adapters ARH and DAB2 as well as the associated AP2 and clathrin via the cytoplasmic NPxY sequence[130,131]. This enables LDL to be incorporated into clathrin-coated vesicles that subsequently pinch off from the plasma membrane and enter the endocytic pathway[12]. In the acidic endosome, LDLR undergoes a conformational change and dissociates from the bound LDL[132]. LDLR is then returned by the COMMD–CCDC22–CCDC93 endosomal recycling complex back to the cell surface for additional rounds of endocytosis[133,134] or is directed to lysosomes for degradation by interaction with PCSK9 (serine protease produced predominantly by the liver; see below). LDLR can also be targeted for lysosomal degradation directly from the plasma membrane in a process regulated by an E3 ligase called inducible degrader of the LDL receptor (IDOL; see below).

Following endocytosis, LDL-carried cholesteryl esters gradually progress through the endo-lysosomal system to finally be hydrolysed by a lysosomal acid lipase to generate cholesterol that is then exported from the lysosomal lumen by concerted actions of NPC2, NPC1 and lysosome-associated glycoprotein LAMP2 (REFS[135,136]). Mutations in *NPC1* or *NPC2* result in a lysosomal lipid storage disorder called NPC disease (TABLE 1). Cholesterol from lysosomes can be delivered to other cellular compartments (notably the ER, where it is sensed as described above), mostly involving non-vesicular transport[1,17]. The lysosome–peroxisome–ER membrane contact sites have been implicated in this process[137–139] (FIG. 3, top). Cholesterol may also be transferred to the ER by the sterol transfer proteins oxysterol-binding protein-related protein 1L (ORP1L) and ORP5 (REFS[140,141]). In addition to the above two pathways, the lysosomal cholesterol can first move to the plasma membrane, via a mechanism involving ORP2 (REF.[142]), and then to the ER[143].

The significance of LDLR in whole-body cholesterol homeostasis is highlighted by familial hypercholesterolaemia, which is caused by impaired LDLR-mediated LDL uptake and the resultant LDL-c build-up in circulation (TABLE 1). In fact, disrupting the LDLR life cycle at any step, such as biosynthesis, surface localization, internalization, recycling and degradation, may affect LDLR numbers or activities and, consequently, LDL clearance. Below, we emphasize IDOL and PCSK9, two regulators that act in an independent but complementary manner to regulate LDLR stability.

***IDOL-induced degradation of LDLR and its regulation.*** IDOL (also known as MYLIP) is an E3 ubiquitin ligase composed of an N-terminal FERM domain that can be divided into three subdomains (F1, F2 and F3), a short linker and a C-terminal RING domain holding the E3 ligase activity. A highly conserved residue (G51 in humans) in the F1 subdomain is critical for IDOL dimerization and stability[144]. The F3 subdomain interacts with membrane phospholipids, the cytoplasmic tails of LDLR and the closely related VLDL receptor and apoE receptor 2 (REFS[145–148]). The RING domain mediates IDOL dimerization and its interaction with the E2 enzyme UBE2D (REF.[149]), and catalyses ubiquitylation of itself and the bound substrates[145–147].

Distinct from the LDLR endocytic pathway that involves the canonical NPxY sequence and clathrin-associated adaptor proteins, IDOL recognizes LDLR at the residues immediately preceding NPxY[147,148], and triggers polyubiquitylation at the two residues following NPxY[145]. The ubiquitylated LDLR is internalized by endocytic adaptor epsin 1 and then sorted — by the activity of ESCRT complexes — to lysosomes for degradation[145,150,151] (FIG. 3, top).

Several factors have been implicated in regulating IDOL-mediated degradation of LDLR. LXRs bind to the *IDOL* promoter and upregulate its expression[145]. Accordingly, activation of the LXR–IDOL axis using synthetic LXR agonists decreases LDLR abundance and limits LDL uptake in cultured cells and livers of non-human primates[145,152,153]. Depletion of MARCH6 induces expression of *IDOL* via LXRs, counteracting LDLR protein increases caused by the activated SREBP2 pathway[97]. Further, broad-spectrum inhibitors of deubiquitylating enzymes can enhance LDLR degradation through inducing transcription of *IDOL* independently of LXR[154]. At the post-translational level, a deubiquitylating enzyme

Foam cells
Cells derived from macrophages that take up too much cholesterol from oxidized low-density lipoproteins and become laden with lipid droplets, giving them a foamy appearance. Foam cells promote the atherosclerotic plaque build-up and inflammation during atherosclerosis.

Lecithin:cholesterol acyltransferase
(LCAT). A lipoprotein-associated enzyme that transfers the fatty acid from the *sn*-2 position of phosphatidylcholine (lecithin) to cholesterol to form a cholesteryl ester.

USP2 was found to interact with IDOL and attenuate its potency in promoting LDLR degradation[155].

***PCSK9-induced degradation of LDLR and its regulation.*** PCSK9 is one of nine proprotein convertases belonging to the secretory serine protease family. It consists of a prodomain and a catalytic domain, followed by a unique C-terminal domain enriched in both Cys and His residues[156,157]. The prodomain is the N-terminal component of a newly synthesized precursor, but is self-cleaved in the ER and non-covalently associates with the rest of the protein thereafter, blocking its protease activity. The processed PCSK9 is further modified by glycosylation, phosphorylation and sulfation[158], and finally released into the extracellular milieu to bind LDLR and other surface proteins[159].

PCSK9 primarily interacts with the EGF-A repeat of LDLR via a portion of the catalytic domain without involving the active site or requiring its dissociation from the inhibitory prodomain[160,161] (which facilitates PCSK9 anchoring at the hepatocyte surface)[162]. The PCSK9–LDLR complex is internalized in clathrin-coated pits (via ARH, at least in some cell types)[163,164], and delivered to endosomes where the C-terminal domain of PCSK9 is induced to establish an interaction with the ligand-binding domain of LDLR by the acidic environment[165]. This increases PCSK9 binding and prevents LDLR from adopting a recycling-competent conformation[166]. LDLR and the bound PCSK9 are eventually degraded in lysosomes[159] (FIG. 3, top). Although the detailed mechanism of this degradative process is still unknown, it is clear that PCSK9, unlike IDOL, employs neither ubiquitylation nor the ESCRT pathway for lysosomal sorting of LDLR[164]. In addition to acting from the extracellular space, in HepG2 cells, the newly synthesized PCSK9 can direct the new pool of LDLR in the *trans*-Golgi towards lysosomes for degradation[167].

*PCSK9* is upregulated by SREBP2 and the liver-enriched transcription activator HNF1α[20,168]. Conversely, increased insulin signalling and mTORC1 activity downregulate PCSK9 expression via repression of HNF4α and HNF1α[169]. Other transcriptional regulators of *PCSK9* include E2F1, FOXO3,SIRT6 and nuclear receptors FXR and PPARs (as reviewed in REFS[159,170–172]). At the post-transcriptional level, three microRNAs have been recently identified to directly bind PCSK9 mRNA and negatively regulate its expression[173].

## Regulation of cholesterol efflux

Although all mammalian cells can produce cholesterol, most (except for hepatocytes, adrenal cells and gonadal cells) are unable to catabolize the molecule and therefore need to dispose the excess out of the cell or store it as cholesteryl esters in lipid droplets (see the next section). Four members belonging to the ATP-binding cassette (ABC) transporter superfamily, ABC subfamily A member 1 (ABCA1) and ABC subfamily G (ABCG) members 1, 5 and 8, are responsible for cholesterol efflux in a cell type-specific manner. In this section, we discuss the mechanisms and regulators of cholesterol efflux mediated by ABCA1 and ABCG1 in macrophages and by ABCG5 and ABCG8 in hepatocytes and enterocytes (FIG. 4).

***ABCA1-mediated cholesterol efflux***

ABCA1 is a full transporter comprising two tandem repeats of the membrane-spanning domains, each of which has six transmembrane segments and a large glycosylated extracellular domain. ABCA1 is widely expressed throughout the body. Mutations in the *ABCA1* gene cause Tangier disease (TABLE 1). Studies on whole-body *Abca1*-knockout mice and human *ABCA1*-transgenic mice in various backgrounds show that ABCA1 has particularly important roles in macrophages, where it promotes removal of excess cholesterol (resulting from their prominent activity in scavenging lipoproteins from circulation), thereby preventing their transformation into foam cells and protecting against atherosclerosis[174,175].

Lipid-free apoA-I is the primary acceptor for cholesterol efflux by ABCA1 (REF.[176]). This generates nascent HDL particles that, under the action of lecithin:cholesterol acyltransferase (LCAT), become mature and competent for acquiring cholesterol from ABCG1 (REF.[177]) (FIG. 4, top). ABCA1 can directly transport or flip several phospholipids across the lipid bilayer[178,179], most likely by recruiting them laterally from the inner leaflet of the membrane[180]. However, the mechanisms by which ABCA1 mediates cholesterol efflux to apoA-I remain controversial. One view is that ABCA1 can interact with apoA-I upon loading with cholesterol and phospholipids in the extracellular domains, thereby passing both lipids directly onto apoA-I[181,182]. Another view is that ABCA1, by promoting phospholipid transport, creates an activated microdomain that protrudes from the cell surface for apoA-I binding[183]. After unfolding of the N terminus by ABCA1 (REF.[184]), apoA-I is then inserted into the membrane and initiates micro-solubilization of the lipid bilayer that leads to the efflux of cholesterol and phospholipids. In addition to these two models, ABCA1 and the associated apoA-I may undergo clathrin-dependent endocytosis from the cell surface to late endosomes and lysosomes, where apoA-I could receive LDL-c via ABCA1 directly from NPC2 (REF.[185]). apoA-I would then be secreted as lipidated particles out of the cell[186]. The physiological importance of this retro-endocytosis pathway is a matter of controversy[183]. In addition, apoA-I binding can prevent ABCA1 from degradation in early endosomes[187], thus promoting its recycling to the cell surface and subsequent nascent HDL biogenesis. These mechanisms are not necessarily exclusive, and given the pathology associated with excess cholesterol, it is not surprising that multiple pathways may operate in parallel to remove cholesterol rapidly from macrophages in the case of cholesterol overloading.

Consistent with a role of ABCA1 in exporting cholesterol, transcription of *ABCA1* is upregulated by LXRs and RXR[188]. In human macrophages, the LXRα–ABCA1 cholesterol efflux pathway is elevated by AMPK[189,190]. An LXR-responsive long non-coding RNA (lncRNA) called MeXis enhances the transcription of *Abca1* in mice[191]. Recently, *ABCA1* was identified as a bona fide target of the major tumour suppressor p53 in liver and colon cancer cells[192]. Transactivation of *ABCA1* by p53 inhibits SREBP2 processing through promoting cholesterol trafficking from the plasma membrane to the ER[193], thereby

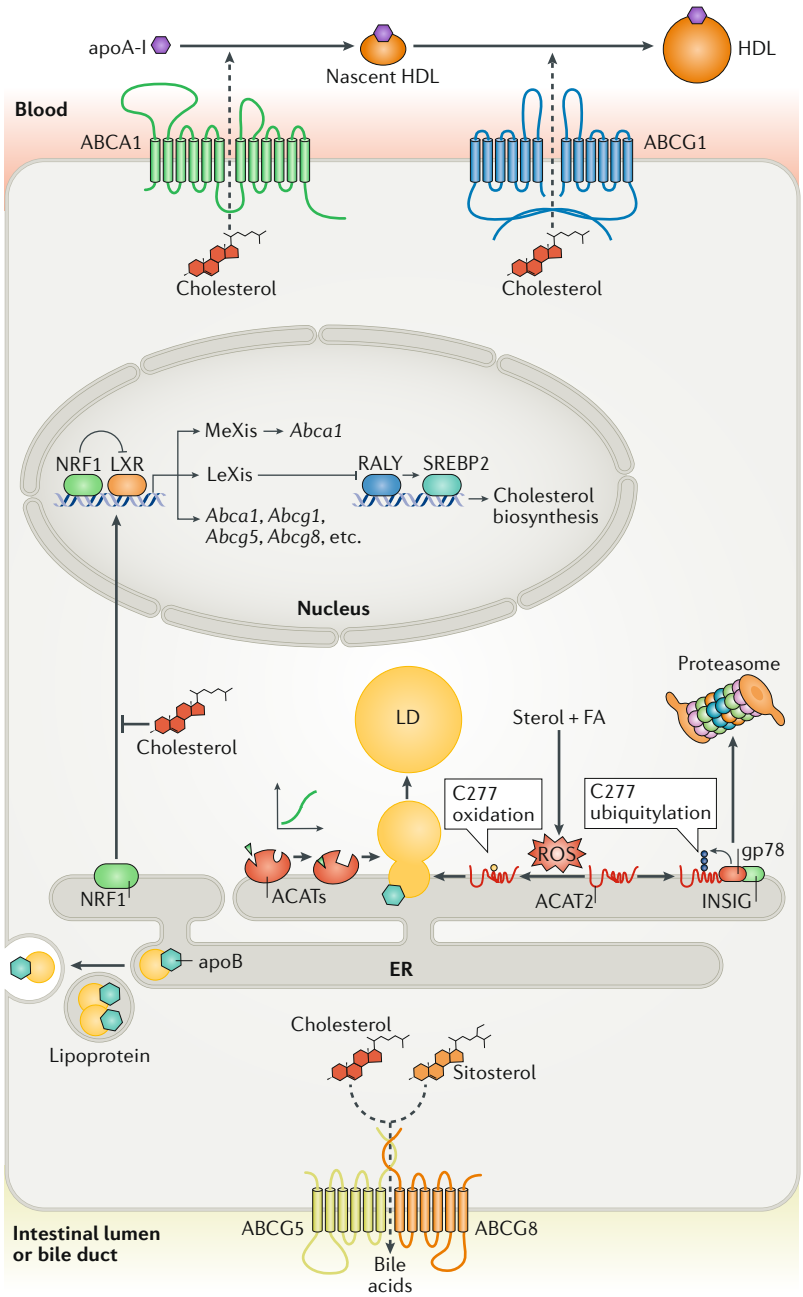



Fig. 4 | **Mechanisms regulating cholesterol efflux and esterification.** Cholesterol export from cells is mediated by ATP-binding cassette (ABC) transporters. ABC subfamily A member 1 (ABCA1) is expressed on the plasma membrane of most cells, including the basolateral surface of enterocytes. ABC subfamily G member 1 (ABCG1) is most abundantly expressed on the surface of macrophages, whereas ABCG5 and ABCG8 are expressed at the apical surface of enterocytes and hepatocytes, forming a heterodimer. Excess cholesterol is esterified by acyl coenzyme A:cholesterol acyltransferases (ACATs; also known as SOATs). ACAT1 is ubiquitously expressed and ACAT2 is predominantly expressed in enterocytes and hepatocytes. Top: ABCA1 mediates cholesterol transport to apolipoprotein A-I (apoA-I) in the blood. In macrophages, this generates nascent high-density lipoprotein (HDL) that serves as an acceptor for ABCG1-mediated cholesterol efflux, leading to the production of HDL. Bottom: cholesterol and sitosterol can be exported by the ABCG5–ABCG8 heterodimer to the intestinal lumen and bile, where cholesterol is extracted by bile salts. Middle: repletion of cholesterol induces pathways for cholesterol export and storage, and acts to suppress further cholesterol biosynthesis. Cholesterol blocks the nuclear entry of nuclear factor erythroid 2 related factor 1 (NRF1) and subsequently derepresses the activity of liver X receptor (LXR), thereby allowing (as shown in mice) transcription of the long non-coding RNA MeXis (macrophage-expressed LXR-induced sequence), which is a positive regulator of *Abca1* transcription; of LeXis (liver-expressed LXR-induced sequence), which prevents efficient transcription of *Srebp2* and cholesterologenic genes by inhibiting DNA binding of RALY (a heterogeneous ribonucleoprotein that is required for the maximal expression of cholesterologenic genes in mouse liver); and direct regulation of the expression of *Abca1*, *Abcg1*, *Abcg5*, *Abcg8* and other proteins. Excess cholesterol can also allosterically (indicated by the sigmoidal curve) activate ACATs in the endoplasmic reticulum (ER), inducing the conversion of cholesterol to cholesteryl esters for storage in lipid droplets (LDs) or secretion as a major component of lipoproteins (including chylomicrons derived from enterocytes as a result of cholesterol absorption, and very-low-density lipoproteins (VLDLs) produced by hepatocytes as a result of cholesterol biosynthesis). Accumulating sterols and fatty acids (FAs) also induce reactive oxygen species (ROS) that oxidize ACAT2 at the Cys277 (C277) residue, stabilizing the protein by competitively blocking its ubiquitylation and proteasomal degradation, and thereby promoting cholesterol storage and/or export. gp78 (also known as AMFR), a ubiquitin ligase; INSIG, insulin-induced gene; SREBP2, sterol regulatory element-binding protein 2.

suppressing the mevalonate pathway and liver tumorigenesis[192]. How ABCA1 coordinates its role in cholesterol efflux and retrograde transport is worth investigating further. ABCA1 expression is downregulated by miR-33 (co-transcribed with *SREBP* mRNAs)[194–196]. Additional transcriptional and post-transcriptional regulators of ABCA1 have been discussed elsewhere[197–199].

### *ABCG1-mediated cholesterol efflux*

Besides ABCA1, ABCG1 is also abundantly expressed in macrophages and many other cell types but expression is low in hepatocytes and is absent from enterocytes[200]. ABCG1 is a half-size transporter that can dimerize with another ABCG1 or ABCG4 to constitute functional transporters. ABCG1 has an established role in fluxing lipids out of the cell. How it contributes to atherosclerosis development is obscure, with conflicting results reported[174]. Nevertheless, combined depletion of *Abca1* and *Abcg1* induces massive lipid accumulation in macrophage-rich tissues[201]. Furthermore, macrophage deficiency of *Abca1* and *Abcg1* is sufficient to accelerate atherosclerosis in *Ldlr*-knockout mice on a chow diet[202]. These results support macrophage cholesterol efflux by ABCA1 and ABCG1 being directly involved in preventing atherosclerosis.

ABCG1 mediates cholesterol efflux to various extracellular acceptors including HDL, LDL, albumin, methyl-β-cyclodextrin and liposomes, but not to lipid-free apoA-I unless ABCA1 is active[177,200,203,204]. Other than cholesterol, oxysterols such as those oxidized at the C7 position and 25-hydroxycholesterol, and choline

phospholipids such as sphingomyelin, are the transport substrates for ABCG1 as well[204,205].

To date, very little is known about the mechanisms underlying ABCG1-mediated lipid removal. The exact subcellular localization of ABCG1 has been a matter of debate, with one group reporting the restricted distribution in endosomal vesicles[206,207] whereas others detected the protein on the plasma membrane[208,209] and along both secretory and endocytic pathways[210]. ABCG1 in early endosomes and recycling endosomes is postulated to mobilize cholesterol from the ER to the inner leaflet of these vesicles, which, upon fusing with the plasma membrane, distribute cholesterol to the outer leaflet of the plasma membrane[206]. Alternatively, cholesterol in late endosomes may be delivered to the plasma membrane by non-vesicular mechanisms[210]. On the cell surface, ABCG1 is specifically localized to the microdomains that are enriched in cholesterol and sphingomyelin and are associated with flotillin 1 and actin[208,211], and hence may redistribute membrane cholesterol and increase its accessibility to extracellular acceptors[208,212]. This expansion of the free cholesterol pool, together with enhanced desorption from the membrane[210,213], accounts for cholesterol efflux mediated by ABCG1. Notably, the removal of plasma membrane cholesterol may solely require the lipid surface of extracellular acceptors[177,210], which need not necessarily bind ABCG1 (REFS[203,213]).

Like *ABCA1*, the *ABCG1* gene harbours multiple response elements for the LXR and RXR heterodimers[214]. ABCG1 (and ABCA1) is repressed by miR-10b in mouse and human cells[215]. Mouse (but not human) *Abcg1* is also a direct target of miR-33 (REFS[194–196]). AMPK can prolong *Abcg1* mRNA stability by activating the ERK pathway[216]. Recently, ovarian cancer cell-derived hyaluronic acid was found to upregulate the expression of both ABCA1 and ABCG1, promoting plasma membrane cholesterol efflux from tumour-associated macrophages and suppressing their antitumour functions[217]. This study implicates a new function of cholesterol efflux in cancer and the immune system.

### *Cholesterol efflux by ABCG5 and ABCG8*

ABCG5 and ABCG8 are nearly exclusively expressed in the apical surface of hepatocytes and enterocytes, where they function as a heterodimer mediating the excretion of neutral sterols, including plant sterols and cholesterol, into the bile and intestinal lumen, respectively[218]. Single or combined mutations in *ABCG5* or *ABCG8* result in sitosterolemia (TABLE 1). In mice, hepatic ABCG5 and ABCG8 directly promote the efflux of liver sterol into the bile[219–221], whereas the intestinal counterparts are involved in disposing plasma-derived cholesterol into the gut lumen, most likely via the transintestinal cholesterol excretion pathway[222,223].

ABCG5 and ABCG8 are postulated to flop cholesterol from the inner leaflet to the outer leaflet of the canalicular membrane, where it is extracted by bile salts[224] (FIG. 4, bottom). In support of this notion, a putative cholesterol-binding region was recently identified in the interface of the purified human ABCG5–ABCG8 dimer[225]. Phospholipids greatly facilitate biliary cholesterol secretion through increasing its solubilization in bile salt micelles[224]. Other than flipping cholesterol across the membrane bilayer, ABCG5 and ABCG8 may push cholesterol partially into the aqueous phase and facilitate its easy pickup by the bile salt–phosphatidylcholine micelles[226]. The exact mechanism of ABCG5 and ABCG8-mediated cholesterol efflux is yet to be determined.

In line with ABCG5 and ABCG8 forming a functional pair, *ABCG5* and *ABCG8* lie head to head on the opposite DNA strands and share an intergenic region as the common promoter driving the transcription of both genes in opposing directions[227–229]. This region also contains several transcription factor binding sites that confer their responsiveness to LRH1 (REF.[230]), HFN4α (REF.[231]), GATA-binding proteins[229,231], FOXO1 (REF.[232]) and nuclear factor-κB[233]. LXRs have been long known as a positive regulator of *ABCG5* and *ABCG8* in vitro and in vivo[228,234,235]. This transactivation is mediated by two evolutionarily conserved regions distal to the intergenic region[236]. FXR agonists and bile acids can induce *ABCG5* and *ABCG8* expression in human and mouse primary hepatocytes via the FXR response elements outside the intergenic region[237].

## Regulation of esterification

The formation of cholesteryl esters is another important means to prevent free cholesterol accumulation in the cell as this pathway, mediated by ACATs, directs cholesterol for storage or secretion. Esterification is also required for the absorption of cholesterol in the intestine and for maintaining the balance between free cholesterol and cholesteryl esters.

Mammals have two ACAT isozymes, ACAT1 and ACAT2 (REF.[238]). Both are integral membrane proteins with nine transmembrane domains predicted for ACAT1 and two to five domains for ACAT2 (REFS[239–241]). The first ~140 amino acids of ACAT1 reside in the cytoplasmic side of the ER and mediate the formation of a tetramer from two homodimers[242,243]. A conserved His460 residue constitutes, at least in part, the active site of ACAT1 (REFS[241,244]). ACAT2 shares a high sequence homology to ACAT1 in the C terminus and the equivalent His434 is key to its catalytic activity[240]. Whether ACAT2 exists as an oligomer is unknown.

### *Role and regulation of ACAT1*

ACAT1 is present throughout the body, most abundantly in macrophages, epithelial cells and steroid hormone-producing cells[245,246]. High levels of ACAT1 are also found in macrophages of human atherosclerotic lesions[247], suggesting the involvement of ACAT1 in the pathology of atherosclerosis. However, the exact role of ACAT1 in macrophages in the contexts of atherosclerosis is elusive and it is now hotly debated whether the loss of ACAT1 prevents or exacerbates atherosclerosis[248–250]. More recently, blocking ACAT1-mediated cholesterol esterification genetically or pharmacologically has been shown to ameliorate amyloidopathy in mouse models of Alzheimer disease[8] and to arrest tumour growth of pancreatic and prostate cancer[251,252]. ACAT1 inhibition in $CD8^+$ T cells increases plasma membrane cholesterol levels, promoting T-cell receptor clustering and

Transintestinal cholesterol excretion pathway
A process of faecal excretion of plasma-derived cholesterol from the inside of enterocytes to the intestinal lumen.

Micelles
The spherical assembly of amphiphilic molecules dispersed in water solvent.

Immunological synapse
The interface formed between an antigen-presenting cell or target cell and a lymphocyte such as a T cell, B cell or natural killer cell.

Sitosterol
A plant sterol with a chemical structure very similar to that of cholesterol. Sitosterol is poorly absorbed by healthy individuals and may help to lower cholesterol in humans.

Retinoic acid
A metabolite of vitamin A1 (all-*trans*-retinol). Retinoic acid is a ligand of nuclear receptors RAR and RXR and regulates cell growth and differentiation.

immunological synapse formation that eventually enhance the antitumour activity of these cells[253]. Addition of the ACAT1 inhibitor synergistically augments the efficacy of immunotherapy against melanoma[253], highlighting a novel therapeutic potential of ACAT1 in cancer treatments.

The purified ACAT1 protein utilizes cholesterol both as an activator of its enzymatic activity and as a substrate[254]. Upon activation, ACAT1 can accommodate sterols or sterol-like molecules that share a 3β-hydroxyl group, such as 7-ketocholesterol, sitosterol and pregnenolone, as substrates[255–257]. The stereospecific interactions between ACAT1 and activators and substrates suggest an allosteric model of enzymatic activity, in which activator binding probably triggers a conformational change of the enzyme to facilitate substrate binding and enhance the catalytic activity[255,256] (FIG. 4, middle).

The transcriptional regulation of ACAT1 has been reported as well. The human *ACAT1* gene is unique in that it has two promoters, P1 and P7, located in chromosome 1 and 7, respectively[258]. The two transcripts driven by P1 and P7 form a mature mRNA through *trans*-splicing. No binding site for SREBPs or LXRs has been identified in either promoter[258]. Instead, the P1 promoter is activated by the cytokine interferon-γ and all-*trans*-retinoic acid[259], the synthetic glucocorticoid dexamethasone[260] and another cytokine, tumour necrosis factor[261]. Insulin can stimulate *ACAT1* expression as well[262].

### *Role and regulation of ACAT2*

ACAT2 is predominately expressed in enterocytes and to some extent also in hepatocytes[246,263,264]. The formation of cholesteryl esters by ACAT2 directly increases intestinal cholesterol absorption and requires at least one copy of the *Acat2* gene[265]. Global ablation of *Acat2* substantially reduces cholesterol absorption, prevents plasma cholesterol elevation and averts diet-induced hepatic cholesterol accumulation[266,267]. Loss or inhibition of ACAT2 also retards atherosclerosis in hyperlipidaemic mice[268–270]. However, ACAT2 deficiency markedly enhances the expression of ABCA1 regardless of dietary cholesterol levels[267,271], through which cholesterol is exported and partly maintains the residual intestinal cholesterol absorption in *Acat2*-knockout mice[272]. Combined depletion of *Acat2* and *Abca1* further lowers cholesterol absorption compared with the single knockouts[272], without further increasing the free cholesterol content seen in the intestine of *Acat2*-knockout mice[273]. Hence, compared with ACAT1, ACAT2 seems to be a promising therapeutic target for atherosclerosis.

ACAT2 can be potently activated by cholesterol to catalyse the esterification of various sterols or steroids containing a 3β-hydroxyl group with fatty acyl-CoA[246,257,264]. However, it is more efficient at esterifying 25-hydroxycholesterol and bile acid derivatives and less efficient at esterifying cholesterol compared with ACAT1 (REF.[274]). The ability of ACAT2 to distinguish sitosterol from cholesterol is high, and only limited sitosteryl esters can be produced by the enzyme[255,275]. This ability, together with selective uptake of cholesterol over sitosterol by NPC1L1 (REF.[100]) and non-discriminative efflux of cholesterol and sitosterol by ABCG5 and ABCG8, enables efficient absorption and resorption of cholesterol by enterocytes and hepatocytes, respectively[276].

Several transcription factors, including HNF1α, HNF4α and CDX2, have been found to enhance ACAT2 expression in human liver and intestinal cells[277–279]. At low cellular lipid levels, the ACAT2 protein can be ubiquitylated at a highly conserved Cys277 residue and targeted for proteasomal degradation[280]. By contrast, production of reactive oxygen species (ROS) induced by lipid overload causes competitive oxidation of Cys277, resulting in ACAT2 stabilization and increased cholesteryl ester formation[280] (FIG. 4, middle). This post-translational regulation of ACAT2 protein adds another line of defence against lipotoxicity in the cell and represents an important mechanism of sensing ROS.

## Interplay of the regulatory pathways

As is apparent from the above discussion, cholesterol metabolism comprises a network of pathways involving enzymes (for example, HMGCR, squalene monooxygenase, ACAT1 and ACAT2), importers (for example, LDLR and NPC1L1), exporters (for example, ABCA1, ABCG1, ABCG5 and ABCG8), sterol transport proteins (for example, NPC1, NPC2 and ORPs) and, importantly, a large number of regulators including metabolites (for example, cholesterol itself, sterol intermediates, oxysterols, geranylgeraniol) (TABLE 2), proteins (for example, transcription regulators, including the SCAP–SREBP complex and LXRs, as well as post-translational regulators, such as INSIGs and E3 ubiquitin ligases) and RNAs (for example, microRNAs and lncRNAs).

To achieve homeostasis, the molecular machineries regulating cholesterol biosynthesis, uptake, transport (not discussed in detail here), efflux and esterification must be tightly controlled so that sufficient cholesterol is produced for cell growth and function but at the same time avoiding excess cholesterol accumulation. Hence, the system must be inherently sensitive to cellular sterol levels (TABLE 2). Indeed, various components can directly sense sterol fluctuations and trigger adaptive responses. These factors include the SCAP–SREBP complex, INSIGs, HMGCR, ACATs, LXRs and the recently identified nuclear factor erythroid 2-related factor 1 (NRF1) that regulates cholesterol efflux in the presence of high cholesterol via derepression of the LXR pathway[281] (FIG. 4).

A site for cholesterol biosynthesis and esterification, and the centre of negative feedback regulation, the ER has surprisingly low levels (about 3% of total ER lipids in rat hepatocytes)[282] of cholesterol at the steady state. Therefore, cholesterol generated from or arriving at the ER needs to be rapidly conveyed to other organelles, such as endosomes, the *trans*-Golgi, mitochondria and lipid droplets, and to the plasma membrane by vesicles or sterol transport proteins, or converted into the esterified form by ACATs. Accumulating ER lanosterol, cholesterol or its derivatives can induce rapid degradation of HMGCR and squalene monooxygenase. High levels of cholesterol also inhibit the SREBP pathway by triggering INSIGs to bind SCAP. This prevents transcription of a battery of genes involved in cholesterol biosynthesis (for example, *HMGCR* and *NADPH*) and uptake

Table 2 | **Homeostatic regulation of cholesterol metabolism by sterols**

| Signals | Function[a] | | Effect on cholesterol homeostasis[b] |
|---|---|---|---|
| Lanosterol, oxysterols, geranylgeraniol | HMGCR degradation | | Biosynthesis ↓ |
| Cholesterol | SM degradation | | Biosynthesis ↓ |
| Cholesterol | SREBP pathway inactivation | Cholesterologenic genes ↓ | Biosynthesis ↓ |
| | | *LDLR* ↓ | Uptake ↓ |
| | | *NPC1L1* ↓ | Uptake ↓ |
| | | miR-33 ↓ and thus *ABCA1* ↑ | Efflux ↑ |
| Oxysterols, desmosterol | LXR activation | *ABCA1*, *ABCG1*, *ABCG5*, *ABCG8* ↑ | Efflux ↑ |
| | | *MeXis* ↑ and thus *ABCA1* ↑ | Efflux ↑ |
| | | *LeXis* ↑ and thus *SREBP2* and its target genes ↓ | Biosynthesis ↓ |
| | | *IDOL* ↑ and thus LDLR ↓ | Uptake ↓ |
| | | *RNF145* ↑ and thus HMGCR and SCAP ↓ | Biosynthesis ↓ |
| | | *SREBP1c* ↑ and thus FA biosynthesis ↑ | –[c] |
| Cholesterol | NRF1 retention in the ER and thus derepression of the LXR pathway | | Efflux ↑ |
| Cholesterol, oxysterols | ACAT1 and ACAT2 allosteric activation | | Esterification ↑ |
| | ACAT2 stabilization | | Esterification ↑ |

ABCA1, ATP-binding cassette subfamily A member 1; ABCG, ATP-binding cassette subfamily G member; ACAT, acyl-coenzyme A:cholesterol acyltransferase; ER, endoplasmic reticulum; FA, fatty acid; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; IDOL, inducible degrader of the LDL receptor; LDL, low-density lipoprotein; LDLR, LDL receptor; LeXis, liver-expressed LXR-induced sequence; LXR, liver X receptor; MeXis, macrophage-expressed LXR-induced sequence; NPC1L1, Niemann–Pick type C1-like 1 protein; NRF1, nuclear factor erythroid 2 related factor 1; RNF145, RING-finger protein 145; SCAP, SREBP-cleavage activating protein; SM, squalene monooxygenase; SREBP, sterol regulatory element-binding protein. [a]Changes (upregulation ↑ or downregulation ↓) in the expression of genes (italicized) and proteins induced by specific sterols. [b]Associated changes in cholesterol metabolic pathways that ensure maintenance of cholesterol homeostasis in the presence of sterols. [c]FA produced upon LXR activation is esterified with cholesterol to form cholesteryl ester.

(for example, *LDLR* and *NPC1L1*). Expression of miR-33, the negative regulator of ABCA1, is downregulated upon cholesterol accumulation as well[283]. Oxysterols and desmosterol[284] can activate the LXR pathway and upregulate transcription of *ABCA1*, *ABCG1*, *ABCG5* and *ABCG8*. LXR activation also enhances expression of *ABCA1* via MeXis, and inhibits the expression of *SREBP2* via LeXis[285]. Additionally, LXR causes upregulation of IDOL and RNF145, which mediate the degradation of LDLR and that of HMGCR and SCAP, respectively. Further, ACAT1 and ACAT2 are allosterically activated by excess cholesterol, which, together with fatty acids produced as a result of LXR-mediated upregulation of *SREBP1c*, is converted into less toxic cholesteryl esters for storage. The stabilization of ACAT2 by sterols and saturated fatty acids contributes to cholesterol esterification as well[280].

Cholesterol homeostasis at systemic levels requires collaboration between various tissues, which ensures a balance between cholesterol absorption (in the intestine) and cholesterol biosynthesis (mostly in the liver) with its release into the bloodstream and subsequent uptake (and removal if necessary) by cells in the body. In the bloodstream, cholesterol is transported as various lipoproteins, mostly LDLs. As LDLR is nearly ubiquitously expressed, LDLs can be taken up by the liver and extrahepatic tissues, including the small intestine, via the LDLR pathway. The liver and small intestine can also acquire cholesterol in an NPC1L1-dependent manner. Cholesterol surpassing the cellular capacity follows three fates depending on its location: it can be stored as it is (in adipocytes); it can be effluxed from the cell (by ABCA1 and ABCG1 in macrophages; by ABCA1 from the basolateral surface of enterocytes and other ABCA1-expressing cells such as hepatocytes and pneumocytes; and by ABCG5 and ABCG8 from the apical surface of enterocytes and hepatocytes); and it can be esterified (by ACAT1 in all cells, and by ACAT2 in enterocytes and hepatocytes). Cholesterol efflux from peripheral cells to plasma apoA-I generates HDLs, which are then transported back to the liver for scavenger receptor class B type I-mediated uptake. Cholesterol released from the liver can be either resorbed or excreted from the body.

## Conclusions and perspectives

Cholesterol homeostasis has been extensively investigated in the last century and is still gaining much attention today owing to the intimate implication in a growing list of diseases beyond cardiovascular disease, such as the peroxisome disorders[137], Alzheimer disease[8] and cancers[192,251–253,286]. Over the past 5 years, many structures of key regulators (the SCAP–SREBP complex[22], INSIG homologue[287], squalene monooxygenase catalytic domain[89], NPC1 (REF.[288]), ABCA1 (REF.[180]), ABCG5 and ABCG8 (REF.[225])) have been resolved; additional mechanisms have been delineated (for example, demonstration of the roles of ABCG5 and ABCG8 in cholesterol excretion[219,222,223], and of NPC1L1 in intestinal cholesterol absorption[103]); other mechanisms

have been refined with new details (for example, demonstration of cholesterol-induced conformational changes in SCAP)[289]; new regulators of cholesterol biosynthesis (RNF145 (REFS[34,61]), MARCH6 (REF.[93]) and UBIAD1 (REFS[67,290])), uptake (LIMA1 (REF.[104])), efflux (NRF1 (REF.[281]), LeXis[285] and MeXis[191]) and esterification (ROS)[280] and new pathways (for example, transintestinal cholesterol excretion)[222] have been identified; and new functions of known proteins (for example, ABCA1 in retrograde sterol transport)[193] have been characterized. These advances provide invaluable mechanistic insights into whole-body cholesterol metabolism under physiological and pathophysiological conditions, opening new possibilities for therapeutic interventions for the treatment of cholesterol-related diseases (TABLE 1).

At present, cholesterol metabolism is widely targeted in the context of cardiovascular disorders. Currently, statins, which function as HMGCR inhibitors, have been widely used for primary and secondary prevention of cardiovascular disease. However, statin efficacy can be limited by compensatory increases in HMGCR protein. Ezetimibe and the PCSK9 inhibitors further decrease LDL-c levels and improve cardiovascular outcomes in hyperlipidaemic individuals on statins[291,292]. Inspired by lanosterol-induced HMGCR degradation, a compound structurally analogous to lanosterol was recently developed to combat statin-associated HMGCR elevation and prevent atherosclerotic plaque formation in mice[293]. Other than cardiovascular disease, cholesterol lowering seems to be a promising strategy for the treatment of cancers[9] and congenital diseases (for example, the cholesterol mobilizer 2-hydroxypropyl-β-cyclodextrin has been demonstrated to ameliorate symptoms of NPC disease)[294–296]. However, more work is needed to lessen the side effects and to search for more effective and safer agents that modify cholesterol metabolism.

Several important questions pertaining to cholesterol metabolism remain unanswered. The current models and theory about cholesterol metabolism are mostly based on work on peripheral tissues, which are separated from the brain by the blood–brain barrier. Whether cholesterol levels are maintained in a similar way in the brain is still uncertain, but this knowledge will be key to understand the association of cholesterol with neurodegenerative disorders. It will also be interesting to study cholesterol metabolism in the less explored cell types (for example, stem cells, immune cells, neurons and so forth). How cholesterol metabolism responds to additional signals other than lipids needs investigation. The new functions of cholesterol, sterol intermediates of the mevalonate pathway and cholesterol derivatives are also important directions to follow.

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