The synthesis of cholesterol requires more than 20 enzymes, many of which are intricately regulated. Post-translational control of these enzymes provides a rapid means for modifying flux through the pathway. So far, several enzymes have been shown to be rapidly degraded through the ubiquitin–proteasome pathway in response to cholesterol and other sterol intermediates. Additionally, several enzymes have their activity altered through phosphorylation mechanisms. Most work has focused on the two rate-limiting enzymes: 3-hydroxy-3-methylglutaryl CoA reductase and squalene monooxygenase. Here, we review current literature in the area to define some common themes in the regulation of the entire cholesterol synthesis pathway. We highlight the rich variety of inputs controlling each enzyme, discuss the interplay that exists between regulatory mechanisms, and summarize findings that reveal an intricately coordinated network of regulation along the cholesterol synthesis pathway. We provide a roadmap for future research into the post-translational control of cholesterol synthesis, and no doubt the road ahead will reveal further twists and turns for this fascinating pathway crucial for human health and disease.

Like most animals, humans need cholesterol, but too much can be harmful. Therefore, multiple layers of intricate interwoven mechanisms have evolved to keep cholesterol levels in check. Cholesterol is essential as the precursor for steroid hormones, bile acids, and oxysterols (Fig. 1) and as an important component of cell and organelle membranes. Providing strength and fluidity to the membrane, cholesterol acts as a barrier to permeability and an organizer of specialized membrane domains, notably lipid rafts, which can serve as signaling hubs (1). However, excess cholesterol contributes to disease, notably atherosclerotic cardiovascular disease (2), but it is also increasingly recognized to play a role in some cancers (3) and in neurodegenerative disease (4). Therefore, balancing cholesterol to ensure levels are adequate but not excessive is crucial.

Omnivores ingest some cholesterol, but humans generally synthesize considerably more cholesterol than we consume (5). Building from acetyl-CoA and harnessing more than 20 enzymes, cholesterol synthesis is an especially energy-intensive process, providing another reason why exquisite regulation is required (5). Many of these mechanisms are feedback loops, triggered when cholesterol levels accumulate sufficiently that no more needs to be made. Thus, almost all genes in the cholesterol synthesis pathway are induced by the sterol-regulatory element binding protein-2 transcription factor when cholesterol levels are low and down-regulated when cholesterol levels are high (6). This relatively well-studied transcriptional program allows gradual oscillations of control. Layered on top is the more acute post-translational regulation, including inactivation and demolition of individual enzymes, for instance by phosphorylation and the ubiquitin–proteasome system, respectively.

Cholesterol is not the only significant sterol in humans, and like oxysterols (7), intermediates in cholesterol synthesis often exhibit biological activities very different from cholesterol itself (8). Indeed, in recent years intermediates in the cholesterol synthesis pathway have been implicated in a range of biological and pathological contexts, including immunity (9), neurological conditions (10), and cancers (11). This provides yet more impetus to better understand how flux through the pathway is controlled.

**Cholesterol synthesis pathway**

The cholesterol synthesis pathway (Table 1) encompasses 22 enzymes and can be divided almost equally into the early sterol synthesis pathway up to lanosterol (the first true sterol) and the post-lanosterol pathway, which has two parallel branches leading to cholesterol (Fig. 1). The early pathway contains HMGCR, the classic control point and well-studied target of the statins, first-line therapy for cardiovascular disease. Also in the early pathway is a second rate-limiting enzyme, SM, which is exquisitely regulated, but in different ways than HMGCR (Fig. 2). The post-lanosterol enzymes also have several interesting modes of regulation that provide yet more control mechanisms regulating this highly intricate pathway.

From lanosterol, the pathway can take one of two intertwined routes, creating a long and winding road to cholesterol through various branch points and side tracks (Fig. 1). Lanosterol can be acted upon by LDM to enter the Bloch pathway, or DHCR24 to enter the Kandutsch–Russell pathway, both of which use the same enzymes to ultimately produce cholesterol, via distinct intermediates (Fig. 2). DHCR24 can theoretically act on any intermediate from lanosterol through to desmosterol to transfer intermediates from the Bloch to the Kandutsch–Russell pathway. A modified Kandutsch–Russell pathway (13) is believed to be more widely utilized, in which DHCR24 converts zymosterol to zymostenol as the major step.
Post-translational control of cholesterol synthesis

Cholesterol synthesis occurs largely in the endoplasmic reticulum (ER), and this is where most enzymes are localized. In fact, there is some evidence that they interact with each other to optimize efficiency of cholesterol synthesis.

In 2013, we reviewed the control of cholesterol synthesis by enzymes beyond the classic control point, HMGCR (12). At the time, only degradation of HMGCR and SM was known, but we now know that at least four additional enzymes are subject to controlled degradation. In 2013, degradation by E3 ligases was only partially explored for HMGCR, now we know that at least four additional enzymes are subject to degradation. In 2013, we reviewed the control of cholesterol synthesis by enzymes beyond the classic control point, HMGCR (12). At the time, only degradation of HMGCR and SM was known, but we now know that at least four additional enzymes are subject to controlled degradation. In 2013, degradation by E3 ligases was only partially explored for HMGCR, but now we know that five E3 ligases may be involved in its degradation, including MARCHF6, which also degrades at least three more cholesterol synthesis enzymes. This expanding field of research is uncovering many complexities that involve intricate regulation of a large number of enzymes along the pathway. We anticipate that each enzyme will have its own story to tell in due course. Here, we delve into the latest developments on the post-translational control of cholesterol synthesis, notably the degradation of its enzymes. We begin by introducing some post-translational modifications and then explore in depth the regulatory mechanisms of the enzymes in the pathway.

Post-translational modifications

Post-translational regulation of enzymes often occurs by post-translational modifications (PTMs), such as ubiquitination (also called ubiquitylation) and phosphorylation, which typically lead to degradation or altered activity, respectively. To survey the PTM landscape of the cholesterol synthesis pathway, we utilized several databases (notably, PhosphoSite Plus (14)), alongside targeted literature searches.

Of the >450 known PTMs (15), the most commonly reported on cholesterol synthesis enzymes are ubiquitination, phosphorylation, and acetylation, in that order. There are a handful of enzymes that are SUMOylated, monomethylated, and/or succinylated on specific lysine residues. Only one enzyme is known to be glycosylated (N-linked glycosylation on HMGCR (16), and one is both O-GalNAc and O-GlcNac modified (EBP (17, 18)). Although one might expect modification with lipid anchors such as prenylation or long-chain fatty acylation given the downstream location of most of the enzymes, no such modifications are reported.

When examining the extent of PTMs in the context of the total human proteome (14, 19), cholesterol synthesis enzymes tended to be more heavily ubiquitinated but less phosphorylated, particularly at serine and threonine residues (Fig. 3; for details of modified sites, please refer to Table S1). PTMs are continually being added to the PhosphoSite database, and so there are likely many more modifications on these enzymes that are yet to be discovered. This is particularly true for enzymes that are likely to be under-represented in large-scale proteomic studies, such as IDI2, which has low expression except in muscle (20), and DHCR14, which is very low in many tissues (21), as well as being particularly hydrophobic (Fig. 3), which may hinder mass spectrometric analysis. Another important note is that each enzyme is very likely to have a large number of “proteoforms,” a term adopted to refer to the complexity of proteins taking into account each possible variation, including its modifications (22).

Ubiquitin–proteasome system (UPS)

The UPS involves the conjugation of ubiquitin to substrates through an E1 activating enzyme, E2 conjugating enzyme, and an E3 ubiquitin ligase. The mammalian genome encodes two E1s and ~40 E2s, but many hundreds of E3s (23). Therefore, the E3 ligases provide specificity toward particular target proteins and are classified into one of three families: the majority comprise the RING ligases, and the remainder are either HECT or U-box ligases. Ubiquitin is typically conjugated to lysine residues of the target protein but can less commonly be attached to noncanonical residues (serine, threonine, and cysteine), as well as to the N-terminal amine. To add another level of sophistication, there are ~100 deubiquitinating enzymes, with the ability to remove, or at least trim, the polyubiquitin chains (24).

There are several E3 ligases implicated in the control of HMGCR (Gp78, TRC8, HRD1, MARCHF6, and RNF145) (25–28), and one E3 ligase, MARCHF6, targets four enzymes in the cholesterol synthesis pathway (SM and HMGCR (28), LDM and DHCR24 (29)). These all lead to degradation of the target enzymes. Deubiquitinases are involved in the regulation of SM,
Table 1
Cholesterol synthesis enzymes
The cholesterol synthesis enzymes and their UniProt (19) identifiers. We have chosen commonly used abbreviations for enzymes throughout this review to maximize clarity.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Protein name (UniProt)</th>
<th>UniProt identifier</th>
<th>EC</th>
<th>Gene symbol</th>
</tr>
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<tbody>
<tr>
<td>Early pathway</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACAT2</td>
<td>Acetyl-CoA acetyltransferase, cytosolic</td>
<td>Q9BW01</td>
<td>2.3.1.9</td>
<td>ACAT2</td>
</tr>
<tr>
<td>HMGCS</td>
<td>Hydroxymethylglutaryl-CoA synthase, cytoplasmic</td>
<td>Q01581</td>
<td>2.3.3.10</td>
<td>HMGCS1</td>
</tr>
<tr>
<td>HMGCR</td>
<td>3-Hydroxy-3-methylglutaeryl-coenzyme A reductase</td>
<td>P06035</td>
<td>1.1.3.4</td>
<td>HMGCR</td>
</tr>
<tr>
<td>MVK</td>
<td>Mevalonate kinase</td>
<td>Q03426</td>
<td>2.7.1.36</td>
<td>MVK</td>
</tr>
<tr>
<td>PMVK</td>
<td>Phosphomevalonate kinase</td>
<td>Q15126</td>
<td>2.7.4.2</td>
<td>PMVK</td>
</tr>
<tr>
<td>MVD</td>
<td>Diphosphomevalonate decarboxylase</td>
<td>P53602</td>
<td>4.1.1.34</td>
<td>MVD</td>
</tr>
<tr>
<td>ID1/ID1</td>
<td>Isopentenyl-diphosphate Δ5-isomerase 1/2</td>
<td>Q13907/Q9BS1</td>
<td>5.3.3.2</td>
<td>ID1/2</td>
</tr>
<tr>
<td>FDPS</td>
<td>Farnesyl pyrophosphate synthase</td>
<td>P14324</td>
<td>2.5.1.1, 2.5.1.10</td>
<td>FDPS</td>
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<td>Geranylgeranyl pyrophosphate synthase</td>
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<td>GGPPS</td>
</tr>
<tr>
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<td>Squalene synthase</td>
<td>P37268</td>
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<td>FFFT1</td>
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<tr>
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<td>Squalene monooxygenase</td>
<td>Q14334</td>
<td>1.14.13.132</td>
<td>SLE</td>
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<td>LDM</td>
<td>Lanosterol synthase</td>
<td>P48449</td>
<td>5.9.97</td>
<td>LSS</td>
</tr>
<tr>
<td>Post-lanosterol pathway</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDM</td>
<td>Lanosterol 14-α-deethylase</td>
<td>Q16850</td>
<td>1.14.13.70</td>
<td>CYP51A1</td>
</tr>
<tr>
<td>DHCR14</td>
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<td>O76082</td>
<td>1.3.1.70</td>
<td>TM7SF2</td>
</tr>
<tr>
<td>LBR</td>
<td>Δ4-Sterol reductase LBR</td>
<td>Q14739</td>
<td>1.3.1.70</td>
<td>LBR</td>
</tr>
<tr>
<td>SC4MOL</td>
<td>Methylsterol monooxygenase 1</td>
<td>Q15800</td>
<td>1.14.13.72</td>
<td>SCAMOL</td>
</tr>
<tr>
<td>NSDHL</td>
<td>Sterol-4-α-carboxylic 3-dehydrogenase, decarboxylating</td>
<td>Q15738</td>
<td>1.1.1.170</td>
<td>NSDHL</td>
</tr>
<tr>
<td>HSD17B7</td>
<td>3-Keto-sterol reductase</td>
<td>P56937</td>
<td>1.1.1.270</td>
<td>HSD17B7</td>
</tr>
<tr>
<td>EBP</td>
<td>3-β-Hydroxysteroid-Δ5,Δ7-isomerase</td>
<td>Q15123</td>
<td>5.3.3.5</td>
<td>EBP</td>
</tr>
<tr>
<td>SCSD</td>
<td>Lathosterol oxidase</td>
<td>O75845</td>
<td>1.14.21.6</td>
<td>SCSD</td>
</tr>
<tr>
<td>DHCR7</td>
<td>7-Dehydrocholesterol reductase</td>
<td>Q9U877M</td>
<td>1.3.1.21</td>
<td>DHCR7</td>
</tr>
<tr>
<td>DHCR24</td>
<td>Δ24-Sterol reductase</td>
<td>Q15392</td>
<td>1.3.1.72</td>
<td>DHCR24</td>
</tr>
</tbody>
</table>

although their identities remain unknown (30). The deubiquitinase USP19 has been implicated in the regulation of both HRD1 (31) and MARCHF6 (32), suggesting an indirect role in controlling cholesterol synthesis.

Many ubiquitination sites on cholesterol synthesis enzymes (Table S1) are yet to be explored for their functional consequences. Several enzymes are rapidly degraded by the UPS, typically in response to changing sterol conditions, although the sterol intermediates promoting degradation can be different for each enzyme. In general, the earlier intermediates feedback more effectively on the earlier enzymes, and the later intermediates affect the later enzymes (Fig. 2). Thus, the first sterol lanosterol can feed back to accelerate degradation of the first rate-limiting enzyme, HMGCR, whereas cholesterol itself feeds back to signal destruction of the second rate-limiting enzyme, SM. This segmental control would enable HMGCR to continue making essential isoprenoids when cholesterol levels are high (33).

Phosphorylation
Phosphorylation adds a phosphate group to a serine, threonine, or tyrosine residue by a kinase. The best-understood and most commonly known post-translational modification, phosphorylation is typically associated with signaling pathways and rapid control of downstream targets. In the context of cholesterol synthesis, there are at least three enzymes that are controlled by their phosphorylated residues: HMGCR (34), DHCR7 (35), and DHCR24 (36). These modifications lead to a change in the activity of the enzyme with consequences for cholesterol synthesis.

Acetylation
Acetylation is the conjugation of acetate to lysine residues or the N-terminal amine. Protein acetylation, long known for regulating transcription in the nucleus, is increasingly being recognized to play important regulatory roles in metabolism. Virtually every enzyme in several metabolic processes (e.g. glycolysis, the urea cycle, fatty acid metabolism, and glycogen metabolism) is acetylated in human liver tissue (37). Furthermore, the effects of acetylation appear to be coordinated to simultaneously shunt metabolic flux down specific pathways and away from others. Interestingly, acetylation is over-represented on the initial two enzymes of cholesterol synthesis (Fig. 3) immediately downstream of acetyl-CoA (Fig. 2). Considering that acetyl-CoA is the starting material for cholesterol synthesis, reversible acetylation may well contribute to flux control through the pathway.

**Interplay between PTMs**
The various PTMs do not doubt interact to varying degrees, helping integrate diverse signals and multiplying their regulatory potential (38). For instance, of the 48 acetylated residues recorded to date across all cholesterol synthesis enzymes, 41 (85%) are also reported to be ubiquitinated, which may indicate competitive cross-talk (39). Conceivably, direct competition between acetylation and ubiquitination for modification of the same lysine residues (38) could reduce degradation and hence preserve enzyme levels. This may favor the increased cholesterol synthesis found in the fed state (40) in which the starting material acetyl-CoA accumulates in the cytosol (38). SUMOylation, methylation, and succinylation may additionally compete on the same lysine residue, further expanding the repertoire of regulation. Phosphorylation and ubiquitination may also functionally interact (41), and indeed we observed 44% of phosphorylated residues and 27% of ubiquitinated residues within 3 amino acids of another modification among the cholesterol synthesis enzymes (Table S1). Proximity to other modified residues is considered a good predictor of functional sites (42), although what proportion of modified residues overall can be considered functional is unclear. Further work is needed to decode the clamorous cross-talk between PTMs across the cholesterol synthesis pathway. However, rather than the high-
throughput methods that first identified the PTMs, low-throughput methods are required to pinpoint effects on individual enzymes. This has presented a serious bottleneck for phosphorylation studies, which will be eased by innovations like machine-based learning approaches that help to prioritize likely functional sites (43). PTMs are major inputs in the regulation of cholesterol synthesis, and below we will discuss how these modifications affect individual enzymes, as well as other regulatory mechanisms.

**Early pathway enzymes**

We will focus on the enzymes that have had the most detailed research into their regulation. For the early enzymes,
only HMGCR and SM have significant work behind them, with only limited studies into the other enzymes, perhaps because they are not known to be rate-limiting and therefore have not been considered worthy of investigation. Interestingly, phosphomevalonate kinase is the smallest enzyme and has all of its lysines ubiquitinated (Fig. 3), although it is currently unknown whether these have any functional consequences. MVK is best known for its role in mevalonate kinase deficiency (44), although this is linked to the decreased production of isoprenoids in a branch pathway (Fig. 1) rather than the production of cholesterol. Some pathogenic mutations in MVK cause rapid proteasomal degradation of the enzyme (45). MVK has also been identified as the luteinizing hormone receptor mRNA-binding protein, for which its role is regulated by SUMOylation, with no known consequence for cholesterol synthesis (46). Building on studies in Caenorhabditis elegans (47), it has been proposed that regulated SUMOylation of HMGCS, along with its ubiquitin–proteasomal degradation, controls the activity of this enzyme with age. Further research into the early enzymes is warranted to uncover regulatory mechanisms that for example control flux entering the sterol versus isoprenoid branches of the pathway. Considering the large number of PTMs on these enzymes (Fig. 3), we believe that many worthwhile findings will eventuate.

The classic control enzyme: HMGCR

The largest of the enzymes (Fig. 3), HMGCR catalyzes the first rate-limiting step of the mevalonate pathway, converting HMG-CoA to mevalonate. In a quintessential example of metabolic feedback regulation, its proteasomal degradation is accelerated by the accumulation of pathway intermediates and cholesterol derivatives (Fig. 2). These include C4-dimethylated sterols, in particular lanosterol and its C24-saturated derivative 24,25-dihydrolanosterol (48–50), as well as side-chain oxyterols such as 25- and 27-hydroxycholesterol (49, 51). However, cholesterol itself does not promote HMGCR turnover (51).

The sterol-induced degradation of HMGCR has been intensely studied and is initiated through a tripartite mechanism. First, HMGCR contains a sterol-sensing transmembrane region (52) that includes ubiquitination sites required for its degradation (Lys-89 and Lys-248) (53). A homologous sterol-sensing domain exists in Scap (sterol-regulatory element binding protein-cleavage activating protein), which directly binds to its regulator cholesterol (54), but interaction with lanosterol or other sterols is yet to be confirmed for HMGCR. Second, ER-resident Insig (insulin-induced gene) proteins are recruited to the HMGCR sterol-sensing domain (55). This provides a scaffold for the binding and activity of cognate E3 ligases, the third component of the cascade. A suite of E3 ligases are implicated in sterol-mediated degradation of HMGCR, and each E3 ligase may help fine-tune the metabolic regulation of this critical enzyme. Most recently, CRISPR/Cas9 screens identified RNF145 and gp78 as the major regulators of HMGCR, with Hrd1 playing a minor role only when the former two proteins are absent (26). RNF145 transcription is sterol-regulated (26, 56), whereas gp78 targets Insigs for degradation in the absence of sterols (57, 58), adding further intricacy to the regulatory circuit controlling HMGCR abundance. It is also proposed that the E3 ligases Trc8 (25) and MARCHF6 (28) affect HMGCR turnover, although the former result is contested by some researchers (27), and the latter may occur through indirect mechanisms (28). It seems likely that unique cellular or tissue-specific contexts will dictate which E3 ligase is the major regulator of HMGCR levels at any one time.

Importantly, the sterol-regulatory network that governs HMGCR levels has been confirmed to be functional in vivo in mouse models. Knockout of gp78 stabilizes HMGCR and up-regulates its activity in mouse liver (58), whereas intake of dimethylated sterol analogues leads to depletion of hepatic HMGCR and reduced serum cholesterol (59). Furthermore, levels of a transgenic protein comprising the sterol-sensing domain of HMGCR are increased or decreased by statin or cholesterol feeding, respectively (2020) 295(51) 17549–17559 17553

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leads to its accumulation in the liver and impedes its down-regulation during cholesterol feeding (60). This collective understanding of sterol-induced HMGCR degradation will undoubtedly be of importance in the future development of cholesterol-lowering therapeutics. Such treatments may be useful in augmenting or substituting statins, which can lose efficacy over time or lead to severe withdrawal effects (61) because of compensatory up-regulation of HMGCR gene expression and a decline in degradation-promoting sterol intermediates (59).

Following its ubiquitination, HMGCR is extracted from the ER membrane by the sequential actions of the AAA+ ATPase VCP and the 19S regulatory particle of the proteasome (62), enabling its degradation. Interestingly, this process is augmented by geranylgeraniol, a derivative of the endogenous isoprenoid geranylgeranyl pyrophosphate (Fig. 1) (53, 62, 63). The effects of this molecule are mediated at least in part by the prenyltransferase UBIAD1, which binds HMGCR and impedes its membrane extraction and subsequent degradation (64). In the presence of its substrate geranylgeranyl pyrophosphate, UBIAD1 is sequestered to the Golgi and no longer able to inhibit HMGCR degradation (64). Therefore, nonsterol mevalonate pathway products also control HMGCR levels, consistent with its key position upstream of the isoprenoid branch of the pathway.

The importance of this accessory mode of regulation in whole-body metabolism is demonstrated by the embryonic lethality of UBIAD1 deficiency in mice, which can be rescued by the knock-in of degradation-resistant HMGCR (65).

Beyond ubiquitination, HMGCR is also subject to regulation by phosphorylation. Here, the energy sensor AMPK phosphorylates Ser-872 within the HMGCR catalytic domain (66), inactivating the enzyme and curtailing flux through the energy-intensive mevalonate pathway (67). Ser-872 phosphorylation does not affect the sterol-induced degradation of HMGCR (67), presumably allowing for HMGCR activity to be rapidly halted when ATP levels fall, irrespective of the current sterol status of the cell.

A second control enzyme: SM

SM controls flux through the cholesterol synthesis pathway downstream of HMGCR as recently reviewed (68). The regulatory domain of SM, the first 100 amino acids (dubbed N100) is highly responsive to sterols, and increased cholesterol or desmosterol levels lead to rapid destruction of the protein, in a feedback loop from cholesterol (33). In brief, excess cholesterol ejects a spring-loaded amphipathic helix in the N100 domain from the ER membrane, initiating its proteasomal degradation (69) by ubiquitination of atypical residues (serines rather than lysines) (70). MARCHF6 is the E3 ubiquitin ligase that targets SM for proteasomal destruction (28) and, in a first for an E3 ligase, is itself stabilized by cholesterol and some intermediates, e.g. desmosterol and lanosterol (71). SM also senses abundance of its substrate squalene via N100 to increase the metabolic capacity at this step (72). This is the first reported example of stabilization of an enzyme by its substrate binding to a site other than its active site. Deubiquitinases also play a role in SM regulation, although the precise DUBs involved are not yet known (30). Intriguingly, deubiquitinase inhibitors had opposing effects on full-length SM and its regulatory domain N100, decreasing the former but increasing the latter (30). The details of this complex interplay require further investigation.

Post- lanosterol enzymes

Lanosterol can be acted upon by LDM or DHCR24 to enter the Bloch or Kandutsch–Russell pathway, respectively. The step beyond LDM is the only step in the late pathway that can be performed by either of two enzymes, DHCR14 or LBR (Fig. 2). Interestingly, of these four enzymes around this critical point of the pathway, only DHCR14 protein levels are subjected to feedback inhibition by sterols (21, 29, 73). At the end of the pathway, either DHCR7 or DHCR24 acts as the final enzyme that produces cholesterol. DHCR7 protein levels (74), but not DHCR24 (73), are decreased in the presence of sterols. Most work on these later enzymes has related to the genes, including transcriptional regulation and genetic mutations, for example, the relatively common Smith–Lemli–Opitz syndrome results from mutations in the DHCR7 gene. By comparison, the post-translational regulation of the remaining enzymes has been relatively neglected and is deserving of further research to explore the regulatory mechanisms, both to fully understand the control of the pathway and as potential future therapeutic targets.

Gateway enzymes: LDM and DHCR24

LDM (also commonly known as CYP51A1) and DHCR24 can each act on lanosterol to control entry into either the Bloch pathway or the classic Kandutsch–Russell pathway. Although the action of LDM is restricted to this point, DHCR24 can transfer any intermediate from the Bloch to the Kandutsch–Russell pathway, although it predominantly does this at the point of zymosterol (13) (Fig. 1). DHCR24 also performs the final step to produce cholesterol from desmosterol.

LDM and DHCR24 protein levels are both unaffected by cholesterol (29, 73) but interestingly are both regulated by the E3 ligase MARCHF6 (29). LDM is additionally targeted by the newly uncovered RNF185–membralin complex (75), again highlighting that multiple E3 ligases can target the same substrate. LDM is turned over in the presence of nitric oxide (29), although the physiological relevance of this is unclear. Although logical candidates, sterols and hypoxia (which would make sense in light of the high oxygen requirements of LDM) do not promote degradation of LDM (29). Further research is warranted to uncover a clear physiological signal.

DHCR24 protein levels are similarly unaffected by sterols like cholesterol and 24,25-epoxycholesterol (73) but are decreased by pregnenolone and some tyrosine-kinase inhibitors (77). However, these effects on protein levels were observed following 24 h of treatment and may be attributable to transcriptional changes, which tend to occur over longer timeframes. Interestingly, 24,25-epoxycholesterol (73) and progesterone (78) inhibit the activity of DHCR24 without affecting its protein levels. Similarly, phosphorylation and signaling play important roles in regulating DHCR24 activity, again without affecting protein levels (36). The anti-arrhythmic drug amiodarone inhibits the activity of DHCR24 (79, 80), perhaps via a similar mechanism as triparanol, the archetypal DHCR24.
DHCR7 appears to be activated by the energy sensor AMPK, which has some structural features in common (80). This indicates that there are multiple ways to inhibit the activity of DHCR24, without needing to adjust the levels of the enzyme. This may be somewhat specific to DHCR24, because we are currently unaware of any other cholesterol synthesis enzymes that are comparatively quite stable (74), yet their activity is very highly regulated. In a fascinating mode of regulation, a protease encoded by hepatitis C virus cleaves DHCR24 to prevent its activity, thereby promoting desmosterol accumulation and its own replication (81).

Twin enzymes: DHCR14 and LBR

The $\Delta^{14}$ double bond of FF-MAS can be reduced to form T-MAS by either DHCR14 or LBR. These twin enzymes have a shared function and appear to have complementary levels of expression in different tissues (21) but vastly different modes of regulation. Like most cholesterol synthesis enzymes, DHCR14 is located in the ER, whereas LBR is found in the inner nuclear membrane, which is contiguous with the ER. Cholesterol and early sterol intermediates lead to rapid degradation of DHCR14 (21). Like HMGCR and SM, this occurred through the proteasome and ubiquitination, but the ubiquitination site and major E3 ligase remain elusive (21). In contrast, LBR was not rapidly turned over nor affected by sterol levels. This is similar to the transcriptional regulation of the two enzymes; LBR is unchanged by sterol conditions, whereas DHCR14 is highly regulated by sterols (21). LBR has several phosphorylated serine residues (82, 83) that affect its chromatin-binding function (84), but there is no information on whether this has any effect on its $\Delta^{14}$-reductase activity. LBR is described as a chimeric protein in which its N-terminal domain is needed for chromatin binding, but its C-terminal domain consisting of transmembrane regions is essential for its role in cholesterol synthesis (85). It remains unclear why LBR has retained its cholesterol synthesis function in the presence of the dedicated DHCR14 enzyme.

Terminal enzyme: DHCR7

DHCR7 is the final enzyme in the Kandutsch–Russell pathway, converting 7-dehydrocholesterol to cholesterol. Like SM and DHCR14, DHCR7 is subject to rapid proteasomal degradation in the presence of cholesterol or desmosterol (74). Like DHCR14, the E3 ligase and ubiquitination sites remain unknown. 7-Dehydrocholesterol (7DHC) is also the precursor to vitamin D (Fig. 1), and therefore there are numerous studies examining links between DHCR7 and vitamin D. Interestingly, vitamin D promotes degradation of DHCR7 in keratinocytes (74), where vitamin D is synthesized. Reduction in DHCR7 protein levels and activity leads to an accumulation of 7DHC, from which more vitamin D can be produced (74).

DHCR7 is well-known as the mutated gene in Smith–Lemli–Opitz syndrome, and the most common mutations destabilize DHCR7 protein, which can be rescued by statin treatment (74), a promising therapy in Smith–Lemli–Opitz syndrome treatment (86). Somewhat similar to DHCR24, DHCR7 activity is also regulated by phosphorylation and signaling (35). Notably, DHCR7 appears to be activated by the energy sensor AMPK, which as mentioned deactivates the earlier key enzyme HMGCR. Possibly these opposing actions of AMPK may serve to save energy by turning off the pathway early but allowing its completion to prevent accumulation of potentially harmful intermediates (35).

A mediator of multiple steps: MARCHF6

The E3 ligase MARCHF6 controls protein levels of at least four enzymes in cholesterol synthesis: the rate-limiting enzymes HMGCR and SM (28) and the gateway enzymes LDM and DHCR24 (29). Together with the finding that cholesterol stabilizes MARCHF6, this indicates that MARCHF6 is intricately involved in cholesterol metabolism (87). MARCHF6 may play a role in the degradation of other enzymes in the pathway as well. Other tested enzymes, DHCR7 (74), DHCR14 (21), EBP, and lanosterol synthase (29), are unlikely to be MARCHF6 targets, suggesting that MARCHF6 regulates the pathway in a very specific and controlled manner. Future work should determine which of the remaining cholesterol synthesis enzymes are similarly degraded by MARCHF6 to fully understand its intricate control over the pathway. It will also be interesting to determine why and how MARCHF6 targets some enzymes but not others. The current four targets are all either rate-limiting or control entry into the separate branches of the pathway, suggesting that these may be particularly critical enzymes to control.

Localization and interactions between cholesterol synthesis enzymes

Cholesterol synthesis occurs largely in the membrane of the ER, likely because of the highly hydrophobic nature of the substrates and products starting from around squalene (88). As such, these hydrophobic enzymes (Fig. 3) are largely localized to the ER, where they are likely to interact with each other and indeed may form something of a metabolon or “cholestone,” a coordinated chain of enzymes to harness substrate channeling benefits. The two terminal enzymes DHCR7 and DHCR24 are known to interact in mammals (89), DHCR7 and EBP interact (90), and in yeast, there is an “ergosome” in which a number of ergosterol-synthesizing enzymes interact (91). Determining which enzymes interact with which others in cholesterol synthesis would help confirm or disprove the existence of a cholestone.

Some cholesterol synthesis enzymes can be found on lipid droplets, such as SM, lanosterol synthase, and sterol-4-carboxylate 3-dehydrogenase (92). There is some suggestion from work in both yeast (93) and mammalian cells (94) that lipid droplet formation could sequester these enzymes and shut down cholesterol synthesis, maybe as a feedback response opposing excess lipid synthesis and storage. Interestingly, DHCR7 has also been observed in the Golgi, whereas DHCR24 and EBP were not (95). All three enzymes were colocated in the ER along with the earlier enzyme SC5D, which was additionally localized to the nucleus, which may suggest that it has additional functions beyond sterol synthesis (95). Under what physiological conditions DHCR7 and SC5D may be relocated needs to be established. Although yet to be tested, it is entirely
possible that the vast array of PTMs on cholesterol synthesis enzymes may influence their cellular localization.

Outstanding questions and concluding remarks

Our understanding of the post-translational control of cholesterol has deepened considerably over the past decade, but many questions remain to be answered. Does MARCHF6 target other enzymes in the cholesterol synthesis pathway? Which other cholesterol synthesis enzymes are controlled by changing sterol levels? What inputs control the other enzymes? How do the multitude of PTMs interact? Does regulation of the enzymes inform the effects of disease mutations and vice versa? How are the enzymes differently regulated in different cellular and physiological contexts? How do these various regulatory inputs affect flux through the pathway?

Although studies of cultured cells are indispensable for the discovery and characterization of enzyme regulatory mechanisms, it is vital that the physiological relevance of these mechanisms is verified at the organismal level, as has been done for HMGCR. Such advances would inform the development of alternative strategies to therapeutically target cholesterol synthesis. In addition to HMGCR, SM is perhaps the next best-understood pathway enzyme and thus an ideal candidate for in vivo studies of its sterol regulation. However, further fundamental insights into less well-characterized enzymes will open a multitude of avenues for modulating pathway activity and broader cholesterol homeostasis.

Cholesterol synthesis enzymes display a myriad of PTMs (Fig. 3). The vast majority of these have been gleaned from high-throughput screens. Further studies will no doubt add to the number, variety, and certainty of these PTMs. In particular, low-throughput studies are crucial to determine the intricate details of these PTMs and the consequent regulation of the enzymes. Nevertheless, a picture emerges of an ornate overlay of PTMs, suggestive of a complex control panel of signals, exquisitely responsive to changing metabolic conditions, especially with respect to energy demands. Phosphates are added or removed from a multitude of sites on successive enzymes, like lights blinking on and off. Acetylation lights up the beginning of the pathway before flickering at discrete points downstream, and sterol-mediated ubiquitination, as some sort of master switch, shuts down the entire energy-intensive pathway via proteasomal degradation at multiple steps. We look forward to further efforts to decode the complex signals that control traffic along this long and winding road to cholesterol.

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Abbreviations—The abbreviations used are: HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; ER, endoplasmic reticulum; PTM, post-translational modification; UPS, ubiquitin–proteasome system; MVK, mevalonate kinase; DHC, dehydrocholesterol; DHCR, dehydrocholesterol reductase; SM, squalene monoxygenase.

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