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Regulation of lipid metabolism: a tale of two yeasts

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Abstract

Eukaryotic cells synthesize multiple classes of lipids by distinct metabolic pathways in order to generate membranes with optimal physical and chemical properties. As a result, complex regulatory networks are required in all organisms to maintain lipid and membrane homeostasis as well as to rapidly and efficiently respond to cellular stress. The unicellular nature of yeast makes it particularly vulnerable to environmental stress and yeast has evolved elaborate signaling pathways to maintain lipid homeostasis. In this article we highlight the recent advances that have been made using the budding and fission yeasts and we discuss potential roles for the unfolded protein response (UPR) and the SREBP-Scap pathways in coordinate regulation of multiple lipid classes.

Introduction

Phospholipids, sphingolipids and sterols are the primary structural components of eukaryotic cell membranes and hence, organisms have evolved complex regulatory mechanisms to control their synthesis. The plasma membrane defines the outer most boundary of the cell while the internal organelles compartmentalize the chemical reactions that constitute cellular metabolism, and not surprisingly their lipid composition is tailored to suit their individual functions. The budding and fission yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have proven to be excellent models for the study of regulation of lipid metabolism, a result of a synergism of genetic and biochemical approaches, and decades of hard work. Much of this work laid the foundation for our understanding of the fundamental lipid metabolic pathways, and now, recent work in the yeasts is providing novel insights into the mechanisms underlying homeostatic control of lipid synthesis and in response to cellular stresses.

Regulation of phospholipid and sphingolipid synthesis

Yeast synthesize phospholipids by three pathways, a *de novo* CDP-DAG pathway [1], and by two salvage pathways, the Kennedy pathway [1] and the recently described exogenous

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lysolipid metabolism (ELM) pathway [2]. These pathways are largely conserved among all eukaryotes, with a few differences arising between fungi, plants and animals. The CDP-DAG and Kennedy pathways (Figure 1) are regulated primarily at the transcriptional level through a DNA element called UAS_{INO}, that is present in the promoters of nearly 30 of the phospholipid synthesis genes [3]. Transcriptional control is maintained by Opi1, which binds and represses the Ino2-Ino4 activator complex in the nucleus. Opi1 is repressed through its sequestration on the ER by binding the signaling lipid phosphatidic acid (PA) [1,4]. Binding of Opi1 to PA is regulated by intracellular pH, in which the lipid acts as the pH sensor, defining an unprecedented role for lipids as pH biosensors [5]. PA is a precursor in the synthesis of both phospholipids and neutral lipids and comprises only ~1% of ER phospholipids. Thus, the concentration of PA in the ER, together with intracellular pH, plays a central role in coordinate regulation of phospholipid synthesis by Opi1 [6]. In mammals, the PA hydrolases of the lipin family are emerging as critical regulators of lipid homeostasis [7••] and have been linked to metabolic diseases including type 2 diabetes [8], suggesting PA might also play a signaling role regulating lipid metabolism in humans.

Regulation of sphingolipid synthesis, until very recently, has been largely undefined in any organism [9]. The highly conserved Orm family of phosphoproteins, which have been linked to childhood asthma [10], have now been identified as negative regulators of serine palmitoyltransferase (SPT), which catalyses the first and rate-limiting step in the sphingolipid biosynthetic pathway [11] (Figure 1). Yeast Orm1 and Orm2 form a stable complex with SPT, and when dephosphorylated, repress SPT activity via an unknown mechanism [11]. Phosphorylation of Orm1 and Orm2 by the conserved kinase Ypk1 in response to sphingolipid deficiency relieves Orm repression of SPT, thus providing feedback control of sphingolipid production [12]. Ypk1 is in turn activated through phosphorylation by TOR kinase in response to sphingolipid levels. An exciting next step will be to determine if TOR senses lipid levels directly and which lipid acts as the signal. Interestingly, TOR kinase is a PA binding protein [13] and PA levels are altered in *orm* mutants [14], suggesting PA might play a role in regulation of sphingolipid metabolism.

Lipid stress sensing by the UPR

Lipid homeostasis is perturbed by different environmental stresses. But how do cells respond to these stresses? It has been recently found that deletion of several key phospholipid synthesis genes in both the CDP-DAG and Kennedy pathways results in activation of the unfolded protein response (UPR) in yeast [15]. The UPR is an ER stress response pathway that detects misfolded proteins in the ER and activates transcription of genes that facilitate proper protein folding [16]. The UPR also upregulates key phospholipid synthesis genes [17,18], thus providing a potential mechanism to respond to lipid stress (Figure 1). Tellingly, some of these target genes are the very same genes that when deleted also activate the UPR. Similarly, loss of Orm1 and Orm2 in yeast activates the UPR [14], and Orm2 is a UPR target gene [18], suggesting that dysregulated sphingolipid synthesis is another form of lipid stress detected by the UPR. Cholesterol overloading of the macrophage ER [19], and altered PC synthesis in the ER of livers from obese mice fed a high-fat diet [20], also cause ER stress and UPR activation, suggesting that lipid stress sensing by the

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UPR might provide coordinated feedback control of multiple lipid classes to balance membrane lipid composition.

How might the UPR detect lipid stress? The ER stress sensor Ire1 is an integral membrane protein of the ER that directly detects misfolded proteins in the ER lumen and activates the UPR [21]. Recent work now indicates that the lumenal domain of Ire1 that binds misfolded proteins is dispensable for UPR activation under conditions of lipid stress [22]. A truncated form of Ire1 containing only the transmembrane and cytoplasmic domains is sufficient to activate the UPR under starvation conditions for the lipid precursor inositol and in a CDP-DAG pathway mutant. This implies that Ire1 detects lipid stress through a mechanism distinct from protein misfolding, although it does not rule out the possibility that other domains in Ire1 also detect protein misfolding under these conditions.

An innovative new assay that is capable of monitoring levels of misfolded proteins in the ER of living cells suggests lipid stress-sensing does not occur via accumulation of unfolded proteins. Lajoie *et al.* measured protein misfolding under a variety of conditions that induce the UPR by monitoring the mobility of the ER chaperone Kar2 tagged with GFP [23]. Under conditions known to cause protein misfolding, Kar2-GFP mobility decreases as a result of its interaction with client proteins. However, no such decrease in Kar2-GFP mobility is observed under conditions of UPR activation by inositol starvation [23], suggesting the UPR is activated through a separate mechanism in response to lipid stress.

Is the lipid stress signal a lipid?

A clue for a possible mechanism for lipid stress sensing comes from experiments that link the UPR to synthesis of inositol. Inositol is a potent regulator of phospholipid metabolism in yeast, because its incorporation into phosphatidylinositol (PI) depletes the ER of PA [4] (Figure 1). Lowered PA releases Opi1 from the ER and its subsequent translocation to the nucleus represses phospholipid synthesis genes. *INO1* is the gene most highly regulated by Opi1 and encodes the rate-limiting enzyme in inositol synthesis. *INO1* is also one of the genes most highly induced by the UPR [18], which occurs via repression of Opi1 [24], suggesting that UPR activation correlates with ER PA levels. Consistent with this, addition of inositol leads to rapid inactivation of the UPR [25], which is strikingly similar to the rate at which it depletes PA in the ER [4]. However, this inactivation of the UPR is independent of Opi1 [26]. PA acts upstream of Opi1 in regulation of phospholipid synthesis genes, indicating the possibility that accumulation of PA in the ER signals lipid stress to the UPR. In support of this idea, loss of Pah1, the major ER PA hydrolase in yeast and a member of the conserved lipin family, results in both elevated PA [27] and activation of the UPR [15]. Such a signaling role for PA is not unprecedented. In plants, PA plays a central role in stress response signaling through its rapid generation and direct activation of a variety of stress effector proteins [28], although a role in UPR activation has yet to be demonstrated.

Sterol biosynthesis is under multivalent regulatory control

Sterols play essential roles in cell physiology, from membrane building blocks [29] to Hedgehog signaling during development [30], and consequently sterol synthesis is subject to regulation at multiple levels. Feedback mechanisms that control cholesterol biosynthesis and

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uptake through transcriptional control and regulated enzyme degradation are well-established in mammalian cells [31]. Ergosterol is the fungal analog of cholesterol, and both the budding yeast S. *cerevisiae* and fission yeast *S. pombe* have proven to be powerful models in which to study sterol biosynthesis. In this section, we discuss sterol regulatory mechanisms in these yeasts in relationship to those operating in mammalian cells.

Transcriptional control of sterol synthesis

Production of cholesterol synthesis enzymes in mammalian cells is tightly regulated at the transcriptional level through a feedback mechanism that responds to the end product cholesterol (Figure 2). The key player in this pathway is an ER membrane-bound transcription factor, SREBP (Sterol Regulatory Element Binding Protein) [31, 32]. SREBP consists of a N-terminal transcription factor domain and a C-terminal domain that forms a complex with the sterol sensing protein, SREBP cleavage activating protein (Scap) [31]. When the ER membrane is sterol-rich, Scap binds cholesterol, and the SREBP-Scap complex is retained in the ER by binding to the resident protein Insig [33]. When ER cholesterol drops, Scap undergoes a conformational change and SREBP-Scap enters COPII vesicles for transport to the Golgi [34,35•]. SREBP is cleaved sequentially in the Golgi by the Site-1 and Site-2 proteases to generate the functional transcription factor that travels to nucleus [31]. Nuclear SREBP activates transcription of cholesterol synthesis enzymes and the LDL receptor to increase sterol supply and restore homeostasis.

The SREBP pathway is functionally conserved in fungi like *S. pombe* [36], *Cryptococcus neoformans* [37,38], and *Aspergillus fumigatus* [39]. *S. pombe* SREBP, called Sre1, binds the Scap homolog, Scp1, and the mechanism of sterol regulation is conserved [36]. Interestingly, *S. pombe* and other ascomyceteous fungi like *Aspergillus* lack homologs of the Site-1 and Site-2 proteases necessitating a different processing mechanism [40]. Recently, two independent genetic screens identified a 5 subunit, membrane-bound Golgi ubiquitin E3 ligase, named the Dsc (defective for SREBP cleavage) E3 ligase, that is required for Sre1 processing [41•,42]. Bioinformatic analysis suggests that the Dsc E3 ligase does not possess protease activity, but reveals structural and organizational similarities with the Hrd1 E3 ligase complex involved in degradation of misfolded proteins by the ER-associated degradation (ERAD) pathway [43]. Genetic experiments show that Sre1 processing requires the 26S proteasome, but how these proteins mediate Sre1 proteolysis and activation requires further study.

Budding yeast *S. cerevisiae* lacks SREBP, and two homologous $Zn(II)_2Cys_6$ binuclear cluster transcription factors, Upc2 and Ecm22, function in ergosterol regulation [44,45]. The DNA binding domains of Upc2 and Ecm22 bind conserved sequences in promoters of ergosterol synthesis genes to activate transcription under conditions of sterol depletion [45,46]. Both Upc2 and Ecm22 contain nuclear localization signals, and Upc2 displays both nuclear and cytoplasmic localization in genome-wide localization studies [47,48]. While it has been proposed that Upc2 and Ecm22p function like SREBP [44,49], no sterol sensor has been identified and whether activation requires proteolysis is unknown.

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Post-translational regulation of sterol synthesis

HMG-CoA reductase (HMGR) is a rate-limiting enzyme in sterol synthesis that catalyzes the conversion of HMG-CoA to mevalonate. In mammalian cells, HMGR is regulated at the level of transcription by SREBP, enzyme activity by AMP-activated protein kinase, and degradation by ERAD (recently reviewed in [50,51]) (Figure 2). HMGR degradation by the proteasome is accelerated by the sterol intermediate 24,25-dihydrolanosterol and the isoprenoid geranylgeranyl pyrophosphate (GGPP) through the action of the ER protein Insig and its associated ubiquitin E3 ligases, gp78 and Trc8 [52]. Recent studies demonstrate that squalene monooxygenase is also subject to regulated proteasomal degradation [53•] (Figure 2). However unlike HMGR, degradation of squalene monooxygenase is accelerated by elevated cholesterol.

Budding yeast expresses two isozymes of HMGR, Hmg1 and Hmg2, and either is sufficient for cell growth. Differential regulation of *HMG1* and *HMG2* transcription by oxygen and sterol intermediates has been suggested but no molecular mechanism for this regulation has been described [54]. Hmg2, like mammalian HMGR, undergoes lipid-regulated turnover mediated by the ERAD pathway [54]. Two lipid signals, the isoprenoid GGPP and an oxysterol, control the rate of Hmg2p degradation [55•]. Elevated GGPP alters Hmg2 conformation and stimulates degradation through an N-terminal sterol sensing domain and oxysterol enhances this degradation signal [56]. Unlike mammalian cells, the *S. cerevisiae* Insig homolog Nsg1 stabilizes Hmg2 by direct binding [57].

Fission yeast codes for one HMGR enzyme Hmg1 and the Insig homolog Ins1. In contrast to *S. cerevisiae* and mammalian cells, Hmg1 protein in *S. pombe* is neither regulated by SREBP nor degraded by Insig-mediated ERAD [58]. Rather, Ins1 regulates the catalytic activity of *S. pombe* Hmg1 by controlling phosphorylation of active site residues [58]. Ins1 binding to Hmg1 stimulates its phosphorylation through a stress responsive MAP kinase Sty1 inhibiting enzyme activity. Hmg1 activity is also suppressed by glucose starvation through by the inhibitory effect of Sds23 on the phosphatase Ppe1 [59].

Stress-induced regulation of sterol synthesis

Molecular oxygen is essential for cholesterol and ergosterol synthesis from squalene, requiring 11 and 12 oxygen molecules respectively [60]. Recent work on SREBP in fission yeast highlights the interplay between hypoxic stress and sterol homeostasis [40]. In addition to controlling sterol homeostasis, Sre1 functions as a hypoxic transcription factor by monitoring oxygen-dependent sterol synthesis as an indirect measure of oxygen supply [36]. Sterol synthesis decreases under low oxygen, and Sre1 is proteolytically activated to increase transcription of genes required for ergosterol synthesis and cell growth. Oxygen additionally regulates activity of the Sre1 N-terminal transcription factor by (1) blocking DNA binding and (2) accelerating degradation by the ubiquitin-proteasome system [60,61•]. Both mechanisms require Ofd1, a putative prolyl-4-hydroxylase of the 2-OG-Fe(II) dioxygenase family, and its inhibitor Nro1 [62]. The catalytic role of Ofd1 is not fully understood, but enzyme activity allows Ofd1 to function as an oxygen sensor to regulate Sre1-dependent transcription.

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Anaerobic gene expression in *S. cerevisiae* is centrally mediated by the transcriptional repressor Rox1 [63]. Upc2, a target gene of Rox1, is derepressed under hypoxic conditions and activates a large number of anaerobically expressed genes in budding yeast, including ergosterol biosynthesis genes. In addition, Upc2 controls the expression of two sterol transporters Aus1 and Pdr11. Unlike *S. pombe* that cannot import exogenous sterol, *S. cerevisiae* takes up sterol under low oxygen when biosynthesis is compromised [36,64]. Recently, two studies identified pathways for ergosterol synthesis gene activation under extracellular stress. During hypoxic growth when heme and ergosterol levels are low, the Hog1 MAP kinase cascade is activated leading to Upc2 activation [65]. Conversely, under hyperosmotic stress Hog1 activates the repressors Rox1 and Mot3. Mot3 acts through Ecm22 to repress sterol synthesis [66].

Regulatory crosstalk?

Studies of lipid synthesis regulation have largely focused on individual classes of lipids: phospholipids, sterols and sphingolipids. But molecular interactions among these lipids seemingly necessitate coordinated control of their supply. Emerging data hints at co-regulation of these lipid classes [67]. In addition to sterols, the major phospholipids PE and PC have been shown to regulate activity of SREBP in *Drosophila* and mammalian cells, respectively [68••,69]. Pah1 is a PA hydrolase and regulator of phospholipid synthesis in budding yeast [6] (Figure 1). Lipin1, the mammalian Pah1 homolog, is a key regulator of phospholipid and neutral lipid balance in cells and coordinates mTORC1-dependent nutrient signaling to SREBP [7••]. Future studies will be aimed at understanding the crosstalk between these lipids and how established homeostatic mechanisms are employed to respond to diverse environmental stresses.

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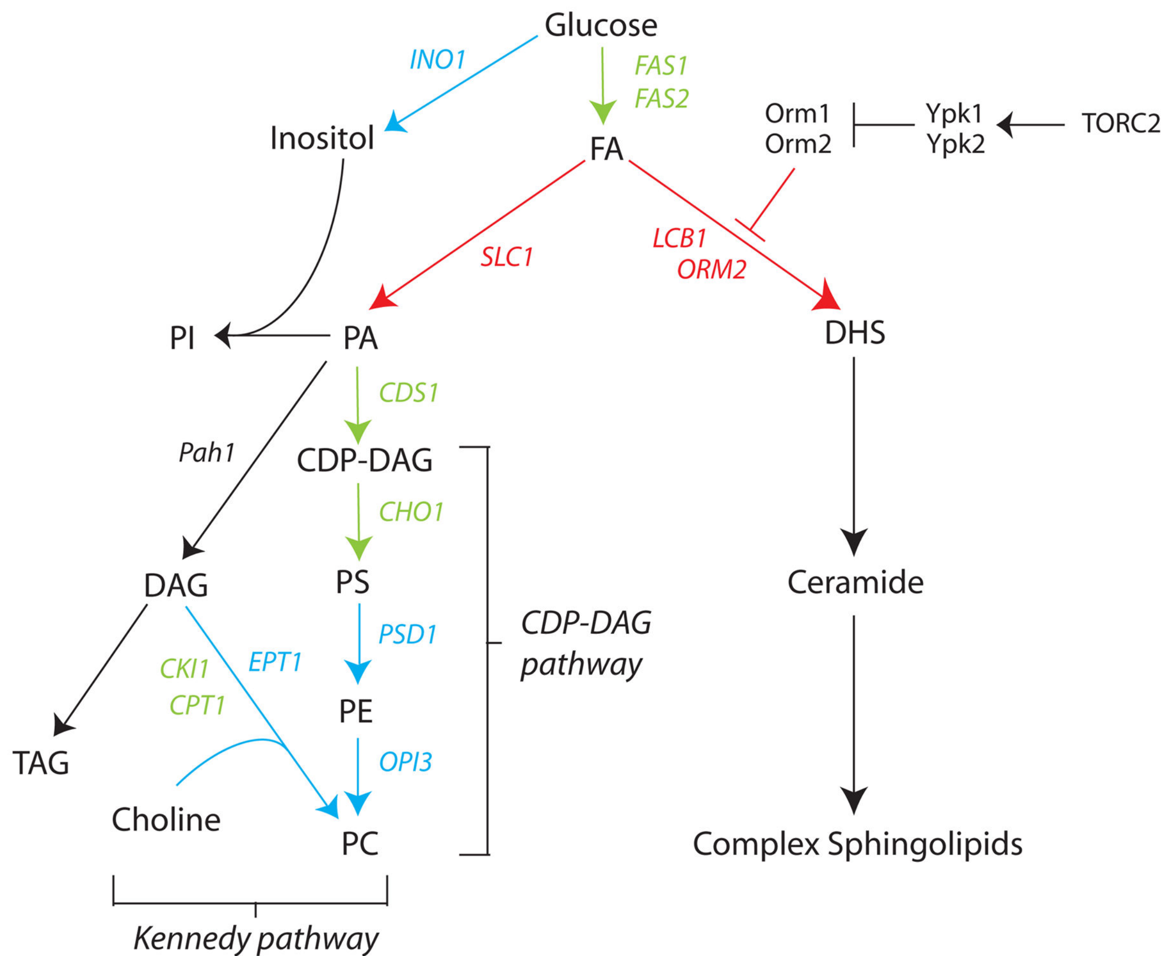

Figure 1. Outline of pathways of phospholipid and sphingolipid metabolism in budding yeast

Pathways and genes co-regulated by Opi1 and the UPR are in blue; those activated by the UPR are in red; and those repressed by Opi1 in green. Steps are not necessarily direct. Abbreviations: FA = fatty acids; PI = phosphatidylinositol; PA = phosphatidic acid; DAG = diacylglycerol; PS = phosphatidylserine; PE = phosphatidylethanolamine; TAG = triacylglycerol; PC = phosphatidylcholine; DHS = dihydro-sphingosine; TORC2 = Target of Rapamycin Complex 2, SPT = serine palmitoyltransferase.

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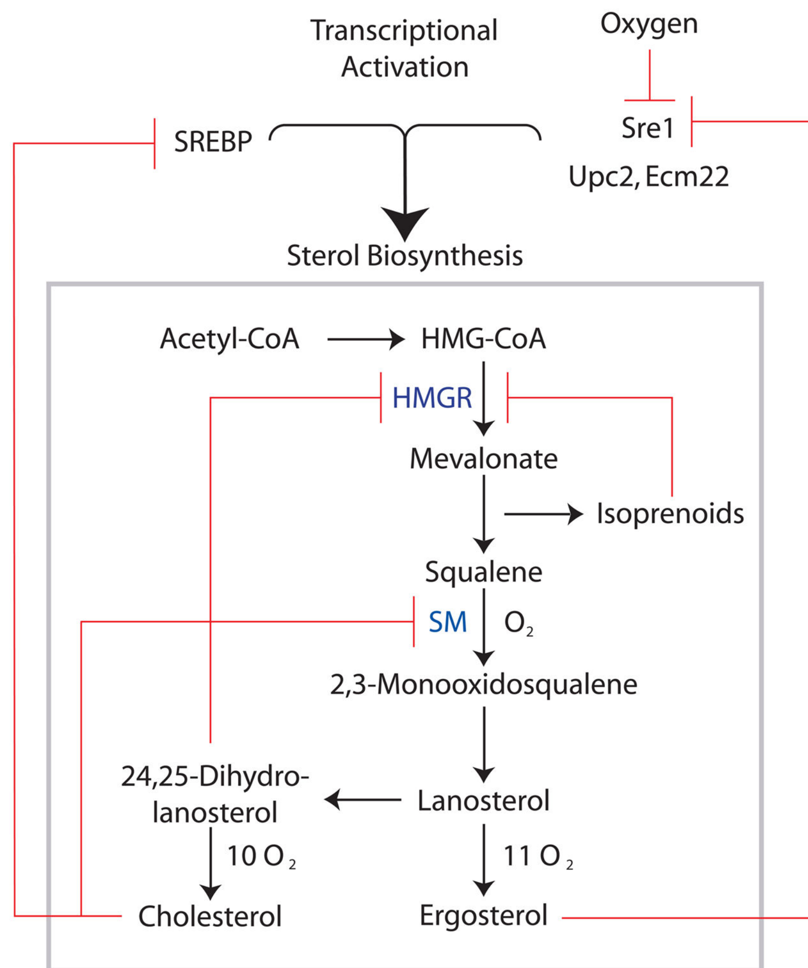

Figure 2. Multivalent regulation of sterol homeostasis

Cholesterol (ergosterol in fungi) synthesis requires more than 20 enzymes and is controlled by negative feedback. The biosynthetic pathway is outlined inside the box showing key intermediates and regulated enzymes. Upon sterol depletion, transcription of sterol synthesis enzymes is activated by sterol-regulated transcription factors, SREBP in mammals, Sre1 in *S. pombe*, and Upc2 and Ecm22 in *S. cerevisiae*. In sterol-rich conditions, the end product reduces biosynthesis by inhibiting transcription factor activity. In *S. pombe*, low oxygen additionally activates Sre1 and sterol enzymes. In mammalian cells, two enzymes, 3-hydroxymethyl-3-methylglutaryl-coenzyme A reductase (HMGR) and squalene monooxygenase (SM) are subject to sterol-dependent degradation. HMGR catalyzes the synthesis of mevalonate from HMG-CoA and is degraded by ERAD in response to 24,25-dihydrolanosterol and an isoprenoid derived from mevalonate. SM catalyzes the first oxygenation reaction of the pathway and is regulated by cholesterol-dependent proteasomal degradation.