Regulation of Sterol Synthesis in Eukaryotes

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cholesterol synthesis, endoplasmic reticulum, SREBP, Scap, Insig, HMG-CoA reductase, yeast

Abstract

Cholesterol is an essential component of mammalian cell membranes and is required for proper membrane permeability, fluidity, organelle identity, and protein function. Cells maintain sterol homeostasis by multiple feedback controls that act through transcriptional and posttranscriptional mechanisms. The membrane-bound transcription factor sterol regulatory element binding protein (SREBP) is the principal regulator of both sterol synthesis and uptake. In mammalian cells, the ER membrane protein Insig has emerged as a key component of homeostatic regulation by controlling both the activity of SREBP and the sterol-dependent degradation of the biosynthetic enzyme HMG-CoA reductase. In this review, we focus on recent advances in our understanding of the molecular mechanisms of the regulation of sterol synthesis. A comparative analysis of SREBP and HMG-CoA reductase regulation in mammals, yeast, and flies points toward an equilibrium model for how lipid signals regulate the activity of sterol-sensing proteins and their downstream effectors.

INTRODUCTION

First discovered as a component of gallstones, cholesterol and its synthesis have held the attention of scientists for more than 200 years. This interest in cholesterol stems from its essential function in cell membranes, its complex four ring structure (**Figure 1**), its role as a precursor to steroids and bile acids, and the fact that disorders in cholesterol metabolism lead to human disease (78).

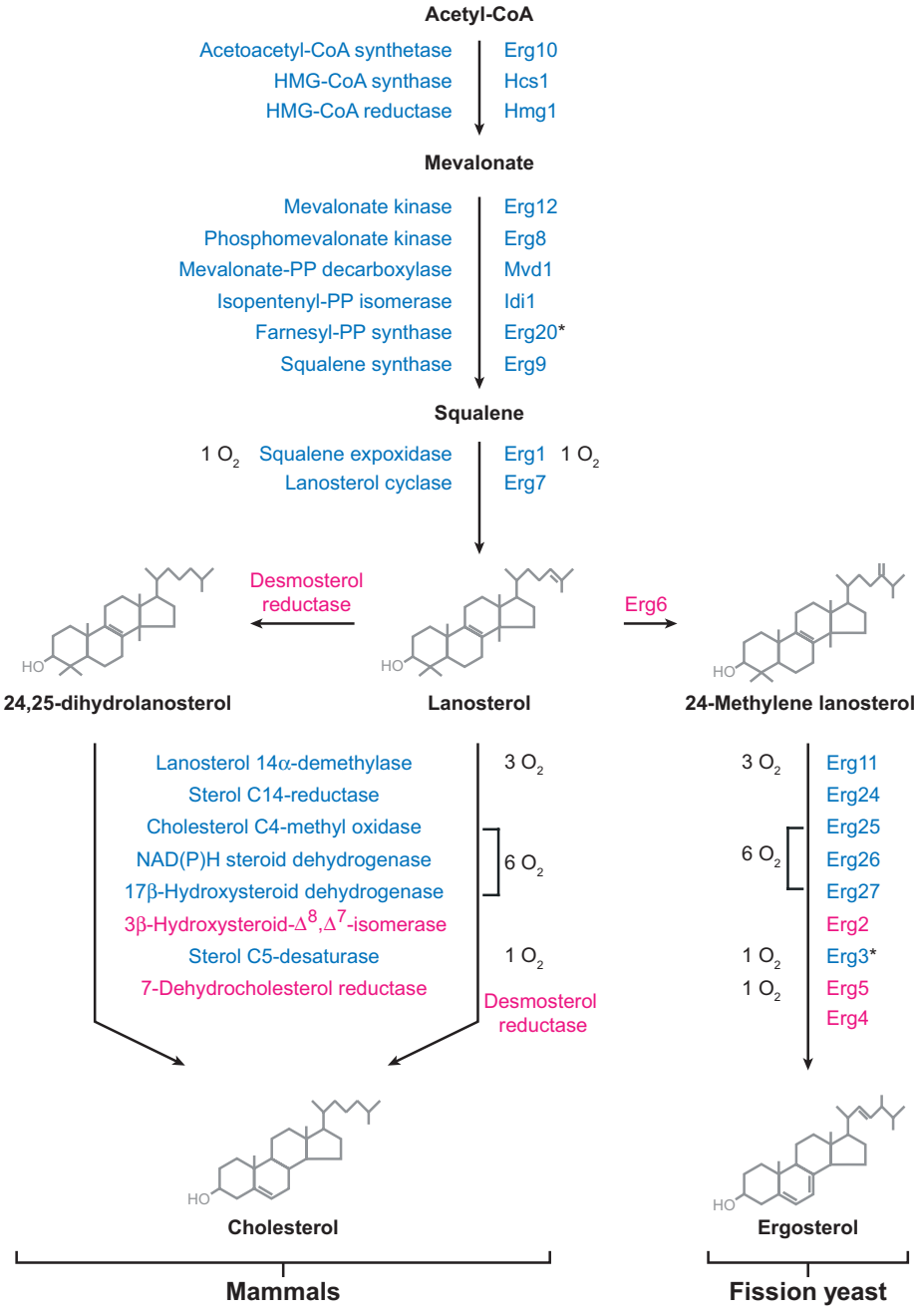

Figure 1

The sterol biosynthetic pathway in mammals and fission yeast. Mammalian enzymes are listed by full name and enzymes of *Schizosaccharomyces pombe* are named based on homology with genes in *Saccharomyces cerevisiae*. Enzymes in blue are conserved between yeast and mammals, while those in magenta are unique to each pathway. Oxygen requirements for oxygen-dependent enzymes are listed. *The fission yeast genome encodes two Erg20 and two Erg3 homologs.

Mammalian cholesterol synthesis was first appreciated to be under the control of end product feedback regulation by Schoenheimer in 1933 (102). In the 1950s, Gould et al. demonstrated this regulation in the liver, while Bloch and others discerned the biosynthetic reactions that convert acetyl-CoA to cholesterol (46, 120). These early discoveries set the stage for the application of molecular biology to the study of cholesterol homeostasis.

Our understanding of the molecular control of mammalian cellular cholesterol homeostasis comes largely from the work of Drs. Michael Brown and Joseph Goldstein and their colleagues at UT-Southwestern Medical Center in Dallas. This historic collaboration has resulted in the description of the endocytic pathway that mediates cellular uptake of low-density lipoprotein (LDL) (12), the purification and cloning of the transcription factors that control sterol-regulated expression of cholesterol biosynthetic enzymes (8, 122), and most recently the identification of the sterol sensors that mediate feedback control of sterol synthesis (43).

Here, we present our current knowledge of the regulation of cellular sterol synthesis in eukaryotes. We focus primarily on studies using cultured cell systems and model genetic organisms, but in many cases these findings have been corroborated in vivo using transgenic and knockout animals (56). A review of the recent progress in this field reveals that we may be nearing an answer to the fundamental question: How do cells measure the level of cholesterol, an insoluble membrane-embedded lipid, and transmit signals to control its supply?

TRANSCRIPTIONAL CONTROL OF STEROL SYNTHESIS IN MAMMALS

Sterol Regulatory Element Binding Proteins

Mammalian cells receive cholesterol primarily from two sources: (*a*) new synthesis from acetyl-CoA (**Figure 1**) or (*b*) from outside the cell via receptor-mediated endocytosis of LDL (12). Activity of these two pathways is controlled by cholesterol at the level of transcription through classic end product feedback inhibition. When cells are depleted of sterols, transcription of genes required for the synthesis and uptake of cholesterol, such as HMG-CoA reductase and LDL receptor, is upregulated through the action of a transcription factor binding to DNA elements in gene promoters called sterol regulatory elements (SRE). Membrane-bound transcription factors called sterol regulatory element binding proteins, SREBPs, recognize these sequences and function as central regulators of cellular lipid homeostasis (43). In sterol-overloaded cells, SREBP is inactive and transcription of these genes falls, returning sterol levels to normal.

SREBP: sterol regulatory element binding protein

Two genes code for three SREBP isoforms [~1150 amino acids (aa)], named SREBP-1a, SREBP-1c/ADD1, and SREBP-2 (13, 119). SREBP-2 preferentially regulates genes involved in cholesterol homeostasis, including all of the sterol biosynthetic enzymes and the LDL receptor (55). In contrast, SREBP-1c/ADD1 activates genes in fatty acid synthesis, while SREBP-1a regulates all SREBP-responsive genes. As a group, SREBPs are transcriptional activators and stimulate transcription of more than 30 genes involved in the uptake and synthesis of cholesterol, fatty acids, triglycerides, and phospholipids (56). Despite differences in their transcriptional targets, the proteolytic activation of each SREBP isoform is regulated by cholesterol through a common mechanism (77, 90). Thus for the purpose of this review, we refer to these proteins collectively as SREBP.

Newly synthesized SREBPs are inserted into the endoplasmic reticulum membrane in a hairpin orientation with the N and C termini extending into the cytosol (**Figure 2**) (34, 89). The N-terminal domain of SREBP is a transcription factor of the basic helix-loop-helix leucine zipper family and the C terminus binds tightly to the C terminus of SREBP

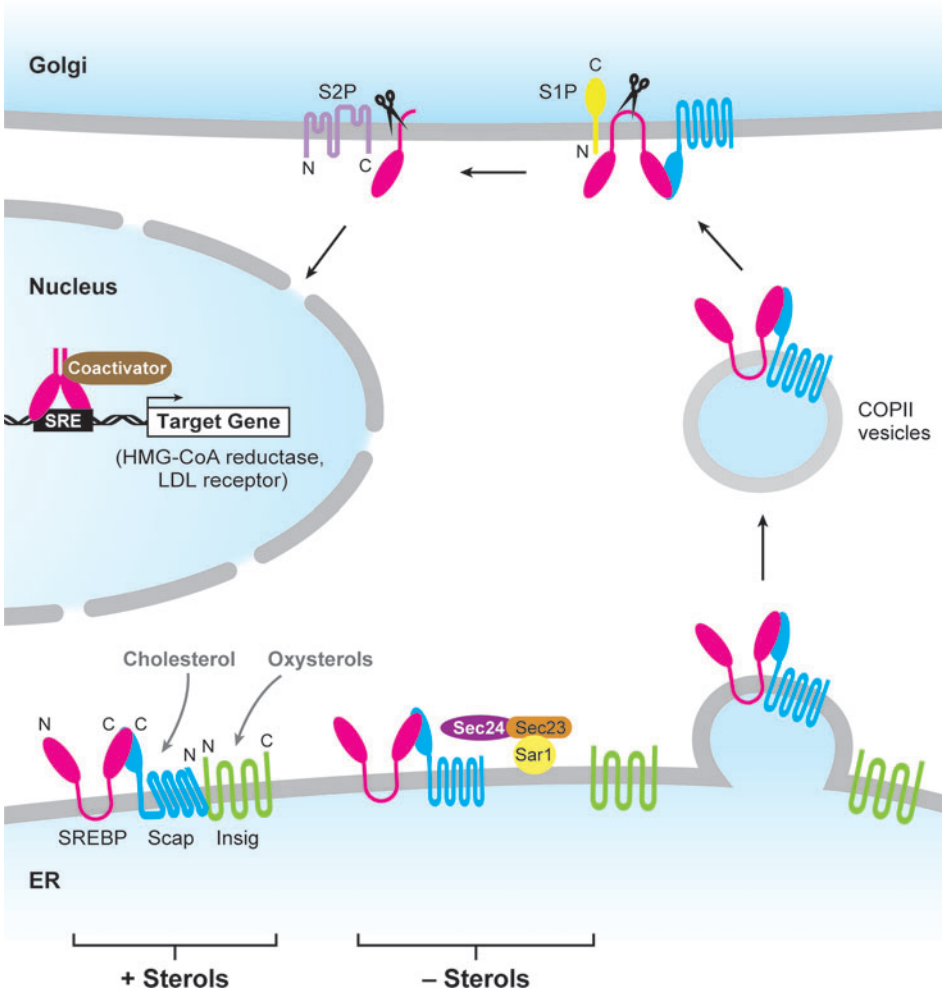

Figure 2

The SREBP pathway in mammals. In the presence of cholesterol or oxysterols, SREBP-Scap is retained in the ER by binding to Insig. In the absence of sterols, Insig no longer binds SREBP-Scap and SREBP-Scap is loaded into COPII vesicles through an interaction with Sec23/24. After transport to the Golgi, the transcription factor domain of SREBP is released from the membrane by two sequential proteolytic cleavage events mediated by the Site-1 (S1P) and Site-2 (S2P) proteases. The nuclear form of SREBP activates target genes such as HMG-CoA reductase and the LDL receptor through binding to sterol regulatory element (SRE) sequences in gene promoters.

cleavage activating protein (Scap) (**Figure 2**). To activate nuclear gene expression, the N terminus must be proteolytically cleaved from the membrane. In sterol-replete cells, the SREBP-Scap complex remains in the ER and is compartmentally separated from the Golgi-localized proteases, the Site-1 protease (S1P) and the Site-2 protease (S2P), which process SREBP (81). ER retention of SREBP-Scap is mediated by the sterol-dependent binding of SREBP-Scap to the ER resident protein Insig (131). Scap functions as the cholesterol sensor in this system. When cells have sufficient sterol, Scap binds cholesterol, which promotes binding to Insig and prevents ER exit (87).

Scap: SREBP cleavage activating protein

Insig: insulin-induced gene

In sterol-depleted cells, Scap does not bind to Insig, allowing ER exit of SREBP-Scap via COPII vesicles (43). In the Golgi, SREBP is first cleaved in the luminal loop by S1P, which separates the protein into two halves. Following Site-1 cleavage, the intramembrane protease S2P cleaves the N-terminal transcription factor from the membrane allowing it to enter the nucleus, bind to SRE sequences, and with the help of transcriptional coactivators upregulate genes required for cholesterol supply. Following SREBP cleavage, Scap is thought to recycle from the Golgi to the ER to participate in additional rounds of SREBP proteolysis.

SREBP Proteases

The Site-1 (1052 aa) and Site-2 (510 aa) proteases act sequentially in the Golgi to release the transcription factor domain of SREBP from the membrane (34). The activity of these proteases is not sterol-regulated. Rather, proteolysis of SREBP is regulated by the compartmental separation of the proteases and SREBP (**Figure 2**) (25). S1P, also called SKI-1, is a member of the subtilisin/kexin family of serine proteases (99, 105). The active Golgi form of S1P is generated from an inactive proenzyme through autocatalytic cleavage (35). S1P cleaves after the consensus sequence RXXL in the luminal loop of SREBPs (19, 29). Following S1P cleavage, the zinc metalloprotease S2P cleaves a Leu-Cys bond within the membrane (30, 91). Cleavage by S2P requires prior cleavage by S1P, but the structural basis for this requirement is unknown (98). S1P and S2P act in tandem on additional substrates, such as the stress response transcription factors, ATF6 and CREBH (104, 132, 134). These two transcription factors contain only one transmembrane segment.

Scap

The sterol-regulated activation of SREBP is controlled by its binding partner Scap (1277 aa) (57, 90). In the presence of sterols, Scap binds to Insig and is unable to enter ER transport vesicles (**Figure 2**). In the absence of sterols, Scap escorts SREBP from the ER to the Golgi where it is cleaved. These functions of Scap are separated into two distinct domains. The C terminus of Scap contains multiple WD-40 repeats and forms a stable complex with the C terminus of SREBP. The N terminus of Scap contains 8 transmembrane segments that form a tetramer and mediate binding to Insig (87, 131). The N terminus of Scap is also the regulatory domain. Somatic cell genetic experiments defined transmembrane segments 2–6 as a sterol-sensing domain (**Figure 3**) (44). Single amino acid substitutions (Y298C, L315F, or D443N) in this domain of Scap render cells unable to sense sterol and result in constitutive SREBP activation, even in cells overloaded with cholesterol (65, 129). Consistent with this domain functioning in sterol sensing, biochemical studies demonstrate that the N terminus of Scap directly binds cholesterol (87). The sterol-sensing mutations block binding to Insig, but thus far do not appear to affect binding to cholesterol (129, 131).

Sterol binding to Scap regulates the activation of SREBP by controlling ER exit of the SREBP-Scap complex in COPII transport vesicles (**Figure 2**) (36). Cargo molecules are selected for incorporation into COPII vesicles through interactions with a subcomplex of COPII proteins consisting of the small GTPase Sar1 and the cargo recognition complex Sec23/24 (**Figure 2**) (48). COPII binding to Scap is sterol-regulated and requires the hexapeptide sequence MELADL in the cytosolic loop between transmembrane segments 6 and 7 (**Figure 3**) (114). Scap most likely binds directly to the Sec24 subunit, which is the cargo binding subunit of Sec23/24 (80). Biochemical assays that monitor the structure of Scap demonstrate that the protein undergoes a sterol-regulated conformational change that requires Insig (2, 9). Recent studies suggest that the distance of the MELADL sequence of Scap from the

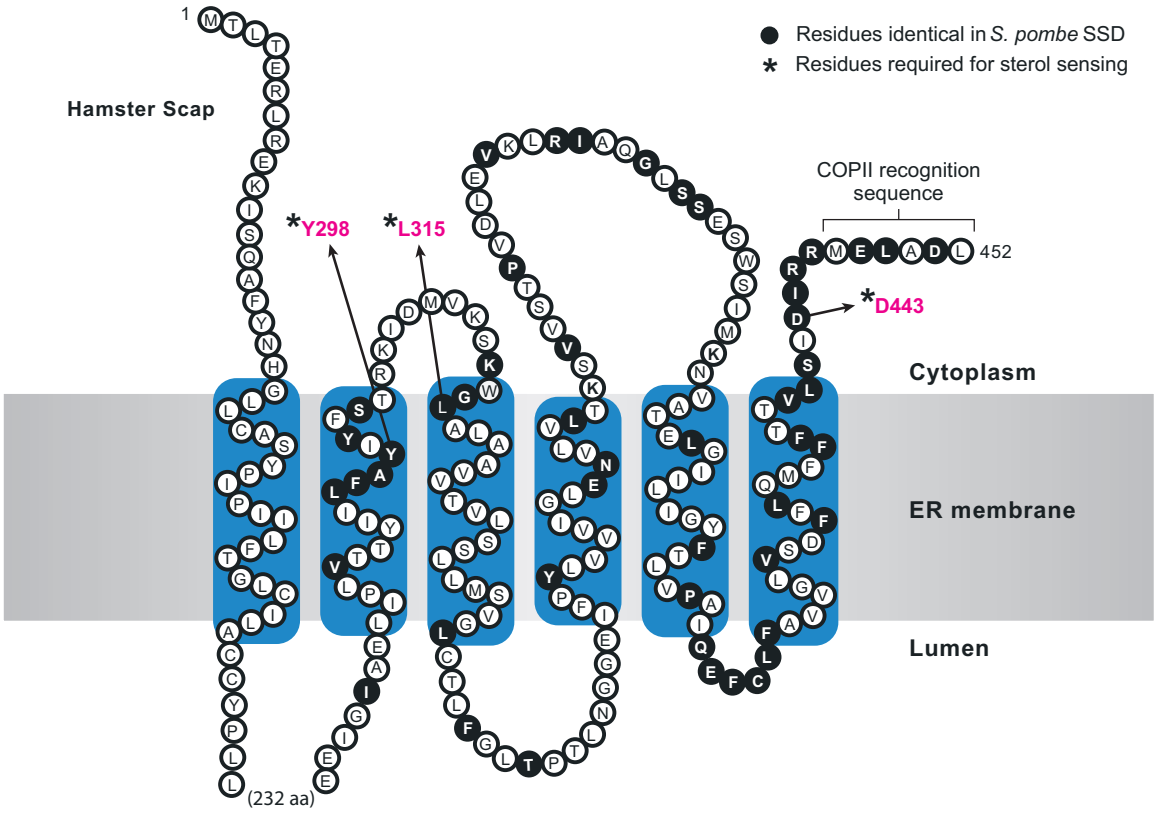

Figure 3

Topology of Scap sterol-sensing domain. Membrane topology of hamster Scap TM 1-6 (aa 1-452) is shown. Residues highlighted in black are identical in fission yeast Scp1. MELADL COPII recognition sequence (aa 447-452) is bracketed. Asterisks denote residues that when mutated confer sterol resistance to Scap and Scp1. Figure was adapted from (114).

membrane regulates access of COPII proteins and ER exit (115). These data support a model in which binding of Scap to sterol and Insig induces a conformational change in the N-terminal transmembrane region of Scap that makes the MELADL sequence inaccessible to COPII (**Figure 2**). The inability of COPII to bind Scap prevents incorporation of SREBP-Scap into ER transport vesicles and activation by Golgi-localized proteases (115).

Insig

Insig-1 was initially identified as an insulin induced gene in a transcriptional study of regenerating liver (84). Mammalian genomes contain two Insig genes that code for Insig-1 (277 aa) and Insig-2 (225 aa), which display a similar tissue expression pattern (127). Purified Insig-2 is a dimer and Insigs contain 6 transmembrane segments (**Figure 4**) (37, 86). Insig-1, but not Insig-2, is an SREBP target gene and has a short half-life due to degradation by the proteasome (45, 70). However, to date these ER resident proteins are functionally interchangeable and for simplicity will be referred to as Insig (127).

The function of Insig in cholesterol homeostasis was first recognized when it was identified as a Scap binding protein that inhibited SREBP cleavage when overexpressed (32, 131). Subsequent characterization revealed

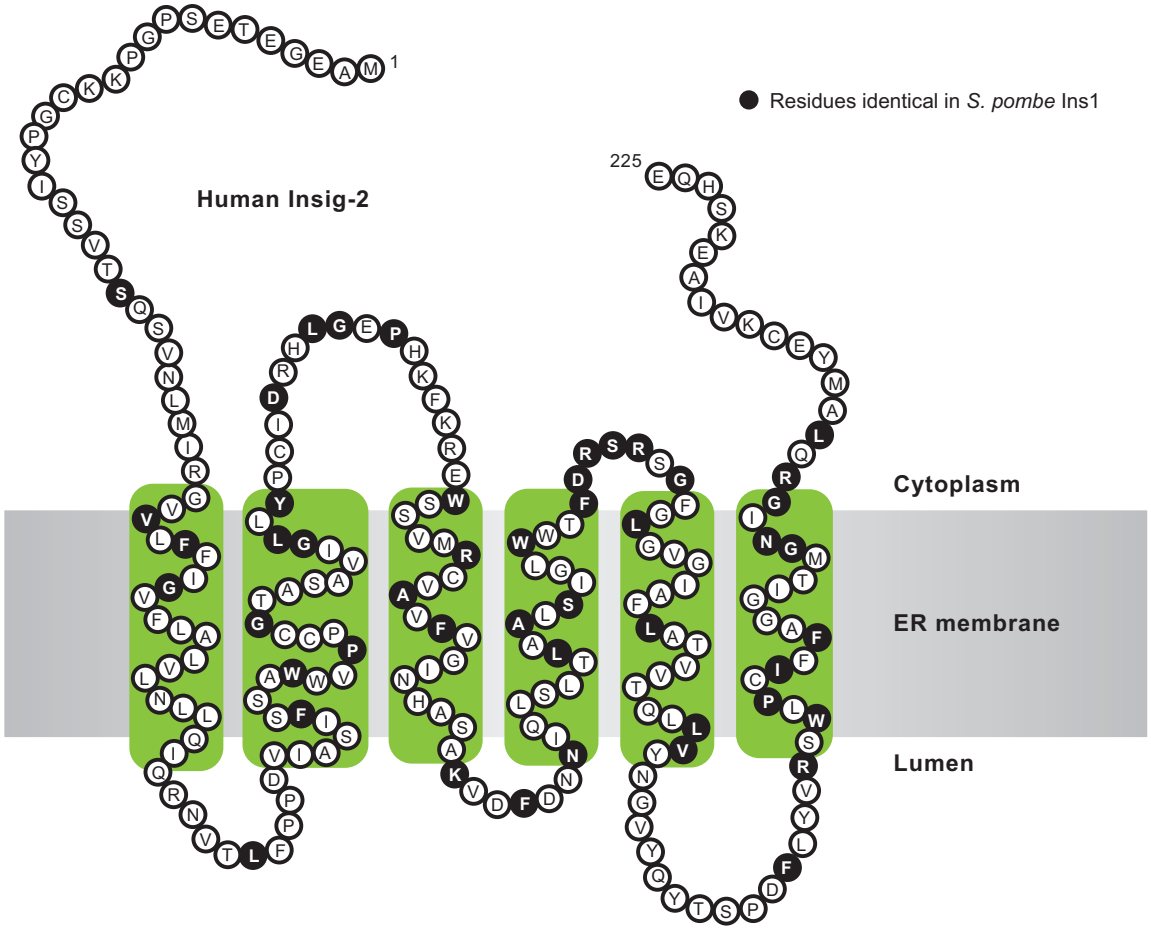

Figure 4

Topology of Insig. Membrane topology of human Insig-2 (225 aa) is shown. Residues highlighted in black are identical in fission yeast Insig. Figure was adapted from (86).

that Insig is required for the ER retention of the SREBP-Scap complex in the presence of sterols (**Figure 2**). Tissue culture cells and mouse hepatocytes lacking Insig show constitutive activation of SREBP (33, 71). Consistent with the function of Insig as a Scap retention protein, the sterol-sensing domain mutations in Scap that cause constitutive SREBP activation block binding to Insig (129).

Cellular cholesterol levels are controlled not only by the end product cholesterol, but also by oxygenated sterols, called oxysterols, which contain additional hydroxyl or keto groups at the 7 position of the B ring or at the 24-, 25-, or 27- positions on the sterol side chain (7, 97). Oxysterols are intermediates in bile acid synthesis and function in elimination of excess sterol from tissues. Oxysterols are present at low concentrations in cells relative to cholesterol and play an important role in the regulation of systemic cholesterol homeostasis (4, 62). Oxysterols, such as 25-hydroxycholesterol (25-HC), have long been recognized as potent inhibitors of sterol synthesis (10, 17, 63, 64), but the molecular mechanism by which oxysterols control SREBP has become clear only recently.

Sre1: fission yeast SREBP

Scp1: fission yeast Scap

Both cholesterol and oxysterols induce binding of Scap to Insig and prevent incorporation of SREBP-Scap into COPII vesicles (2, 114). However, unlike cholesterol, 25-HC does not bind to Scap, nor does 25-HC induce a conformational change in Scap in cells lacking Insig (86, 115). An explanation for the differential effects of cholesterol and oxysterols comes from two recent studies that identified Insig as an oxysterol binding protein that mediates the action of oxysterols on SREBP cleavage (86, 115). In these studies, recombinant purified Insig was shown to specifically bind to oxysterols and not cholesterol. Importantly, the binding specificity of Insig to different oxysterols directly correlated with the ability of these compounds to inhibit SREBP cleavage (86). Tissue culture cells lacking Insigs were used to demonstrate that Insig is preferentially required for inhibition of SREBP proteolysis by 25-HC and not cholesterol (115). Finally, Insig mutants that are defective in oxysterol binding fail to inhibit SREBP cleavage in response to 25-HC. Consistent with these recent findings, previous reconstitution of oxysterol-regulated proteolysis of human SREBP in insect cells, which lack Insig, required coexpression of human Insig (27). Taken together, these data suggest that cholesterol and oxysterol act through different intracellular receptors to induce binding of Scap to Insig. Cholesterol binds to Scap and induces binding to Insig, while oxysterols bind to Insig, and then cause Insig to bind Scap. Binding to Insig in both cases results in a conformational change in Scap that prevents access of COPII proteins to the MELADL sequence, resulting in ER retention of SREBP-Scap (**Figure 2**) (115).

TRANSCRIPTIONAL CONTROL OF STEROL SYNTHESIS IN FUNGI

In addition to their interesting biology, fungi are relatively simple eukaryotic organisms in which genetics and molecular biology can be applied to understand biological mechanisms conserved in mammals. Although fungi do not synthesize cholesterol, the close structural similarity between cholesterol and the fungal sterol ergosterol has made fungi useful model organisms for understanding sterol homeostasis (**Figure 1**) (51, 92). Research in this area has been further fueled by the fact that many antifungal therapies target the ergosterol pathway. In the past several decades, the sterol biosynthetic pathway has been studied primarily in the budding yeast *S. cerevisiae*. Discoveries resulting from this work include: the cloning of new sterol synthesis enzymes and their subcellular localization (22, 72), identification of regulatory transcription factors (121), and the isolation of genes required for the degradation of HMG-CoA reductase and other ER proteins (49). More recently, studies of SREBP orthologs in other fungi have yielded important insights into the core regulatory mechanisms of sterol homeostasis in mammals (34).

Schizosaccharomyces pombe

Completion of the *S. pombe* genome project revealed that unlike *S. cerevisiae*, fission yeast contain homologs of SREBP and Scap, named Sre1 and Scp1, respectively (60). From a structural standpoint, these proteins show little overall sequence identity, but the topologies and functionally important domains of SREBP and Scap are conserved in the fission yeast proteins. Sre1 shares high sequence identity with the basic helix-loop-helix DNA binding domain of SREBP and consequently can bind to a mammalian sterol regulatory element (SRE) such as that found in the LDL receptor promoter (118). Likewise, Scp1 is predicted to contain eight transmembrane segments and a C-terminal SREBP-binding domain. Notably, what little sequence identity Scp1 and Scap share flanks the three conserved, sterol-sensing domain residues required for ER retention of Scap in mammalian cells (**Figure 3**). Characterization of Sre1 and Scp1 revealed that these proteins are functional homologs of SREBP and Scap. Sre1 and

Scp1 form a stable complex, and Sre1 is proteolytically cleaved in response to sterol depletion by a Scp1-dependent mechanism (60). Mutation of the three sterol-sensing residues in Scp1 to the corresponding sterol-resistant amino acids results in constitutive activation of Sre1, indicating that Scp1 and Scap share structural requirements for sterol sensing (58). Furthermore, activation of Sre1 likely requires COPII-mediated ER exit as proteolysis of Sre1 requires the conserved MELADL COPII binding sequence in Scap (LELQDF in Scp1) (A. Hughes & P. Espenshade, unpublished observations). Thus, Sre1 and Scp1 are orthologs of mammalian SREBP and Scap.

Considering this conservation between the mammalian and yeast proteins, it came as a surprise that fission yeast SREBP functions in an oxygen-sensing pathway, mediating adaptation of cells to low oxygen growth (34, 60, 118). Yeast cultured in atmospheric oxygen (21% oxygen) have low levels of active, nuclear Sre1. Incubation of cells under anaerobic conditions leads to activation of Sre1 and a dramatic accumulation of nuclear Sre1. Genome-wide expression-profiling experiments revealed that under low oxygen Sre1 is a transcriptional activator, and Sre1 upregulates expression of genes required for low oxygen growth, including enzymes in oxygen consumptive pathways (118). In particular, Sre1 activates expression of a number of oxygen-dependent enzymes in ergosterol, heme, sphingolipid, and ubiquinone biosynthesis. Sre1-dependent transcription is essential for adaptation to low oxygen as *sre1*Δ and *scp1*Δ cells fail to grow under these conditions. While Sre1 does not control all low oxygen gene expression, such as the induction of glycolytic enzymes, two thirds of upregulated genes require Sre1 (118).

The ability of Sre1 to respond to changes in oxygen concentration derives from the conserved function of Sre1-Scp1 to sense sterols. Ergosterol synthesis is highly oxygen-consumptive; each molecule of ergosterol requires 12 molecules of molecular oxygen for its synthesis (**Figure 1**) (95). Therefore, changes in oxygen availability directly affect sterol synthesis. Indeed, when cells are shifted to low oxygen ergosterol synthesis is initially inhibited prior to an adaptive response that restores sterol synthesis to normal levels (60). Sre1 mediates this adaptive response, most likely through the upregulation of oxygen-dependent enzymes in sterol synthesis. Collectively, these data have led to a model in which Sre1-Scp1 monitors sterol synthesis as an indirect measure of oxygen availability.

What then is the signal for low oxygen activation of Sre1? As outlined in **Figure 1**, a drop in oxygen should lead to both an accumulation of substrates for oxygen-dependent enzymes and a decrease in ergosterol production. Experiments with sterol synthesis inhibitors clearly demonstrated that Sre1-Scp1 is activated in response to an overall decrease in ergosterol (60). However, recent experiments investigating the nature of the physiological signal for Sre1 activation under low oxygen indicate that the accumulation of the sterol intermediate lanosterol signals to activate Sre1 through Scp1 (58). A combination of genetic and biochemical experiments demonstrated that under low oxygen lanosterol and 24-methylene lanosterol, which are substrates for the cytochrome P450 enzyme Erg11, accumulate in cells and lead to activation of Sre1. Overexpression of Erg11 to levels that suppress the accumulation of these intermediates blocks low oxygen activation of Sre1 (58). Consistent with these findings, addition of exogenous lanosterol activates Sre1. These experiments suggest that fission yeast Scp1 responds to a different sterol signal than mammalian Scap, which binds cholesterol and not lanosterol (87). Sre1 controls transcription of genes in multiple biosynthetic pathways, such as heme and sphingolipids (118). Insomuch as Sre1 is subject to feedback regulation by sterols, products or intermediates from these other oxygen-dependent pathways may also regulate Sre1 activity. Presently, the mechanism by which the N-terminal transcription factor domain of Sre1 is released from the membrane is unknown as fission

yeast lack identifiable homologs of S1P and S2P.

Ins1: fission yeast Insig

Sequence database searches also revealed the existence of a fission yeast Insig homolog, called Ins1 (**Figure 4**) (60, 75). As described above, Insig binds the sterol-sensing domain of Scap and is required for the ER retention of SREBP-Scap complex. If Ins1 performed a similar function in *S. pombe*, loss of Ins1 would result in constitutive activation and unregulated proteolysis of Sre1. However, in *ins1*△ cells basal Sre1 activity remained low and Sre1 was regulated normally (60). Ins1 does not bind Scp1. Rather, Ins1 forms a stable complex with *S. pombe* HMG-CoA reductase, called Hmg1, and Ins1 functions as a negative regulator of Hmg1 activity (J. Burg & P. Espenshade, unpublished observations). These results indicate that in fission yeast sterol regulation of Scap does not require Insig, suggesting that sterol sensing and regulation of SREBP ER exit may be an intrinsic property of yeast Scap. Alternatively, an Insig-like protein may exist to facilitate ER retention of the Sre1-Scp1 complex.

Cryptococcus neoformans

Recent studies in the pathogenic fungus *Cryptococcus neoformans* demonstrated that the function of SREBP in hypoxic adaptation and regulation of sterol synthesis is conserved across fungal phyla (18, 20). *C. neoformans* is a basidiomycetous fungus that upon inhalation disseminates to the brain and causes lethal meningoencephalitis in immunocompromised individuals (15). *C. neoformans* contains homologs of both SREBP and Scap. Characterization of these genes showed that Sre1p is also activated in response to low oxygen and sterol depletion in this organism and that both *SRE1* and *SCP1* are required for low oxygen growth (18). As in fission yeast, Sre1p activation is regulated by levels of lanosterol and is required for transcriptional regulation of sterol synthesis by directly activating expression of sterol genes under low oxygen (58). Consistent with this function of Sre1p, cells lacking *SRE1* or *SCP1* have defects in sterol synthesis and are sensitive to inhibitors of the sterol pathway (18). Virulence studies in mice demonstrated that *SRE1* is essential for brain infection and virulence in a tail vein model of infection, suggesting that adaptation to host tissues requires *SRE1*. Parallel results were obtained in studies by Madhani and colleagues using a *C. neoformans* strain of a different serotype (20). This group additionally found that *SRE1* and *SCP1* are required for pulmonary infection in mice.

C. neoformans lacks an Insig homolog, but contains an apparent S2P homolog, named *STP1*. Cells lacking *STP1* show phenotypes similar to cells lacking *SRE1* or *SCP1* (20) (C. Bien & P. Espenshade, unpublished observations). Other fungi with apparent SREBP homologs include *Aspergillus fumigatus*, *A. nidulans*, *Neurospora crassa*, and *Candida albicans*, raising the question whether SREBP also functions in response to oxygen limitation and regulates sterol synthesis in these organisms.

Saccharomyces cerevisiae

The fungal ergosterol biosynthetic pathway was largely elucidated through genetic and biochemical studies in the budding yeast *S. cerevisiae* (72). Although sterol synthesis is under transcriptional control in *S. cerevisiae*, budding yeast lacks SREBP and Scap (26, 109). Instead, Upc2p and Ecm22p, two transcription factors that are homologous to each other but not to SREBP, control expression of ergosterol biosynthetic genes in response to sterol depletion (121). As in fission yeast, low oxygen activates transcription of sterol synthesis genes through a sterol-dependent mechanism, which in *S. cerevisiae* is mediated by Upc2p (23). How Upc2p responds to sterol depletion under low oxygen conditions is not known. Additionally under low oxygen, Upc2p induces expression of two genes, *AUS1* and *PDR11*, that code for ATP binding cassette (ABC) transporters that facilitate exogenous sterol uptake (125). Expression of these proteins is repressed under conditions

when oxygen is sufficient for sterol synthesis. *S. pombe* lacks detectable homologs of these sterol transporters and thus is unable to import exogenous cholesterol under low oxygen (60). Transcription of sterol biosynthetic genes in *S. cerevisiae* is independently regulated by oxygen through the heme-dependent transcription factor Hap1p and a transcriptional repressor Rox1p (67, 68). Thus, both Upc2p and Hap1p appear to monitor levels of oxygen-dependent molecules, ergosterol and heme, respectively, as an indirect measure of environmental oxygen. However, while both fission and budding yeast monitor sterol synthesis as a measure of oxygen supply, these two fungi use structurally unrelated transcription factors to achieve this regulation. These findings highlight the importance of regulating sterol synthesis in response to differential oxygen supply.

Although mechanistic conservation exists in the regulation of sterol synthesis between fungi and mammals, there are likely to be significant differences in these systems that will uncover new regulatory paradigms. A few unanswered questions already hint at this possibility. For example, although database searches have identified a candidate S2P in *C. neoformans*, neither *S. pombe* nor *C. neoformans* contains an obvious homolog of S1P. In addition, *S. pombe* lacks an S2P homolog. Understanding the details of how Sre1 is released from the membrane in these two organisms will likely advance our understanding of regulated intramembrane proteolysis (RIP), a signaling mechanism in which regulatory proteins are generated from cleavage of membrane precursors (14, 76).

REGULATION OF SREBP IN FLIES AND WORMS

The function of SREBP has been examined in two additional organisms, *D. melanogaster* and *C. elegans*. Both of these organisms do not synthesize sterols and are thus sterol auxotrophs. What then is the function of SREBP in these systems and what lipid does Scap sense? Detailed studies in *D. melanogaster* revealed that flies contain orthologs of SREBP, Scap, S1P, and S2P, but lack a recognizable Insig homolog (96, 103). Characterization of dSREBP/HLH106 in *Drosophila* S2 cells revealed that proteolysis of dSREBP requires dScap, dS1P, and dS2P (103). Proteolysis of dSREBP is regulated by levels of phosphatidylethanolamine (PE), the predominant phospholipid in insect cells, and not by sterols (28). Consistent with PE acting in end product feedback inhibition, dSREBP directs transcription of genes required for fatty acid synthesis, such as acetyl-CoA carboxylase and fatty acid synthase (103). In whole flies, dSREBP is primarily required during larval development for production of fatty acids. Flies deficient for SREBP are fatty acid auxotrophs and die during larval development (66). Fly development can be rescued by supplementing the diet with fatty acids, such as oleate.

Less is known about the regulation of SREBP activation in *C. elegans*. Worm SREBP, named SPD-1/LPD-1, is highly expressed in the intestine and is required for fat storage (3, 79, 130). Worms contain clear homologs of SREBP, Scap, and S2P, but also lack an Insig gene. As in flies, SPD-1 regulates genes involved in fatty acid synthesis, such as acetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase (79, 130). To date, the molecular signals controlling SPD-1 proteolysis are unknown. Thus in sterol auxotrophs such as flies and worms, SREBP primarily regulates fatty acid synthesis. Importantly, this regulation is Insig-independent.

EQUILIBRIUM MODEL FOR SREBP-SCAP REGULATION

Evidence from mammals, fission yeast, and flies discussed above can be assembled into a speculative model for the sterol regulation of SREBP-Scap exit from the ER and subsequent activation of SREBP. **Figure 5** depicts a model that is an extension of concepts recently put forth by Brown & Goldstein

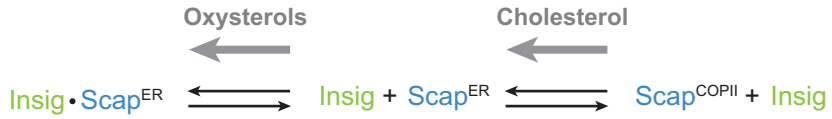

Figure 5

Equilibrium model for regulation of SREBP-Scap ER exit. $Scap^{COPII}$ and $Scap^{ER}$ denote the ER exit and ER retained forms of Scap, respectively. Cholesterol affects the equilibrium between $Scap^{COPII}$ and $Scap^{ER}$ by inducing the $Scap^{ER}$ conformation. Insig binds to the $Scap^{ER}$ conformation, further shifting the equilibrium from $Scap^{COPII}$. Cholesterol and oxysterols inhibit the activation of SREBP by binding to Scap or Insig, respectively, and shifting the conformational equilibrium of Scap away from $Scap^{COPII}$.

(115). In this model, Scap exists in equilibrium between two conformations: $Scap^{COPII}$ and $Scap^{ER}$. $Scap^{COPII}$ leads to ER exit of SREBP-Scap because the MELADL sequence of Scap is available for COPII binding, and $Scap^{ER}$ is retained in the ER because the MELADL sequence is inaccessible to COPII. Thus, the equilibrium between $Scap^{COPII}$ and $Scap^{ER}$ dictates the level of SREBP activation. In this model, multiple factors influence the equilibrium between $Scap^{COPII}$ and $Scap^{ER}$ either directly or indirectly. First, cholesterol binding to Scap shifts the equilibrium to $Scap^{ER}$. Second, binding of Scap to Insig stabilizes $Scap^{ER}$. Third, oxysterol stimulates binding of Insig to $Scap^{ER}$. In this way, both cholesterol and oxysterols, acting through Scap and Insig, respectively, inhibit SREBP activation by shifting the equilibrium away from the $Scap^{COPII}$ conformation.

This model relies on three key assumptions for which different amounts of experimental evidence exist:

1 The ability to assume two functionally distinct conformations ($Scap^{COPII}$ and $Scap^{ER}$) is an intrinsic property of Scap and does not require Insig. This concept is supported by the fact that cholesterol added in vitro alters the conformation of Scap and cholesterol blocks SREBP proteolysis in Insig-deficient cells (115). In addition, regulation of SREBP-Scap in fission yeast, *C. neoformans*, and flies does not require Insig (18, 60).

2 Insig binds preferentially to $Scap^{ER}$. In support of this idea, single amino acid mutations in the sterol-sensing domain of Scap that result in constitutive SREBP activation shift the conformation to $Scap^{COPII}$ (1, 9). Importantly, these mutant Scap proteins fail to bind to Insig (129, 131).

3 Oxysterol binding to Insig promotes Insig-Scap binding. This assumption is supported by the recent demonstration that Insig is an oxysterol binding protein and that Insig mutants that do not bind oxysterol fail to bind Scap (115).

This model defines a sterol-sensing function for Scap that is independent of Insig. However, the model also highlights at least two advantages cells gain by the addition of Insig regulation to the SREBP-Scap pathway: (*a*) the ability to sense and respond to oxysterols and (*b*) a fine-tuning mechanism that alters the sensitivity of Scap to sterols. Oxysterols play an important role in the systemic regulation of cholesterol homeostasis in mammals (4, 62). By directly acting on Insig to induce $Scap^{ER}$, oxysterols enable cells to sense whole-body sterol levels in addition to monitoring the status of cellular cholesterol supply. This is an important layer of regulation that potentially allows mammalian cells to monitor two different sources of cellular cholesterol. Supporting this idea, neither oxysterols nor Insig control the regulation of fission yeast SREBP-Scap, whose only source of sterol is de novo synthesis (58, 60). Knowledge about the physiological regulation of oxysterols in mammals will be important for a complete understanding of systemic cholesterol homeostasis.

The incorporation of Insig into the SREBP-Scap pathway in mammals allows

for additional layers of regulation that act by affecting Insig protein levels. The model predicts that levels of Insig protein should influence the $Scap^{ER}$-$Scap^{COPII}$ equilibrium. Indeed, overexpression of Insig retains SREBP-Scap and blocks SREBP activation even in the absence of exogenous sterol (131). In this way, Insig protein levels may alter the sensitivity of SREBP activation to cholesterol by regulating the equilibrium between $Scap^{COPII}$ and $Scap^{ER}$. This property of the system would facilitate regulation of SREBP-Scap by signals other than sterols. Consistent with this concept, recent studies have shown that cell stress induced by hypotonicity or thapsigargin reduce Insig-1 protein levels and activate SREBP-Scap even in the presence of sterols (70). Additionally, the presence of two Insig proteins, which possess different turnover properties and whose expression is differentially regulated by hormones such as insulin, provides a platform for SREBP-Scap regulation in different tissues or in response to environmental stimuli (69, 128).

Although extensive experimentation is certainly required to test its validity, this model represents an attempt to incorporate existing data from multiple organisms into a single testable model, assuming an evolutionarily conserved regulatory mechanism.

REGULATED DEGRADATION OF HMG-CoA REDUCTASE

Cholesterol biosynthesis is a tightly regulated pathway that employs multiple feedback mechanisms to maintain homeostasis (42). Much work over the past several decades has centered on the regulation of a rate-limiting enzyme HMG-CoA reductase in this pathway (HMGR). HMGR uses two molecules of NADPH to convert one molecule of HMG-CoA to mevalonate (**Figure 1**). This is the first committed step in the mevalonate pathway that leads to the production of cholesterol and isoprenoids, which are required for production of dolichol, tRNA, heme A, ubiquinone, and prenylated proteins such as Ras and Rabs (42). Products of the isoprenoid pathway are also required for proper germ cell migration during development (100). As a key enzyme in this pathway, HMGR is regulated by feedback mechanisms operating at multiple levels: transcription, translation, posttranslational modification, and protein degradation (42). Among the best studied are the regulation of HMGR transcription by SREBP and the sterol-dependent degradation of the enzyme (43). Surprisingly, both of these mechanisms require Insig.

HMGR: HMG-CoA reductase

HMGR (888 aa) is an ER membrane protein that shares an overall topology and domain structure with Scap (**Figure 6**) (43). The N-terminal domain contains 8 transmembrane segments and the cytosolic C terminus contains the catalytic portion of the enzyme (74, 93). Like Scap, transmembrane segments 2–6 of HMGR contain a sterol-sensing domain (57). In sterol-depleted cells, SREBP stimulates HMGR transcription and HMGR protein displays a long half-life, thereby maximizing mevalonate production for sterol and isoprenoid synthesis. Products from both of these pathways feed back to control HMGR activity. Accumulating cholesterol promotes Insig binding to Scap. Consequently, SREBP proteolysis is inhibited and transcription of HMGR decreases. At the same time, products of both the sterol and isoprenoid pathway accelerate the degradation of HMGR by a ubiquitin-proteasome dependent pathway that requires Insig (**Figure 6**) (11, 88, 94).

Although it has been known for some time that products of the mevalonate pathway control the stability of HMGR (11), DeBose-Boyd and colleagues have recently uncovered the molecular details behind this posttranslational feedback regulation (43). A key discovery was the finding that the sterol intermediates lanosterol and 24,25-dihydrolanosterol (**Figure 1**), rather than the end product cholesterol, stimulate HMGR degradation (112). Lanosterol is the first sterol synthesized from mevalonate and thereby serves as a measure of mevalonate flux into the sterol pathway (**Figure 1**). A series of elegant genetic

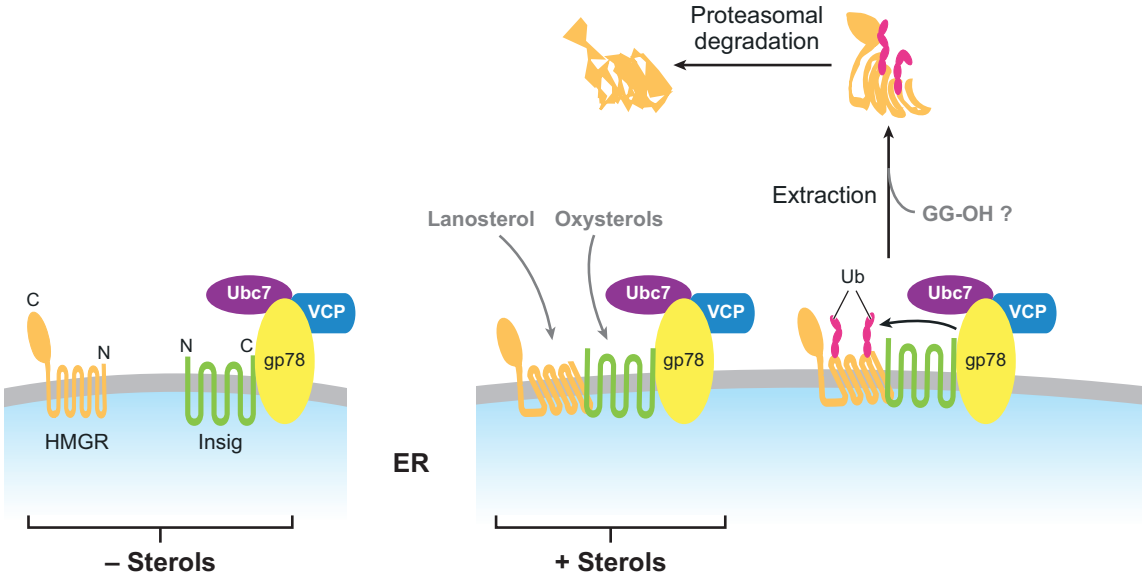

Figure 6

Regulated degradation of HMG-CoA reductase in mammals. In the absence of sterols, HMGR does not bind Insig and is present at high levels. In the presence of lanosterol or oxysterols, Insig mediates the ubiquitinylation (Ub) and subsequent degradation of HMGR by the proteasome through interactions with the E2 conjugating enzyme Ubc7, the E3 ubiquitin ligase gp78, and the ATPase VCP/p97. Geranylgeraniol (GG-OH) enhances HMGR degradation through an unknown mechanism that acts downstream of ubiquitinylation. Figure was adapted from (43).

and biochemical studies have led to the model outlined in **Figure 6** (43, 113). Accumulation of lanosterol stimulates binding of HMGR to Insig, resulting in the ubiquitinylation of HMGR on two cytosolic lysine residues and subsequent degradation by the 26S proteasome. Insig binds to the membrane domain of HMGR and is required for HMGR degradation by virtue of the fact that it forms a complex with the E3 ubiquitin ligase gp78 (113). The multidomain structure of gp78 (643 aa) allows this ER membrane protein to organize the sterol-dependent degradation of HMGR. The N-terminal membrane attachment domain of gp78 binds to Insig. The remainder of the protein consists of (*a*) a RING finger consensus sequence conferring E3 ubiquitin ligase activity; (*b*) a binding domain for the E2 ubiquitin conjugating enzyme Ubc7; and (*c*) a domain that binds to VCP/p97, an ATPase involved in delivery of ubiquitinylated membrane proteins to the proteasome (133, 135). Further studies are required to understand how VCP/p97 extracts ubiquitinylated HMGR from the membrane and targets the protein to the proteasome. Notably, the degradation mechanism for mammalian HMGR shares many similarities with that first described in *S. cerevisiae* for the turnover of yeast HMGR (49).

The differential regulation of Scap and HMGR by cholesterol and lanosterol, respectively, adds additional flexibility to the regulation of sterol synthesis. Scap monitors production of the end product cholesterol and through SREBP controls transcription of all enzymes in the pathway (55). Sterol intermediates containing methyl groups at the 4-position of the sterol A-ring cannot support growth of mammalian cells and may be toxic (126). By monitoring levels of lanosterol, the first 4-methylated sterol in the pathway, cells can avoid the accumulation of these intermediates by down-regulating HMGR activity

and flux into the top portion of the sterol pathway. In addition to lanosterol, oxysterols promote the ubiquitinylation and degradation of HMGR in both intact and permeabilized cells (110, 112). Although it remains to be proven, oxysterols are presumably acting through their ability to bind Insig and to stimulate Insig binding to HMGR (86). The ability of both fission yeast Scap (Scp1) and HMGR to respond to lanosterol suggests that the HMGR and Scp1 sterol-sensing domains share properties. Insomuch as Scp1 regulation does not require Insig in yeast, the ability of lanosterol to signal to HMGR may also not require Insig directly.

High concentrations of mevalonate are required for the rapid degradation of HMGR. Lanosterol addition stimulates the ubiquitinylation and degradation of HMGR, but the complete and rapid degradation of HMGR requires an additional nonsterol isoprenoid signal (112). The requirement for this signal can be filled by addition of geranylgeraniol, a 20-carbon isoprenyl alcohol, but not the 15-carbon alcohol farnesol (107). Presumably, the action of geranylgeraniol requires its conversion to the pathway intermediate geranylgeranyl pyrophosphate (GG-PP). However, it is not known at which step GG-PP or a metabolite of GG-PP is acting to control HMGR degradation. GG-PP has been proposed to act at a postubiquitinylation step, since geranylgeraniol alone did not stimulate HMGR ubiquitinylation in a permeabilized cell system (43, 107). One possibility is that GG-PP is required for the synthesis of a prenylated protein required for the extraction and degradation of HMGR (107).

The recognition that lanosterol regulates HMGR activity raises questions about the physiological control of lanosterol levels and the subsequent regulation of HMGR activity. Lanosterol is metabolized to 4,4-dimethylcholesta-8,14,24-trienol in a demethylation reaction carried out by the cytochrome P450 Cyp51, which requires NADPH, heme, and oxygen (**Figure 1**). Changes in the supply of these substrates may decrease Cyp51 activity and result in lanosterol accumulation. In addition, the heme binding protein PGRMC1 was recently identified as a positive regulator of Cyp51 and provides another potential regulatory point for HMGR activity (24, 59). HMGR is the target for the widely used cholesterol-lowering drugs called statins (117). The high therapeutic interest in HMGR has led to the discovery of additional agents that inhibit HMGR activity. Two of these hypocholesterolemic compounds are vitamin E (tocotrienols) and the bisphosphonate ester SR-12813 (6, 83). Recent studies demonstrate that both of these compounds accelerate degradation of HMGR by mimicking the lanosterol signal (106, 111). Importantly, SR-12813 does not affect regulation of SREBP-Scap complex by Insig and therefore is specific to HMGR. This property indicates that SR-12813 likely signals through HMGR and not Insig (106). Collectively, these findings suggest that multiple signals can influence the rate of cholesterol synthesis by affecting the stability of HMGR.

In *S. cerevisiae*, HMG-CoA reductase, Hmg2p, is also regulated by proteasomal degradation in response to the isoprenoid farnesyl pyrophosphate (F-PP), or a signal derived from it (40). Insomuch as F-PP is a precursor for GG-PP, the isoprenoid signals may be conserved between yeast and mammals. Budding yeast Insig, called Nsg1p, forms a complex with Hmg2p and participates in the control of Hmg2p degradation (38). However, in contrast to mammalian Insig, Nsg1p is a positive regulator of Hmg2p stability. Nsg1p is proposed to act as a chaperone, promoting correct Hmg2p folding and decreasing ubiquitinylation and degradation. Oxysterols have been implicated as a positive signal for Hmg2p degradation in budding yeast (41). It remains to be tested whether Nsg1p plays a role in this regulation. Likewise, fission yeast Insig forms a complex with HMGR, but similar to mammals, fission yeast Insig functions as a negative regulator of HMGR activity (J. Burg & P. Espenshade, unpublished observations).

PHYSIOLOGICAL REGULATION OF CELLULAR STEROL HOMEOSTASIS

Feedback Control by Cholesterol, Lanosterol, and SREBP Transcriptional Targets

Multiple layers of feedback regulation exist to control cellular sterol homeostasis and these are summarized in **Figure 7**. In response to sterol depletion, cells activate SREBP proteolysis, resulting in transcription of SREBP target genes. In addition to upregulating sterol biosynthetic enzymes and the LDL receptor, SREBP activates transcription of Insig-1 (**Figure 7**, Step 1). Following activation of SREBP, the resultant increase in cholesterol supply (Step 2) combines with elevated levels of Insig to inhibit SREBP proteolysis (Steps 3 and 4). This regulatory mechanism is termed convergent feedback inhibition (45). In this model, complete inhibition of SREBP proteolysis requires both cholesterol and Insig signals. In this way, the cell may ensure that downstream transcriptional targets have been upregulated prior to repressing SREBP activity (43). In a similar fashion, HMGR activity ultimately leads to the production of lanosterol (Step 5), which when elevated negatively regulates HMGR protein levels in an Insig-dependent manner (Steps 6 and 7). Regardless of the

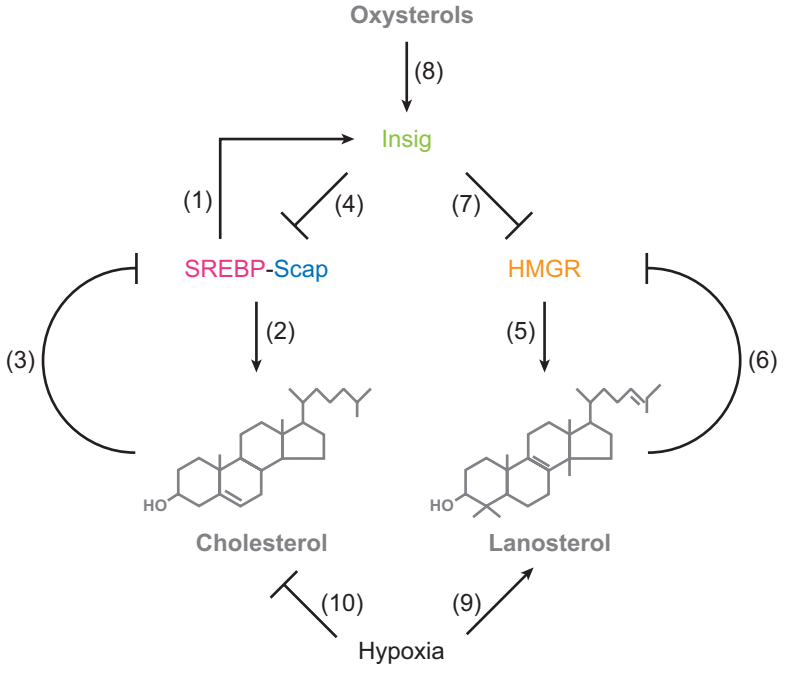

Figure 7

Feedback control of sterol synthesis in mammals. In the absence of sterols, SREBP is activated, leading to increased levels of Insig (*Step 1*) and cholesterol (*Step 2*) through an upregulation of biosynthetic enzymes and the LDL receptor. Cholesterol and Insig together feed back and inhibit SREBP activation (*Steps 3 and 4*). In a similar fashion, HMGR activity increases lanosterol (*Step 5*), which together with Insig negatively regulates HMGR protein levels (*Steps 6 and 7*). Oxysterols act through Insig to negatively regulate both SREBP-Scap and HMGR (*Step 8*). Studies from fission yeast and the oxygen requirements of the sterol pathway (**Figure 1**) predict that hypoxia should lead to an increase in lanosterol and a decrease in cholesterol, respectively (*Steps 9 and 10*). These changes in lanosterol and cholesterol levels may lead to degradation of HMGR and activation of SREBP under low oxygen conditions.

levels of lanosterol and cholesterol in the cell, oxysterols are able to inhibit both SREBP-Scap and HMGR in an Insig-dependent fashion, thus serving as a dominant signal to reduce total cellular sterol levels (Step 8).

The SREBP pathway is also controlled by positive feedback regulation insomuch as SREBPs activate their own transcription (56). This positive transcriptional feedback regulation is also present in *S. pombe*, *C. neoformans*, and *D. melanogaster* (18, 103, 118). In *C. neoformans*, Sre1 also upregulates transcription of the candidate S2P, presumably to promote more efficient processing of the membrane-bound transcription factor (18). In mammalian cells, *PCSK9* is a SREBP target gene that encodes a soluble serine protease (82). Pcsk9 functions as a posttranslational, negative regulator of LDL receptor, and by down-regulating cholesterol uptake, Pcsk9 is a positive regulator of SREBP activity (54). These examples of SREBP target genes as pathway regulators suggest that the lists of SREBP transcriptional targets may be a rich source of candidate regulators.

Adaptation to Environmental Oxygen

One fundamental principle emerging from the studies in fungi is that regulatory mechanisms exist to control sterol homeostasis in response to changes in environmental oxygen. Changes in oxygen supply directly impact sterol synthesis rate due to the presence of multiple oxygen-requiring enzymes in the pathway (**Figure 1**) (95). While this has been demonstrated in fungi, similar mechanisms must exist in mammalian cells to permit cells to adjust the rate of sterol synthesis to match oxygen availability in different tissues. In fission yeast, lanosterol demethylation is the proximal rate-limiting reaction when oxygen becomes limiting (**Figure 1**). Cells grown in 0.5% oxygen show elevated levels of lanosterol, but not the upstream intermediate squalene that is converted to squalene epoxide in an oxygen-dependent reaction (58). In fungi, lanosterol signals to activate Sre1 and upregulate the lanosterol demethylase enzyme Erg11/Cyp51, thereby restoring flux through the pathway. In mammals, a similar hypoxic accumulation of lanosterol would be expected to induce degradation of HMGR, thereby reducing sterol synthesis and the accumulation of lanosterol, a potentially toxic intermediate (**Figure 7**, Step 9). The drop in sterol synthesis should activate SREBP-Scap (**Figure 7**, Step 10), leading to increased Cyp51 and LDLR expression to relieve the low oxygen block at Cyp51 and to stimulate uptake of extracellular cholesterol. Through this feedback regulation, the cell would reestablish sterol homeostasis, but in a hypoxic environment. Consistent with this model, hypoxia stimulates the degradation of HMGR (R. DeBose-Boyd, personal communication).

Insig must play an important role in low oxygen adaptation as the central mediator of feedback regulation in this system. Tissues in which sterol homeostasis is particularly influenced by oxygen supply may display more pronounced dysregulation of cholesterol synthesis in Insig knockout mice (33). One tissue in which this feedback regulation may play a prominent role is the brain, which is a relatively low-oxygen environment (108). Brains of Insig-deficient mice show increased accumulation of lanosterol and defects in craniofacial development (31). Based on these concepts, we propose that one important physiological function of this elaborate feedback control is to allow cells in different tissues to match the demand for sterol synthesis with that of oxygen supply.

Cellular Stress

The importance of maintaining lipid homeostasis in response to changing cellular environments is further emphasized by the growing number of stress conditions that induce SREBP activation. Conditions shown to activate SREBP include: hypotonic shock, thapsigargin-induced ER stress, bacterial pore-forming toxin, and sheer stress (47, 70, 73). Although the molecular signals leading to

SREBP cleavage are incompletely understood in these cases, SREBP cleavage requires S1P and/or S2P, suggesting that these conditions induce ER exit of SREBP-Scap complex. Activation of SREBP in response to hypotonic shock and thapsigargin correlated with the loss of Insig-1 protein, suggesting that loss of ER retention activated SREBP (70). Activation of SREBP in response to bacterial toxin treatment required caspase-1 in addition to S1P and S2P; under these conditions SREBP activation increased cell survival (47). Each of these stresses may signal to SREBP by a novel, independent mechanism. Alternatively, these stresses may act through known regulators of SREBP. For example, in fission yeast hydrogen peroxide stimulates Sre1 activation through inhibition of sterol synthesis (58). Chemical inhibition of sterol synthesis at lanosterol demethylase in mammalian cells causes ER stress and activation of the integrated stress response (50), suggesting that the sterol pathway may also play a role in sensing cell stress. Finally, SREBP was among the first characterized substrates of caspase-3 (124). SREBP-1 and SREBP-2 are cleaved prior to the first transmembrane segment in response to apoptotic signals, producing an active transcription factor (52). The physiological role of this regulation is unclear.

Regulated Degradation of Nuclear SREBP

To maintain the proper supply of cholesterol for expansion of cell membranes, cholesterol synthesis must be coordinated with cell growth and nutrient supply. For example, cells actively internalizing membrane during phagocytosis compensate for this membrane loss by upregulating lipid synthesis (16). In addition, the rate of cholesterol synthesis is coupled to the energy status of the cell through the direct down-regulation of HMGR activity by AMP-activated kinase (21, 101). Another way in which nutrient signals may control sterol homeostasis is through the regulated degradation of nuclear SREBP. The cleaved nuclear form of SREBP is rapidly degraded by a proteasome-dependent pathway (53, 123). While sterols do not appear to affect the turnover of SREBP, recent studies demonstrated the phosphorylation-dependent degradation of SREBP in response to signals from the kinase GSK-3 (116). Phosphorylation of SREBP by GSK-3 promotes binding to the SCF^{Fbw7} ubiquitin E3 ligase and subsequent degradation (85). GSK-3 activity is inhibited by insulin, suggesting a mechanism by which glucose supply could regulate SREBP (39). Additionally, SREBP activity may be regulated through the action of transcriptional coactivators such as CBP/p300, Mediator, and PGC-1β that function in SREBP-dependent transcription (**Figure 2**) (5, 130). In fission yeast, nuclear Sre1 is heavily phosphorylated and has a half-life of ~10 min (60) (B. Hughes & P. Espenshade, unpublished observations), providing an opportunity for additional signals to impact oxygen-dependent transcription. Indeed, additional modes of SREBP regulation are likely to emerge as we understand more about this important pathway.

UNANSWERED QUESTIONS

The past 15 years have yielded tremendous advances in our understanding of the molecular control of sterol synthesis. However, each new finding brings with it a new set of questions. The identification of Scap and Insig as sterol binding proteins in mammalian cells added a new level of molecular detail to SREBP pathway regulation. But, how do sterols interact and control the activity of these two proteins? The equilibrium model presented in **Figure 5** makes several assumptions that need to be tested in vitro using purified Scap and Insig proteins. How does cholesterol alter the conformation of Scap? Does HMGR undergo a similar conformational change in response to lanosterol? Does HMGR bind lanosterol? Determining the crystal structures of the membrane domains of

Scap, Insig, and HMGR should provide important insights into these issues.

Insig controls both the activation of SREBP through Scap and the degradation of HMGR. How can Insig binding to these two proteins result in such different outcomes, ER retention and ubiquitinylation? What are the functional differences between Insig-1 and Insig-2? Are there additional levels of tissue-specific regulation of Insigs? In targeting HMGR for ubiquitinylation, Insig functions as a substrate recognition adaptor. Does Insig target other ER proteins for degradation by the proteasome? Following ubiquitinylation, HMGR is extracted from the ER membrane through unknown mechanisms. How is this accomplished and what is the role for the nonsterol isoprenoid signal, geranylgeraniol in this process?

Experiments in fungi highlight the importance of oxygen supply for sterol synthesis. Tissues experience a wide range of oxygen tensions under homeostatic conditions as well as acute changes in oxygen supply. How do mammalian cells regulate sterol synthesis in response to oxygen availability? Does SREBP function in an oxygen sensing pathway in mammals as it does in fungi? In order to monitor sterol as an indirect measure of oxygen supply, new oxygen-dependent synthesis must be the only source of cellular sterol. The delivery of cholesterol to mammalian cells through lipoproteins would be expected to short-circuit this oxygen sensing mechanism. Perhaps, sterol synthesis is used as a measure of oxygen availability when lipoprotein is not a major source of cholesterol, such as early in development.

Finally, what role if any does oligomerization play in the regulation of the sterol-sensing domain proteins, Scap and HMGR? Purification of Scap revealed that it is a tetramer (87). Likewise, the crystal structure for HMGR showed that its catalytic domain exists as a tetramer (61). Purified Insig is a dimer and dimerization of SREBP is mediated by its leucine zipper domain in the N terminus (86). Thus, complexes between SREBP-Scap and Insig have the potential to be extremely large. It is possible that the assembly and stoichiometry of these complexes play a critical role in the sorting of SREBP-Scap into COPII vesicles and the ubiquitinylation of HMGR.

The next five years are likely to produce exciting answers to many of these questions. The establishment of fungal models for SREBP regulation raises the hope that the powerful genetics of these organisms will reveal novel regulators of sterol synthesis and serve as a complement to the elegant use of biochemistry and somatic cell genetics employed to dissect the regulation of mammalian cholesterol homeostasis.

SUMMARY POINTS

1. SREBP is a principal transcriptional regulator of sterol and other lipid biosynthetic enzymes in eukaryotes. Activity of SREBP is controlled by the sorting of the SREBP-Scap complex into COPII transport vesicles.
2. Current data regarding the control of cellular sterol synthesis can be assembled into a testable equilibrium model in which multiple regulatory inputs affect the conformation of Scap and control its binding to COPII proteins.
3. Insig is a central regulator of mammalian cellular sterol homeostasis. Insig controls transcription of sterol biosynthetic genes by regulating Scap, and Insig acts directly to accelerate HMGR degradation by the proteasome.
4. Cholesterol, lanosterol, and oxysterols feed back to inhibit cholesterol synthesis by signaling through three different proteins: Scap, HMGR, and Insig, respectively.

5. Sterol synthesis is highly oxygen consumptive. Cells must regulate sterol homeostasis in response to changes in oxygen availability. Fungi take advantage of this oxygen requirement and monitor sterol synthesis as an indirect measure of oxygen supply.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Adams CM, Goldstein JL, Brown MS. 2003. Cholesterol-induced conformational change in SCAP enhanced by Insig proteins and mimicked by cationic amphiphiles. *Proc. Natl. Acad. Sci. USA* 100:10647–52
2. Adams CM, Reitz J, De Brabander JK, Feramisco JD, Li L, et al. 2004. Cholesterol and 25-hydroxycholesterol inhibit activation of SREBPs by different mechanisms, both involving SCAP and Insigs. *J. Biol. Chem.* 279:52772–80
3. Ashrafi K, Chang FY, Watts JL, Fraser AG, Kamath RS, et al. 2003. Genome-wide RNAi analysis of *Caenorhabditis elegans* fat regulatory genes. *Nature* 421:268–72
4. Beaven SW, Tontonoz P. 2006. Nuclear receptors in lipid metabolism: targeting the heart of dyslipidemia. *Annu. Rev. Med.* 57:313–29
5. Bengoechea-Alonso MT, Ericsson J. 2007. SREBP in signal transduction: cholesterol metabolism and beyond. *Curr. Opin. Cell Biol.* 19:215–22
6. Berkhout TA, Simon HM, Patel DD, Bentzen C, Niesor E, et al. 1996. The novel cholesterol-lowering drug SR-12813 inhibits cholesterol synthesis via an increased degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J. Biol. Chem.* 271:14376–82
7. Bjorkhem I. 2002. Do oxysterols control cholesterol homeostasis? *J. Clin. Invest.* 110:725–30
8. Briggs MR, Yokoyama C, Wang XD, Brown MS, Goldstein JL. 1993. Nuclear-protein that binds sterol regulatory element of low-density-lipoprotein receptor promoter. I. Identification of the protein and delineation of its target nucleotide sequence. *J. Biol. Chem.* 268:14490–96
9. **Brown AJ, Sun LP, Feramisco JD, Brown MS, Goldstein JL. 2002. Cholesterol addition to ER membranes alters conformation of SCAP, the SREBP escort protein that regulates cholesterol metabolism. *Mol. Cell* 10:237–45**
10. Brown MS, Goldstein JL. 1974. Suppression of 3-hydroxy-3-methylglutaryl coenzyme-A reductase-activity and inhibition of growth of human fibroblasts by 7-ketocholesterol. *J. Biol. Chem.* 249:7306–14

Using an in vitro protease sensitivity assay, the authors demonstrate that cholesterol can alter the conformation of Scap in vitro.

11. Brown MS, Goldstein JL. 1980. Multivalent feedback-regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell-growth. *J. Lipid Res.* 21:505–17
12. Brown MS, Goldstein JL. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science* 232:34–47
13. Brown MS, Goldstein JL. 1997. The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* 89:331–40
14. Brown MS, Ye J, Rawson RB, Goldstein JL. 2000. Regulated intramembrane proteolysis: A control mechanism conserved from bacteria to humans. *Cell* 100:391–98
15. Casadevall A, Perfect JR. 1998. *Cryptococcus Neoformans*. Washington, DC: ASM Press. 541 pp.
16. Castoreno AB, Wang Y, Stockinger W, Jarzylo LA, Du H, et al. 2005. Transcriptional regulation of phagocytosis-induced membrane biogenesis by sterol regulatory element binding proteins. *Proc. Natl. Acad. Sci. USA* 102:13129–34
17. Chang TY, Limanek JS. 1980. Regulation of cytosolic acetoacetyl coenzyme A thiolase, 3-hydroxy-3-methylglutaryl coenzyme A synthase, 3-hydroxy-3-methylglutaryl coenzyme A reductase, and mevalonate kinase by low density lipoprotein and by 25-hydroxycholesterol in Chinese hamster ovary cells. *J. Biol. Chem.* 255:7787–95
18. Chang YC, Bien CM, Lee H, Espenshade PJ, Kwon-Chung KJ. 2007. Sre1p, a regulator of oxygen sensing and sterol homeostasis, is required for virulence in *Cryptococcus neoformans*. *Mol. Microbiol.* 64:614–29
19. Cheng D, Espenshade PJ, Slaughter CA, Jaen JC, Brown MS, et al. 1999. Secreted site-1 protease cleaves peptides corresponding to luminal loop of sterol regulatory element-binding proteins. *J. Biol. Chem.* 274:22805–12
20. Chun CD, Liu OW, Madhani HD. 2007. A link between virulence and homeostatic responses to hypoxia during infection by the human fungal pathogen *Cryptococcus neoformans*. *PLoS Pathog.* 3:e22
21. Clarke PR, Hardie DG. 1990. Regulation of HMG-CoA reductase: identification of the site phosphorylated by the AMP-activated protein kinase in vitro and in intact rat liver. *EMBO J.* 9:2439–46
22. Czabany T, Athenstaedt K, Daum G. 2007. Synthesis, storage and degradation of neutral lipids in yeast. *Biochim. Biophys. Acta* 1771:299–309
23. Davies BS, Rine J. 2006. A role for sterol levels in oxygen sensing in *Saccharomyces cerevisiae*. *Genetics* 174:191–201
24. DeBose-Boyd RA. 2007. A helping hand for cytochrome P450 enzymes. *Cell Metab.* 5:81–88
25. **DeBose-Boyd RA, Brown MS, Li WP, Nohturfft A, Goldstein JL, et al. 1999. Transport-dependent proteolysis of SREBP: Relocation of Site-1 protease from Golgi to ER obviates the need for SREBP transport to Golgi. *Cell* 99:703–12**
26. Dimster-Denk D, Rine J. 1996. Transcriptional regulation of a sterol-biosynthetic enzyme by sterol levels in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 16:3981–89
27. Dobrosotskaya IY, Goldstein JL, Brown MS, Rawson RB. 2003. Reconstitution of sterol-regulated ER-to-Golgi transport of SREBP-2 in insect cells by coexpression of mammalian SCAP and insigs. *J. Biol. Chem.* 278:35837–42
28. **Dobrosotskaya IY, Seegmiller AC, Brown MS, Goldstein JL, Rawson RB. 2002. Regulation of SREBP processing and membrane lipid production by phospholipids in *Drosophila*. *Science* 296:879–83**

Demonstrates that Scap functions to control SREBP proteolysis by regulating the ER-to-Golgi transport of SREBP.

Demonstrates the regulation of SREBP-Scap by the phospholipid phosphatidylethanolamine, not sterols, in *Drosophila* cells.

29. Duncan EA, Brown MS, Goldstein JL, Sakai J. 1997. Cleavage site for sterol-regulated protease localized to a Leu-Ser bond in the lumenal loop of sterol regulatory element-binding protein-2. *J. Biol. Chem*. 272:12778–85
30. Duncan EA, Dave UP, Sakai J, Goldstein JL, Brown MS. 1998. Second-site cleavage in sterol regulatory element-binding protein occurs at transmembrane junction as determined by cysteine panning. *J. Biol. Chem*. 273:17801–9
31. Engelking LJ, Evers BM, Richardson JA, Goldstein JL, Brown MS, et al. 2006. Severe facial clefting in Insig-deficient mouse embryos caused by sterol accumulation and reversed by lovastatin. *J. Clin. Invest*. 116:2356–65
32. Engelking LJ, Kuriyama H, Hammer RE, Horton JD, Brown MS, et al. 2004. Overexpression of Insig-1 in the livers of transgenic mice inhibits SREBP processing and reduces insulin-stimulated lipogenesis. *J. Clin. Invest*. 113:1168–75
33. Engelking LJ, Liang G, Hammer RE, Takaishi K, Kuriyama H, et al. 2005. Schoenheimer effect explained—feedback regulation of cholesterol synthesis in mice mediated by Insig proteins. *J. Clin. Invest*. 115:2489–98
34. Espenshade PJ. 2006. SREBPs: sterol-regulated transcription factors. *J. Cell Sci*. 119:973–76
35. Espenshade PJ, Cheng D, Goldstein JL, Brown MS. 1999. Autocatalytic processing of site-1 protease removes propeptide and permits cleavage of sterol regulatory element-binding proteins. *J. Biol. Chem*. 274:22795–804
36. Espenshade PJ, Li WP, Yabe D. 2002. Sterols block binding of COPII proteins to SCAP, thereby controlling SCAP sorting in ER. *Proc. Natl. Acad. Sci. USA* 99:11694–99
37. Feramisco JD, Goldstein JL, Brown MS. 2004. Membrane topology of human Insig-1, a protein regulator of lipid synthesis. *J. Biol. Chem*. 279:8487–96
38. Flury I, Garza R, Shearer A, Rosen J, Cronin S, et al. 2005. INSIG: a broadly conserved transmembrane chaperone for sterol-sensing domain proteins. *EMBO J*. 24:3917–26
39. Frame S, Cohen P. 2001. GSK3 takes centre stage more than 20 years after its discovery. *Biochem. J*. 359:1–16
40. Gardner RG, Hampton RY. 1999. A highly conserved signal controls degradation of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in eukaryotes. *J. Biol. Chem*. 274:31671–78
41. Gardner RG, Shan H, Matsuda SP, Hampton RY. 2001. An oxysterol-derived positive signal for 3-hydroxy-3-methylglutaryl-CoA reductase degradation in yeast. *J. Biol. Chem*. 276:8681–94
42. Goldstein JL, Brown MS. 1990. Regulation of the mevalonate pathway. *Nature* 343:425–30
43. Goldstein JL, DeBose-Boyd RA, Brown MS. 2006. Protein sensors for membrane sterols. *Cell* 124:35–46
44. Goldstein JL, Rawson RB, Brown MS. 2002. Mutant mammalian cells as tools to delineate the sterol regulatory element-binding protein pathway for feedback regulation of lipid synthesis. *Arch. Biochem. Biophys*. 397:139–48
45. Gong Y, Lee JN, Lee PC, Goldstein JL, Brown MS, et al. 2006. Sterol-regulated ubiquitination and degradation of Insig-1 creates a convergent mechanism for feedback control of cholesterol synthesis and uptake. *Cell Metab*. 3:15–24
46. Gould RG, Taylor CB, Hagerman JS, Warner I, Campbell DJ. 1953. Cholesterol metabolism. I. Effect of dietary cholesterol on the synthesis of cholesterol in dog tissue in vitro. *J. Biol. Chem*. 201:519–28

47. Gurcel L, Abrami L, Girardin S, Tschopp J, Van Der Goot FG. 2006. Caspase-1 activation of lipid metabolic pathways in response to bacterial pore-forming toxins promotes cell survival. *Cell* 126:1135–45
48. Gurkan C, Stagg SM, LaPointe P, Balch WE. 2006. The COPII cage: unifying principles of vesicle coat assembly. *Nat. Rev. Mol. Cell Biol*. 7:727–38
49. Hampton RY. 2002. Proteolysis and sterol regulation. *Annu. Rev. Cell Dev. Biol*. 18:345–78
50. Harding HP, Zhang Y, Khersonsky S, Marciniak S, Scheuner D, et al. 2005. Bioactive small molecules reveal antagonism between the integrated stress response and sterol-regulated gene expression. *Cell Metab*. 2:361–71
51. Henneberry AL, Sturley SL. 2005. Sterol homeostasis in the budding yeast, *Saccharomyces cerevisiae*. *Semin. Cell Dev. Biol*. 16:155–61
52. Higgins ME, Ioannou YA. 2001. Apoptosis-induced release of mature sterol regulatory element-binding proteins activates sterol-responsive genes. *J. Lipid. Res*. 42:1939–46
53. Hirano Y, Yoshida M, Shimizu M, Sato R. 2001. Direct demonstration of rapid degradation of nuclear sterol regulatory element-binding proteins by the ubiquitin-proteasome pathway. *J. Biol. Chem*. 276:36431–37
54. Horton JD, Cohen JC, Hobbs HH. 2007. Molecular biology of PCSK9: its role in LDL metabolism. *Trends Biochem. Sci*. 32:71–77
55. Horton JD, Goldstein JL, Brown MS. 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest*. 109:1125–31
56. Horton JD, Shah NA, Warrington JA, Anderson NN, Park SW, et al. 2003. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. *Proc. Natl. Acad. Sci. USA* 100:12027–32
57. Hua XX, Nohturfft A, Goldstein JL, Brown MS. 1996. Sterol resistance in CHO cells traced to point mutation in SREBP cleavage-activating protein. *Cell* 87:415–26
58. Hughes AL, Lee CS, Bien CM, Espenshade PJ. 2007. 4-Methyl sterols regulate fission yeast SREBP-Scap under low oxygen and cell stress. *J. Biol. Chem*. 282:24388–96
59. Hughes AL, Powell DW, Bard M, Eckstein J, Barbuch R, et al. 2007. Dap1/PGRMC1 binds and regulates cytochrome P450 enzymes. *Cell Metab*. 5:143–49
60. Hughes AL, Todd BL, Espenshade PJ. 2005. SREBP pathway responds to sterols and functions as an oxygen sensor in fission yeast. *Cell* 120:831–42
61. Istvan ES, Palnitkar M, Buchanan SK, Deisenhofer J. 2000. Crystal structure of the catalytic portion of human HMG-CoA reductase: insights into regulation of activity and catalysis. *EMBO J*. 19:819–30
62. Kalaany NY, Mangelsdorf DJ. 2006. LXRS and FXR: the yin and yang of cholesterol and fat metabolism. *Annu. Rev. Physiol*. 68:159–91
63. Kandutsch AA, Chen HW. 1973. Inhibition of sterol synthesis in cultured mouse cells by 7alpha-hydroxycholesterol, 7beta-hydroxycholesterol, and 7-ketocholesterol. *J. Biol. Chem*. 248:8408–17
64. Kandutsch AA, Chen HW, Heiniger HJ. 1978. Biological activity of some oxygenated sterols. *Science* 201:498–501
65. Korn BS, Shimomura I, Bashmakov Y, Hammer RE, Horton JD, et al. 1998. Blunted feedback suppression of SREBP processing by dietary cholesterol in transgenic mice expressing sterol-resistant SCAP(D443N). *J. Clin. Invest*. 102:2050–60
66. Kunte AS, Matthews KA, Rawson RB. 2006. Fatty acid auxotrophy in *Drosophila* larvae lacking SREBP. *Cell Metab*. 3:439–48
67. Kwast KE, Burke PV, Poyton RO. 1998. Oxygen sensing and the transcriptional regulation of oxygen-responsive genes in yeast. *J. Exp. Biol*. 201:1177–95

Expression cloning of Scap cDNA from a mutant CHO cell line resistant to oxysterol regulation.

Characterization of yeast SREBP-Scap revealed a function for this pathway in adaptation to low oxygen environment.

68. Kwast KE, Lai LC, Menda N, James DT III, Aref S, et al. 2002. Genomic analyses of anaerobically induced genes in *Saccharomyces cerevisiae*: functional roles of Rox1 and other factors in mediating the anoxic response. *J. Bacteriol.* 184:250–65
69. Lee JN, Gong Y, Zhang X, Ye J. 2006. Proteasomal degradation of ubiquitinated Insig proteins is determined by serine residues flanking ubiquitinated lysines. *Proc. Natl. Acad. Sci. USA* 103:4958–63
70. Lee JN, Ye J. 2004. Proteolytic activation of sterol regulatory element-binding protein induced by cellular stress through depletion of Insig-1. *J. Biol. Chem.* 279:45257–65
71. Lee PC, Sever N, DeBose-Boyd RA. 2005. Isolation of sterol-resistant Chinese hamster ovary cells with genetic deficiencies in both Insig-1 and Insig-2. *J. Biol. Chem.* 280:25242–49
72. Lees ND, Bard M, Kirsch DR. 1999. Biochemistry and molecular biology of sterol synthesis in *Saccharomyces cerevisiae*. *Crit. Rev. Biochem. Mol. Biol.* 34:33–47
73. Lin T, Zeng L, Liu Y, DeFea K, Schwartz MA, et al. 2003. Rho-ROCK-LIMK-cofilin pathway regulates shear stress activation of sterol regulatory element binding proteins. *Circ. Res.* 92:1296–304
74. Liscum L, Finermoore J, Stroud RM, Luskey KL, Brown MS, et al. 1985. Domain-structure of 3-hydroxy-3-methylglutaryl coenzyme-A reductase, a glycoprotein of the endoplasmic-reticulum. *J. Biol. Chem.* 260:522–30
75. Loewen CJ, Levine TP. 2002. Cholesterol homeostasis: not until the SCAP lady INSIGs. *Curr. Biol.* 12:R779–81
76. Makinoshima H, Glickman MS. 2006. Site-2 proteases in prokaryotes: Regulated intramembrane proteolysis expands to microbial pathogenesis. *Microbes Infect.* 8:1882–88
77. Matsuda M, Korn BS, Hammer RE, Moon YA, Komuro R, et al. 2001. SREBP cleavage-activating protein (SCAP) is required for increased lipid synthesis in liver induced by cholesterol deprivation and insulin elevation. *Genes Dev.* 15:1206–16
78. Maxfield FR, Tabas I. 2005. Role of cholesterol and lipid organization in disease. *Nature* 438:612–21
79. McKay RM, McKay JP, Avery L, Graff JM. 2003. *C. elegans*: a model for exploring the genetics of fat storage. *Dev. Cell* 4:131–42
80. Miller EA, Beilharz TH, Malkus PN, Lee MC, Hamamoto S, et al. 2003. Multiple cargo binding sites on the COPII subunit Sec24p ensure capture of diverse membrane proteins into transport vesicles. *Cell* 114:497–509
81. Nohturfft A, Yabe D, Goldstein JL, Brown MS, Espenshade PJ. 2000. Regulated step in cholesterol feedback localized to budding of SCAP from ER membranes. *Cell* 102:315–23
82. Park SW, Moon YA, Horton JD. 2004. Post-transcriptional regulation of low density lipoprotein receptor protein by proprotein convertase subtilisin/kexin type 9a in mouse liver. *J. Biol. Chem.* 279:50630–38
83. Parker RA, Pearce BC, Clark RW, Gordon DA, Wright JJ. 1993. Tocotrienols regulate cholesterol production in mammalian cells by post-transcriptional suppression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J. Biol. Chem.* 268:11230–38
84. Peng Y, Schwarz EJ, Lazar MA, Genin A, Spinner NB, et al. 1997. Cloning, human chromosomal assignment, and adipose and hepatic expression of the CL-6/INSIG1 gene. *Genomics* 43:278–84
85. Punga T, Bengoechea-Alonso MT, Ericsson J. 2006. Phosphorylation and ubiquitination of the transcription factor sterol regulatory element-binding protein-1 in response to DNA binding. *J. Biol. Chem.* 281:25278–86

86. Radhakrishnan A, Ikeda Y, Kwon HJ, Brown MS, Goldstein JL. 2007. Sterol-regulated transport of SREBPs from endoplasmic reticulum to Golgi: Oxysterols block transport by binding to Insig. *Proc. Natl. Acad. Sci. USA* 104:6511–18

87. Radhakrishnan A, Sun LP, Kwon HJ, Brown MS, Goldstein JL. 2004. Direct binding of cholesterol to the purified membrane region of SCAP: mechanism for a sterol-sensing domain. *Mol. Cell* 15:259–68

88. Ravid T, Doolman R, Avner R, Harats D, Roitelman J. 2000. The ubiquitin-proteasome pathway mediates the regulated degradation of mammalian 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J. Biol. Chem*. 275:35840–47

89. Rawson RB. 2003. The SREBP pathway—insights from Insigs and insects. *Nat. Rev. Mol. Cell Biol*. 4:631–40

90. Rawson RB, DeBose-Boyd R, Goldstein JL, Brown MS. 1999. Failure to cleave sterol regulatory element-binding proteins (SREBPs) causes cholesterol auxotrophy in Chinese hamster ovary cells with genetic absence of SREBP cleavage-activating protein. *J. Biol. Chem*. 274:28549–56

91. Rawson RB, Zelenski NG, Nijhawan D, Ye J, Sakai J, et al. 1997. Complementation cloning of S2P, a gene encoding a putative metalloprotease required for intramembrane cleavage of SREBPs. *Mol. Cell* 1:47–57

92. Reiner S, Micolod D, Schneiter R. 2005. *Saccharomyces cerevisiae*, a model to study sterol uptake and transport in eukaryotes. *Biochem. Soc. Trans*. 33:1186–88

93. Roitelman J, Olender EH, Bar-Nun S, Dunn WA Jr, Simoni RD. 1992. Immunological evidence for eight spans in the membrane domain of 3-hydroxy-3-methylglutaryl coenzyme A reductase: implications for enzyme degradation in the endoplasmic reticulum. *J. Cell Biol*. 117:959–73

94. Roitelman J, Simoni RD. 1992. Distinct sterol and nonsterol signals for the regulated degradation of 3-hydroxy-3-methylglutaryl-CoA reductase. *J. Biol. Chem*. 267:25264–73

95. Rosenfeld E, Beauvoit B. 2003. Role of the nonrespiratory pathways in the utilization of molecular oxygen by *Saccharomyces cerevisiae*. *Yeast* 20:1115–44

96. Rosenfeld JM, Osborne TF. 1998. HLH106, a *Drosophila* sterol regulatory element-binding protein in a natural cholesterol auxotroph. *J. Biol. Chem*. 273:16112–21

97. Russell DW. 2000. Oxysterol biosynthetic enzymes. *Biochim. Biophys. Acta* 1529:126–35

98. Sakai J, Duncan EA, Rawson RB, Hua X, Brown MS, et al. 1996. Sterol-regulated release of SREBP-2 from cell membranes requires two sequential cleavages, one within a transmembrane segment. *Cell* 85:1037–46

99. Sakai J, Rawson RB, Espenshade PJ, Cheng D, Seegmiller AC, et al. 1998. Molecular identification of the sterol-regulated luminal protease that cleaves SREBPs and controls lipid composition of animal cells. *Mol. Cell* 2:505–14

100. Santos AC, Lehmann R. 2004. Germ cell specification and migration in *Drosophila* and beyond. *Curr. Biol.* 14:R578–89

101. Sato R, Goldstein JL, Brown MS. 1993. Replacement of serine-871 of hamster 3-hydroxy-3-methylglutaryl-CoA reductase prevents phosphorylation by AMP-activated kinase and blocks inhibition of sterol synthesis induced by ATP depletion. *Proc. Natl. Acad. Sci. USA* 90:9261–65

102. Schoenheimer R, Breusch F. 1933. Synthesis and destruction of cholesterol in the organism. *J. Biol. Chem*. 103:439–48

103. Seegmiller AC, Dobrosotskaya I, Goldstein JL, Ho YK, Brown MS, et al. 2002. The SREBP pathway in *Drosophila*: regulation by palmitate, not sterols. *Dev. Cell* 2:229–38

References 86 and 87 describe biochemical experiments that demonstrate the direct binding of cholesterol and oxysterols to Scap and Insig, respectively.

104. Seidah NG, Khatib AM, Prat A. 2006. The proprotein convertases and their implication in sterol and/or lipid metabolism. *Biol. Chem*. 387:871–77
105. Seidah NG, Mowla SJ, Hamelin J, Mamarbachi AM, Benjannet S, et al. 1999. Mammalian subtilisin/kexin isozyme SKI-1: a widely expressed proprotein convertase with a unique cleavage specificity and cellular localization. *Proc. Natl. Acad. Sci. USA* 96:1321–26
106. Sever N, Lee PC, Song BL, Rawson RB, DeBose-Boyd RA. 2004. Isolation of mutant cells lacking Insig-1 through selection with SR-12813, an agent that stimulates degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J. Biol. Chem*. 279:43136–47
107. Sever N, Song BL, Yabe D, Goldstein JL, Brown MS, et al. 2003. Insig-dependent ubiquitination and degradation of mammalian 3-hydroxy-3-methylglutaryl-CoA reductase stimulated by sterols and geranylgeraniol. *J. Biol. Chem*. 278:52479–90
108. Sharp FR, Bernaudin M. 2004. HIF1 and oxygen sensing in the brain. *Nat. Rev. Neurosci*. 5:437–48
109. Smith SJ, Crowley JH, Parks LW. 1996. Transcriptional regulation by ergosterol in the yeast *Saccharomyces cerevisiae*. *Mol. Cell Biol*. 16:5427–32
110. Song BL, DeBose-Boyd RA. 2004. Ubiquitination of 3-hydroxy-3-methylglutaryl-CoA reductase in permeabilized cells mediated by cytosolic E1 and a putative membrane-bound ubiquitin ligase. *J. Biol. Chem*. 279:28798–806
111. Song BL, DeBose-Boyd RA. 2006. Insig-dependent ubiquitination and degradation of 3-hydroxy-3-methylglutaryl coenzyme a reductase stimulated by delta- and gamma-tocotrienols. *J. Biol. Chem*. 281:25054–61
112. Song BL, Javitt NB, DeBose-Boyd RA. 2005. Insig-mediated degradation of HMG-CoA reductase stimulated by lanosterol, an intermediate in the synthesis of cholesterol. *Cell Metab*. 1:179–89
113. Song BL, Sever N, DeBose-Boyd RA. 2005. Gp78, a membrane-anchored ubiquitin ligase, associates with Insig-1 and couples sterol-regulated ubiquitination to degradation of HMG CoA reductase. *Mol. Cell* 19:829–40
114. Sun LP, Li L, Goldstein JL, Brown MS. 2005. Insig required for sterol-mediated inhibition of Scap/SREBP binding to COPII proteins in vitro. *J. Biol. Chem*. 280:26483–90
115. Sun LP, Seemann J, Goldstein JL, Brown MS. 2007. Sterol-regulated transport of SREBPs from endoplasmic reticulum to Golgi: Insig renders sorting signal in Scap inaccessible to COPII proteins. *Proc. Natl. Acad. Sci. USA* 104:6519–26
116. Sundqvist A, Bengoechea-Alonso MT, Ye X, Lukiyanchuk V, Jin J, et al. 2005. Control of lipid metabolism by phosphorylation-dependent degradation of the SREBP family of transcription factors by SCF(Fbw7). *Cell Metab*. 1:379–91
117. Tobert JA. 2003. Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. *Nat. Rev. Drug Discov*. 2:517–26
118. Todd BL, Stewart EV, Burg JS, Hughes AL, Espenshade PJ. 2006. Sterol regulatory element binding protein is a principal regulator of anaerobic gene expression in fission yeast. *Mol. Cell Biol*. 26:2817–31
119. Tontonoz P, Kim JB, Graves RA, Spiegelman BM. 1993. ADD1: a novel helix-loop-helix transcription factor associated with adipocyte determination and differentiation. *Mol. Cell Biol*. 13:4753–59
120. Vance DE, Van Den Bosch H. 2000. Cholesterol in the year 2000. *Biochim. Biophys. Acta* 1529:1–8
121. Vik A, Rine J. 2001. Upc2p and Ecm22p, dual regulators of sterol biosynthesis in *Saccharomyces cerevisiae*. *Mol. Cell Biol*. 21:6395–405

Demonstrates that lanosterol, and not cholesterol, is the signal for Insig-dependent degradation of HMG-CoA reductase.

Demonstrates that Insig mediates the action of oxysterols on SREBP by regulating the conformation of Scap and access of COPII proteins to the MELADL ER exit signal.

122. Wang XD, Briggs MR, Hua XX, Yokoyama C, Goldstein JL, et al. 1993. Nuclear-protein that binds sterol regulatory element of low-density-lipoprotein receptor promoter.2. purification and characterization. *J. Biol. Chem*. 268:14497–504

Biochemical purification and characterization of SREBP from HeLa cells.

123. Wang XD, Sato R, Brown MS, Hua XX, Goldstein JL. 1994. Srebp-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. *Cell* 77:53–62
124. Wang XD, Zelenski NG, Yang JX, Sakai J, Brown MS, et al. 1996. Cleavage of sterol regulatory element binding proteins (SREBPs) by CPP32 during apoptosis. *EMBO J.* 15:1012–20
125. Wilcox LJ, Balderes DA, Wharton B, Tinkelenberg AH, Rao G, et al. 2002. Transcriptional profiling identifies two members of the ATP-binding cassette transporter superfamily required for sterol uptake in yeast. *J. Biol. Chem*. 277:32466–72
126. Xu F, Rychnovsky SD, Belani JD, Hobbs HH, Cohen JC, et al. 2005. Dual roles for cholesterol in mammalian cells. *Proc. Natl. Acad. Sci. USA* 102:14551–56
127. Yabe D, Brown MS, Goldstein JL. 2002. Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. *Proc. Natl. Acad. Sci. USA* 99:12753–58
128. Yabe D, Komuro R, Liang GS, Goldstein JL, Brown MS. 2003. Liver-specific mRNA for Insig-2 down-regulated by insulin: implications for fatty acid synthesis. *Proc. Natl. Acad. Sci. USA* 100:3155–60
129. Yabe D, Xia ZP, Adams CM, Rawson RB. 2002. Three mutations in sterol-sensing domain of SCAP block interaction with insig and render SREBP cleavage insensitive to sterols. *Proc. Natl. Acad. Sci. USA* 99:16672–77
130. Yang F, Vought BW, Satterlee JS, Walker AK, Jim Sun ZY, et al. 2006. An ARC/Mediator subunit required for SREBP control of cholesterol and lipid homeostasis. *Nature* 442:700–4

131. Yang T, Espenshade PJ, Wright ME, Yabe D, Gong Y, et al. 2002. Crucial step in cholesterol homeostasis: Sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. *Cell* 110:489–500

Purification and isolation of Insig as a Scap binding protein that negatively regulates SREBP proteolysis.

132. Ye J, Rawson RB, Komuro R, Chen X, Dave UP, et al. 2000. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol. Cell* 6:1355–64
133. Ye Y, Shibata Y, Kikkert M, van Voorden S, Wiertz E, et al. 2005. Recruitment of the p97 ATPase and ubiquitin ligases to the site of retrotranslocation at the endoplasmic reticulum membrane. *Proc. Natl. Acad. Sci. USA* 102:14132–38
134. Zhang K, Shen X, Wu J, Sakaki K, Saunders T, et al. 2006. Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. *Cell* 124:587–99
135. Zhong X, Shen Y, Ballar P, Apostolou A, Agami R, et al. 2004. AAA ATPase p97/valosin-containing protein interacts with gp78, a ubiquitin ligase for endoplasmic reticulum-associated degradation. *J. Biol. Chem*. 279:45676–84

RELATED REVIEWS

Chang TY, Chang CCY, Ohgami N, Yamauchi Y. 2006. Cholesterol sensing, trafficking, and esterification. *Annu. Rev. Cell Dev. Biol.* 22:129–57

Kalaany NY, Mangelsdorf DJ. 2006. LXRS AND FXR: the yin and yang of cholesterol and fat metabolism. *Annu. Rev. Physiol.* 68:159–91

Wang DQ. 2007. Regulation of intestinal cholesterol absorption. *Annu. Rev. Physiol*. 69:221–48