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Protein Sensors for Membrane Sterols

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Cholesterol is an essential component of animal cell membranes, and its concentration is tightly controlled by a feedback system that operates at transcriptional and posttranscriptional levels. Here, we discuss recent advances that explain how cells employ an ensemble of membrane-embedded proteins to monitor sterol concentrations and adjust sterol synthesis and uptake.

Introduction

Among the known feedback control systems in animal cells, the cholesterol system is unique in that the regulated end-product—cholesterol—is sequestered entirely within cell membranes. To monitor levels of membrane sterols, cells employ two membrane-embedded proteins of the endoplasmic reticulum (ER)—Scap and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase). These proteins share a polytopic intramembrane sequence called the sterol-sensing domain. Through this domain, sterols cause Scap and HMG CoA reductase to bind to Insigs, newly characterized membrane proteins of the ER. Upon binding Scap and reductase, Insigs exert actions that limit cholesterol synthesis. By controlling Scap and reductase, Insigs stand at the crossroads between the transcriptional and posttranscriptional regulatory mechanisms that assure cholesterol homeostasis.

Figure 1 illustrates the domains of the membrane proteins discussed in this review. One of these proteins, Scap, is an escort protein for sterol regulatory element binding proteins (SREBPs), membrane bound transcription factors that activate all of the genes required to produce cholesterol (Brown and Goldstein, 1997; Horton et al., 2002). Scap binds to SREBPs and escorts them from the ER to the Golgi apparatus where the SREBPs are proteolytically processed to yield active fragments that enter the nucleus (Brown and Goldstein, 1999). This processing is mediated by the action of two Golgi-associated membrane bound proteases: Site-1 protease, a serine protease of the subtilisin family (Espenshade et al., 1999) and Site-2 protease, a Zn^{2+} metalloprotease (Zelenski et al., 1999). When cholesterol builds up in ER membranes, the Scap/SREBP complex fails to exit the ER, proteolytic processing of SREBPs is abolished, and transcription of target genes declines (Goldstein et al., 2002).

HMG CoA reductase, the rate-controlling enzyme in cholesterol biosynthesis, is also embedded in ER membranes (Figure 1). When certain sterols accumulate, the enzyme is rapidly degraded, thereby helping to terminate sterol synthesis (Gil et al., 1985; Skalnik et al., 1988). The effects of sterols on Scap and HMG CoA reductase are mediated through their respective sterol-sensing domains (denoted by SSD in Figure 1; Hua et al., 1996a; Yang et al., 2000; Sever et al., 2003b). In both cases, sterols cause these domains to bind to Insigs (Yang et al., 2002; Sever et al., 2003b). Insig binding has dramatically different consequences for Scap and HMG CoA reductase. Upon binding Insig, Scap is retained in the ER, whereas the reductase is ubiquitinated and degraded.

Role of Insig in Regulating Scap Transport

The manner in which Insigs regulate Scap transport is illustrated in Figure 2. Mammalian cells produce three closely related isoforms of SREBP, called SREBP-1a, SREBP-1c,

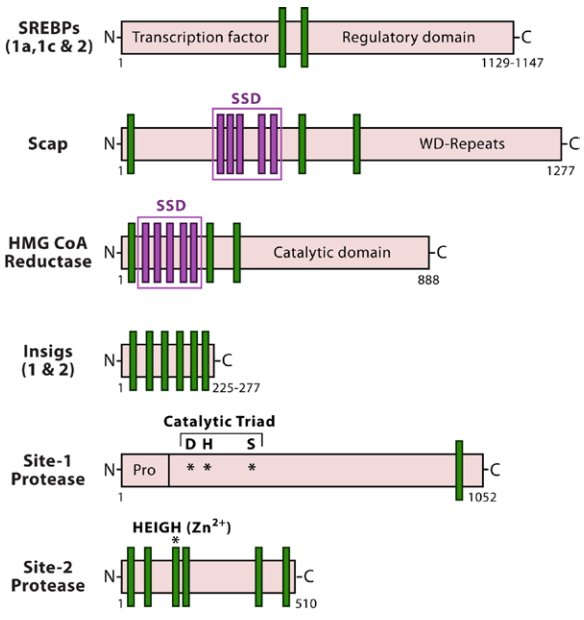

Figure 1. Domain Structures of Membrane Proteins that Regulate Cholesterol

Amino acid numbers refer to human proteins. Vertical bars denote transmembrane domains identified by hydropathy plots and verified by topology mapping (see text for references). SSD denotes sterol-sensing domain. Pro in Site-1 protease denotes propeptide sequence. The catalytic triad in Site-1 protease is the hallmark of a serine protease. The HExxH motif in Site-2 protease is the hallmark of a Zn^{2+} metalloprotease.

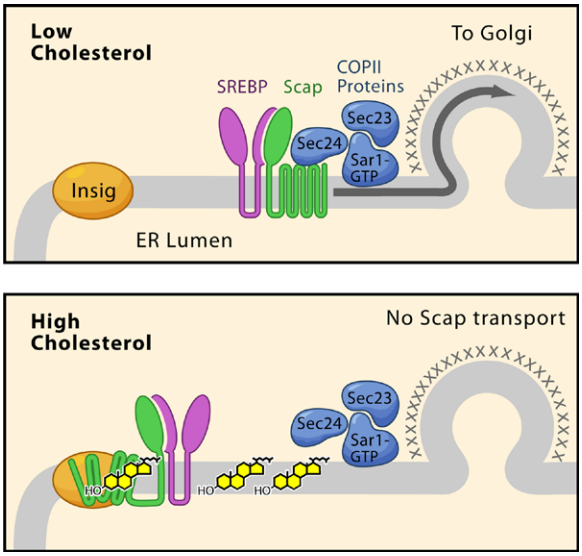

Figure 2. Insig-Mediated Regulation of Scap/SREBP Transport

(Top panel) When animal cells are deprived of sterols, Scap escorts SREBPs from the ER to Golgi by binding to Sec24, a component of the Sar1/Sec23/Sec24 complex of the COPII protein coat. Once in the Golgi, the SREBPs are proteolytically processed to generate their nuclear forms that activate genes for cholesterol synthesis and uptake.

(Bottom panel) Cholesterol negatively regulates ER-to-Golgi transport by binding to Scap, thereby changing its conformation and triggering the binding of Scap to Insig, an ER anchor protein. Insig prevents the binding of Scap to COPII proteins, thereby halting transport of SREBPs to the Golgi.

and SREBP-2 (Brown and Goldstein, 1997). SREBP-1a and -1c are produced from the same gene through the use of different promoters and alternative splicing. All three SREBPs are oriented in ER membranes in a hairpin fashion with both the NH_2-terminal transcription factor domains and the COOH-terminal regulatory domain facing the cytosol. These domains are separated by two membrane-spanning helices that flank a short loop that projects into the ER lumen (Figures 1 and 2). Immediately after their synthesis on ER membranes, the SREBPs bind to Scap through an interaction between the COOH-terminal regulatory domain of the SREBP and the cytosolically-oriented COOH-terminal WD-repeat domain of Scap (Sakai et al., 1997).

Scap is embedded in ER membranes through its NH_2-terminal domain, which is composed of eight transmembrane (TM) helices separated by hydrophilic loops (Nohturfft et al., 1998b; Figure 1). TM helices 2–6 comprise the sterol-sensing domain (Hua et al., 1996a; Nohturfft et al., 1998a). In sterol-depleted cells, the Scap/SREBP complex exits the ER in COPII-coated vesicles that bud from ER membranes (Nohturfft et al., 2000; Espenshade et al., 2002; Sun et al., 2005). Scap mediates this exit by binding to COPII coat proteins through the general mechanism defined for yeast and mammalian membrane proteins that move from ER to Golgi (Antonny and Schekman, 2001; Aridor et al., 1998; Barlowe, 2002). COPII binding is triggered by Sar1, a small GTP binding protein that attaches to ER membranes upon exchanging GTP for GDP (Figure 2). Membrane bound Sar1 then attracts Sec23/24, a cytosolic heterodimer. Upon binding to membranes, the Sec24 component binds to cargo proteins (such as Scap), and the Sec23 component attracts other proteins to form the coat of the budding vesicle. In the case of Scap, Sec24 binds to a hexapeptide sequence, MELADL, located in the cytoplasmic loop between TM helices 6 and 7 of Scap. Mutations within this sequence abolish Sec23/24 binding to Scap and prevent the Scap/SREBP complex from exiting the ER (Sun et al., 2005).

When cholesterol builds up in ER membranes, the sterol binds to Scap, and this triggers a conformational change that causes Scap to bind to Insig. When Scap binds to Insig, the Sar1/Sec23/24 complex can no longer bind to Scap, and therefore Scap remains in the ER along with its attached SREBP (Sun et al., 2005). Saturable and specific binding of cholesterol to Scap has been studied in vitro using the purified recombinant membrane domain of Scap (TM helices 1–8) and [^{3}H]cholesterol, both of which are added to the assay tube in detergent micelles (Radhakrishnan et al., 2004). In these assays, Scap behaves like a standard receptor for cholesterol, although the kinetics of the binding reaction are unusual in that both the ligand and the receptor must be added in separate detergent micelles. In this in vitro reaction, the rate-limiting step is the transfer of the [^{3}H]cholesterol from the donor micelles to the acceptor micelles that contain Scap.

The cholesterol-triggered conformational change in Scap has been monitored in membranes isolated from cholesterol-treated cells and in membranes from sterol-depleted cells that were incubated with cholesterol in vitro (Brown et al., 2002; Adams et al., 2003, 2004). In these experiments, the conformation of Scap is measured by exposing sealed membrane vesicles to an impermeant protease such as trypsin. When cholesterol has been added to Scap-containing membranes, the tryptic digestion pattern of Scap is altered. A new protease fragment is generated through cleavage at arginine-505, which is in the cytosolic loop between TM helices 6 and 7 (Brown et al., 2002). In membranes from sterol-depleted cells, this arginine is protected from tryptic cleavage, perhaps because it is close to the membrane surface. When cholesterol is added to intact cells or to sterol-depleted membranes in vitro, arginine-505 becomes exposed, possibly because it is pushed away from the membrane. Interestingly, arginine-505 is located in the same cytoplasmic loop as the MELADL sequence that binds to COPII proteins. Arginine-505 is at the COOH-terminal end and MELADL is at the NH_2-terminal end of this 77 amino acid loop (Sun et al., 2005). Conformational changes within this loop may be crucial in the regulation of Scap transport.

In its cholesterol bound conformation, Scap binds to Insig proteins (Adams et al., 2003; Adams et al., 2004). The role of Insigs in Scap transport was first disclosed through biochemical studies in cultured cells. In early experiments, we noted that overexpression of Scap by transfection led to constitutive ER-to-Golgi transport that could no longer

be prevented by sterols (Hua et al., 1996b). We reasoned that the overexpressed Scap had saturated an ER protein that was responsible for holding Scap back in the ER and that the excess Scap was no longer hindered in moving to the Golgi. The same effect could be obtained by simply expressing part of the membrane domain of Scap (TM helices 1–6), which contains the sterol-sensing domain (Yang et al., 2000). To isolate the putative ER retention protein, we created permanent lines of human kidney 293 cells that overexpressed either the TM1–6 fragment of Scap or, as a control, the TM1–5 fragment, which did not liberate Scap from the ER in the presence of sterols (Yang et al., 2000). The cells were incubated with sterols, membranes were isolated, and affinity chromatography techniques were used to purify the Scap TM fragments and any associated proteins. The latter were identified by high-sensitivity mass spectroscopy. A protein that copurified with Scap(TM1–6), but not with Scap(TM1–5), was identified as one of two isoforms of Insig, which we designated Insig-1 (Yang et al., 2002). When we subsequently found in transfection experiments that Insig-1 coprecipitated with Scap(TM1–6), but not Scap(TM 1–5), and observed that coprecipitation of full length Scap and Insig-1 occurred only in sterol-enriched membranes, we were encouraged to believe that Insig-1 binding was a physiologically relevant event. This conclusion was confirmed through studies of Scaps with point mutations in the sterol-sensing domain that abolish regulation by sterols.

Our laboratory identified three sterol-resistant Scaps (D443N, Y298C, and L315F) in unbiased screens designed to isolate mutant cells that fail to turn off SREBP processing in the presence of cholesterol or other sterols (Hua et al., 1996a; Nohturfft et al., 1998a; Yabe et al., 2002b). After the discovery that Insig-1 bound Scap, we showed that each of these mutant Scaps had lost the ability to bind to Insigs and that this binding defect rendered the cells resistant to the effect of cholesterol in blocking SREBP processing (Yang et al., 2002; Yabe et al., 2002b; Adams et al., 2003). The requirement for the Scap/Insig interaction was confirmed in a stringent fashion through studies of SRD-15 cells, a line of mutant Chinese hamster ovary (CHO) cells that lacks all Insig proteins (Lee et al., 2005). In these cells, cholesterol cannot block Scap exit from the ER. Regulation is restored by transfecting cells with cDNAs encoding either of the two Insig isoforms, Insig-1 or Insig-2.

Insig-1 and Insig-2: Similarities and Differences

The Insig-1 mRNA was originally identified in 1991 as a transcript (designated CL-6) that increased in regenerating rat liver and in cultured rat H35 hepatoma cells after treatment with insulin (Mohn et al., 1991). Later, CL-6 was renamed Insig1 to denote "insulin-induced gene" (Peng et al., 1997). Our laboratory rediscovered the Insig-1 mRNA as a target of SREBP action in a differential expression screen of cultured cells (Luong, 2000). In reinvestigating the insulin induction of Insig-1 mRNA, we found that the insulin effect was indirect, resulting from insulin's potent stimulation of *SREBP-1c* gene transcription, which in turn led to a secondary activation of *Insig-1* transcription (discussed below). Insig-2 was discovered in 2002 through bioinformatics as a closely related isoform that resembled Insig-1 in its Scap binding function but differed in its pattern of regulation (Yabe et al., 2002a).

Human Insig-1 and Insig-2 contain 277 and 225 amino acids, respectively (Figure 1). Both Insigs are extremely hydrophobic. Topology studies suggest that most of the protein consists of six transmembrane helices separated by short hydrophilic loops (Feramisco et al., 2004). Only short sequences at the NH_2 and COOH termini project into the cytoplasm. These sequences contain the major differences between the two Insigs. The NH_2-terminal sequence of Insig-2 is 50 residues shorter than the sequence in Insig-1. The membranous regions of the two proteins (corresponding to amino acids 84–269 of Insig-1) are 85% identical. Both Insigs bind to cholesterol-loaded Scap, and both retain the Scap/SREBP complex in the ER (Yabe et al., 2002a; Adams et al., 2003).

Insigs appear to enhance the response to cholesterol by supporting cholesterol binding to Scap. Overexpressing Insig-1 or Insig-2 lowers the threshold for producing the conformational changes in Scap that are detectable by trypsin digestion of membrane preparations (Adams et al., 2003). The most likely interpretation is that cholesterol dissociates readily from Scap, and dissociation is retarded when cholesterol-loaded Scap binds to Insigs, thereby locking cholesterol into the Insig/Scap/SREBP complex. Further insights into this process may emerge when the in vitro cholesterol binding assay can be performed using purified recombinant Scap with or without purified Insigs. This is technically challenging because it requires reconstituting the Scap/Insig interaction in detergent micelles rather than in membrane bilayers.

So far, the major differences between Insig-1 and Insig-2 relate to the regulation of their expression (Yabe et al., 2002a). Insig-1 is an obligatory SREBP target gene (Luong, 2000; Janowski, 2002; Horton et al., 2002). Thus, the Insig-1 mRNA is produced only when cleaved SREBP is present in the nucleus, and transcription falls dramatically when SREBP processing is blocked. In contrast, Insig-2 is expressed at a low but constitutive level, at least in cultured cells. It is not regulated by SREBPs (Yabe et al., 2002a).

Inasmuch as *Insig-1* transcription is dependent upon SREBPs, Insig-1 mRNA levels are regulated by cholesterol. Thus, Insig-1 mRNA rises when cells are sterol depleted, and it falls when cholesterol accumulates. Remarkably, the level of Insig-1 protein varies oppositely to the level of its mRNA, owing to sterol-regulated degradation. When cells are deprived of sterols, the Insig-1 protein is rapidly ubiquitinated and degraded by proteasomes with a half-life of less than 30 min (Gong et al., 2006). When cholesterol is present, cholesterol-loaded Scap binds to Insig-1, thereby stabilizing Insig-1 and preventing its ubiquitination. Thus, in sterol-overloaded cells, Insig-1 has a relatively long half-life (>2 hr). This stabilization is totally dependent upon Scap. In Scap-deficient cells, cholesterol fails to stabilize Insig-1, and stability is restored by transfection of a cDNA encod-

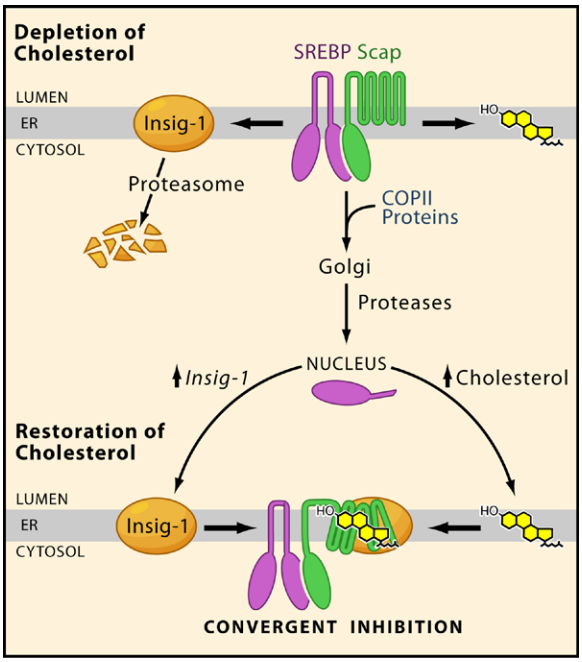

Figure 3. Model for Convergent Feedback Inhibition of Cholesterol Synthesis and Uptake

The essential feature of the model is that SREBP processing will not be blocked by sterols unless two SREBP-induced products converge on Scap simultaneously: (1) newly synthesized Insig-1, and (2) newly synthesized or acquired cholesterol. This convergence assures that SREBP processing will not be terminated before the needs of the cell for cholesterol and nonsterol isoprenoids are met (Gong et al., 2006).

ing wild-type Scap, but not any of the three mutant Scaps that cannot bind Insigs. In contrast to Insig-1, Insig-2 has a much longer half-life (Lee and Ye, 2004) that is not regulated by sterols (Gong et al., 2006).

Convergent Feedback Inhibition

The regulation of Insig-1 at two levels (gene transcription and protein stability) sets the stage for a process that we have called "convergent feedback inhibition" (Figure 3). When cells have abundant cholesterol, Scap binds to Insig-1, stabilizing the protein and leading to an accumulation of a stable Insig-1/Scap/SREBP complex. The nuclear content of SREBP falls and thus Insig-1 mRNA levels decline. The stable Insig-1/Scap/SREBP complex serves as a reservoir for SREBP. When cells are acutely deprived of sterols, Scap/SREBP dissociates from Insig-1, whereupon the latter is ubiquitinated and degraded in proteasomes (Gong et al., 2006). The liberated Scap/SREBP complex binds COPII proteins and moves to the Golgi complex where the SREBP is processed to its nuclear form. The nuclear SREBP turns on the genes for cholesterol biosynthesis and uptake through low-density lipoprotein (LDL) receptors. At the same time, the nuclear SREBP turns on the gene for Insig-1. This increases the amount of Insig-1 synthesis, but the protein will be rapidly degraded unless sufficient cholesterol has accumulated to bind to Scap, allowing Scap

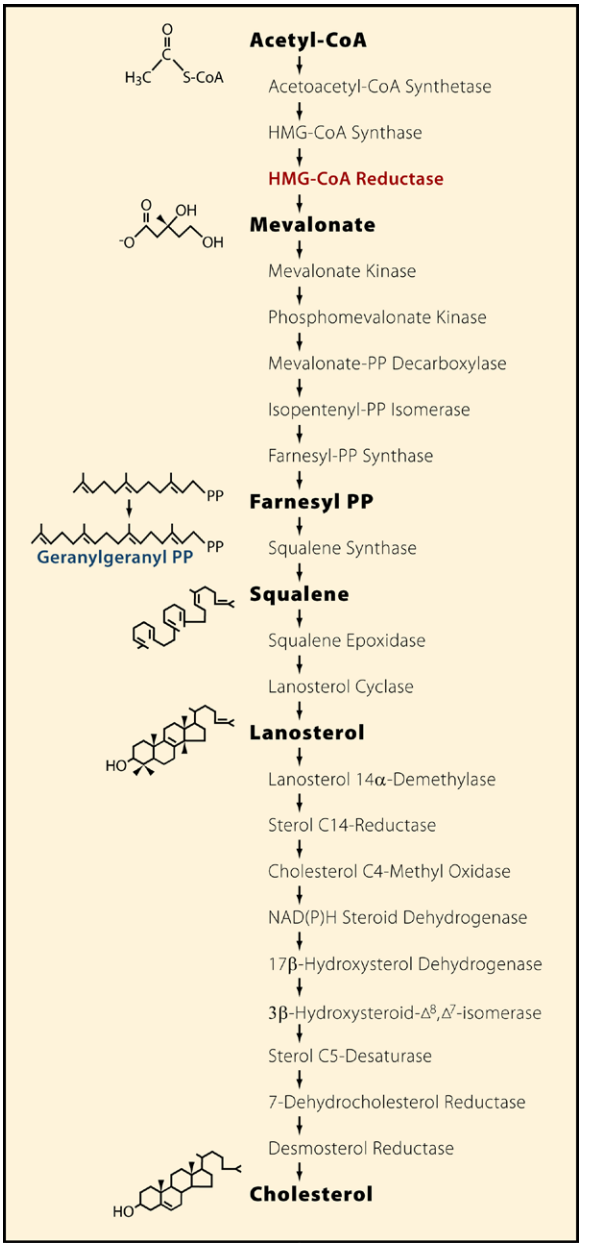

Figure 4. Mevalonate Pathway in Mammalian Cells

Acetyl-CoA is converted to cholesterol by at least 20 enzymes, all of whose genes are regulated by the SREBP pathway. Four key intermediates (mevalonate, farnesyl pyrophosphate, squalene, lanosterol) are highlighted in black boxes. Geranylgeranyl pyrophosphate, highlighted in the blue box, is a nonsterol isoprenoid derived from farnesyl pyrophosphate. Other nonsterol isoprenoids derived from farnesyl pyrophosphate (dolichol, heme A, ubiquinone) and from isopentyl pyrophosphate (isopentyl group of tRNAs) are not shown.

to stabilize Insig-1. Blocking of SREBP processing therefore requires the convergence of two molecules—newly supplied cholesterol (either from synthesis or uptake) and newly synthesized Insig-1 (Gong et al., 2006).

The reason for this convergence is not yet fully apparent.

It likely relates to the fact that SREBPs regulate the mevalonate pathway, which produces vital isoprenoid end products in addition to cholesterol (Goldstein and Brown, 1990; Figure 4). If cholesterol alone could block SREBP processing, and if cholesterol accumulated rapidly, the mevalonate pathway might be blocked before the other isoprenoids have accumulated to sufficient levels. The requirement for concurrent Insig-1 synthesis might be a failsafe mechanism that confirms that SREBP has entered the nucleus and turned on all of the genes of the mevalonate pathway.

Insigs Mediate Sterol-Triggered Degradation of HMG CoA Reductase

HMG CoA reductase is the first committed enzyme in the mevalonate pathway (Figure 4). As such, it is subjected to an extraordinary degree of regulation that maintains a steady flow of substrates for synthesis of nonsterol isoprenoids while preventing overproduction of cholesterol and potentially toxic cholesterol precursors (Goldstein and Brown, 1990). At the transcriptional level, the reductase is controlled by SREBPs, and gene transcription is thus suppressed by cholesterol through the sterol-regulated transport mechanism described above. At the posttranscriptional level, the reductase is regulated by sterol-accelerated degradation. Remarkably, both processes are mediated by Insigs (Sever et al., 2003b).

HMG CoA reductase is bound to ER membranes through its NH_2-terminal domain that contains eight TM helices (Roitelman et al., 1992; Figure 1). TM helices 2–6 comprise the sterol-sensing domain (Hua et al., 1996a; Nohturfft et al., 1998a; Sever et al., 2003a). The COOH-terminal domain of the reductase projects into the cytosol and contains the entire catalytic activity (Liscum et al., 1985). It is attached to the membrane domain by a proline-rich sequence that is predicted to be flexible and is readily proteolyzed in vitro upon cell homogenization. The substrate and product of the enzyme are both water soluble, and the reason for membrane attachment is purely for regulation (Gil et al., 1985).

In sterol-deprived cells, HMG CoA reductase is degraded slowly with a half-life exceeding 12 hr. When sterols and other isoprenoids accumulate, the enzyme is degraded rapidly with a half-life of less than 1 hr. Degradation is mediated entirely by the membrane-attachment domain (Gil et al., 1985). Indeed, expression of the truncated soluble catalytic domain of the reductase fully restores cholesterol synthesis to reductase-deficient mutant CHO cells, but the soluble enzyme has a long half-life that is no longer accelerated by sterols (Gil et al., 1985). In contrast, the membrane domain undergoes sterol-accelerated degradation, even when the catalytic domain is deleted (Sever et al., 2004).

The mechanism for sterol-regulated HMG CoA reductase degradation has been elucidated recently through studies of wild-type and mutant CHO cells. Remarkably, cholesterol is only a weak inducer of this degradation. The cholesterol precursor, lanosterol, is much more potent (Song et al., 2005a; Figure 4). A potential reason for this specificity

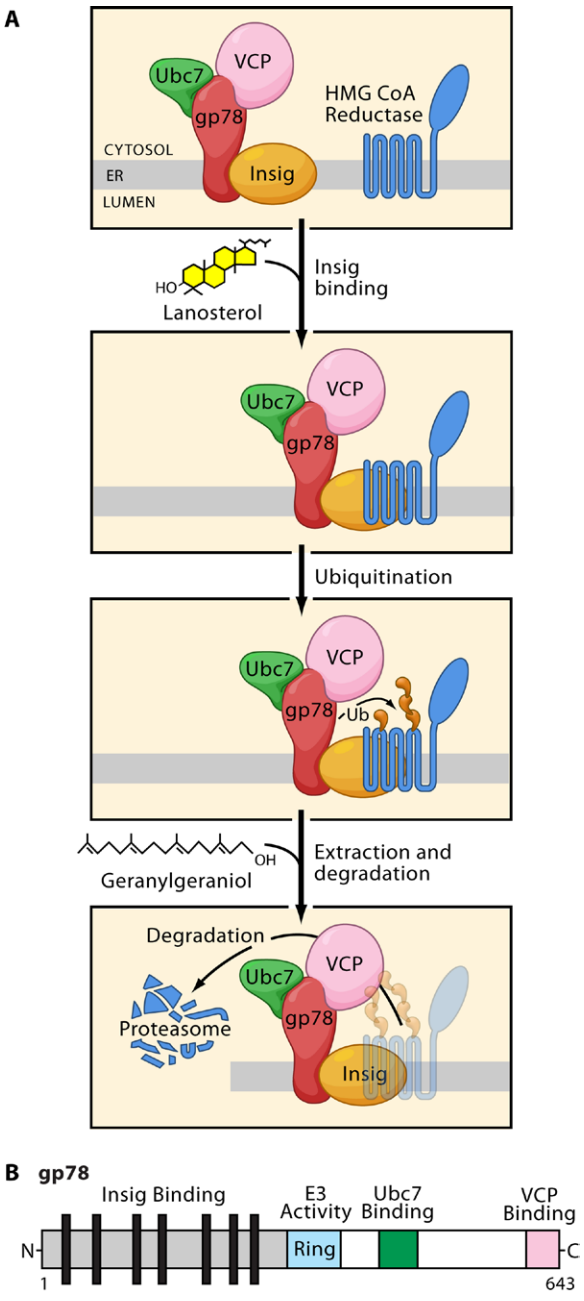

Figure 5. Regulated Degradation of HMG CoA Reductase in Animal Cells

(A) The first step occurs when lanosterol triggers the binding of HMG CoA reductase to Insig-1 or Insig-2. Insig exists in a complex with a membrane-embedded E3 ubiquitin ligase, gp78. In turn, gp78 is bound to the E2 ubiquitin-conjugating enzyme Ubc7 and to VCP, an ATPase that plays a role in extracting ubiquitinated proteins from membranes. In the second step, the E3 activity of gp78 ubiquitinates the reductase. In the third step, the ubiquitinated HMG CoA reductase is extracted from the membrane by VCP and delivered to the proteasome for degradation. This postubiquitination step is stimulated by geranylgeraniol through an undefined mechanism that likely involves a geranylgeranylated protein, such as one of the Rab proteins.

(B) Domain structure of gp78. The location of transmembrane helices is predicted from hydropathy plots, but this topology has not been verified biochemically (Shimizu et al., 1999; Fang et al., 2001; Zhong et al., 2004).

is discussed in the next section. Accelerated degradation begins when lanosterol accumulates in ER membranes (Figure 5). This accumulation induces the reductase to bind to Insig-1, as demonstrated by coimmunoprecipitation (R.D.-B., unpublished data). By analogy to Scap regulation, it seems likely that lanosterol binds directly to the sterol-sensing domain of HMG CoA reductase. However, so far it has been impossible to demonstrate this binding in vitro using the direct binding assay that was used to measure cholesterol binding to Scap. Moreover, we have not been able to detect a change in the tryptic cleavage pattern of the reductase upon lanosterol addition that is analogous to the change detected with Scap. Therefore, it remains possible that lanosterol does not bind to HMG CoA reductase directly, but rather to some other protein that induces the reductase to bind to Insigs.

HMG CoA reductase binding to Insigs requires the sequence YIYF in its sterol-sensing domain (Sever et al., 2003a). Remarkably, the first tyrosine of this sequence is equivalent to tyrosine 298 of Scap, which is required for Scap binding to Insig (Nohturfft et al., 1998a; Adams et al., 2003). Indeed, transfection-mediated overexpression of Scap's sterol-sensing domain competitively inhibits the Insig-mediated, sterol-accelerated degradation of the reductase, suggesting that the sterol-sensing domains from Scap and the reductase bind to the same sites on Insigs (Sever et al., 2003a). Like Scap, HMG CoA reductase binds to both Insig-1 and Insig-2 (Sever et al., 2003a).

How does Insig binding lead to degradation of HMG CoA reductase in animal cells? An early clue came from the observation that inhibitors of the proteasome block sterol-stimulated degradation of the reductase, leading to the accumulation of ubiquitinated reductase within cells (Ravid et al., 2000). This ubiquitination was subsequently shown to be absolutely dependent on the action of either Insig-1 or Insig-2 (Sever et al., 2003a). Ubiquitination of the reductase occurs on cytosolically exposed lysines 89 and 248, which lie immediately adjacent to transmembrane helices 3 and 7 (Figure 1). There is no evidence for ubiquitination of the catalytic domain of the reductase. Indeed, as mentioned above, the catalytic domain is not required for sterol-regulated degradation.

Coimmunoprecipitation experiments revealed that a fraction of Insig-1 molecules are bound constitutively to a protein named gp78 (Song et al., 2005b). As illustrated in Figure 5B, gp78 is a 643 amino acid protein with four identifiable domains (Shimizu et al., 1999; Fang et al., 2001): (1) an NH_2-terminal membrane attachment region of 298 amino acids that binds Insigs; (2) a 43-amino acid region that includes a RING finger consensus sequence conferring E3 ubiquitin ligase activity; (3) a 42 amino acid region homologous to Cue1p, an ER membrane protein in yeast that serves as a membrane anchor for Ubc7p, a cytosolic ubiquitin-carrying E2 protein (Ponting, 2000); and (4) a 48 amino acid region that mediates the interaction of gp78 with VCP (Valosin-containing protein, also known as p97), an ATPase implicated in the postubiquitination steps of ER-associated protein degradation (ERAD; Zhong et al., 2004; Ye et al., 2005). ERAD is the process by which denatured ER proteins are retrotranslocated across the ER membrane for degradation by cytosolic proteasomes (Meusser et al., 2005).

Addition of lanosterol to intact cells triggers the binding of HMG CoA reductase to the Insig-1/gp78 complex (Figure 5A). Several lines of evidence indicate that this sterol-triggered reductase/Insig-1/gp78 complex is essential for the ubiquitination and subsequent degradation of the reductase. First, reduction of gp78 levels by RNA interference (RNAi) abrogated sterol-accelerated reductase ubiquitination and degradation (Song et al., 2005b). Second, a truncated gp78 containing only its membrane domain and lacking its RING finger domain bound to Insig-1, displaced full-length gp78, and acted as a dominant-negative inhibitor with respect to sterol-accelerated degradation of the reductase (Song et al., 2005b). These data indicate that the Insig-1/gp78 interaction occurs though the binding of the TM domains of the two proteins.

In addition to functioning as an E3 ubiquitin ligase, gp78 also performs two other functions in HMG CoA reductase degradation. The Cue1p-like domain of mammalian gp78 is known to bind Ubc7, an E2-conjugating enzyme (Fang et al., 2001). Preliminary evidence in the reductase system indicates that Ubc7 is indeed the E2 that supplies activated ubiquitin to the RING finger domain of gp78 (B.-L. Song and R.D.-B., unpublished data). The second function of gp78 is to link ubiquitination to degradation through its association with VCP, the ATPase that is implicated in ERAD. Indeed, VCP was recovered in the sterol-induced HMG CoA reductase/Insig-1/gp78 immunoprecipitate. Moreover, reduction of VCP levels with RNAi prevented the acceleration of reductase degradation in the presence of lanosterol (Song et al., 2005b).

Considered together, the current data on HMG CoA reductase degradation lead to the model shown in Figure 5A. A fraction of membrane bound Insig-1 molecules are pre associated with gp78 and its attached Ubc7 and VCP molecules. Addition of lanosterol causes HMG CoA reductase to bind to this complex, whereupon the RING finger domain of gp78 transfers ubiquitin from Ubc7 to lysines 89 and 248 of reductase (Sever et al., 2003a). Once sufficient ubiquitins have been transferred, VCP somehow extracts reductase from the ER membrane and delivers it to proteasomes for degradation (Song et al., 2005b).

The complete extraction and degradation of ubiquitinated HMG CoA reductase requires a nonsterol isoprenoid derived from mevalonate (Goldstein and Brown, 1990; Sever et al., 2003a). Thus, if the mevalonate pathway is blocked by the reductase inhibitor compactin, lanosterol can still induce the binding of reductase to Insig-1 and the subsequent ubiquitination. However, the ubiquitinated reductase is not rapidly extracted from the ER and degraded unless the cells are also supplied with mevalonate, the product of the reductase reaction. The mevalonate requirement can be bypassed by supplying the cells with geranylgeraniol (GG-OH), a 20 carbon isoprenoid, but not with 15 carbon farnesol (Sever et al., 2003a). It

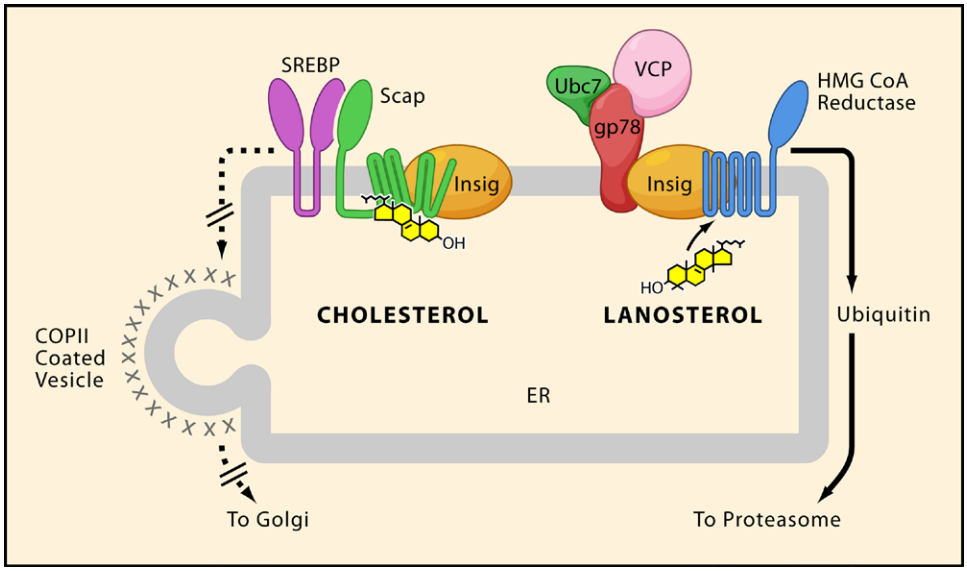

Figure 6. Two Actions of Insigs in Cholesterol Homeostasis
Insigs regulate ER-to-Golgi transport of Scap/SREBP in a process that is inhibited by cholesterol. Insigs also regulate the ubiquitin-mediated proteasomal degradation of HMG CoA reductase in a process that is stimulated by lanosterol.

is likely that GG-OH is converted to GG-pyrophosphate (GG-PP). The major product of GG-PP is the GG group that is attached covalently to selected cellular proteins. Such geranylgeranylated proteins include Rab proteins, which are known to participate in vesicular transport (Seabra et al., 2002).

An unsolved puzzle is the mechanism by which the eight membrane-spanning helices of HMG CoA reductase are extracted from the membrane and degraded in proteasomes without ever releasing the catalytic domain into the cytosol. All of the ubiquitination occurs in the membranous domain of the reductase (Sever et al., 2003a), and the proteasomes presumably degrade this domain first. If an interior clip were made in the protease-susceptible linker between the membrane domain and the catalytic domain, the long-lived catalytic domain would be released into the cytosol, defeating the purpose of sterol-accelerated degradation. The problem is compounded by the observation that the cytosolic domain of the reductase forms a tight tetramer, as determined by X-ray crystallography (Istvan et al., 2000). How does the proteasome unwind this tetramer? Are multiple proteasomes involved? Does the postulated requirement for a geranylgeranylated Rab protein indicate that a vesicular budding or fusion reaction is required? These questions may be answered when methods are developed to study the degradation of ubiquitinated reductase in vitro.

Insig Effects on Scap and HMG CoA Reductase: Parallels and Paradoxes

Figure 6 contrasts the effects of Insigs on Scap and HMG CoA reductase. In both cases, sterols trigger the binding of the respective sterol-sensing domains to Insigs, but that is where the similarity ends. The Scap/Insig interaction blocks the binding of COPII proteins to Scap, allowing Scap/SREBP to remain in the ER in a stable complex with Insigs. Far from inducing degradation, the Scap/Insig complex protects Insig-1 from ubiquitination and degradation. On the other hand, the reductase binds to a form of Insig-1 that exists in a complex with gp78 and VCP. Binding leads to ubiquitination and degradation of the reductase through a mechanism that appears similar to the ERAD pathway for degradation of denatured ER proteins.

Why does Insig-1 binding not lead to the degradation of Scap as it does for HMG CoA reductase? One possibility for the lack of ubiquitination and degradation of Scap is that it binds to a fraction of Insig-1 that is not attached to gp78/VCP. Another possibility is that binding of Scap displaces gp78/VCP from Insig-1. Alternatively, binding of Scap to the Insig-1/gp78/VCP complex may somehow lead to the inhibition of the attached gp78. Finally, when the reductase binds to Insig-1, the membrane domain may be partially unfolded in a manner that allows it to be recognized by gp78 as a denatured protein. On the other hand, Scap may remain in a tightly folded conformation that prevents it from being recognized as a gp78 substrate. Consistent with the latter hypothesis is the evidence that the reductase in yeast becomes unfolded when nonsterol metabolites bind to it, allowing the protein to be degraded through the standard ERAD mechanism (Shearer and Hampton, 2005). Each of these possibilities should be distinguishable with available tools of cell biology and protein biochemistry.

A significant difference in the regulation of Scap and HMG CoA reductase relates to sterol specificity (Figure 6). Scap transport is regulated by the end-product cho-

lesterol, and the biosynthetic intermediate, lanosterol, has no effect on this process. In contrast, degradation of the reductase is regulated preferentially by lanosterol. An analysis of the mevalonate pathway provides a rationalization (Figure 4). The rationalization assumes that cells cannot tolerate the accumulation of lanosterol, which is known to be toxic (Xu et al., 2005). SREBPs stimulate transcription of all of the genes in the pathway, including the enzymes after lanosterol as well as the enzymes prior to lanosterol (Horton et al., 2003). If lanosterol were to accumulate and if this blocked SREBP processing, there would be a reduction in the enzymes necessary to convert lanosterol to cholesterol. This might actually increase the lanosterol accumulation. It seems more advantageous, therefore, to allow lanosterol to specifically stimulate the degradation of the reductase, which would prevent additional lanosterol synthesis while permitting high levels of the lanosterol-metabolizing enzymes. On the other hand, if the end-product cholesterol accumulates, the processing of SREBPs is blocked, and this shuts down the whole pathway.

Oxysterol Regulation of Insig Binding to Scap and HMG CoA Reductase

In addition to cholesterol and lanosterol, the mevalonate pathway is regulated potently by sterols that contain additional polar groups that increase hydrophilicity. Small amounts of oxysterols with hydroxyls at the 24, 25, or 27 position are synthesized from cholesterol in various tissues (Russell, 2000). The hydroxyl groups render the sterols more water soluble than cholesterol, and hence they facilitate their excretion from cells. When added to cultured cells, 25-hydroxycholesterol causes both Scap (Adams et al., 2004) and HMG CoA reductase (Sever et al., 2003b) to bind to Insigs and thereby triggers all of the regulatory events. With regard to Scap, 25-hydroxycholesterol seems to act by an indirect mechanism. In contrast to cholesterol, 25-hydroxycholesterol does not bind to recombinant Scap in the in vitro binding assay (Radhakrishnan et al., 2004), nor does it trigger the trypsin-detectable conformational change in Scap (Adams et al., 2004). Moreover, a photoactivated derivative of cholesterol, but not 25-hydroxycholesterol, crosslinks to Scap when added to cells or to isolated membranes (Adams et al., 2004). We postulate, therefore, that a separate 25-hydroxycholesterol binding protein mediates the effect of 25-hydroxycholesterol on Scap. It is possible that this same postulated protein also mediates oxysterol effects on the reductase.

Several years ago our laboratory purified and cloned a cytosolic oxysterol binding protein (OSBP-1; Dawson et al., 1989) that translocates to Golgi membranes upon binding of 25-hydroxycholesterol (Ridgway et al., 1992). Attempts to demonstrate a role for this protein in Scap regulation have been unsuccessful. RNAi directed against OSBP-1 reduced the protein level by more than 80%, but it did not affect 25-hydroxycholesterol inhibition of SREBP processing. Overexpression of the predicted dominant-negative fragments of this protein also had no effect. More than 14 relatives of OSBP have been identified in the mammalian genome (Olkkonen, 2004), and it is possible that one of these relatives is a true regulator of Scap and/or HMG CoA reductase interaction with Insigs.

In the past, we have considered the possibility that oxysterols do not act on Scap either directly or indirectly, but rather they act by increasing the cholesterol content of ER membranes (Brown et al., 2002). Indeed, in early studies, we showed that addition of 25-hydroxycholesterol to cultured cells leads to an increase in the esterification of cholesterol by an ER enzyme, implying that cholesterol has accumulated in the ER (Brown et al., 1975). A similar conclusion was reached by Lange and Steck (1997). Recent data renders this mechanism less likely to account for the oxysterol effect on Scap and HMG CoA reductase. If 25-hydroxycholesterol were acting by increasing ER cholesterol, then we should have found an altered conformation of Scap as determined by the trypsin-cleavage assay. However, when 25-hydroxycholesterol was added to cells, no such change was observed, whereas a change was easily demonstrated when cholesterol was added (Brown et al., 2002; Adams et al., 2004). Moreover, when added to cells, the concentration of 25-hydroxycholesterol required to activate cholesterol esterification is orders of magnitude higher than that required to block SREBP processing or to accelerate reductase degradation. For this reason, we favor the idea of a specific (not-yet-identified) 25-hydroxycholesterol binding protein.

Reciprocal Regulation of Insig-1 and -2 in Liver

In mammals, the liver is the organ most active in lipid synthesis, and here SREBPs play special roles. In addition to cholesterol, the liver synthesizes large amounts of fatty acids, not only for its own membranes, but also for export to other tissues in lipoproteins. SREBPs control both processes (Horton et al., 2002). The two prominent isoforms of SREBP in liver, SREBP-2 and SREBP-1c, have divergent but partially overlapping functions. SREBP-2 is primarily involved in stimulating cholesterol synthesis, whereas SREBP-1c primarily stimulates fatty acid synthesis. In achieving the latter effect, SREBP-1c increases transcription of all of the genes necessary to convert acetyl CoA to long-chain unsaturated fatty acids. In addition, SREBP-1c activates genes encoding the three enzymes that produce NADPH, which is required for synthesis of cholesterol as well as fatty acids, and the enzymes necessary to incorporate fatty acids into triglycerides and phospholipids (Horton et al., 2002).

Fatty acid synthesis in liver is controlled by two pancreatic hormones: insulin (which stimulates) and glucagon (which inhibits) (McGarry, 1998). The transcriptional effects of both hormones are mediated by changes in the amount of SREBP-1c. Insulin stimulates transcription of the *SREBP-1c* gene (Kim et al., 1998; Horton et al., 1998; Shimomura et al., 1999; Foufelle and Ferre, 2002), and glucagon blocks this effect (Shimomura et al., 2000). Indirect evidence suggests that insulin may also increase the processing of the SREBP-1c precursor to the mature form (Hegarty et al.,

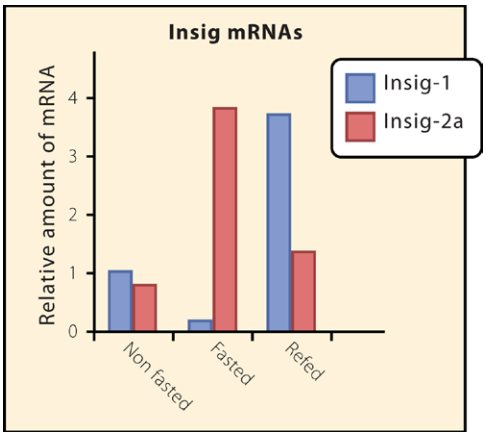

Figure 7. Reciprocal Changes in Expression of Insig-1 and Insig-2 in Livers of Mice Subjected to Fasting and Refeeding
The relative levels of Insig-1 and Insig-2a mRNA are shown from real-time PCR quantification (replotted from Figure 5 in Engelking et al. [2004]). The levels of Insig-1 and Insig-2a are similar in nonfasted mice. When mice are fasted, the expression of Insig-2a goes up dramatically, whereas expression of Insig-1 is decreased. Likewise, upon refeeding following a 12 hr fast, Insig-1 expression is greatly increased compared to the expression of Insig-2a. Similar trends were observed for Insig protein expression.

2005). In liver, transcription of the *SREBP-1c* gene is also activated by nuclear receptors known as Liver X Receptors (LXRs) (Repa et al., 2000; Chen et al., 2004). In cultured cells, LXRs are potently activated by three oxysterols: 24(*S*),25-epoxycholesterol, 22(*R*)-hydroxycholesterol, and 27-hydroxycholesterol (Repa and Mangelsdorf, 2000). Two of these oxysterols (24(*S*),25-epoxycholesterol and 27-hydroxycholesterol) also block SREBP processing. On the other hand, the most potent inhibitor of SREBP processing, 25-hydroxycholesterol, is a relatively weak activator of LXRs. By increasing SREBP-1c levels in situations of sterol overload, the LXRs may assure a sufficient supply of fatty acids to allow storage of excess cholesterol as cholesteryl ester (Repa et al., 2000).

In keeping with its regulatory role in lipid synthesis, insulin has profound effects on Insig isoforms in the liver. The liver produces an organ-specific isoform of Insig-2, designated Insig-2a (Yabe et al., 2003). The Insig-2a transcript differs from the ubiquitous transcript (Insig-2b) because it arises from an upstream promoter that produces a different 5′ noncoding exon. This exon splices into the same coding exon that initiates Insig-2b. Thus, the Insig-2a and Insig-2b transcripts encode the same protein, but they differ in their pattern of regulation. *Insig-2b* is expressed at an extremely low level in liver. *Insig-2a* is transcribed at high levels only in liver, and only when insulin levels are low. The Insig-2a transcript is strongly repressed by insulin (Yabe et al., 2003).

The insulin-mediated regulation of Insigs in liver sets the stage for reciprocal regulation of Insig-1 and Insig-2 under conditions of fasting and feeding (Engelking et al., 2004). In fasted mice, insulin levels are low. As a result, the *SREBP-1c* gene is not actively transcribed, nuclear SREBP-1c levels are low, and Insig-1 mRNA and protein levels are low. In contrast, Insig-2 levels are high, owing to the Insig-2a transcript. Upon feeding, insulin levels rise. This leads to a rapid reduction in Insig-2 mRNA and protein levels, owing to insulin-mediated repression of *Insig-2a* transcription. At the same time, insulin induces *SREBP-1c* transcription, nuclear SREBP-1c activates the *Insig-1* gene, and Insig-1 mRNA and protein levels rise to higher levels than in the basal nonfasting state. The net result is a replacement of Insig-2 by Insig-1. The striking reciprocal relation between Insig-1 and Insig-2 is illustrated in Figure 7.

In the fed state under the influence of insulin, SREBP-1c is actively processed to its nuclear form in order to convert excess carbohydrate to fatty acids. Under these conditions, the purpose of fatty acid synthesis is energy storage rather than membrane maintenance, and it seems likely that the liver has specific mechanisms to override the SREBP feedback control mechanism. The Insig-2 to Insig-1 switch may play a role in this override system.

To begin exploring these possibilities, knockout mice have been created in which the *Insig-1* and *Insig-2* genes are disrupted in liver through recombination (Engelking et al., 2005). Simultaneous germline disruption of both Insig genes was embryonically lethal. Therefore, the *Insig-2* gene was disrupted in the germline, and the *Insig-1* gene was disrupted specifically in liver through an inducible Cre recombinase in adult animals. The resultant mice (designated *L-Insig-1*$^{-/-}$;*Insig-2*$^{-/-}$) appeared grossly normal. However, their livers were enlarged and full of cholesterol and triglycerides. Despite this lipid accumulation, the livers continued to synthesize fatty acids and cholesterol, owing to inappropriately high levels of mRNAs encoded by SREBP target genes and a dramatic increase (>100-fold) in HMG CoA reductase protein. The disproportionate increase in reductase protein is likely caused by slowed degradation in the absence of Insigs. Feeding of dietary cholesterol failed to reduce nuclear SREBPs and reductase protein and to suppress cholesterol synthesis in these livers, whereas prompt suppression was observed in matched wild-type animals. These findings confirmed that Insigs are required for feedback regulation of SREBP processing and reductase degradation in liver, as they are in cultured cells. More detailed studies will be required to define the specific roles of each Insig protein and to determine the metabolic consequences of their reciprocal regulation.

Summary

It is noteworthy that the Scap/SREBP transport system and the HMG CoA reductase degradation system both make use of general mechanisms that cells use for many other proteins. Thus, Scap/SREBP is transported by classic COPII-coated vesicles, and the reductase appears to be degraded by the classic ERAD pathway. The unique feature of both the Scap/SREBP and reductase processes is regulation. In regulating cellular sterol concentrations, nature has engrafted a specific control mechanism upon two general processes. It is remarkable that the central regulator in both processes is the same protein—Insig.

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REFERENCES

Adams, C.M., Goldstein, J.L., and Brown, M.S. (2003). Cholesterol-induced conformational change in SCAP enhanced by Insig proteins and mimicked by cationic amphiphiles. Proc. Natl. Acad. Sci. USA *100*, 10647–10652.

Adams, C.M., Reitz, J., DeBrabander, J.K., Feramisco, J.D., Brown, M.S., and Goldstein, J.L. (2004). Cholesterol and 25-hydroxycholesterol inhibit activation of SREBPs by different mechanisms, both involving SCAP and insigs. J. Biol. Chem. *279*, 52772–52780.

Antonny, B., and Schekman, R. (2001). ER export: public transportation by the COPII coach. Curr. Opin. Cell Biol. *13*, 438–443.

Aridor, M., Weissman, J., Bannykh, S.I., Nuoffer, C., and Balch, W.E. (1998). Cargo selection by the COPII budding machinery during export from the ER. J. Cell Biol. *141*, 61–70.

Barlowe, C. (2002). COPII-dependent transport from the endoplasmic reticulum. Curr. Opin. Cell Biol. *14*, 417–422.

Brown, M.S., and Goldstein, J.L. (1997). The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell *89*, 331–340.

Brown, M.S., and Goldstein, J.L. (1999). A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. Proc. Natl. Acad. Sci. USA *96*, 11041–11048.

Brown, M.S., Dana, S.E., and Goldstein, J.L. (1975). Cholesteryl ester formation in cultured human fibroblasts: Stimulation by oxygenated sterols. J. Biol. Chem. *250*, 4025–4027.

Brown, A.J., Sun, L., Feramisco, J.D., Brown, M.S., and Goldstein, J.L. (2002). Cholesterol addition to ER membranes alters conformation of SCAP, the SREBP escort protein that regulates cholesterol metabolism. Mol. Cell *10*, 237–245.

Chen, G., Liang, G., Ou, J., Goldstein, J.L., and Brown, M.S. (2004). Central role for liver X receptor in insulin-mediated activation of *Srebp-1c* transcription and stimulation of fatty acid synthesis in liver. Proc. Natl. Acad. Sci. USA *101*, 11245–11250.

Dawson, P.A., Ridgway, N.D., Slaughter, C.A., Brown, M.S., and Goldstein, J.L. (1989). cDNA cloning and expression of oxysterol binding protein, an oligomer with a potential leucine zipper. J. Biol. Chem. *264*, 16798–16803.

Engelking, L.J., Kuriyama, H., Hammer, R.E., Horton, J.D., Brown, M.S., Goldstein, J.L., and Liang, G. (2004). Overexpression of Insig-1 in the livers of transgenic mice inhibits SREBP processing and reduces insulin-stimulated lipogenesis. J. Clin. Invest. *113*, 1168–1175.

Engelking, L.J., Liang, G., Hammer, R.E., Takaishi, K., Kuriyama, H., Evers, B.M., Li, W.-P., Horton, J.D., Goldstein, J.L., and Brown, M.S. (2005). Schoenheimer Effect explained—feedback regulation of cholesterol synthesis in mice mediated by Insig proteins. J. Clin. Invest. *115*, 2489–2498.

Espenshade, P.J., Cheng, D., Goldstein, J.L., and Brown, M.S. (1999). Autocatalytic processing of Site-1 protease removes propeptide and permits cleavage of sterol regulatory element-binding proteins. J. Biol. Chem. *274*, 22795–22804.

Espenshade, P.J., Li, W.-P., and Yabe, D. (2002). Sterols block binding of COPII proteins to SCAP, thereby controlling SCAP sorting in ER. Proc. Natl. Acad. Sci. USA *99*, 11694–11699.

Fang, S., Ferrone, M., Yang, C., Jensen, J.P., Tiwari, S., and Weissman, A.M. (2001). The tumor autocrine motility factor receptor, gp78, is a ubiquitin protein ligase implicated in degradation from the endoplasmic reticulum. Proc. Natl. Acad. Sci. USA *98*, 14422–14427.

Feramisco, J.D., Goldstein, J.L., and Brown, M.S. (2004). Membrane topology of human insig-1, a protein regulator of lipid synthesis. J. Biol. Chem. *279*, 8487–8496.

Foufelle, F., and Ferre, P. (2002). New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a role for the transcription factor sterol regulatory element binding protein-1c. Biochem. J. *366*, 377–391.

Gil, G., Faust, J.R., Chin, D.J., Goldstein, J.L., and Brown, M.S. (1985). Membrane-bound domain of HMG CoA reductase is required for sterol-enhanced degradation of the enzyme. Cell *41*, 249–258.

Goldstein, J.L., and Brown, M.S. (1990). Regulation of the mevalonate pathway. Nature *343*, 425–430.

Goldstein, J.L., Rawson, R.B., and Brown, M.S. (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*, 139–148.

Gong, Y., Lee, J.N., Lee, P.C.W., Goldstein, J.L., Brown, M.S., and Ye, J. (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.

Hegarty, B.D., Bobard, A., Hainault, I., Ferre, P., Bossard, P., and Foufelle, F. (2005). Distinct roles of insulin and liver X receptor in the induction and cleavage of sterol regulatory element-binding protein-1c. Proc. Natl. Acad. Sci. USA *102*, 791–796.

Horton, J.D., Bashmakov, Y., Shimomura, I., and Shimano, H. (1998). Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. Proc. Natl. Acad. Sci. USA *95*, 5987–5992.

Horton, J.D., Goldstein, J.L., and Brown, M.S. (2002). SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J. Clin. Invest. *109*, 1125–1131.

Horton, J.D., Shah, N.A., Warrington, J.A., Anderson, N.N., Park, S.W., Brown, M.S., and Goldstein, J.L. (2003). Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. Proc. Natl. Acad. Sci. USA *100*, 12027–12032.

Hua, X., Nohturfft, A., Goldstein, J.L., and Brown, M.S. (1996a). Sterol resistance in CHO cells traced to point mutation in SREBP cleavage activating protein (SCAP). Cell *87*, 415–426.

Hua, X., Sakai, J., Brown, M.S., and Goldstein, J.L. (1996b). Regulated cleavage of sterol regulatory element binding proteins (SREBPs) requires sequences on both sides of the endoplasmic reticulum membrane. J. Biol. Chem. *271*, 10379–10384.

Istvan, E.S., Palnitkar, M., Buchanan, S.K., and Deisenhofer, J. (2000). Crystal structure of the catalytic portion of human HMG-CoA reductase: Insights into regulation of activity and catalysis. EMBO J. *19*, 819–830.

Janowski, B.A. (2002). The hypocholesterolemic agent LY295427 upregulates INSIG-1, identifying the INSIG-1 protein as a mediator of cholesterol homeostasis through SREBP. Proc. Natl. Acad. Sci. USA *99*, 12675–12680.

Kim, J.B., Sarraf, P., Wright, M., Yao, K.M., Mueller, E., Solanes, G., Lowell, B.B., and Spiegelman, B.M. (1998). Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. J. Clin. Invest. *101*, 1–9.

Lange, Y., and Steck, T.L. (1997). Quantitatnion of the pool of cholesterol associated with acyl-CoA: Cholesterol acyltransferase in human fibroblasts. J. Biol. Chem. *272*, 13103–13108

Lee, J.N., and Ye, J. (2004). Proteolytic activation of sterol regulatory element-binding protein induced by cellular stress through depletion of

Insig-1. J. Biol. Chem. *279*, 45257–45265.

Lee, P.C.W., Sever, N., and DeBose-Boyd, R.A. (2005). Isolation of sterol-resistant Chinese hamster ovary cells with genetic deficiencies in both Insig-1 and Insig-2. J. Biol. Chem. *280*, 25242–25249.

Liscum, L., Finer-Moore, J., Stroud, R.M., Luskey, K.L., Brown, M.S., and Goldstein, J.L. (1985). Domain structure of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a glycoprotein of the endoplasmic reticulum. J. Biol. Chem. *260*, 522–530.

Luong, A. (2000). Identification of three novel SREBP-activated target genes: acetyl CoA synthetase, 3-β-hydroxysterol dehydrogenase, and CL-6/INSIG1. Ph.D. dissertation. (Dallas: University of Texas Southwestern Medical Center at Dallas), pp. 1–137.

McGarry, J.D. (1998). Glucose-fatty acid interactions in health and disease. Am. J. Clin. Nutr. *67* (*Suppl.*), 500S–504S.

Meusser, B., Hirsch, C., Jarosch, E., and Sommer, T. (2005). ERAD: the long road to destruction. Nat. Cell Biol. *7*, 766–772.

Mohn, K.L., Laz, T.M., Hsu, J.-C., Melby, A.E., Bravo, R., and Taub, R. (1991). The immediate-early growth response in regenerating liver and insulin-stimulated H-35 cells: comparison with serum-stimulated 3T3 cells and identification of 41 novel immediate-early genes. Mol. Cell. Biol. *11*, 381–390.

Nohturfft, A., Brown, M.S., and Goldstein, J.L. (1998a). Sterols regulate processing of carbohydrate chains of wild-type SREBP cleavage-activating protein (SCAP), but not sterol-resistant mutants Y298C or D443N. Proc. Natl. Acad. Sci. USA *95*, 12848–12853.

Nohturfft, A., Brown, M.S., and Goldstein, J.L. (1998b). Topology of SREBP cleavage-activating protein, a polytopic membrane protein with a sterol-sensing domain. J. Biol. Chem. *273*, 17243–17250.

Nohturfft, A., Yabe, D., Goldstein, J.L., Brown, M.S., and Espenshade, P.J. (2000). Regulated step in cholesterol feedback localized to budding of SCAP from ER membranes. Cell *102*, 315–323.

Olkkonen, V.M. (2004). Oxysterol binding protein and its homologues: new regulatory factors involved in lipid metabolism. Curr. Opin. Lipidol. *15*, 321–327.

Peng, Y., Schwarz, E.J., Lazar, M.A., Genin, A., Spinner, N.B., and Taub, R. (1997). Cloning, human chromosomal assignment, and adipose and hepatic expression of the CL-6/INSIG1 gene. Genomics *43*, 278–284.

Ponting, C.P. (2000). Proteins of the endoplasmic-reticulum-associated degradation pathway: domain detection and function prediction. Biochem. J. *351*, 527–535.

Radhakrishnan, A., Sun, L.-P., Kwon, H.J., Brown, M.S., and Goldstein, J.L. (2004). Direct binding of cholesterol to the purified membrane region of SCAP: mechanism for a sterol-sensing domain. Mol. Cell *15*, 259–268.

Ravid, T., Doolman, R., Avner, R., Harats, D., and Roitelman, J. (2000). The ubiquitin-proteasome pathway mediates the regulated degradation of mammalian 3-hydroxy-3-methylglutaryl-coenzyme A reductase. J. Biol. Chem. *275*, 35840–35847.

Repa, J.J., and Mangelsdorf, D.J. (2000). The role of orphan nuclear receptors in the regulation of cholesterol homeostasis. Annu. Rev. Cell Dev. Biol. *16*, 459–481.

Repa, J.J., Liang, G., Ou, J., Bashmakov, Y., Lobaccaro, J.-M.A., Shimomura, I., Shan, B., Brown, M.S., Goldstein, J.L., and Mangelsdorf, D.J. (2000). Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRα and LXRβ. Genes Dev. *14*, 2819–2830.

Ridgway, N.D., Dawson, P.A., Ho, Y.K., Brown, M.S., and Goldstein, J.L. (1992). Translocation of oxysterol binding protein to Golgi apparatus triggered by ligand binding. J. Cell Biol. *116*, 307–319.

Roitelman, J., Olender, E.H., Bar-Nun, S., Dunn, W.A., Jr., and Simoni, R.D. (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*, 959–973.

Russell, D.W. (2000). Oxysterol biosynthetic enzymes. Biochim. Biophys. Acta *1529*, 126–135.

Sakai, J., Nohturfft, A., Cheng, D., Ho, Y.K., Brown, M.S., and Goldstein, J.L. (1997). Identification of complexes between the COOH-terminal domains of sterol regulatory element binding proteins (SREBPs) and SREBP cleavage-activating protein (SCAP). J. Biol. Chem. *272*, 20213–20221.

Seabra, M.C., Mules, E.H., and Hume, A.N. (2002). Rab GTPases, intracellular traffic and disease. Trends Mol. Med. *8*, 23–30.

Sever, N., Song, B.-L., Yabe, D., Goldstein, J.L., Brown, M.S., and DeBose-Boyd, R.A. (2003a). Insig-dependent ubiquitination and degradation of mammalian 3-hydroxy-3-methylglutaryl-CoA reductase stimulated by sterols and geranylgeraniol. J. Biol. Chem. *278*, 52479–52490.

Sever, N., Yang, T., Brown, M.S., Goldstein, J.L., and DeBose-Boyd, R.A. (2003b). Accelerated degradation of HMG CoA reductase mediated by binding of Insig-1 to its sterol-sensing domain. Mol. Cell *11*, 25–33.

Sever, N., Lee, P.C.W., Song, B.-L., Rawson, R.B., and DeBose-Boyd, R.A. (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*, 43136–43147.

Shearer, A.G., and Hampton, R.Y. (2005). Lipid-mediated, reversible misfolding of a sterol-sensing domain protein. EMBO J. *24*, 149–159.

Shimizu, K., Tani, M., Watanabe, H., Nagamachi, Y., Niinaka, Y., Shiroishi, T., Ohwada, S., Raz, A., and Yokota, J. (1999). The autocrine motility factor receptor gene encodes a novel type of seven transmembrane protein. FEBS Lett. *456*, 295–300.

Shimomura, I., Bashmakov, Y., Ikemoto, S., Horton, J.D., Brown, M.S., and Goldstein, J.L. (1999). Insulin selectively increases SREBP-1c mRNA in livers of rats with streptozotocin-induced diabetes. Proc. Natl. Acad. Sci. USA *96*, 13656–13661.

Shimomura, I., Matsuda, M., Hammer, R.E., Bashmakov, Y., Brown, M.S., and Goldstein, J.L. (2000). Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and *ob/ob* mice. Mol. Cell *6*, 77–86.

Skalnik, D.G., Narita, H., Kent, C., and Simoni, R.D. (1988). The membrane domain of 3-hydroxy-3-methylglutaryl-coenzyme A reductase confers endoplasmic reticulum localization and sterol-regulated degradation onto β-galactosidase. J. Biol. Chem. *263*, 6836–6841.

Song, B.-L., Javitt, N.B., and DeBose-Boyd, R.A. (2005a). Insig-mediated degradation of HMG CoA reductase stimulated by lanosterol, an intermediate in the synthesis of cholesterol. Cell Metab. *1*, 179–189.

Song, B.-L., Sever, N., and DeBose-Boyd, R.A. (2005b). Gp78, a membrane-anchored ubiquitin ligase, associates with Insig-1 and couples sterol-regulated ubiquitination to degradation of HMG CoA reductase. Mol. Cell *19*, 829–840.

Sun, L.-P., Li, L., Goldstein, J.L., and Brown, M.S. (2005). Insig required for sterol-mediated inhibition of Scap/SREBP binding to COPII proteins in vitro. J. Biol. Chem. *280*, 26483–26490.

Xu, F., Rychnovsky, S.D., Belani, J.D., Hobbs, H.H., Cohen, J.C., and Rawson, R.B. (2005). Dual roles for cholesterol in mammalian cells. Proc. Natl. Acad. Sci. USA *102*, 14551–14556.

Yabe, D., Brown, M.S., and Goldstein, J.L. (2002a). 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*, 12753–12758.

Yabe, D., Xia, Z.-P., Adams, C.M., and Rawson, R.B. (2002b). 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*, 16672–16677.

Yabe, D., Komuro, R., Liang, G., Goldstein, J.L., and Brown, M.S. (2003). Liver-specific mRNA for Insig-2 down-regulated by insulin: Implications for fatty acid synthesis. Proc. Natl. Acad. Sci. USA *100*, 3155–3160.

Yang, T., Goldstein, J.L., and Brown, M.S. (2000). Overexpression of membrane domain of SCAP prevents sterols from inhibiting SCAP/SREBP exit from endoplasmic reticulum. J. Biol. Chem. *275*, 29881–29886.

Yang, T., Espenshade, P.J., Wright, M.E., Yabe, D., Gong, Y., Aebersold, R., Goldstein, J.L., and Brown, M.S. (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.

Ye, Y., Shibata, Y., Kikkert, M., van Voorden, S., Wiertz, E., and Rapoport, T.A. (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*, 14132–14138.

Zelenski, N.G., Rawson, R.B., Brown, M.S., and Goldstein, J.L. (1999). Membrane topology of S2P, a protein required for intramembranous cleavage of sterol regulatory element-binding proteins. J. Biol. Chem. *274*, 21973–21980.

Zhong, X., Shen, Y., Ballar, P., Apostolou, A., Agami, R., and Fang, S. (2004). AAA ATPase p97/valosin-containing protein interacts with gp78, a ubiquitin ligase for endoplasmic reticulum-associated degradation. J. Biol. Chem. *279*, 45676–45684.