

# Cholesterol homeostasis: **ESCAPE** from the ER

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**Transcriptional regulation and membrane traffic have traditionally been quite separate fields of biology, but they have been brought under the same roof by recent advances in understanding the cellular control of cholesterol metabolism.**

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Mammalian cells tightly regulate the levels of many proteins responsible for cholesterol synthesis and uptake [1], including enzymes of sterol synthesis, such as HMG-CoA reductase, and the LDL receptor that mediates endocytosis of cholesterol-rich LDL particles. A substantial component of this parallel regulation of sterol synthesis and uptake occurs at the level of transcription. Abundant cholesterol decreases transcription of the relevant target genes, whereas a scarcity of cholesterol promotes their transcription. This is only part of the fascinating biology of sterol regulation, but the focus of this dispatch will be on transcriptional regulation. For close to thirty years, Brown, Goldstein and colleagues have been committed to understanding how sterols regulate transcription [2]. Their

studies have revealed an astonishing number of cellular insights and unlikely connections. The latest surprise is an unexpected link between transcriptional regulation and cellular membrane traffic [3].

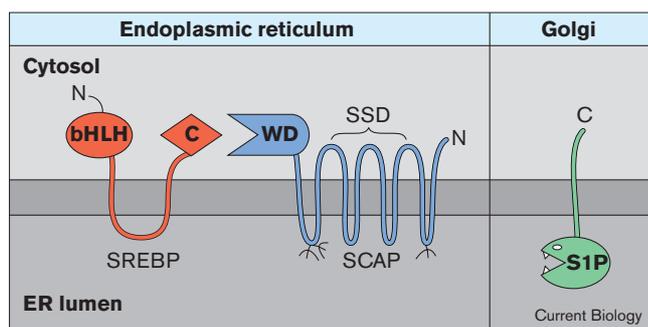
## **SREBP: ER bound for glory**

The promoters of all sterol-regulated genes include a regulatory sequence known as the sterol response element (SRE), which will faithfully impart physiologically correct cholesterol regulation to heterologous reporter genes. So far, this sounds like any number of transcriptionally regulated genes. But the identification and study of the proteins responsible for transcription and sterol control yielded unexpected connections to membrane traffic. As might be expected, a transcription factor exists that specifically binds SRE — not surprisingly, this is known as the SRE binding protein (SREBP). The isolation of SREBP was a prodigious task, requiring numerous approaches and the efforts of many talented people [4].

There are actually two isoforms of this protein, SREBP1 and SREBP2, and three splice variants of isoform 1. All have similar structure and behavior, and in this work we will refer to a ‘consensus’ protein SREBP. SREBP has an unusual structure that plays a central role in regulation [5] (Figure 1). The amino-terminal half of SREBP is sufficient to mediate SRE-dependent transcription, and the sequence of this part of the protein shows that SREBP is a member of the large basic-helix–loop–helix/leucine zipper family of transcription factors. In the full-length protein, this transcription factor region is connected to a membrane anchor with two transmembrane spans, a small luminal loop, and a large cytosolic carboxy-terminal region. Full-length SREBP is attached to the endoplasmic reticulum (ER), with the amino-terminal region facing the cytosol.

The transcriptionally active amino-terminal portion of SREBP must be cleaved from the membrane anchor in order to enter the nucleus and activate transcription. It is the production of this soluble, active form that is regulated by sterols [4]. High cholesterol levels inhibit cleavage and so decrease the amount of active, soluble SREBP, whereas low cholesterol levels promote cleavage and so increase the amount of soluble SREBP. Fancy, and novel. Release of the amino-terminal region actually occurs by two proteolytic cleavages at specific sites in the membrane-bound SREBP [6]. The first cleavage takes place in the luminal loop, and the second occurs in the first membrane span, near enough to the cytosolic face to release the soluble portion. Only the first cleavage is regulated by sterols, and the second one can only proceed after the first has taken place.

**Figure 1**



Key players in cholesterol-regulated transcription. The proteins SREBP, SCAP and S1P are shown with key features. SREBP: the basic-helix–loop–helix (bHLH) region is an active transcription factor when released from the ER membrane anchor. The carboxy-terminal region mediates binding to SCAP. SCAP: the WD domain binds the carboxy-terminal region of SREBP (C); the ‘birds feet’ represent glycosylation sites that were important in initial formulation of the regulated-transport model; and SSD is the sterol-sensing domain. S1P: the bulk of the protein, including the protease catalytic region, is in the ER lumen. In all cases, N and C refer to the amino terminus and the carboxyl terminus, respectively.

The identity of SREBP was brought to light by heroic biochemistry. The other players (so far) in sterol-regulated processing of SREBP were discovered through the use of somatic cell genetics. Over the past 20 years, a variety of mammalian cell lines have been derived with abnormal cholesterol homeostasis. Analysis of these lines, along with generation of new ones, led to identification of mutants deficient in specific aspects of SREBP processing, including the first, sterol-regulated cleavage [7], the second cleavage [8], and the sterol regulation of normal cleavage [9]. Through the use of thoroughly clever expression cloning strategies, the wild-type gene defective in each mutant line was cloned. These efforts identified the two distinct membrane-bound proteases responsible for the first and second cleavage reactions, called S1P and S2P, respectively, and ‘SREBP cleavage activating protein’ (SCAP), the key protein in the coupling of SREBP cleavage rate to cellular levels of sterols.

#### SCAP: a guide to the Golgi

SCAP resides in the ER, and consists of a hydrophobic amino-terminal domain with eight transmembrane spans, and a carboxy-terminal region with five WD repeats [9] (Figure 1). SCAP binds tightly to SREBP *in vivo*, through interaction between the WD domains and the SREBP carboxy-terminal region [10]. The membrane anchor of SCAP has a region called the ‘sterol-sensing domain’ that is implicated in cholesterol interactions in a variety of proteins. The sterol-sensing domain of SCAP is required for sterol-regulated cleavage of SREBP — a single residue change in this region causes the sterol-insensitivity observed in the cell line from which SCAP was cloned. The original model derived from these studies was that SCAP orchestrates S1P-catalyzed cleavage of SREBP by forming a complex whose efficiency is altered by sterols [11].

A critical hint that things could actually be different arose in the study of SCAP biochemistry [12,13]. SCAP has two *N*-linked glycosylations on one of its luminal loops (Figure 1). Despite the bulk ER localization of SCAP, these decorations are partially resistant to endoglycosidase H, a biochemical hallmark of a visit to the early Golgi compartment, and studies with inhibitors and other enzymes confirmed that the unexpected endoglycosidase H resistance is indeed due to Golgi enzymes. Furthermore, this biochemical feature of SCAP is affected by cellular sterol content. When sterols are abundant, the SCAP glycosylations are endoglycosidase H sensitive. Conversely, when cellular sterols are low, SCAP glycosylations are modified to endoglycosidase H resistance by Golgi enzymes. These observations were interpreted to mean that SCAP cycles between the early Golgi and the ER, and that high sterol content specifically blocks the ER-to-Golgi movement of SCAP by interacting with, or somehow affecting, the sterol-sensing domain (Figure 2a).

As mentioned above, SREBP resides in the ER. But as shown by the recent work of DeBose-Boyd *et al.* [3], the active form of S1P, called S1P-C, resides in a Golgi-like compartment where it is activated. As SREBP binds to SCAP, and SCAP cycles through the Golgi in a sterol-regulated manner, it seemed possible that S1P cleavage of SREBP could be regulated through SCAP-mediated delivery of SREBP to the Golgi, where it would meet active S1P. In the latest addition to the story, DeBose-Boyd *et al.* [3] have tested these ideas with an elegant set of experiments. The ‘escort’ model of SCAP action makes several strong predictions. If SCAP serves only to bring SREBP to the compartment where active S1P resides, then bringing S1P to where most of the SREBP resides should override both sterol regulation and SCAP-dependence of SREBP processing.

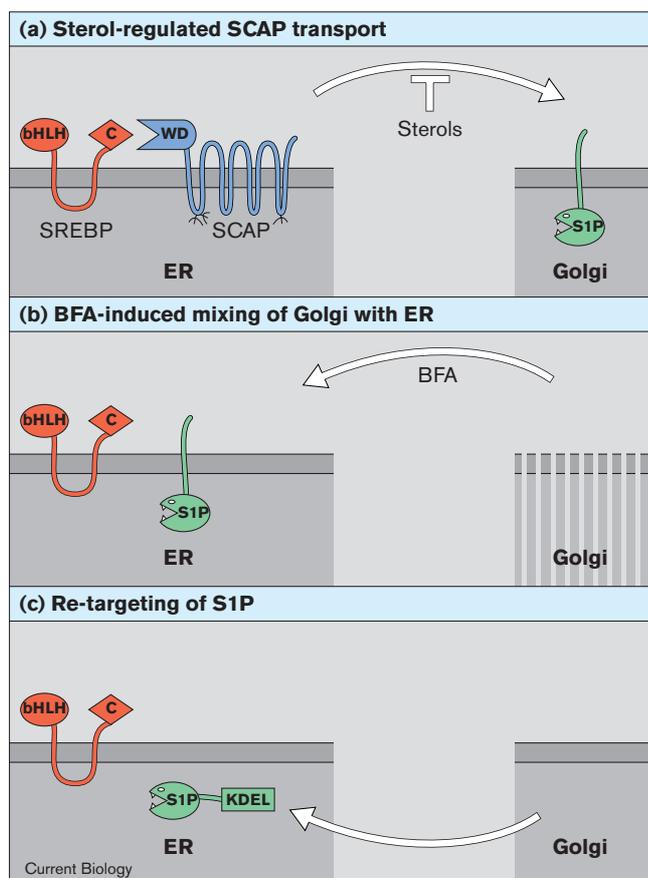
#### S1P meets its match: location, location, location

This ‘mountain to Mohammed’ experiment was done both by pharmacological and molecular biological means. The drug brefeldin A (BFA) causes disassembly of the Golgi, and rapid mixing of Golgi and ER contents. If exposure to Golgi-localized S1P determines SREBP cleavage, then BFA treatment should cause S1P-catalyzed cleavage (Figure 2b). DeBose-Boyd *et al.* [3] found that indeed it does — in fact, it causes complete processing of SREBP to the active nuclear form. BFA-induced processing of SREBP was unaffected by high sterol levels, and thus was not regulated. Importantly, BFA-induced processing of SREBP still occurred in a mutant cell line missing SCAP, but failed to occur in a line missing S1P. Thus, SCAP was not needed for SREBP processing in conditions where S1P and SREBP were allowed to mingle.

To test if SREBP processing could be brought about by mislocalization of only S1P, DeBose-Boyd *et al.* [3] took advantage of a well-known cellular ‘traffic law’. The KDEL — Lys-Asp-Glu-Leu — sequence is a luminal ER retention signal, which mediates retrieval of proteins from the early Golgi and delivery back to the ER. The authors engineered a modified version of S1P, S1P-KDEL, consisting of the soluble, luminal domain of S1P with an added KDEL sequence at the carboxyl terminus. Because of the added signal, S1P-KDEL goes to the Golgi to be activated, but it ultimately resides in the ER (Figure 2c). By the SCAP-traffic model, expression of S1P-KDEL should allow SREBP processing that is not inhibited by elevated sterols, and is not dependent on SCAP. And this is exactly what was found to occur! Interestingly, mislocalization of only active S1P to the ER results in complete processing of SREBP, indicating that either some S2P resides in the ER, or that S1P-cleaved SREBP can go to where S2P does reside.

The work of DeBose-Boyd *et al.* [3] clearly shows that exposure of SREBP to activated S1P is the critical event in

Figure 2



Regulating SREBP cleavage by exposure to S1P. **(a)** SCAP movement to the Golgi brings SREBP along for the ride (arrow), exposing it to S1P. SCAP movement to the Golgi is inhibited by high sterol content (T bar). **(b)** Brefeldin A (BFA) induces mixing of Golgi and ER contents, overriding SCAP-mediated, sterol control of S1P cleavage of SREBP. **(c)** KDEL-modified S1P makes a visit to the Golgi where it is activated, and then takes up residence in the ER, again overriding regulation of SREBP cleavage.

cholesterol regulation of transcription. SCAP's role appears to be to ferry SREBP to this protease, and neither cholesterol nor SCAP seem to be involved with either cleavage. There are several important issues to be explored in the framework of this new model. The view that SREBP is moved to the Golgi where it is exposed to active S1P is quite reasonable, as the bulk of active S1P appears to reside in the Golgi, the bulk of SREBP resides in the ER, SCAP moves between them in a sterol-regulated manner, and SCAP binds SREBP but apparently not S1P.

Nevertheless, it still is possible that molecules of active, Golgi-resident S1P are moved to the ER by SCAP, and efficiently directed to SREBP by SCAP's ability to bind the substrate. It will be important to directly examine the transport of SREBP, either by biochemical or other means, as a key test of the favored — SREBP to Golgi — model. A

related question concerns the fate of the cleaved, carboxy-terminal portion of SREBP. Does it move to the Golgi? Does it ride back to the ER with SCAP? Or is it perhaps degraded by the secretory pathway quality-control mechanisms? This is a potentially important question, as an excess of the leftover carboxy-terminal region could possibly compete with full-length SREBP for binding to SCAP, as it does when produced by molecular biological means [10].

Understanding the controlled transit of SCAP and SREBP (or S1P?) is likely to provide many important insights relevant to cholesterol regulation, and will address numerous issues of specificity and targeting in membrane traffic. It would be quite impressive and useful to harness the newly available optical tools — such as green fluorescent protein (GFP) and deconvoluting confocal microscopy — to directly observe sterol-regulated transport *in vivo*.

#### Where SCAP might take us: from sterols to soil, and beyond

The sterol-sensing domain appears to contain information that mediates cholesterol regulation of SCAP. Related sterol-sensing domains are found in several other proteins associated with cholesterol [14]. These include the sterol-synthetic enzyme HMG-CoA reductase [15], the hedgehog receptor Patched and its homologue Dispatched [16–18], and the Neimann-Pick C1 gene product [19,20]. How does the sterol sensing-domain operate? Proteins with sterol-sensing domains seem to have quite varied connections to cholesterol: SCAP undergoes altered intracellular transport; HMG-CoA reductase is subjected to sterol-regulated degradation; Patched is a receptor for the only known ligand, Hedgehog, that has a covalently attached cholesteryl moiety; Dispatched appears to be involved in presenting or processing that ligand; and the Neimann-Pick C1 protein is important for intracellular movement of endocytosed cholesterol.

These disparate connections to cholesterol do not immediately suggest a unified action for the sterol-sensing domain. Most popular is the model that the sterol-sensing domain serves as a sterol-binding site, the ligation of which causes protein-specific consequences — such as transport, degradation, ligand binding or presentation, or sterol transport. Alternatively, the sterol-sensing domain might allow a protein to respond to some sterol-sensitive features of the membrane, or perhaps it interacts with as yet undiscovered sterol-binding proteins. Whatever the mechanism of sterol-sensing domain action, the easily-observed readout of this domain's function provided by sterol-regulated SCAP transport should go a long way towards providing an understanding of this widely used yet mysterious motif.

Perhaps the most exciting feature of this new view of SCAP action is that it may represent a general regulation strategy. Because the intracellular transport and localization

of proteins in the eukaryotic secretory pathway can be brought about by small sequence modules that function with relative autonomy from the proteins they are targeting, it is easy to imagine that this strategy of regulation could be widely employed in the evolution of cellular regulatory mechanisms. Even in prokaryotes, where discrete secretory compartments are not maintained, there are numerous examples of targeted localization to specific membranes. With this in mind, it is intriguing that the *Bacillus subtilis* transcription factor  $\sigma^K$  has striking similarities to SREBP, undergoing regulated proteolytic activation by an S2P homologue, SpoIVFB [21]. Considering the distance between a mammalian cell and a humble soil bacterium, this observation bodes well for the idea that regulation by compartmentalization will turn out to be a very big deal.

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